

SELECTIVE CONTROL OF EGYPTIAN BROOMRAPE (*OROBANCHE AEGYPTIACA*
PERS.) BY GLYPHOSATE AND ITS AMINO ACID STATUS IN RELATION TO
SELECTED HOSTS

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Keywords: Egyptian broomrape, *Orobancha aegyptiaca*, Glyphosate, Amino acid

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Abstract

Broomrapes are achlorophyllous holoparasites of many economically important dicotyledonous crops. As weeds, they cause reductions in crop yield, adversely affect crop quality, and result in loss of cultivated land due to reduced crop alternatives. Few effective control measures exist for broomrapes. One of the most promising approaches is the use of low rates of glyphosate in hosts with tolerance to the herbicide. Recently, availability of glyphosate-resistant crops has provided an alternative in broomrape infested areas. Knowledge about the nitrogen status of broomrapes is essential for developing new control strategies. Broomrapes have two potential sources of amino acids. First, the haustorium aids in the translocation of amino acids from the host plant to the parasites. Second, broomrapes may be able to synthesize some amino acids themselves and obtain the rest from the host. However, the relative importance of these two modes of acquiring amino acids by broomrapes is not clear. Osmotic stress has been implicated as a possible reason for inhibition of broomrape germination by nitrogen. To date, there has been no attempt to correlate osmotic potential with nitrogen induced inhibition of broomrape germination. Optimum temperatures for conditioning and germination are different among broomrape species. Although temperature is known to influence germination in broomrape, its effect on subsequent development of the parasitic seedling has not been studied. Studies were conducted to determine the use of glyphosate in controlling broomrape in common vetch that is tolerant to low rates of glyphosate, and to compare this response with broomrape control in oilseed rape that has been genetically engineered for glyphosate resistance. Glyphosate dose response studies using a commercial formulation and patterns of absorption, translocation, and metabolism, using ^{14}C -glyphosate, were determined for both host crops. Glyphosate significantly reduced the growth of broomrape at 0.18 and 0.36 kg ae ha⁻¹ in common vetch

and 0.25 to 0.75 kg ha⁻¹ in oilseed rape. More than 25% of translocated ¹⁴C-glyphosate in both host crops accumulated in broomrape tubercles. Broomrape parasitism caused a redistribution of translocated ¹⁴C-glyphosate in the roots of both host crops. Glyphosate was metabolized up to 25% in common vetch, but remained intact in oilseed rape. Studies were conducted to analyze amino acid composition of both nonparasitized and broomrape-parasitized hosts and associated broomrape after hydrolysis and phenylisothiocyanate derivatization of amino acids. Results indicated that amino acid concentrations of leaves of parasitized carrot plants were lower than those of the leaves of nonparasitized carrot plants. Broomrape tubercles had equal or higher amino acid concentrations compared to those of the leaves of nonparasitized carrot plants. Levels of free alanine and arginine concentrations of broomrape callus were higher than those of any other tissue of either carrot or broomrape. The effect of glyphosate on the host-broomrape interaction regarding amino acid metabolism was examined. Glyphosate generally increased the amino acid concentrations in common vetch and oilseed rape plants, and broomrape attachments. The aromatic amino acids, phenylalanine and tyrosine, did not differ from this pattern. Concentrations of certain amino acids in broomrape were similar to those of parasitized common vetch and parasitized oilseed rape, whereas levels of several others, were higher in broomrape attachments compared to the host plants. *In vitro* studies were conducted to determine the influence of osmotic potential and temperature on broomrape germination. Osmotic potential significantly affected germination and radicle elongation of broomrapes. No correlation was found between osmotic potential and ammonium-induced inhibition of germination of broomrapes. Temperature significantly influenced germination and radicle elongation of all broomrape species tested.

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1. LITERATURE REVIEW

1.1 INTRODUCTION

Parasitic plants are those that are dependent on other autotrophic plants for survival, for part or all of their life cycle. They have an unusual biology with respect to autotrophic plants and are economically important. Parasitism has evolved independently in higher plants (Kuijt 1969). Parasitic plants belong to 17 different families, but only eight of these contain plants that are considered weeds. Witchweed (*Striga* spp.) and broomrape (*Orobanche* spp.) are the most economically important parasitic weeds in cultivated crops. The following discussion is focused primarily on management of broomrapes. However, literature on witchweed and other similar parasitic weeds is cited to illustrate certain aspects of biology of broomrapes where research is lacking.

Broomrapes are phanerogamic holoparasites that attack the roots of many dicotyledonous crops. They lack chlorophyll (Baccarini and Melandri 1967; Saghir et al. 1973) and obtain carbon, nutrients, and water through haustoria which connect the parasites with the host vascular system.

Broomrapes belong to the family Orobanchaceae. The genus *Orobanche* has more than 150 species (Musselman 1980) among which only a few parasitize agronomic crops. Broomrapes vary in host range, some parasitizing a broad range of crops, whereas others are more specific. *O. ramosa* L. has the widest host range, parasitizing many solanaceous crops such as potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.) and tomato (*Lycopersicon esculentum* Mill.), members of Brassicaceae, Leguminaceae, and several other families. *O. aegyptiaca* Pers. has a host range similar to that of *O. ramosa*, and is also parasitic on carrot (*Daucus carota* L.), legumes such as common vetch (*Vicia sativa* L.), and crucifers including oilseed rape (*Brassica napus* L.). In the Middle East, *O. crenata* Forsk. has a debilitating effect on broad bean (*Vicia faba* L.), and also attacks carrot. *O. cernua* Loefl. and *O. cumana* Wallr. are extremely damaging to sunflower (*Helianthus annuus* L.).

1.2 GEOGRAPHIC DISTRIBUTION

The majority of broomrapes are found in the warm and temperate parts of the northern hemisphere, especially the Mediterranean region (Sauerborn 1991), but some species have spread to many other parts of the world. *O. aegyptiaca* occurs mainly in southeastern Europe, northeastern Africa, and the Middle East, whereas *O. ramosa*, which is closely related to *O. aegyptiaca*, is mostly found in the Middle East. *O. cernua* and *O. cumana* are primarily distributed in the Middle East, southern and eastern Europe, and northern Africa. *O. crenata* is restricted to the Middle East.

1.3 ECONOMIC IMPORTANCE

It is no wonder that broomrapes are called ‘halook’ in Egypt, which refers to ancient invaders who ransacked Egypt, and ‘aleket’ in Hebrew, meaning bloodsucking leeches. They cause extensive damage by reducing the yield of parasitized crops. For example, in the former USSR, *O. aegyptiaca* caused a 50% reduction in yield of watermelon [*Citrullus lanatus* (Thunb.) Mansf.] (Panchenko 1974), 13 to 52% in muskmelon (*Cucumis melo* L.), and 15% in tomato (Kabulov and Tashpulatova 1974). Yield losses have also been reported in broad bean (Mesa-García and García-Torres 1982, 1984; Mesa-García et al. 1984; Zaitoun et al. 1991), sunflower (Malykhin 1974; Shalom et al. 1988), tobacco (Emiroglu et al. 1987; Krishnamurthy et al. 1977b), and tomato (Cordas 1973; Hodossy 1981). Although it is hard to make exact estimates of the above yield losses due to the difficulty in creating broomrape-free plots for comparison with infested plots, the potential for loss in crop yield due to broomrape infestations is never over estimated.

Broomrape infestations have been reported to decrease the area under cultivation. Broad bean is a major legume crop in Egypt, Morocco, and the Middle East, and is subject to devastation by *O. crenata*. Zahran et al. (1979, cited in Sauerborn 1991) reported that the actual area under broad bean cultivation decreased by 30% in one decade (between 1968 and 1978) because of *O. crenata* infestations (quoted in Sauerborn 1991). Sauerborn (1991)

estimated that in just four countries, Morocco, Portugal, Spain, and Syria, 63% of the planted area under broad bean (200,000 ha) is infested with *O. crenata*. This area may have been even larger if not for farmers abandoning planting of broad bean in fields known to be infested with broomrape (Parker and Riches 1993). In Yugoslavia, 37% of the sunflower cropping area was lost due to broomrape in the 1950s (Mijatovic and Stojanovic 1973).

Besides causing yield loss and reduction in cultivated area of crops, broomrapes also reduce crop quality. The presence of broomrape plant material in harvested crop produce may reduce the value of the crop or make it unmarketable. For instance, in Israel, the value of hay was reduced due to the presence of broomrape stalks (Foy et al. 1989). Broomrape attack resulted in yield losses as well as reduction in quality of tobacco in Cuba (Parker and Riches 1993) and in India (Krishnamurthy et al. 1977b).

Broomrapes reduce the number of crop alternatives available to farmers. The presence of broomrape in a field may force farmers to plant a less economical, non-host crop or to leave the field fallow. For instance, many productive carrot and solanaceous vegetable growing areas had to be abandoned in Israel due to broomrape infestations and lack of other economically viable crop choices (Foy 1996).

1.4 GROWTH AND DEVELOPMENT OF BROOMRAPES

1.4.1 Seeds

Broomrapes are annuals that reproduce by seeds. Seeds are usually dark brown, oval shaped, measure 0.35 x 0.25 mm (Kadry and Tewfic 1956), and weigh 3 to 6 μg (Parker and Riches 1993). They have a pattern of raised ridges on their surface. There is a hardened testa, surrounding a fatty endosperm that has an undifferentiated embryo at one end (Kadry and Tewfic 1956). The number of seeds per plant varies from 10^5 to 5×10^5 , depending on the species.

1.4.2 Germination

Broomrape seed germination occurs only in response to a chemical signal from the host root. Before germination, broomrape seeds must undergo conditioning under suitable temperature and moisture conditions. The conditioning phase may range from five to several days, depending on the species. The requirement of conditioning of the seeds is not completely understood. Following the conditioning phase, the seed produces a 'germ tube' or radicle in response to a chemical stimulant from the host root. The stability of the chemical stimulant is very short-lived in the soil.

Several factors influence germination of broomrapes in the soil including temperature, moisture, pH, nutrients, soil type, and stimulants produced by host plants. The influence of osmotic stress and temperature on broomrape germination and subsequent development is briefly discussed.

Reports of inhibitory effects of nitrogen on the growth of broomrapes, including germination have been common in the literature for many years (reviewed by Parker and Riches 1993; Sauerborn 1991). Osmotic stress has been implicated as a possible reason for inhibition of broomrape germination by nitrogen (Ernst 1986). Wegmann (1986) observed that broomrape has a lower osmotic potential compared to the host caused by mannitol synthesis, and hence its ability to retain water and nutrients. Germination tests at different osmotic potentials demonstrated an adaptation of the germ tube of *O. ramosa* to dry habitats (Linke 1987). To date, there has been no attempt to correlate osmotic potential with nitrogen induced inhibition of broomrape germination.

Optimum temperatures for conditioning and germination are different among broomrape species. Studies on the effect of temperature on germination of *O. aegyptiaca*, *O. crenata*, and *O. cumana* indicated that every species had a specific optimum temperature range for germination and development which generally reflected its geographical distribution (Sauerborn 1991). Kasasian (1973a) showed that optimum temperatures for both conditioning and germination were about 18 C for *O. crenata* and about 23 C for *O. ramosa*. Similarly, Weldeghiorghis and Murdoch (1996) reported an optimal temperature of 18 C for

O. crenata germination. van Hezewijk et al. (1991b, 1993) reported an optimum conditioning temperature of 15 to 20 C for *O. crenata*. Although temperature is known to influence germination in broomrape, its effect on subsequent development of the parasitic seedling has not been studied.

1.4.3 Radicle Elongation and Attachment to Host

After germination, the radicle elongates by cell division and extension (Parker and Riches 1993), and attaches to host roots mainly in the region of root elongation and absorption (Foy et al. 1989). The tip of the radicle enlarges as soon as it attaches to the host root and forms a 'haustorium'. Subsequently, the haustorial tissue penetrates the host root by enzymatic degradation, rather than mechanical destruction (Dörr and Kollman 1974), and establishes connections with the host vascular system. It is by these connections that the parasite derives its nutrients and water from the host.

Until recently, no direct connections have been reported between the host phloem tissue and the haustorial cells except for contact cells (Dörr and Kollman 1975) or polymorphic cells (Pennypacker et al. 1979) that were tightly appressed to phloem sieve cells of the host. These undifferentiated cells, which are mostly parenchymatous in nature, probably absorb nutrients from the sieve cells via the sieve areas and transport the nutrients to the parasite. Dörr and Kollman (1995) have recently reported interspecific sieve pores derived from interspecific plasmodesmata at the point where broomrape and the host cells differentiate into sieve elements. Direct connections between haustorial tissue and the xylem of the host were observed (Dörr and Kollman 1976; Pennypacker et al. 1979; Saghir et al. 1973; Visser and Dörr 1987).

The part of the broomrape seedling outside the root of the host swells to form a tubercle. After 1 to 2 wk of growth, a shoot bud develops on the tubercle producing a flowering spike which elongates, and emerges above the soil.

1.5 MANAGEMENT OF BROOMRAPES

Management of broomrapes is often difficult due to several reasons. These include the high amount of seed production, viability of seeds in the soil over several years (Cubero and Moreno 1979; Linke and Saxena 1991a; Puzzilli 1983), lack of seed germination in the absence of a chemical trigger from a suitable host, vigorous growth habit after emergence, and close association with the host crop. Several means for managing broomrapes have been tried over the years, albeit with somewhat limited effectiveness.

1.5.1 Mechanical and Physical Methods

1.5.1.1 Hand Weeding and Tillage

Hand weeding is the most commonly practiced method of controlling broomrapes in the developing countries and is recommended only under conditions of light infestations. However, it is time consuming and labor intensive, and only limits seed production. It was reported that three years of hand weeding could control *O. cernua* in tobacco in India (Krishnamurthy and Rao 1976), but the problem remained. Tillage is not a feasible control strategy due to the very late emergence of broomrape shoots in the growing season and the risk of crop injury due to close association of the parasite with the host.

1.5.1.2 Deep Inversion Plowing and Fire

Several strategies, that physically affect broomrape seeds, such as deep inversion plowing, fire, and soil solarization have been tried. Placement of seeds at 20-cm depth was observed to cause little emergence of *O. cernua* (Krishnamurthy et al. 1987). However, the buried seeds could be brought up by subsequent tillage. Parker and Riches (1993) propose

burning of residue from infested crops to reduce carry over of broomrape seeds back to the soil.

1.5.1.3 Soil Solarization

Solarization is defined as the method of covering moist soil with a clear polyethylene sheet and heating the soil by solar radiation. The temperature of the soil is increased, and the polyethylene cover preserves moisture and at the same time prevents temperature loss. This process can increase the temperature of covered soil by 10 C compared to uncovered soil. *O. aegyptiaca* (Jacobsohn et al. 1980; Sauerborn and Saxena 1987), *O. crenata* (Sauerborn and Saxena 1987), and *O. ramosa* (Braun et al. 1987) infestations have been reduced by 90 to 100% using solarization. The biggest limitation to this method however, is the high cost of the polyethylene (Foy et al. 1989). Availability of appropriate machinery and cloud-free sunny days may further restrict use of this method.

1.5.2 Cultural Methods

1.5.2.1 Trap and Catch Crops

Trap and catch crops have been used in crop rotations to reduce the broomrape seed bank in the soil. Trap crops cause germination of broomrape seeds without themselves being attacked (Musselman 1980), whereas catch crops are susceptible to attack by broomrapes. Several trap crops such as flax (*Linum usitatissimum* L.) (Abu-Irmaileh 1982; Kleifeld et al. 1994; Krishnamurthy et al. 1977a), beans (*Phaseolus* spp.) (Abu-Irmaileh 1982), and broad bean, and catch crops like berseem (*Trifolium alexandrinum* L.) (Al-Menoufi 1991) and sweet pepper (*Capsicum frutescens* L.) (Abu-Irmaileh 1982) have been used with varying degrees of success. The use of trap and catch crops to reduce broomrape infestations is limited due to the fact that there are vast amounts of broomrape seeds dispersed in the soil and only a small

proportion may be exposed to germination stimulants in the rhizosphere of trap and catch crops (Foy et al. 1989).

1.5.2.2 Sowing Date and Cropping Density

The degree of infestation by broomrapes is closely related to the sowing date of the host crop (Sauerborn 1991). Delay of the sowing date has resulted in reduced parasitism of broad bean and lentil (*Lens culinaris* Medic.) by *O. aegyptiaca* (Sauerborn 1991). This strategy takes advantage of the optimum seasonal temperatures of broomrape seed germination, but is useful only when early maturing varieties are available to compensate for the loss in yield due to the short vegetation period of a conventional variety under late sowing conditions.

Increasing density of broad bean reduced competition from *O. crenata* (Pieters and Aalders 1986) and number of attachments of *O. crenata* (Manschadi et al. 1997). However, increase in other inputs such as seeds, cultivation, fertilizer, and pesticides may result in higher production costs.

1.5.2.3 Host Plant Resistance/Tolerance

Host plant resistance to broomrapes is another approach to limit broomrape infestations. Most of the work on breeding for resistance to broomrapes has been done in sunflower against *O. cernua* and in broad bean against *O. crenata*. In Russia, certain varieties of sunflower were found to be resistant to *O. cernua* in 1912, but the resistance broke down in the late 1920s due to existence of different races of *O. cernua* (Cubero 1986, 1991; Pustovoit 1967, 1976). Similarly, since the early 1990s, several virulent races of *O. cernua* have emerged in Spain jeopardizing the sunflower industry in Spain (García-Torres et al. 1993). Cubero (1991) has summarized the work done in Italy, Spain, and Egypt which showed various degrees of susceptibility in broad bean to broomrape. Only one broad bean variety with resistance to *O. crenata*, F-402 that was identified in Egypt, has been successfully

used in breeding programs (Nassib et al. 1979, 1982, 1984). Reports of resistance are available in several other crops, including common vetch (Gil et al. 1982, 1984; Goldwasser et al. 1996), eggplant (*Solanum melongena* L.) (Dalela and Mathur 1971a), mustard (*Brassica* spp.) and rape (Dalela and Mathur 1971b), tobacco (Dalela and Mathur 1971a; Racovitza 1973), tomato (Avdeev and Shcherbinin 1976; Dalela and Mathur 1971a). Much effort is required in the study of inheritance of resistance and variability in pathogenicity of the parasite mechanism (Cubero 1991).

1.5.3 Nutrient Management (Nitrogen)

1.5.3.1 Nitrogen Fertilization

During their evolution, parasitic plants have acquired the ability to obtain nutrition from host plants and have adapted to prefer less fertile soil conditions (Sauerborn 1991; ter Borg 1986). Reports of inhibitory effects of nitrogen on the growth of broomrapes, based on field, greenhouse, and laboratories studies, have been common in the literature going back to the 19th century, when farmers were using manure and compost to reduce broomrape growth (Manoja 1854, cited in Ciccarone and Piglionica 1979).

In general, the ammonium form of nitrogen has been found to be more inhibitory on broomrapes than the nitrate form, while urea has an intermediate effect. Pieterse (1991) demonstrated that the germination and growth of *O. crenata* were severely affected when exposed to 4 mM nitrogen in the form of urea or ammonium during conditioning and germination phases, while the nitrate form had no effect. Jain and Foy (1987, 1992) reported similar results with *O. aegyptiaca* when nitrogen was applied during the preconditioning period. Further, van Hezewijk and Verkleij (1996) have shown that application of ammonium sulfate at 8 mM in combination with a nitrification inhibitor during the conditioning phase reduced germination of *O. crenata*. This effect was more pronounced with 4 mM ammonium sulfate applied with a nitrification inhibitor during the germination phase. Reduced germination and radicle length were observed in *O. ramosa*, grown in association with host

crop seedlings in response to application of ammonium nitrate (Abu-Irmaileh 1994).

The inhibitory effect of ammonium was also evident in greenhouse experiments. Abu-Irmaileh (1979, 1981) was able to obtain good suppression of *O. ramosa* in tomato and tobacco with ammonium sulfate at 4 g kg⁻¹ of soil, but yields of tomato were reduced if both phosphorus and potassium were not increased. Similar results were obtained by Jain and Foy (1987, 1992) with *O. aegyptiaca* in tomato, where 1 g N (in the form of ammonium nitrate) kg⁻¹ soil reduced broomrape emergence but inhibited tomato growth; addition of 1 g phosphate (as potassium phosphate) kg⁻¹ soil increased growth of tomato to 3 times that of the infested control.

Some controlled field experiments have been done to investigate the effectiveness of nitrogen fertilization in reducing broomrape infestations. van Hezewijk *et al.* (1991a) demonstrated significant reductions of *O. crenata* in broad bean following applications of ammonium sulfate equivalent to 14 and 28 kg N ha⁻¹. Kukula and Masri (1984) were able to reduce the number of emerging broomrape shoots with 40 kg ha⁻¹ ammonium nitrate and to increase the yield of broad bean significantly. Both the number of *O. ramosa* shoots and their dry weight decreased in tomato when N in the form of ammonium sulfate and ammonium nitrate was increased from 0 to 16 kg ha⁻¹ (Demirkan and Nemli 1994).

In summary, nitrogen has some inhibitory effect on growth of broomrapes. Ammonium form of nitrogen has been found to reduce broomrape parasitism to a greater extent than that caused by either urea or nitrate form. Further field research is required to accurately predict the magnitude of effect of nitrogen on broomrape infestations.

1.5.3.2 Nitrogen Metabolism in Broomrapes

Autotrophic plants receive nitrogen by N₂ fixation and/or absorption of nitrate from the soil. The process of N₂ fixation (reduction) to ammonium is achieved by several prokaryotic microorganisms, the most important of which is *Rhizobium* that is symbiotically associated with legume roots. N₂ fixation is dependent on adenosine triphosphate (ATP), a source of electrons, and an enzyme complex called nitrogenase. Ammonium formed by N₂

fixation is then absorbed by plant roots and assimilated into organic compounds. Nitrate is the primary source of nitrogen for most plants in their natural habitat and most of nitrate reduction to ammonium occurs at the site (roots or shoots) at which the most nitrate reductase activity occurs. Nitrate reduction is catalyzed by two different enzymes, nitrate reductase and nitrite reductase (Hewitt 1975).

The first organic compound into which ammonium is incorporated is glutamine, catalyzed by glutamine synthetase (GS) (Mifflin and Lea 1980). GS occurs in two isoforms in the leaf tissue of higher plants (McNally et al. 1983a, 1983b). One isoform is found in the chloroplasts and the other in the cytoplasm (Hirel et al. 1982; Mann et al. 1979). The primary function of the chloroplastic GS in a higher plant is the reassimilation of ammonia produced in photorespiration (Joy 1988), whereas that of the cytoplasmic GS is ammonia assimilation in the dark (Hirel and Gadal 1980).

During the process of evolution, broomrapes have lost the ability to sustain their own efficient nitrogen metabolism (Sauerborn 1991). Lee and Stewart (1978) were unable to detect nitrate reductase activity in broomrape, which indicates that the parasite is unable to utilize nitrogen in the form of nitrate. Low GS activity was found in broomrape and another holoparasite, *Lathraea* (McNally and Stewart 1987; McNally et al. 1983a, 1983b, 1984). Additionally, in *Lathraea*, nitrate reduction has been shown to be incomplete or to proceed at very low rates (Thalouarn et al. 1987, 1988, 1990). Witchweed, a hemiparasite that is partially photosynthetic, has been found to possess very low activities of the chloroplastic GS compared to a typical C₃ plant (McNally and Stewart 1987; McNally et al. 1983a, 1983b, 1984). Witchweed exhibits low rates of photosynthesis as well as photorespiration, and this is reflected by the low activity of the chloroplastic GS isoform.

Absence or low activity of nitrogen assimilating enzymes in broomrape may indicate that the parasite must have access to organic nitrogen forms from the host plant. Translocation of amino acids and amides has been shown for broomrape (Aber et al. 1983) and other angiospermous parasites (Fer 1979; McNally et al. 1983a; Renaudin and Larher 1981; Thalouarn et al. 1986). Nandakumar et al. (1976) reported lack of the enzyme serine/threonine dehydratase in several holoparasites, which indicates their dependence on host plants for the amino acid isoleucine. Analysis of xylem sap of witchweed parasitizing

sorghum indicated that the main nitrogen components were asparagine, aspartate, and glutamate, with a very low content of nitrate (Stewart 1987). Growth of parasitic plants, *in vitro*, showed that they perform better on an organic rather than an inorganic nitrogen supply suggesting their reliance on hosts for their nitrogen (Hall et al. 1987; Igbinossa and Thalouarn 1996; Okonkwo 1987).

In summary, the weak ability of parasitic plants such as broomrapes to assimilate inorganic nitrogen is indicative of their ability to obtain organic nitrogen from the host. Low or absence of GS activity in broomrapes may contribute to sensitivity to nitrogen fertilization. The mechanism of inhibition of broomrape growth by nitrogen, which is central to practical utilization of this strategy, continues to be elusive. Knowledge about the nitrogen status of broomrapes in relation to their hosts is essential for elucidating nitrogen inhibitory mechanism.

1.5.4 Biological Methods

A fly, *Phytomyza orobanchia* Kalt., has been used for the control of broomrape in the former USSR and eastern Europe (Girling et al. 1979; Klyueva and Pamuchki 1982, 1983; Kovalev 1973; Mihajlovic 1986; Trenchev 1981). Several limitations restrict beneficial effects of *Phytomyza*. Tillage may bury broomrape stalks, containing *Phytomyza* pupae, deeper in the soil, thus preventing emergence of adults. Application of insecticides severely decreases the insect population. Parasites of *Phytomyza* reduce the fly population considerably. Crop rotations may also negatively impact survival of *Phytomyza*.

Fungi such as *Fusarium oxysporum* var. *orthoceras* gave some control of *O. aegyptiaca* (Panchenko 1981) and *O. cernua* (Bedi and Donchev 1991). *F. oxysporum*, along with a complex of fungi, controlled *O. ramosa* in tomato to some extent (Hodosy 1981). Both insects and pathogens such as fungi can be useful in control of broomrape in an integrated management program.

1.5.5 Chemical Methods

Chemical strategies have been used to control broomrapes either directly or indirectly. Direct involvement is by reduction or destruction of broomrape seed reserves in the soil, prevention of or negative influence on the germination of broomrape seeds and attachment to the host root. Measures such as soil fumigation, germination stimulants, and certain preplant or preemergence herbicides act directly on broomrape. Indirect control is aimed at suppressing growth of the parasite after attachment and penetration of the host root. For this purpose foliage applied herbicides and genetically engineered herbicide-resistant crops are useful. Chemical names of herbicides and other chemicals, mentioned in this chapter, are listed in Table 2.1.

1.5.5.1 Soil Fumigation

Soil fumigation involves the application of highly volatile compounds into the soil whereby the soil is sterilized. The chemical permeates the soil and kills all soil-borne pathogens including bacteria, fungi, nematodes, and weed seeds. The seeds must be physiologically active to be killed. Fumigants have been widely tested for use in controlling broomrapes.

Methyl bromide has been recognized as an effective soil fumigant. It has been routinely used in the US to control localized populations of *O. ramosa* before planting tomato (Wilhelm 1962; Wilhelm et al. 1958, 1959). Zahran (1970) has demonstrated use of methyl bromide for controlling *O. crenata* and *O. minor* in Egypt before planting tobacco and broad bean, respectively. There are several limitations that restrict use of methyl bromide over a large scale. The cost of the chemical as well as the polyethylene sheet needed to cover the treated soil are prohibitively high. A well tilled soil that has been kept moist at 70% field capacity and temperature above 10 C are required for productive results after methyl bromide application. Safety gear is recommended for application personnel due to extreme toxicity of the gas. The Environmental Protection Agency (EPA) of the US government has banned the

use of methyl bromide due to its toxicity, and a global ban may not be far off. Parker and Riches (1993) caution regarding the risk of bromine residues in produce from methyl bromide treated areas. Further, methyl bromide may temporarily suppress *Rhizobium*, although other soil microflora recover in a few days after treatment (Parker and Riches 1993).

Although earlier attempts with ethylene dibromide (EDB) were not promising (Piglionica 1975; Piglionica and Spezzacatena 1979), EDB applied alone or in combination with chloropicrin gave effective control of *O. crenata* in pea, but not *O. aegyptiaca* or *O. ramosa* (Jacobsohn et al. 1988). Application of EDB is considerably cheaper compared to methyl bromide because the soil need not be covered with polyethylene sheets after application. Nevertheless, EDB is harmful and has been suspended from use in the US, by the EPA, and several other countries due to human health and environmental considerations (Foy et al. 1989).

Metham-sodium provided good control of broomrape in broad bean (Zahran 1970) and tobacco (James 1976; James and Frater 1977). In Israel, application of metham-sodium through irrigation water ('chemigation') to dry soil and covering with polyethylene sheet during and 1 wk after application has been found to provide consistent control of broomrape (Kleifeld et al. 1991). Successful control of several species of broomrape was obtained with Telone II®, pure 1,3-dichloropropene (Jacobsohn et al. 1991).

Zahran (1970) reported that dazomet or Di-trapex® proved less useful than methyl bromide or metham-sodium. Several other reports on dazomet are available from Bulgaria (Fetvadhiev 1970), Turkey (Nemli et al. 1991), the US (Norris et al. 1992), and Ethiopia (Sherif et al. 1989). Methyl isothiocyanate gave partial control of broomrape in New Zealand (James and Frater 1977). Of all the soil fumigants, methyl bromide is the most effective in controlling broomrape, but the high cost of application coupled with safety concerns have restricted the extent of its usage.

1.5.5.2 Germination Stimulants

Since broomrape seeds must attach to a host root shortly after germination to survive, any means that would cause seed germination in the absence of a suitable host has potential as a control strategy. This stimulation of seed germination in the absence of a susceptible host is called 'suicidal germination' (Eplee 1975). Strigol was isolated from cotton (*Gossypium hirsutum* L.) roots and identified as a germination stimulant of parasitic weed seeds (Cook et al. 1966, 1972). Certain synthetic analogs of strigol have also been produced (Johnson et al. 1976, 1981; Pepperman et al. 1982). Application of strigol or its synthetic analogs did not provide practical control of broomrape due to their short stability in the soil. Both the activity and stability of the germination stimulants is dependent on the soil pH and moisture conditions.

Ethylene has been found to effectively stimulate witchweed seed germination (Eplee 1975). In fact, it has been a major component of an integrated witchweed management program in the US from 1956 to 1996. There was limited success when ethylene was used for stimulation of broomrape seed germination (Parker and Wilson 1986). Foy et al. (1989) reviewed several other compounds including herbicides that have been used to stimulate as well as inhibit germination in broomrape seeds. Germination stimulants, both natural and synthetic, have good potential as effective tools of management of broomrape, but much remains to be learned about their structure, activity, and stability in the soil.

1.5.5.3 Preplant and Preemergence Herbicides

Several herbicides have been tested on broomrape for control during its growth below the soil. Of thirteen herbicides tested in tomato in greenhouse experiments, only dichloral urea gave consistent control of broomrape (Saghir and Abu-Shakra 1971; Saghir and Dastgheib 1978). In a detailed review by Foy et al. (1989), several herbicides were reported to have shown some selectivity against broomrape in a variety of crops. However, none of these had reliability for field applications.

Emergence of two relatively new classes of herbicides, sulfonyleureas and imidazolinones, in the early 1990s provided additional options for broomrape control. García-Torres et al. (1989, 1991) showed that chlorsulfuron and imazethapyr were effective against broomrape. Chlorsulfuron gave better control both in broad bean and sunflower when applied to the soil surface than when incorporated (Kotoula-Syka and Eleftherohorinos 1991). In the same study, some control of *O. ramosa* was obtained in tomato. Kleifeld et al. (1996) were able to control *O. aegyptiaca* effectively in tomato with chlorsulfuron and triasulfuron applied by sprinkler irrigation. *In vitro* application of chlorsulfuron, triasulfuron, and rimsulfuron inhibited germination of *O. aegyptiaca* (Plakhine et al. 1996). Imazethapyr gave good control of broomrape in several legumes (García-Torres and López-Granados 1991a, 1991b; Jacobsohn and Eldar 1992). Good control of broomrape was obtained by coating seeds of broad bean and pea with imazethapyr, and sunflower with propyzamide (García-Torres et al. 1996).

Although sulfonyleureas and imidazolinones have been found to be effective against broomrapes, extensive use is not recommended due to the risk of evolution of resistance in weeds. The problems associated with herbicide resistance in weeds are universally known and can never be over emphasized.

Certain fungicides have been used for suppression of broomrape. Krishnamurthy et al. (1982) reported that tridemorph decreased *O. cernua* growth in tobacco. Petzoldt (1979) claimed that a seed dressing with benomyl resulted in reduced infestation of *O. crenata* in broad bean.

1.5.5.4 Postemergence herbicides

Any herbicide that can translocate, without being metabolized, through a host plant into broomrape attached to the host roots has potential for use in broomrape control. This aspect was first demonstrated by Whitney (1972, 1973) when 2,4-D applied to the host plant accumulated several times more in *O. crenata* than broad bean. However, the damage to the host was extreme. 2,4-DB is usually used selectively for broadleaf weed control in legumes

due to its differential metabolism in legumes and weeds. There are few reports regarding its use for broomrape control, although Foy et al. (1989) mentioned some selectivity against broomrape in peanut (*Arachis hypogea* L.).

Kasasian (1973b) reported for the first time, selective control of broomrape in broad bean with glyphosate, a new nonselective foliar applied herbicide at that time. Rates of 0.2 to 0.3 kg ha⁻¹ gave good selective control when sprayed 6 wk after sowing. Schmitt et al. (1979) demonstrated the first successful application of glyphosate in field trials for controlling broomrape soon after attachment in broad bean. Ever since, there have been several reports of selective control of broomrape in broad bean (Americanos 1983; Jacobsohn and Kelman 1980; Kukula and Masri 1984; Mesa-García et al. 1984; Schluter and Aber 1979; Zahran et al. 1980, 1981).

The duration of the broomrape-broad bean competition is an important factor to be considered in relation to the time when glyphosate should be applied (Mesa-García and García-Torres 1985). Studies with ¹⁴C-labeled glyphosate indicated that pods of broad bean were stronger sinks for glyphosate than other parts of the plant (Arjona-Berral et al. 1990). According to Müller and Distler (1991), glyphosate is best translocated from broad bean to broomrape at the tubercle and bud stage of the parasite.

Glyphosate has been used in several other legume crops, in addition to broad bean. Common vetch was proposed to be tolerant to glyphosate at low rates (Jacobsohn and Levy 1986; Kleifeld 1996). However, further research is required to establish the tolerance of common vetch to glyphosate. Glyphosate was tested for selectivity at very low rates on lentil in Spain and Morocco (Arjona-Berral et al. 1988; Kelili et al. 1983). Jacobsohn and Kelman (1980) reported less selectivity in pea. It was observed that the tolerance of legumes to glyphosate was higher at the pod-bearing stage than in the vegetative stage (Arjona-Berral and García-Torres 1984; Mesa-García et al. 1984).

The potential use of glyphosate has also been investigated in several non-legume crops with varying degrees of success. Broomrape was controlled in Cyprus and Israel when glyphosate was applied at a low rate of 50 g ai ha⁻¹ in carrot and celery (*Apium graveolens* L.) (Americanos 1991; Jacobsohn and Levy 1986). Kasasian (1973b) and Lolas (1986) were unable to obtain consistently good control of broomrape in tobacco. Foy et al. (1988)

screened several tomato cultivars as well as some wild *Lycopersicon* species for possible resistance to glyphosate, but none of the screened varieties provided adequate selectivity for use in broomrape infested areas. *O. cumana* was controlled to a good extent with glyphosate without causing much injury in sunflower (Petzoldt and Sneyd 1986), but Castejón-Muñoz et al (1987, 1990) reported inadequate selectivity.

In summary, glyphosate has good use in broad bean, carrot, and celery. There is insufficient selectivity in most of the other crops including legumes such as pea and lentil, tomato, tobacco, and sunflower. With the availability of glyphosate-resistant crops, glyphosate use may increase rapidly.

Selective control of broomrape by glyphosate in some of the host crops is due to rapid translocation of the herbicide away from the crop foliage to the parasitic attachments on the host roots (Foy et al. 1989). Recently, ^{14}C -glyphosate was shown to translocate from tomato leaves to *O. aegyptiaca* shoots (Jain and Foy 1997). Aber et al. (1983) demonstrated translocation of organic substances, particularly sucrose, to *O. crenata* tubercles from $^{14}\text{CO}_2$ fixation by broad bean plants. It has also been shown that ^{14}C -labeled photoassimilates accumulated in *O. ramosa* after fixation of $^{14}\text{CO}_2$ by tomato plants (Saghir et al. 1973).

The imidazolinone herbicides have been tested for their postemergence use. Imazaquin provided some control of broomrape tubercles in broad bean (Linke 1992; Sauerborn et al. 1987, 1989) and at low doses in pea (Linke 1992) It also had postemergence selectivity in tobacco (Vasilakakis et al. 1988). Imazethapyr was used selectively in broad bean (García-Torres and López-Granados 1991a) and pea (Jacobsohn and Eldar 1992).

Other postemergence chemicals used for broomrape control include maleic hydrazide in tobacco (Evtushenko et al. 1973) and muskmelon (Prokudina 1976; Setdarov 1979). Spraying growth regulators on the host crop provided reduced parasitism of broomrape in broad bean with kinetin (El-Ghamrawy and Neumann 1991) and in tomato with cycocel and gibberellic acid (Hassan et al. 1991). Application of kerosene to broomrape shoots has been reported to have given good control (Krishnamurthy et al. 1976; Linke and Saxena 1991b).

1.5.5.5 Genetically Engineered Herbicide-Resistant Crops

In most broomrape infested areas, there hasn't been widespread acceptance of herbicides as a viable control method because of lack of herbicides that protect crops sufficiently so as to guarantee normal yield (Sauerborn 1991). The presently available herbicides are mainly used for killing subterranean or emerged broomrape and preventing further seed production. It may take several years to obtain significant reduction in the amount of seeds that are present in the soil. The lack of suitable herbicides for effective control of broomrape may be due to the fact that the infestations are regional and herbicide companies are reluctant to produce specialized products that would be targeted at the parasite (Sauerborn 1991). Further, development of a specific herbicide would be difficult due to the complex interaction between broomrape and its hosts. Under these circumstances, availability of genetically engineered herbicide-resistant crops has provided an effective tool for combating the broomrape problem.

The applications of genetically engineered herbicide-resistant crops for broomrape control were first addressed by Foy et al. (1989). Joel (1992) reported the first application of herbicide-resistant crops for broomrape control. Complete control of *O. aegyptiaca* was achieved when transgenic tobacco was treated with chlorsulfuron (Joel 1992). Gressel et al. (1994) have emphasized the immediate need for genetically engineered herbicide-resistant crops for controlling parasitic weeds.

Typically, two kinds of herbicide resistance can be genetically engineered into crop plants (Gressel et al. 1994). First, target-site resistance where the herbicide binding to the target enzyme is prevented. This permits movement of the herbicide 'as is' through the treated host crop to parasitic attachments on the host root. The second resistance mechanism involves metabolism or breakdown of the herbicide by the treated crop to harmless compounds. Metabolic resistance, therefore, is not suitable for systemic herbicides, but applicable only for herbicides applied directly to the parasite below the soil (Joel et al. 1995).

Recently, four examples of genetically engineered resistance, three target-site and one metabolic, were tested for use in controlling broomrape (Joel et al. 1995). Excellent control

of broomrape was obtained with chlorsulfuron in a transgenic tobacco line containing a modified acetolactate synthase enzyme resistant to chlorsulfuron, with glyphosate in oilseed rape plants containing a modified enolphosphate-shikimate phosphate synthase resistant to glyphosate, and with asulam in tobacco plants having a modified dihydropteroate synthase resistant to asulam. A variety of tomato engineered for resistance to glufosinate, an inhibitor of GS, was infested with broomrape in spite of application of glufosinate. This was expected because the transgenic line of tomato metabolized the herbicide. In all of the four cases, control plants were severely infested with broomrape.

Thus, the utility of transgenic herbicide-resistant crops in management of broomrape is clearly evident. However, careful judgment should be exercised while using herbicide-resistant crops to avoid the overuse of the herbicide and the risk of evolution of herbicide resistance in wild weedy relatives of the transgenic crops by interbreeding.

1.6 OBJECTIVES

The objectives of this research were:

a) to determine the use of glyphosate in controlling broomrape in common vetch that is tolerant to low rates of glyphosate, and to compare this response with broomrape control in oilseed rape that has been engineered for glyphosate resistance,

b) to determine the nitrogen status of broomrapes and their hosts by analyzing their respective amino acid profiles,

c) to examine the mode of acquisition of amino acids by broomrape from two selected hosts, common vetch and oilseed rape, and to better understand the impact of broomrape parasitism on amino acid composition of these host crops,

d) to examine osmotic potential as a mechanism for inhibition of germination and subsequent development of broomrapes, and

e) to determine the effect of temperature on the early development of broomrapes.

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Table 2.1. Common and chemical names of compounds listed in this chapter.

Common name	Chemical name
Asulam	methyl[(4-aminophenyl)sulfonyl]carbamate
Benomyl	[methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate]
Chlorsulfuron	2-chloro- <i>N</i> -[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide
2,4-DB	4-(2,4-dichlorophenoxy)butanoic acid
Dazomet	tetrahydro-3,5-dimethyl-2 <i>H</i> -1,3,5-triazine-2-thione
Di-Trapex	dichloropropane + dichloropropene + methyl isothiocyanate
EDB	1,2-dibromoethane
Glufosinate	2-amino-4-(hydroxymethylphosphinyl)butanoic acid
Glyphosate	<i>N</i> -(phosphonomethyl)glycine
Imazaquin	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-[1 <i>H</i> -imidazol-2-yl]-3-quinolinecarboxylic acid
Imazethapyr	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-[1 <i>H</i> -imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid
Maleic hydrazide	1,2-dihydro-3,6-pyridazinedione
Metham-sodium	sodium salt of methylcarbamodithioic acid
Propyzamide	3,5-dichloro-(<i>N</i> -1,1-dimethyl-2-propynyl)-benzamide
Rimsulfuron	<i>N</i> -[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide
Telone II	1,3-dichloropropane
Triasulfuron	2-[[[[[4-(dimethylamino)-6-(2,2,2-difluoroethoxy)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]-3-methylbenzoic acid
Tridemorph	<i>N</i> -tridecyl-2,6-methylmorpholine

2. SELECTIVE CONTROL OF EGYPTIAN BROOMRAPE IN COMMON VETCH AND OILSEED RAPE BY GLYPHOSATE

2.1 INTRODUCTION

Egyptian broomrape (*Orobanche aegyptiaca* Pers.) is an achlorophyllous, phanerogamic holoparasite that attacks the roots of many dicotyledonous crops. It obtains carbon, nutrients, and water through haustoria which connect the parasite to the host vascular system. Broomrape infestations cause extensive reduction in crop yield, adversely affect crop quality, and result in loss of cropping alternatives in infested fields. Commonly affected host crops include legumes such as common vetch (*Vicia sativa* L.) and broad bean (*Vicia faba* L.), cruciferous crops such as oilseed rape (*Brassica napus* L.), and several members of the families Apiaceae, Asteraceae, and Solanaceae.

The management of broomrape is often difficult due to its close association with the host for all of its life cycle. Despite many management strategies tried against broomrape (reviewed by Foy et al. 1989; Parker and Riches 1993; Sauerborn 1991), few methods have been reliable and economical, except for use in high-value crops.

Glyphosate [N-(phosphonomethyl)glycine] is a systemic, nonselective, and foliar-applied herbicide. It is readily translocated to underground parts, immature leaves, and meristems. It has been reported to be very stable in plants (Coupland 1984; Devine and Bandeen 1983; Gottrup et al. 1976). Its mode of action is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase that leads to the production of the aromatic amino acids, phenylalanine, tyrosine, and tryptophan.

Since discovery of its herbicidal properties in 1971 (Baird et al. 1971) and commercial introduction in 1974, glyphosate has been used extensively in both cultivated and noncrop lands. It is generally applied as a preplant treatment or after harvest in cropping areas for broad spectrum weed control. In spite of its nonselectivity, some degree of selectivity in certain crops, some of which are broomrape hosts, has been reported (reviewed by Foy et al. 1989; Parker and Riches 1993).

Most success with glyphosate against broomrape has been achieved in broad bean. Kasasian (1973) first reported the selective control of broomrape, *O. crenata* Forsk., in broad bean with glyphosate. Rates of 0.2 to 0.3 kg ha⁻¹ gave good selective control when sprayed 6 wk after sowing. After application to host foliage, glyphosate is thought to translocate through the host phloem to broomrape attachments on the host roots and exert its toxic effect. Common vetch is an important legume forage crop in the Middle East. It is highly susceptible to attack by broomrape. A variety of common vetch, 'Yovel', has been reported to be tolerant to glyphosate at low rates (Kleifeld 1996).

Although glyphosate has provided good control of broomrape in certain crop situations, the margin of crop safety and applicator's error is very narrow due to its toxicity to the host crop itself. The problem of herbicide toxicity to the host plant can be avoided in crops that are genetically engineered for glyphosate resistance. The possibility of herbicide-resistant crops for broomrape control was first addressed by Foy et al. (1989) and described in detail by Gressel et al. (1994). Joel (1992) reported the first utilization of herbicide-resistant crops for broomrape control, demonstrating complete suppression of the parasite by application of chlorsulfuron to parasitized transgenic chlorsulfuron (2-chloro-*N*-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide)-resistant tobacco (*Nicotiana tabacum* L.).

Recently, four examples of genetically engineered resistance, three target-site and one metabolic, were tested for use in controlling broomrape (Joel et al. 1995). Excellent control of broomrape was obtained with chlorsulfuron in a transgenic tobacco line containing a modified acetolactate synthase enzyme resistant to chlorsulfuron, with glyphosate in oilseed rape plants containing a modified enolphosphate-shikimate phosphate synthase resistant to glyphosate, and with asulam (methyl[(4-aminophenyl)sulfonyl]carbamate) in tobacco plants having a modified dihydropteroate synthase resistant to asulam. A variety of tomato (*Lycopersicon esculentum* Mill.) engineered for resistance to glufosinate (2-amino-4-(hydroxymethylphosphinyl)butanoic acid), an inhibitor of GS, was infested with broomrape in spite of application of glufosinate. This was expected because the transgenic line of tomato metabolized the herbicide. In all of the four cases, control plants were severely infested with broomrape.

The objective of this research was to determine the use of glyphosate in controlling broomrape parasitism in common vetch that is tolerant to low rates of glyphosate, and to compare this response with broomrape control in oilseed rape that has been engineered for glyphosate resistance. Glyphosate dose responses using a commercial formulation and patterns of absorption, translocation, and metabolism using ^{14}C -labeled glyphosate were determined for both host crops.

2.2 MATERIALS AND METHODS

2.2.1 Dose Response

Seeds of common vetch¹ (var. ‘Yovel’) and glyphosate-resistant oilseed rape² were surface sterilized and cleaned, as described for carrot seeds in the ‘Materials and Methods’ section of chapter 3. Four seeds were planted 2.5 cm below the soil surface in 50-ml centrifuge tubes containing a vermiculite:topsoil mixture (3:1 by vol). Three holes were drilled near the bottom of the tubes for drainage. The bottom 20 ml of the tubes was filled with the soil mix. The next 20-ml volume was filled with soil mix infested with broomrape seeds³ at 3 mg seeds ml⁻¹ of soil mix. Five milliliters of non-infested soil were added above the infested soil. Both common vetch and oilseed rape plants emerged within 7 to 10 d of planting and 2-wk-old seedlings were thinned to two per tube. Plants were watered and fertilized as required. Throughout the experiment, plants were placed in a controlled environment room. Light intensity was 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ obtained from fluorescent lighting. The temperature was 24 C during the 12-h light period and 20 C during the 12-h dark period. Initial attachments of broomrape on the host roots were found 15 d after planting. Host plants were dug up periodically to check for parasitic attachments and growth stage of the parasite.

Glyphosate⁴ was applied at 0.09, 0.18, and 0.36 kg ae ha⁻¹ to common vetch, and 0.25, 0.5, and 0.75 kg ae ha⁻¹ to oilseed rape. In a preliminary study, glyphosate was applied to broomrape parasitized-common vetch plants at 0.009, 0.09, and 0.9 kg ha⁻¹. Glyphosate had

no effect on broomrape at the 0.009 kg rate, whereas at the 0.9 kg rate, common vetch plants were killed. The 0.09 kg rate provided some control of broomrape, hence was selected as the low rate. Two- and four-fold increased rates of the 0.09 rate were also included in the actual experiment to get an estimate of broomrape control as well as glyphosate tolerance of common vetch. The glyphosate rates used in oilseed rape were consistent with those that were reported in the literature. In a preliminary experiment, a sample of the oilseed rape seeds were planted, and emerged plants treated with glyphosate at 0.75 kg ha⁻¹ to confirm resistance to glyphosate. Untreated parasitized plants of common vetch and oilseed rape were used as the respective controls. A nonionic surfactant⁵ was added at 0.25% v/v to all glyphosate treatments. All the herbicide treatments were applied 5 wk after planting common vetch and oilseed rape with a greenhouse sprayer equipped with a Teejet 8001E nozzle tip⁶ delivering a volume of 200 L ha⁻¹ at 220 kPa.

Visual observations of host plant injury were not recorded due to lack of injury symptoms. One week after treatment, fresh weights were taken of host shoot and root, and broomrape, and number of live attachments were counted for both common vetch and oilseed rape.

The experiment followed a completely randomized design with 17 replications for common vetch and 12 replications for oilseed rape and was repeated. All data were subjected to analysis of variance using SAS statistical software (SAS 1989). Standard error of treatment means was used for separation of treatment means. Results from the repeated experiment were consistent with the previous results. Data from only one experiment are presented.

2.2.2 Absorption and Translocation

Seeds of common vetch and oilseed rape, cleaned as described before, were planted in sterile vermiculite in a plastic tray. One week after emergence, vermiculite was washed off the roots and the seedlings were transplanted into polyethylene bags (15 by 30 cm) according to Parker and Dixon (1983). Strips of glass-fiber filter paper measuring 14 by 23 cm, were

inserted into the bags and moistened with 10 ml sterile distilled water using a disposable syringe. The top of the bag was folded over a piece of wooden dowel and stapled on both sides. The bag could then be suspended in a rectangular cardboard box. Common vetch and oilseed rape seedlings were introduced into the bag through a 5-cm slit such that the whole root system was below the level of the dowel.

Throughout their growth, plants were watered as required with one-half strength Hoagland's nutrient solution (Hoagland and Arnon 1950) that was injected with a syringe. The bags were placed upright in a cardboard box in a growth room. The light intensity was $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by fluorescent lights placed 45 cm from the top of the polyethylene bags. Temperatures were 24 C during the 12-h light period and 20 C during the dark. One week after transplanting, the main shoots of common vetch were clipped to induce formation of secondary shoots. After development of a wide-spread root system, surface sterilized broomrape seeds were spread on roots of the host plants with a spatula. Broomrape seeds were surface sterilized, as described for common vetch and oilseed rape seeds, following a 20-s dip in 70% (by vol) aqueous ethanol. Eight days after spreading broomrape seeds on host roots, 5 ml of a 10 mg L^{-1} stock of a synthetic germination stimulant, GR-24, a strigol analog, was added to each bag to induce uniform germination of the parasite seeds. Development of broomrape attachments to host roots was observed 1 wk later and broomrape tubercles after 3 wk.

The application rate of glyphosate was $0.18 \text{ kg ae ha}^{-1}$ for common vetch and $0.75 \text{ kg ae ha}^{-1}$ for oilseed rape, both in a spray volume of 200 L ha^{-1} . The ^{14}C -methyl labeled glyphosate⁷ (specific activity = $2035 \text{ kBq } \mu\text{mol}^{-1}$) and technical grade glyphosate⁸ (99.9%) were converted from the acid form to the isopropylamine salt form by addition of an equimolar amount of isopropylamine. Stock solutions were made, by mixing the isopropylamine salt of ^{14}C -methyl labeled and technical grade glyphosate in distilled water, followed by addition of a nonionic surfactant⁹ (cationic polyethoxylated tallow amine) at 0.8% (by vol). A $10\text{-}\mu\text{l}$ droplet of the respective stock solution containing radioactivity was applied with a micro-syringe such that each common vetch plant received $22.94 \text{ kBq } ^{14}\text{C}$ -methyl labeled glyphosate and each oilseed rape plant $34.41 \text{ kBq } ^{14}\text{C}$ -methyl labeled glyphosate. The herbicide solution was applied to both parasitized and nonparasitized plants on the adaxial

surface of the basal leaflets of third or fourth true leaf of common vetch and the third true leaf of oilseed rape. Since the leaf surface of the oilseed rape plants was waxy, herbicide was applied within a 1-cm-diam ring of lanolin. Growth conditions were similar to those described for the dose response experiments. Four days after application of herbicide, both common vetch and oilseed rape plants were harvested and divided into the following parts: treated leaf, shoot above treated leaf, shoot below treated leaf, shoot apex, root, and broomrape attachment (in the parasitized plants). Unabsorbed ^{14}C -glyphosate was washed off the treated leaves with 1 ml distilled water. The harvested parts were dried in an oven at 60 C for 48 h. Dried samples were combusted in a sample oxidizer¹⁰ and assayed for radioactivity by a liquid scintillation counter¹¹. The evolved $^{14}\text{CO}_2$, after combustion of samples, was trapped in a CO_2 absorber¹² and an LSC cocktail¹³ simultaneously added to the oxidized samples.

Treatments were replicated nine times for common vetch and six for oilseed rape, and experiments repeated. The experiment followed a completely randomized design. Means of measured radioactivity in parasitized and nonparasitized plants were compared by Wilcoxon-Mann-Whitney rank sum procedure ($p = 0.05$) using SAS statistical software. Results were consistent between repeated experiments. Data from only one experiment are presented.

2.2.3 Autoradiography

Autoradiography experiments were set up and harvested exactly as described for the absorption and translocation experiments, except that both common vetch and oilseed rape were harvested as whole plants. After harvest, plants were mounted on white glossy paper, avoiding contact of the treated leaf with other parts of the plant, pressed (order of stacking in the press from bottom to top - metal plate, mounted plant, piece of foam rubber, a wire screen, second metal plate, and press held together by large metal binder clips on all four sides), and dried at 60 C for 48 h. Dried plants were exposed to X-ray film¹⁴ for 4 wk. After exposure, film was developed¹⁵ and fixed¹⁶. Experiment was conducted once using four replications. Plants not treated with ^{14}C -glyphosate were used as the control.

2.2.4 Metabolism

2.2.4.1 High Performance Liquid Chromatography (HPLC)

Glyphosate metabolism experiments were set up and harvested as described for the absorption and translocation experiments, except common vetch received 96 kBq per plant and oilseed rape 33.6 kBq ^{14}C -glyphosate per plant. After harvesting the plants, fresh weights of tissues were recorded, the plant material frozen in liquid nitrogen, and subjected to the following extraction procedure as described by Komořa et al. (1992).

Frozen tissues were ground in a mortar with a pestle in 3.75 ml chloroform (CHCl_3) and methanol (CH_3OH) (1:2 by vol) plus 0.5 ml H_2O per 0.5 g fresh weight. Residues were then extracted by sonication¹⁷ with 4.75 ml methylene chloride (CH_2Cl_2), CH_3OH , and H_2O (1:2:0.8 by vol). The remaining residues were washed with 1 ml CH_3OH and twice with 2.5 ml H_2O under sonication. To the combined single phase extracts, 3 ml CH_2Cl_2 were added to separate aqueous and organic phases.

The supernatant was concentrated to 1 ml by rotary evaporation¹⁸ and filtered through a 0.45 μm nylon syringe filter¹⁹ into 2-ml glass vials. Samples were then brought to total dryness under a steady flow of nitrogen. The dry residue was re-suspended in 100 μl of HPLC mobile phase [6.2 mM KH_2PO_4 in 4% methanol (pH 1.9)] and centrifuged at 3000 rpm for 5 min. The supernatant was collected into 250 μl inserts²⁰ and sealed in crimp neck vials²¹ with aluminum seals²² and assayed for the presence of glyphosate and/or its metabolites by HPLC separation. A 2 to 20- μl aliquot of supernatant was injected onto a strong anion exchange column²³. An anion exchange guard column cartridge²⁴ was installed between the HPLC injector and the analytical column. A liquid chromatograph²⁵ equipped with a DR5 pump system and a radioisotope detector²⁶ was used to detect glyphosate and its metabolites. Flow rate of the mobile phase was 1 ml min^{-1} and 4 ml min^{-1} that of the cocktail²⁷. Retention time for glyphosate was determined by using 99.7 % ^{14}C -methyl labeled glyphosate. Treatments were replicated five times for common vetch and oilseed rape, and experiments repeated. Results were identical and combined data from repeated experiments are presented.

The mobile phase was prepared according to Burns (1983). It was a mixture of methanol and water (4:96 by vol) containing 6.2 mM potassium dihydrogen phosphate (KH_2PO_4) and the pH adjusted to 1.9 with 85% phosphoric acid. It was filtered through a 0.45 μm nylon filter²⁸ and degassed before use on the column.

2.2.4.2 Thin Layer Chromatography (TLC)

A procedure outlined by Sprankle et al. (1978) was used. Aliquots (5 to 20 μl) from HPLC sample preparation were spotted on 20 by 20 cm cellulose TLC sheets²⁹ 2 cm from the bottom. ^{14}C -methyl labeled glyphosate was used as standard. Sheets were developed for 17 cm in a solvent system containing 100% ethanol:water:15 N ammonium hydroxide:trichloroacetic acid:17 N acetic acid (55:35:2.5 by vol:3.5 g by wt:2 by vol). The TLC sheets were then dried at 21 C and autoradiographed. After autoradiography, the radioactive spots on the TLC sheets were scraped and radioactivity measured by liquid scintillation spectrometry.

Technical grade (99.9%) standard of glyphosate, and reagent grade aminomethylphosphonic acid (AMPA), glycine, and sarcosine were used for preparation of standards. A stock solution of each of the four compounds was prepared at 1 mg ml^{-1} and a mixture of all four compounds at 1 mg ml^{-1} each was prepared. A 20- μl drop from each of the stock solutions of the four compounds and their mixture were chromatographed on a cellulose sheet for 17 cm. Sheets were visualized by uniformly spraying a solution of 0.5% ninhydrin in butanol and drying in an oven at 100 C for 5 min. Both for standards and samples, the distance from the origin to the center of the spot was used for calculation of Rf values.

2.3 RESULTS AND DISCUSSION

2.3.1 Dose Response

ANOVA indicated a nonsignificant effect with glyphosate on shoot and root growth of common vetch. However, comparison of individual treatment means revealed that glyphosate reduced root growth, whereas shoot growth remained similar between glyphosate treatments and the untreated check (Figure 2.1). Legumes such as broad bean have been found to be tolerant to low rates of glyphosate (Jacobsohn and Kelman 1980; Kasasian 1973; Mesa-García et al. 1984; Schluter and Aber 1979; Schmitt et al. 1979).

Although visual observations of host plant injury were not recorded due to close similarity of leaf appearance between treatments, the shoot growth of oilseed rape was significantly reduced by glyphosate, one week after application (Figure 2.2). Thereafter, the plants recovered from the temporary inhibition of growth. This recovery was not unexpected because the oilseed rape had been engineered for glyphosate resistance. In a similar study, Joel et al. (1995) reported normal growth and flowering in a transgenic glyphosate-resistant oilseed rape variety after application of glyphosate at 0.72 kg ha^{-1} . The shoot growth reduction ranged from 18% of the untreated check with 0.5 kg ha^{-1} glyphosate to 30% of the untreated check with the 0.75 kg ha^{-1} . Root growth of oilseed rape was not significantly affected by application of glyphosate.

Application of glyphosate significantly affected the growth of broomrape in common vetch with all three rates providing greater reduction compared to the untreated check (Figure 2.3). The number of live attachments of broomrape was greatly reduced with glyphosate at 0.18 and 0.36 kg ha^{-1} (Figure 2.3). Two applications of glyphosate at 0.18 kg ha^{-1} may provide more reliable control of broomrape than a single application of 0.36 kg ha^{-1} , but further research is needed to find the optimum rate. Glyphosate has been tried for selective control of *Orobanche* in several legumes. As early as 1973, selective control *O. crenata* in broad bean was obtained with glyphosate at rates of 0.2 to 0.3 kg ha^{-1} applied 6 wk after sowing (Kasasian 1973). Results have not been very encouraging in pea (*Pisum sativum* L.)

(Jacobsohn and Kelman 1980) or lentil (*Lens culinaris* Medic.) (Arjona-Berral et al. 1988; Kelili et al. 1983). However, results from the current research indicate that glyphosate is useful for broomrape control in common vetch.

Glyphosate rate significantly affected the growth of broomrape in oilseed rape. All three rates reduced broomrape growth compared to the untreated check (Figure 2.4). Complete suppression of the parasite was obtained with glyphosate at both 0.5 and 0.75 kg ha⁻¹ (Figure 2.4). Joel et al. (1995) reported complete prevention of broomrape in a transgenic glyphosate-resistant oilseed rape variety after application of glyphosate at 0.72 kg ha⁻¹. The number of live attachments was significantly reduced at the increased rates of glyphosate with no attachments surviving at 0.75 kg ha⁻¹ (Figure 2.4). The reduction in number of live attachments rather than fresh weight of attachments may be a better measure for estimating efficacy of glyphosate or other herbicides for control of broomrape because surviving attachments may be expected to adversely affect the host crop.

2.3.2 Absorption and Translocation

Absorption and translocation studies were conducted with ¹⁴C-glyphosate to investigate the patterns of distribution of glyphosate in broomrape-parasitized common vetch and oilseed rape. A single duration of translocation of 4 d was selected. Jain (1987) and Jain and Foy (1997) reported no differences in the extent of glyphosate absorption and translocation between 3 and 7 d in tomato parasitized by broomrape.

The ¹⁴C recovery was 98% in common vetch. Radiolabeled glyphosate was absorbed similarly by both parasitized and nonparasitized common vetch plants (Table 2.1). After 96 h, 47% of the applied label was recovered in the parasitized and 53% in the nonparasitized plants. In both parasitized and nonparasitized plants, nearly 90% of the absorbed ¹⁴C label was retained in the treated leaf and the remaining translocated to all parts of the plant (Table 2.1).

There was no difference in the extent of translocation of the ¹⁴C label between parasitized and nonparasitized common vetch plants (Table 2.1). Further the amount of

absorbed radioactivity, expressed per unit tissue fresh weight, was similar between parasitized and nonparasitized common vetch plants (Table 2.2). A similar response was visible regarding translocated radioactivity in similar tissues of parasitized and nonparasitized plants, except in the root (Table 2.3). The amount of translocated ^{14}C -glyphosate was more than twice in the nonparasitized root (29%) compared to the parasitized root (14%). Part of this difference is reflected in the high amount of translocated label, 27%, that has accumulated in the broomrape attachments (Table 2.3).

The ^{14}C recovery was 91% in oilseed rape. Absorption of ^{14}C -glyphosate was similar in both parasitized and nonparasitized oilseed rape plants. The amount of ^{14}C -glyphosate was 77% of that applied in the parasitized plants and 66% in nonparasitized plants after 96 h (Table 2.1). In both parasitized and nonparasitized plants, more than 60% of the absorbed ^{14}C label remained in the treated leaf and the rest translocated (Table 2.1).

The amount of total translocated label was similar in both parasitized and nonparasitized oilseed rape plants (Table 2.1). There was no difference between parasitized and nonparasitized oilseed rape plants in the amount of absorbed radioactivity expressed per unit fresh weight of tissue (Table 2.2). However, distribution of radioactivity, when expressed as % translocated, was significantly different between parasitized and nonparasitized plants within corresponding tissues, with the exception of the shoot apex (Table 2.3). Some of this difference can be accounted for by the amount of translocated radioactivity, nearly 33%, to broomrape attachments (Table 2.3). This indicates a change in the distribution of herbicide in the parasitized plants due to broomrape attachments.

Thus, in both common vetch and oilseed rape, broomrape parasitism caused a redistribution of translocated glyphosate, especially in the root. The concentration of ^{14}C -glyphosate was greater in broomrape attachments than in any parts of broad bean or pea except the treated leaf, and much higher than in the roots (Arjona-Berral et al. 1990). The amount of accumulation of translocated radioactivity in broomrape attachments was more than 25% in both common vetch and oilseed rape. Broomrape acts as a sink and competes directly with other actively growing parts of the host plant for photoassimilates, similar to the translocation pattern of ^{14}C -glyphosate observed here.

2.3.3 Autoradiography

X-ray autoradiograms of both common vetch and oilseed rape indicated that ^{14}C -glyphosate applied to the leaves was translocated to all parts of the plants. Of the translocated label, greatest accumulation was observed in the shoot apex and broomrape attachments on the host roots. Recently, ^{14}C -glyphosate was shown to translocate from tomato leaves to broomrape shoots (Jain and Foy 1997). Aber et al. (1983) demonstrated translocation of organic substances, particularly sucrose, to *O. crenata* tubercles from $^{14}\text{CO}_2$ fixation by broad bean plants. It has also been shown that ^{14}C -labeled photoassimilates accumulated in *O. ramosa* L. after fixation of $^{14}\text{CO}_2$ by tomato plants (Saghir et al. 1973).

2.3.4 Metabolism

2.3.4.1 High Pressure Liquid Chromatography (HPLC)

Results indicated metabolism of glyphosate in common vetch. At least three peaks (data not shown) other than the one corresponding to retention time of glyphosate were detected in the treated leaf, root, and broomrape attachments. It is probable that the metabolites in the broomrape attachments on common vetch were translocated from the host and were not due to breakdown of glyphosate by the parasite; current research (see results from dose response experiments in this chapter), and additionally, earlier reports have demonstrated the susceptibility of broomrape to glyphosate application. Oilseed rape plants were not analyzed by HPLC due to limitations of radioactivity in samples, injector volume, and sensitivity of the detector.

2.3.4.2 Thin Layer Chromatography (TLC)

Thin layer chromatographic analysis of extracts from common vetch and broomrape indicated metabolism of glyphosate which ranged from 11 to 27% depending on the tissue (Table 2.4). Three metabolites were detected from extracts of common vetch. The Rf value of radioactive glyphosate was 0.4 and that of its first metabolite was 0.53. These values correspond to the Rf values of technical grade glyphosate and ¹⁴C-glyphosate standard, and reagent grade AMPA, respectively. Komoßa et al. (1992) reported metabolism of glyphosate to AMPA in soybean cell cultures. The other two radioactive spots had much higher Rf values (0.6 to 0.8) and they overlapped, hence making it difficult to determine their exact Rf values and compare with that of technical grade glycine and sarcosine. No metabolism of glyphosate was found in oilseed rape. Approximately, 97% of the extracted radioactivity was in the form of glyphosate (data not shown).

In summary, glyphosate has provided good control of broomrape in both common vetch and oilseed rape. The high amount of ¹⁴C-glyphosate that has translocated to the broomrape attachments in both common vetch and oilseed rape indicates that the parasite acts as a strong sink for accumulation of photoassimilates, similar to the pattern observed with accumulation of radiolabeled glyphosate. This response is also evident from the autoradiograms. Results from the metabolism experiments indicate that tolerance to glyphosate in common vetch is contributed, at least in part, by metabolism of the herbicide. It is not surprising that oilseed rape was resistant to glyphosate not by metabolism, but by possession of an altered target site, i.e., a glyphosate-resistant EPSP synthase. These results indicate that common vetch and glyphosate-resistant oilseed rape can be viable alternatives in broomrape-infested areas.

2.4 SOURCES OF MATERIALS

¹ Common vetch¹ (var. 'Yovel') provided by Dr. Y. Kleifeld, Newe Ya'ar Research Center, Ramat Ishay 30095, Israel.

² Glyphosate-resistant oilseed rape, Monsanto Canada, Inc., 5827-181 St., Edmonton AB, Canada T6M 1V7.

³ Broomrape (*O. aegyptiaca*) seed provided by Dr. Y. Kleifeld, Newe Ya'ar Research Center, Ramat Ishay 30095, Israel.

⁴ Glyphosate, Roundup®, Monsanto Co., St. Louis, MO 63167.

⁵ Nonionic surfactant, X-77®, Valent USA Corp., North Carolina Blvd., Walnut Creek, CA 94596.

⁶ Teejet 8001E® nozzle tip, Spraying Systems Co., Wheaton, IL 60189.

⁷ ¹⁴C-glyphosate, Monsanto Co., St. Louis, MO 63167.

⁸ Technical grade glyphosate (99.9%), Monsanto Co., St. Louis, MO 63167.

⁹ Nonionic surfactant, Mon 0818, Monsanto Co., St. Louis, MO 63167.

¹⁰ Sample oxidizer, Model B306, Packard Instruments, 2200 Warrenville Rd., Dowers Grove, IL 60515.

¹¹ Liquid scintillation counter, Model LS 6500, Beckman Instruments, Inc., 2500 Harbour Blvd., Fullerton, CA 92634.

¹² Carbosorb®, Packard Instruments, 800 Research Pky., Meriden, CT 06450.

¹³ Permafluor E⁺®, Packard Instruments, 800 Research Pky., Meriden, CT 06450.

¹⁴ X-ray film, X-Omat™ AR 5, Eastman Kodak Co., Rochester, New York 14650.

¹⁵ GBX Developer, Eastman Kodak Co., Rochester, New York 14650.

¹⁶ GBX Fixer, Eastman Kodak Co., Rochester, New York 14650.

¹⁷ Sonicator, Model 2210-Branson Ultrasonic Cleaner, Branson Ultrasonics Corp., Eagle Rd., Danbury, CT 06810.

¹⁸ Rotary evaporator, Model RE-121, Buchi Labortechnik AG, Meierseggstr. 40, CH-9230 Flawil, Switzerland.

¹⁹ Nylon syringe filter, Micron Separations, Inc., Westboro, MA 01581.

- ²⁰ Insert, Alltech Associates, Inc., 2051 Waukegan Rd., Deerfield, IL 60015.
- ²¹ Crimp neck vial, Alltech Associates, Inc., 2051 Waukegan Rd., Deerfield, IL 60015.
- ²² Aluminum seal, Alltech Associates, Inc., 2051 Waukegan Rd., Deerfield, IL 60015.
- ²³ Strong anion exchange column, Partisil 10 μ SAX 250 X 4.6 mm, Whatman, Inc., 9 Bridewell Pl., Clifton, NJ 07014.
- ²⁴ Anion exchange guard column cartridge, Whatman Labsales, P.O. Box 1359, Hillsboro, OR 97123.
- ²⁵ LC, Model 1090 M, Hewlett Packard, Waldbronn Analytical Division, Postfach 1280, D-7517 Waldbronn, Germany.
- ²⁶ Radioisotope detector, Model 171, Beckman Instruments, Inc., Scientific Instruments Division, 2500 Harbour Blvd., Fullerton, CA 92634.
- ²⁷ Cocktail, Ready Flow III® cocktail, Beckman Instruments, 846 E. Algonquin Rd., Schaumburg, IL 60173.
- ²⁸ Nylon filter, Lida Manufacturing Corp., 9115 26th Av., Kenosha, WI 53143.
- ²⁹ Cellulose TLC sheet, Eastman Kodak Co., Rochester, New York 14650.

2.5 LITERATURE CITED

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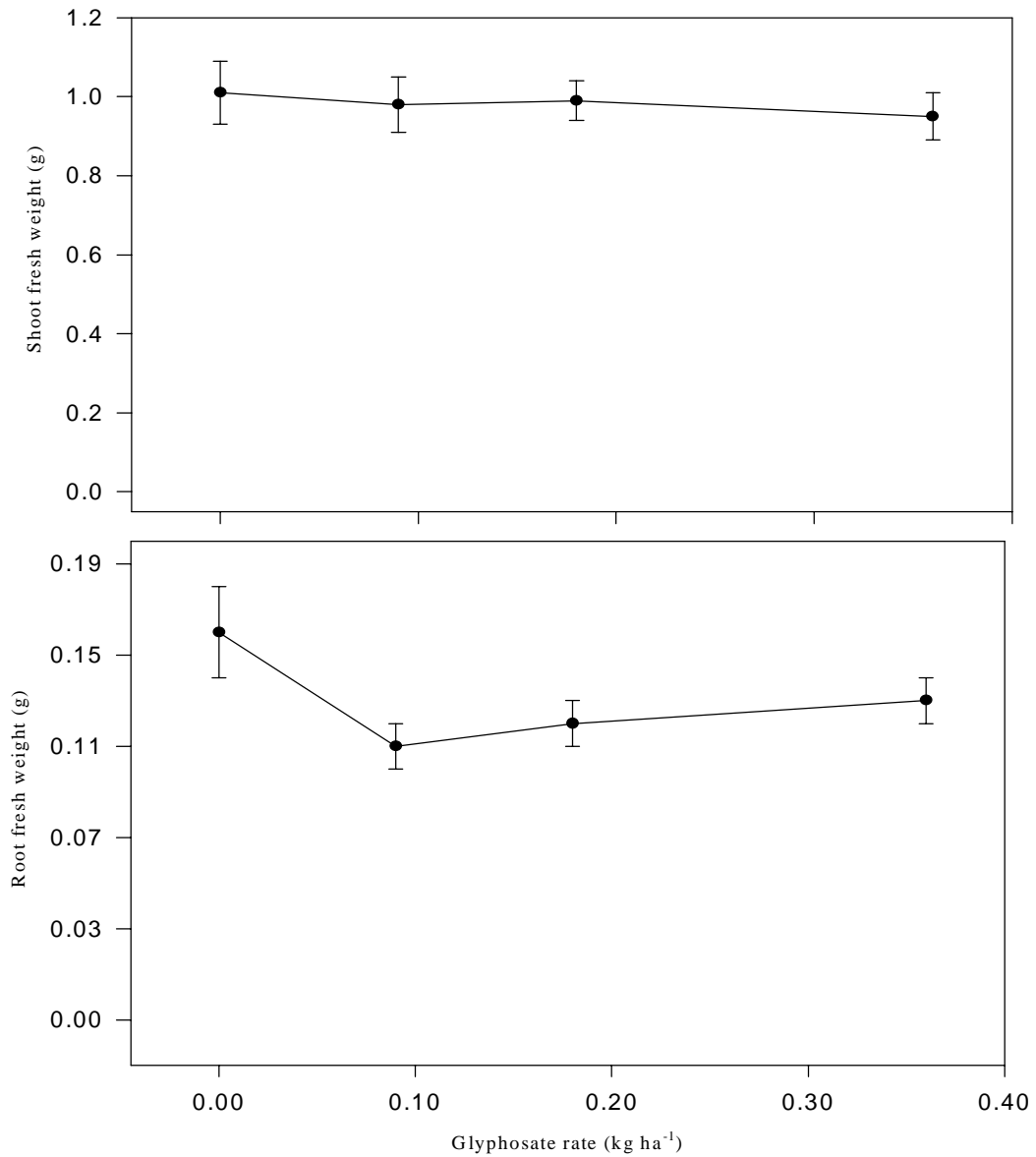


Figure 2.1. Effect of glyphosate on the growth of shoot and root of common vetch. Fresh weights were recorded 1 wk after application of the herbicide. Data are the means of 17 replications and vertical bars represent standard error of means.

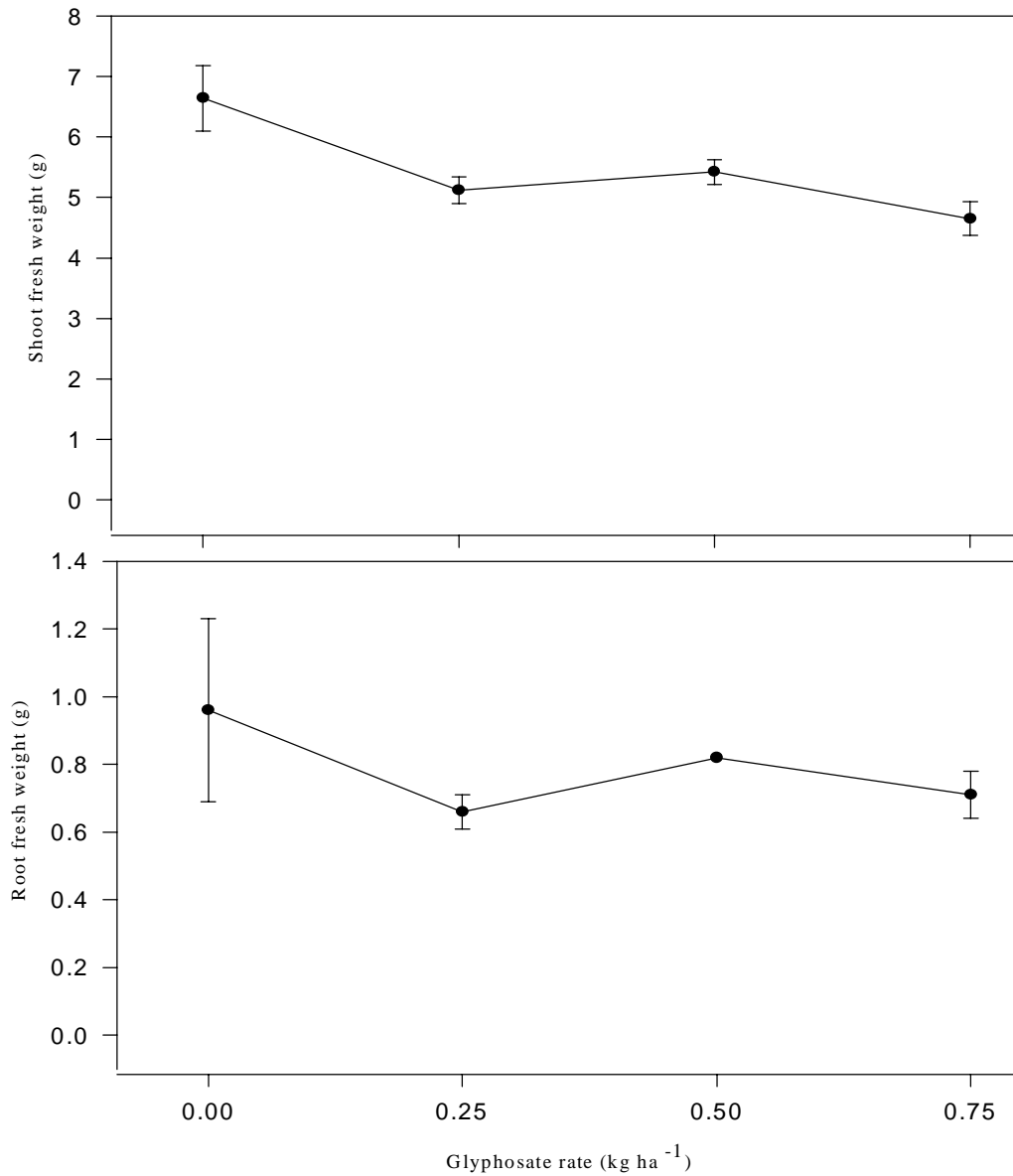


Figure 2.2. Effect of glyphosate on the growth of shoot and root of oilseed rape. Fresh weights were recorded 1 wk after application of the herbicide. Data are the means of 12 replications and vertical bars represent standard error of means.

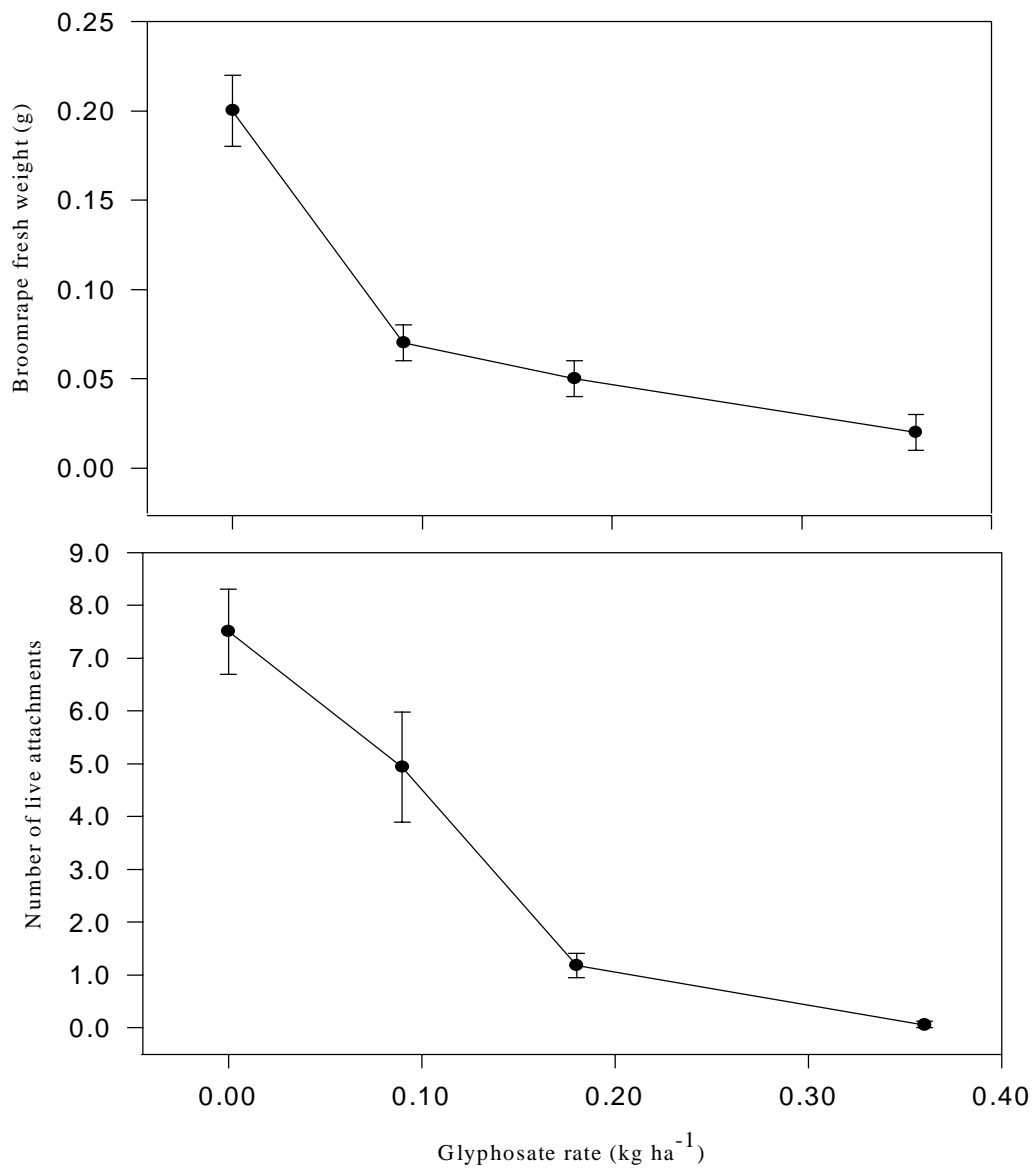


Figure 2.3. Effect of glyphosate on the growth and the number of live broomrape attachments in common vetch. Fresh weight and number of attachments were recorded 1 wk after application of the herbicide. Data are the means of 17 replications and vertical bars represent standard error of means.

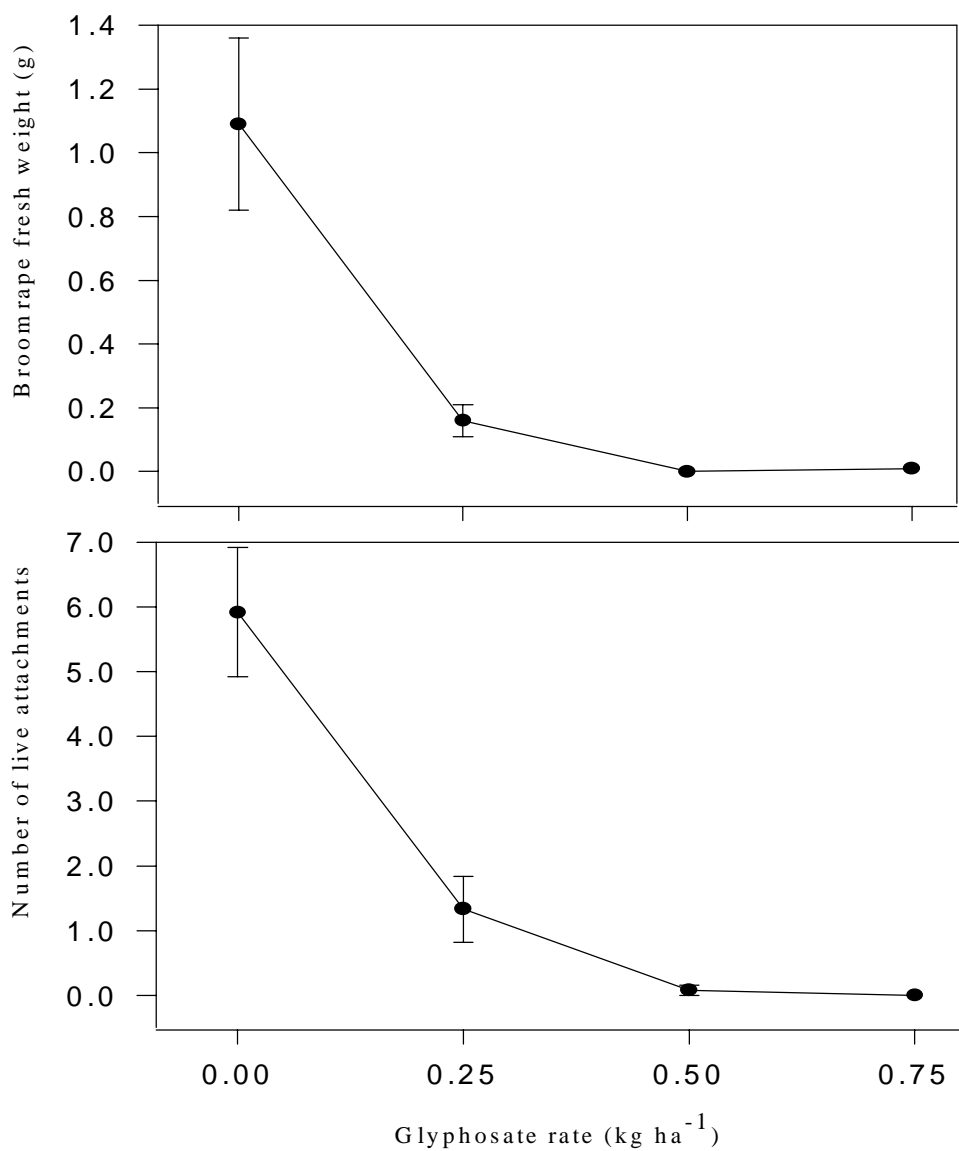


Figure 2.4. Effect of glyphosate on the growth and the number of live broomrape attachments in oilseed rape. Fresh weight and number of attachments were recorded 1 wk after application of the herbicide. Data are the means of 12 replications and vertical bars represent standard error of means.

Table 2.1. Absorption and translocation of foliar-applied ¹⁴C-glyphosate in common vetch and oilseed rape 4 d after treatment.

Plant type	Total absorbed	Treated leaf	Total translocated
	% of applied ^a	% of absorbed ^a	
Common vetch			
Parasitized	47	88	12
Nonparasitized	53	90	10
Significance	NS ^b	NS	NS
Oilseed rape			
Parasitized	77	61	39
Nonparasitized	66	63	37
Significance	NS	NS	NS

^aData are the means of nine replications for common vetch and six replications for oilseed rape.

^bIndicates no significant difference between parasitized and nonparasitized plant means within each column and within each crop according to Wilcoxon-Mann-Whitney's rank sum test ($p = 0.05$).

Table 2.2. Distribution of foliar-applied ^{14}C -glyphosate, expressed as radioactivity in Bq per unit fresh weight, in tissues of common vetch and oilseed rape 4 d after treatment.

Plant type	Shoot above	Shoot below	Shoot apex	Root	Broomrape attachments
	treated leaf	treated leaf			
Bq mg ⁻¹ ^a					
Common vetch					
Parasitized	0.11	0.33	2.62	0.39	4.34
Nonparasitized	0.15	0.30	3.22	0.28	-
Significance ^b	NS	NS	NS	NS	-
Oilseed rape					
Parasitized	2.23	1.39	9.61	0.31	2.82
Nonparasitized	1.39	0.16	10.82	0.59	-
Significance	NS	NS	NS	NS	-

^aData are the means of nine replications for common vetch and six replications for oilseed rape.

^bIndicates no significant difference between parasitized and nonparasitized plant means within each column and within each crop according to Wilcoxon-Mann-Whitney's rank sum test ($p = 0.05$).

Table 2.3. Distribution of foliar-applied ¹⁴C-glyphosate, expressed as % of translocated, in tissues of common vetch and oilseed rape, 4 d after treatment.

Plant type	Shoot above	Shoot below	Shoot apex	Root	Broomrape attachments
	treated leaf	treated leaf			
% of translocated ^a					
Common vetch					
Parasitized	19	16	24	14	27
Nonparasitized	29	17	25	29	-
Significance ^b	NS	NS	NS	*	-
Oilseed rape					
Parasitized	9	9	15	36	31
Nonparasitized	20	14	19	47	-
Significance	*	*	NS	*	-

^aData are the means of nine replications for common vetch and six replications for oilseed rape.

^bA significant difference between parasitized and nonparasitized plant means within each column and within each crop is indicated by * and a nonsignificant difference by NS, according to the Wilcoxon-Mann-Whitney's rank sum test ($p = 0.05$).

Table 2.4. Proportion of ¹⁴C-glyphosate and its metabolites in tissues of common vetch, 4 d after treatment, analyzed by thin layer chromatography according to Sprankle et al. (1978).

Tissue	% of extracted activity ^a	
	Glyphosate	Metabolites
Treated leaf	89	11
Shoot above treated leaf	77	23
Shoot below treated leaf	73	27
Shoot apex	81	19
Root	76	24
Broomrape attachments	79	21

^aAverage of two replicated experiments.

3. INFLUENCE OF EGYPTIAN BROOMRAPE PARASITISM ON AMINO ACID COMPOSITION OF CARROT

3.1 INTRODUCTION

Egyptian broomrape (*Orobanche aegyptiaca* Pers.) is a phanerogamic holoparasite that attacks the roots of many dicotyledonous crops. It lacks chlorophyll (Baccarini and Melandri 1967; Saghir et al. 1973) and obtains carbon, nutrients, and water through haustoria which connect the parasite with the host's vascular system. Broomrape parasitism has a detrimental effect on the growth and yield of several economically important host crops, including carrot (*Daucus carota* L.) and legumes such as common vetch (*Vicia sativa* L), in the Mediterranean region (Foy et al. 1989; Goldwasser et al. 1997).

Due to its close association with the host plant throughout its life cycle and its growth under the soil surface, many conventional weed-control strategies targeted at broomrape have been futile. Anecdotal reports suggest that nitrogen, in the form of either manure or inorganic fertilizer, has some inhibitory effect on broomrape parasitism. This observation is supported by findings from controlled greenhouse and laboratory studies (reviewed by Parker and Riches 1993; Sauerborn 1991). Understanding the mechanism of nitrogen inhibition of broomrape is central to effective and practical utilization of the above strategy. Knowledge about the nitrogen, amino acids as a starting point, status of broomrape is essential for elucidating the mechanism of nitrogen inhibition of its growth.

Some reports on the nitrogen status of broomrape exist. Lee and Stewart (1978) were unable to detect nitrate reductase activity in broomrape, which indicates that the parasite is unable to utilize nitrogen in the form of nitrate. Low activity of glutamine synthetase, the enzyme that catalyzes the incorporation of ammonium into organic compounds, was found in broomrape and another holoparasite, *Lathraea* (McNally and Stewart 1987; McNally et al. 1983a, 1983b, 1984). Additionally, in *Lathraea*, nitrate reduction has been shown to be incomplete or to proceed at very low rates (Thalouarn et al. 1987, 1988, 1990).

Absence or low activity of nitrogen assimilating enzymes in broomrape may indicate

that the parasite must have access to organic nitrogen in the host plant. Translocation of amino acids and amides has been shown for broomrape (Aber et al. 1983) and other angiospermous parasites (Fer 1979; McNally et al. 1983a; Renaudin and Larher 1981; Thalouarn et al 1986). Broomrape seeds were able to absorb and metabolize radioactive leucine (Leu) both during conditioning and germination, and a part of the Leu was metabolized (Bar Nun and Mayer 1993). Determination of the amino acid profiles of broomrape and its hosts will provide a better understanding of the biochemistry and physiology of the host-parasite interaction, and perhaps, help elucidate the basis of suppression of growth of broomrape by nitrogen.

The objective of this research was to determine the nitrogen status of broomrape in relation to its host, carrot, by investigating the effect of broomrape parasitism on the amino acid composition of carrot, and by comparing amino acid profiles of different growth stages of broomrape.

3.2 MATERIALS AND METHODS

3.2.1 Plant Material

Carrot (variety 'Nantes Coreless'¹) seeds were surface sterilized for 15 min by soaking in a solution containing 1% (by vol) sodium hypochlorite [NaOCl (commercial bleach)] and Tween 20^{®2} (1:100 by vol), rinsed with sterile distilled water, and dried. Seeds were then planted in sterile vermiculite. Seedlings emerged after 10 d. After appearance of the second true leaf, vermiculite was washed off the roots and the seedlings were transplanted into polyethylene bags³ containing strips of filter paper⁴. Transplanting, fertilization, and watering procedures, and growth conditions were similar to those described in the Materials and Methods section of chapter 2.

Broomrape⁵ seeds were surface sterilized, as described for carrot seeds, following a

20-s dip in 70% (by vol) aqueous ethanol. Seeds were conditioned and germinated according to Mangnus et al. (1992). Approximately 50 surface-sterilized broomrape seeds were placed on a 1-cm diam glass-fiber filter paper⁶ (GFFP) disk and covered with a second disk. Disk sandwiches were placed in 9-cm-diam Petri dishes, 60 per dish, and moistened with sterile distilled water, 7 mL per dish. Dishes sealed with parafilm, were incubated at 25 C in the dark for 1 wk. After the conditioning period, disks were blotted dry on a paper towel for 1 h to remove excess moisture. Disks were then treated with a 10 mg L⁻¹-stock solution of GR-24,⁷ a synthetic analog of strigol, a germination stimulant, at 75 µl per disk. Two rings of laboratory-cut Whatman no. 1 filter paper⁸ of 9 cm outer diam and 7 cm inner diam were placed inside the lid of the Petri dish and wetted with 2 ml sterile distilled water to prevent the seeds from drying. GR-24-treated disks were returned to the Petri dishes, that were then sealed with parafilm and incubated at 25 C for 8 d. Seeds were considered to have germinated when the radicles had emerged. Embryos of both dry and germinated seeds were devitalized by soaking the seeds in a film of 100% ethanol for 8 h at 25 C in 9-cm Petri dishes. Ethanol was allowed to evaporate overnight and then seeds were harvested.

With the development of a widespread carrot root system two wk after transplanting, conditioned seeds of broomrape were spread on the roots with a spatula. Carrot plants were checked periodically under a microscope for broomrape germination and attachments on carrot roots. Initial attachments were found 7 d after conditioned seeds were spread on the roots. Tubercles were observed 4 wk after formation of the initial attachments and were harvested 2 wk after formation. Floral spikes of broomrape were collected 2 wk after emergence from the tubercle. Leaves and roots from both parasitized and nonparasitized carrot plants were harvested 7 wk after the seedlings were transplanted into the polyethylene bags.

Callus cultures of broomrape were developed and maintained according to Ben-Hod et al. (1991b). Surface sterilized and conditioned broomrape seeds were placed on a medium containing one-half strength Murashige and Skoog (1962) basal salt mixture,⁹ 2% (by wt) sucrose,¹⁰ 100 mg L⁻¹ myo-inositol,¹¹ 0.5 mg L⁻¹ pyridoxine,¹² 0.1 mg L⁻¹ thiamine,¹³ and 0.5 mg L⁻¹ nicotinic acid.¹⁴ The medium was solidified with 0.8% (by wt) agar¹⁵. Seeds were then wetted with filter-sterilized GR-24 solution at 10 mg L⁻¹ to stimulate germination. Two

weeks after germination, broomrape seed calli were transferred to a fresh medium containing naphthylacetic acid¹⁶ and benzyl adenine,¹⁷ both at concentrations of 0.1 mg L⁻¹. Calli were subcultured initially at 2-wk intervals for 6 wk, and, thereafter transferred to fresh medium every 4 wk. Cultures were always placed in the dark. Friable seed calli were harvested every 4 wk.

Immediately after harvest, all tissues were quickly frozen in liquid nitrogen and lyophilized or stored at -80 C until lyophilized. Dried tissues, except dry and germinated seed, collected over several months and stored at -20 C were pooled and ground to pass a 1-mm screen using a cyclone mill¹⁸ and stored at -20 C until analyzed. Subsamples were used for chemical analyses, and results are expressed on an “as is” dry weight basis.

3.2.2 Chemical Analyses

Total N, C, and S of duplicate subsamples of each tissue sample, were determined simultaneously by combustion and gas chromatography techniques using a Fison Instruments C-H-N-S analyzer¹⁹ according to Pella and Colombo (1978). Amino acids were analyzed as phenylthiocarbamyl derivatives using a modification of the procedure of Bidlingmeyer et al. (1984) which is described below. Protein amino acid concentrations were derived by subtracting the values obtained from free-amino acid analyses from total-amino acid values obtained from analysis of the corresponding hydrolysates.

3.2.3 Preparation of Samples for Amino Acid Analysis

For extraction of free amino acids, the procedure described by Foster (1990) was used. A 0.5 g subsample of each sample was placed in a cellulose thimble²⁰ in a Soxhlet extractor²¹; 250 µl of a 20 mM stock solution of norvaline²² [NVa (internal standard)] was added to each thimble; and samples were extracted with 35% (by vol) aqueous ethanol for 90 min. Flasks containing extracts were transferred to a 35 C water bath and evaporated to

dryness with a stream of air. The dry residue was resuspended in 6 ml of 35% (by vol) aqueous ethanol. After passage of the resuspended residue through an activated C18 Sep-Pak cartridge²³ and filtration through a 0.2 µm nylon syringe filter,²⁴ extracts were stored at -20 C until derivatized.

For total amino acid determinations, subsamples of 0.02 g of each tissue were weighed into 5-ml glass ampules²⁵. After addition of 3 ml of 6 N hydrochloric acid (HCl), ampules were immediately purged with grade-5 nitrogen and sealed. Samples were then hydrolyzed for 6 h in an autoclave²⁶ set at 132 C. For quantification of S-containing amino acids, samples were oxidized prior to hydrolysis. Subsamples weighing 0.02 g were oxidized for 16 h with 0.7 ml performic acid (Spindler et al., 1984). As described by Elkin and Griffith (1985), performic acid was prepared by mixing 30% hydrogen peroxide (H₂O₂) and 88% formic acid (FA) containing phenol (5.56 mg ml⁻¹) in the ratio 1 part H₂O₂ to 9 parts FA. The oxidation process was terminated by addition of 0.1 ml cold 48% hydrobromic acid (HBr). After 30 min, three drops of 1-octanol were added to each ampule to minimize foaming. Samples were reduced to dryness in a Savant concentrator/evaporator²⁷ and hydrolyzed as described above. Hydrolysates were transferred to 5-ml volumetric flasks, 1 ml of internal standard solution [α -aminobutyric acid²⁸ (AAB) in 0.1 N HCl, 5 µmol ml⁻¹] was added, and the volume brought to 5 ml with MilliQ²⁹ water. Aliquots filtered through a 0.45 µm Teflon syringe filter³⁰ were used for the amino acid derivatization sequence. Tryptophan, which is destroyed by acid hydrolysis, regardless of oxidative pretreatment, was not analyzed.

3.2.4 Derivatization Procedure

Ten microliters of hydrolysates, and 20 µl of free-amino acid extracts and standards were added to labeled Pyrex® culture tubes tubes³¹ (6 by 50 mm) and dried in a Savant concentrator/evaporator. Two redrying steps were performed by adding, in each step, 10 µl of the redrying reagent [methanol, deionized water, and triethylamine³² (2:2:1 by vol)] under vacuum. Twenty microliters of the derivatization reagent [methanol, deionized water, triethylamine, and phenylisothiocyanate³³ (7:1:1:1 by vol)] was added to each sample tube; the

reaction was allowed to proceed for 10 min; then the liquid was dried under vacuum. Derivatives were stored frozen at -20 C until analyzed. Two hundred microliters of diluent (710 mg of disodium hydrogen phosphate dissolved in 1 L of MilliQ water, pH adjusted to 7.4 with 10% phosphoric acid, and acetonitrile³⁴ added at 5% by vol) was added to each culture tube with a Hamilton syringe. After the tubes were centrifuged for 30 min at low speed (1200 rpm) in a Savant concentrator, supernates were transferred to autosampler vials with a Pasteur pipette.

3.2.5 Preparation of Amino Acid Standards

Stock solutions, 2.5 $\mu\text{mol ml}^{-1}$, of individual amino acids³⁵ [AAB, cysteic acid (Cya), hydroxyproline (Hyp), methionine sulfone (MetSO₂), and norleucine (Nle)] were prepared in 0.1 N HCl. A mixture containing 0.2 ml each of the individual amino acid stock solutions was prepared. A 0.2-ml aliquot of this mixture was added to a 0.2-ml aliquot of the protein amino acid standard solution³⁶ (Standard H). Ten microliters of this mix was used for the derivatization procedure.

3.2.6 Analytical Procedure

High performance liquid chromatographic analysis of phenylisothiocyanate (PITC)-derivatized samples was performed using a system consisting of a Perkin Elmer Series 200 LC pump³⁷, a Beckman Instruments model 165 variable wavelength detector³⁸ (254 nm), a Thermo Separation Products Spectra System model AS3000 autosampler³⁹ equipped with a refrigerated sample compartment, and a Waters Pico-Tag Free Amino Acid analysis column⁴⁰ (3.9 x 30 cm). Column temperature was maintained at 36 C with a metal-block column heater⁴¹. Separation of sample components was accomplished using the gradient listed in Table 3.1. Data acquisition and peak quantification were accomplished using a PE Nelson Turbochrome 4 chromatographic data system⁴². Amino acid concentrations are expressed as $\mu\text{mol per g}$ dry weight of tissue.

3.3 RESULTS AND DISCUSSION

3.3.1 Elemental Analysis

Elemental analysis of the carrot and broomrape tissues revealed differences in the C, N, and S composition of the tissues (Table 3.2). Total C concentrations, expressed as a percentage of the dry weight of the tissues, were similar in leaves of nonparasitized (40.6%) and parasitized (42.0%) carrot plants. Parasitized carrot roots had a slightly higher C concentration (39.4%) compared to the nonparasitized roots (33.4%). This difference may be due to the higher demand for organic compounds in the parasitized roots due to broomrape attachments. It also reflects the contribution of the broomrape attachments to the composition of the parasitized root samples. The total C concentrations in broomrape tubercles (42.6%) and shoots (43.2%) were higher than that in the nonparasitized roots.

Seeds of broomrape exhibited higher concentrations of C (52.1% in dry seeds and 55.1% in germinated seeds) than other broomrape tissues (42.6% to 45.3%). Similar concentrations in the two seed types suggest that the rate of metabolism may not be as high in germinating seeds as it is in the actively growing tubercle or shoot. The concentration of C in broomrape callus (45.3%) was comparable to those in the tubercles and shoot, indicating that the parasite, in the form of callus, is able to sustain its carbon requirement in the absence of the host.

Total N and total S analyses gave similar results for leaves of nonparasitized (4.3% N and 0.5% S) and parasitized (4.2% N and 0.5% S) carrot plants. On a dry weight basis, both N and S concentrations were lower in the parasitized roots (2.8% N and 0.3% S) than in the nonparasitized roots (3.8% N and 0.4% S). These values are consistent with a dilution effect associated with the presence of broomrape tubercles (2.8% N and 0.6% S) and shoots (2.1% N and 0.2% S) in the parasitized root sample, but may also reflect an adverse effect of the parasite on N and S incorporation by the host plants. Although the tubercle N concentration was similar to that of the parasitized root, the tubercle S concentration was higher than that in the parasitized root and all other tissues (carrot or broomrape) (0.2% to 0.5%) analyzed.

These results indicate that the tubercle is an actively growing stage of the parasite and has a high demand for N- and S-containing compounds. Tubercle N and S concentrations were higher than those in the shoots, but similar to those in the callus (3.0% N and 0.5% S). Thus, broomrape appears to have the ability to sustain its nitrogen requirement in the presence of an organic N source. Parasitic plants, when grown on an artificial medium containing an organic N source, performed better compared to an inorganic N source (Hall et al. 1987; Igbinossa and Thalouarn 1996; Okonkwo 1987). Total N and S concentrations of dry (1.9% N and 0.2% S) and germinated (1.7% N and 0.2% S) broomrape seeds were similar and lower than those observed in all of the other tissues, both carrot and broomrape.

3.3.2 Amino Acid Composition

The amino acid composition of hydrolysates of carrot and broomrape tissues is presented in Figures 3.1, 3.2, and 3.3. Amino acid profiles of roots from nonparasitized and parasitized carrot plants were similar (Figure 3.1). Concentrations of individual amino acids were generally higher in leaves of nonparasitized carrot plants than in leaves of parasitized plants. In particular, concentrations of alanine (Ala), valine (Val), Leu, and lysine (Lys) of leaves of nonparasitized plants (67, 35.5, 52.6, and 38.2 $\mu\text{mol g}^{-1}$, respectively) were higher than those in the leaves of parasitized plants (46.2, 19.4, 26.4, and 20.7 $\mu\text{mol g}^{-1}$, respectively). Although total N (expressed as % on dry weight basis) was similar in leaves of nonparasitized (4.3%) and parasitized (4.2%) carrot plants (Figure 3.1), amino acid concentrations were lower in leaves of parasitized plants compared to those in leaves of nonparasitized plants. This suggests that N has been incorporated into compounds other than amino acids. Also, broomrape parasitism may have altered the functioning of certain enzymes which may have caused metabolism of amino acids and subsequent incorporation of N into other compounds such as alkaloids, ureides, nitrogenous bases of DNA and RNA, and precursors of chlorophyll.

With the exception of Hyp, Ala, Val, isoleucine (Ile), Leu and Lys, levels of individual amino acids in leaves were equal to or greater than their corresponding levels in the roots,

whether or not the plants were parasitized. Differences in the amino acid composition of the leaves of the nonparasitized and parasitized plants may reflect broomrape parasitism.

A particularly noteworthy observation is that tubercles had similar or higher concentrations of amino acids (25% more Ala, increase in Val and Ile by two-fold or more, more than 50% Leu, and more than 75% Lys), except glycine (Gly), arginine (Arg), proline (Pro), and tyrosine (Tyr), compared to leaves of nonparasitized carrot plants (Figures 3.1 and 3.3). It is possible that broomrape parasitism has altered the activity of certain enzymes in carrot plants by inactivation, inhibition, or reduction of enzyme protein. Some of the inactivated enzymes may be directly or indirectly linked to amino acid biosynthesis. Stewart (1987) has shown that activity of several enzymes was decreased in *Sorghum vulgare* L. due to parasitization by *Striga hermonthica* L., a hemi-parasitic weed. The activity of ribulose biphosphate carboxylase and phosphoenolpyruvate carboxylase in parasitized sorghum plants was reduced by 80 and 50%, respectively, compared to nonparasitized plants (Stewart 1987). Amino acids such as glutamate (Glu), aspartate (Asp), asparagine (Asn), and Ala are translocated through the xylem and phloem to different parts of the plant serving as sources of N. Disruption of enzyme activity in carrot plants by broomrape parasitism may have had an adverse effect on translocation of amino acids in carrot and further metabolism of amino acids. Effect of broomrape on host plants is unlike other interactions such as plants and mycorrhizae. Mycorrhizae are symbiotic and mutualistic associations between nonpathogenic or weakly pathogenic fungi and living root cells, primarily cortical and epidermal cells. The fungi improve the mineral and water-absorbing capacity of roots and receive organic nutrient from the plant in return.

Among the tissues of broomrape, concentrations of individual amino acids, except Glu, were similar in dry and germinated seeds (Figure 3.2). Glu was higher in the dry (63.4 $\mu\text{mol g}^{-1}$) compared to the germinated seeds (45.6 $\mu\text{mol g}^{-1}$). This amino acid, which contributes carbon skeletons for the synthesis of several other amino acids, may have been metabolized during germination. The tubercle, shoot, and callus had higher amino acid content compared to the dry and germinated seeds (Figures 3.2 and 3.3), indicating a higher rate of metabolic activity in the former compared to the latter. The amino acid composition of the callus was comparable to that of the tubercle, in spite of the absence of a host. Profiles of

enzymes involved in amino acid synthesis in the callus will provide more information on its biosynthetic capability and N regulation of broomrape. Typically, broomrape callus, derived from either seed or plant tissue, grows into an unorganized and nondifferentiated form (personal observation). However, Ben-Hod et al. (1991a) were able to parasitize tomato roots with partially differentiated broomrape calli developed from seeds (Ben-Hod et al. 1991b) and obtained broomrape shoots. The callus of broomrape, which is grown in absence of a host, could serve as an ideal model system for studying specific biochemical and physiological processes in broomrape.

Cya, derived from cysteine and cystine, was present at similar concentrations in all host and parasite samples analyzed. Leaves of nonparasitized and parasitized carrot plants, and tubercles of broomrape (19.4 to $23.2 \mu\text{mol g}^{-1}$) contained similar concentrations of methionine (Met), analyzed as MetSO_2 . These concentrations were higher than those in other tissues (7.6 to $14.7 \mu\text{mol g}^{-1}$) investigated (Figures 3.1, 3.2, and 3.3). In plants, the S-containing amino acids are mostly incorporated into proteins.

3.3.3 Free Amino Acid Composition

Differences in the free amino acid composition of the tissues of carrot and broomrape were notable. In carrot tissues, the concentration of Arg was greatest, followed by Asp, Ala, serine (Ser), and Glu (Figure 3.4). Concentrations of Asn and glutamine (Gln) in carrot tissues (data not shown) were comparable to Glu. These amides tend to convert to Asp and Glu, respectively, during storage, handling and analysis. As a consequence, Asp and Glu concentrations overestimate concentrations while Asn and Gln concentrations are underestimates. The sum of the concentrations for the acidic amino acids and their corresponding amides provide a value that can be related to the Asp and Glu values from hydrolysates.

Among tissues of broomrape, certain trends were apparent. Free amino acid concentrations of the tubercle were similar to those of the roots of parasitized carrot plants (Figures 3.4 and 3.6). This is evidence of the close association between the root and

broomrape tubercle. The contents of Asp, Asn (data not shown), and Glu of broomrape tissues (tubercle and shoot) (Figure 3.6) were generally higher than those of the other amino acids except Ser in callus ($5 \mu\text{mol g}^{-1}$), Arg and Ala in the tubercle (6.7 and $3.9 \mu\text{mol g}^{-1}$) and callus (27.3 and $17.7 \mu\text{mol g}^{-1}$). Analysis of xylem sap of *S. hermonthica* parasitizing *S. vulgare* indicated that the main nitrogen components were Asp, Asn, and Glu (Stewart 1987). The levels of Arg and Ala in broomrape callus (27.3 and $17.7 \mu\text{mol g}^{-1}$) were remarkably higher than any other amino acid in any tissue (Figures 3.4, 3.5, and 3.6). In mammals, Arg is a precursor to urea. Arg may serve as a storage form of organic nitrogen in broomrape. If nitrogen fertilization were to increase levels of Arg in broomrape, it may indicate the accumulation of the amino acid as a detoxifying mechanism of nitrogen by the parasite. Ser and Val levels in the callus (5 and $2.6 \mu\text{mol g}^{-1}$), though lower than Arg and Ala in the callus, were higher than any of the carrot or parasitic tissues.

3.3.4 Protein Amino Acid Composition

The protein amino acid composition of tissues of both carrot and broomrape reflected the respective total amino acid profiles, except the levels of Arg and Ala in the callus (Figures 3.7, 3.8, and 3.9). Levels of free pools of the S-containing amino acids, cystine (Cys2) and Met (data not shown) were almost negligible and almost all of Cys2 and Met was incorporated into protein. All of hydroxyproline was incorporated into protein (Figures 3.7, 3.8, and 3.9). There were negligible levels of free hydroxyproline. Hydroxyproline is an important constituent of plant cell wall proteins known as extensins or hydroxyproline-rich glycoproteins. The synthesis of these cell wall proteins is under strict developmental regulation and is known to be environmentally induced by pathogen attack (Keller 1993). Hydroxyproline was included in the analyses to note any change in its concentration in carrot plants due to broomrape attack.

3.4 SOURCES OF MATERIALS

¹ Carrot variety 'Nantes Coreless', American Seed, South Easton, MA 02375.

² Tween 20® polyoxyethylene sorbitan monolaurate, a surfactant. ICN Biomedicals, Inc., 1263 South Chillicothe Rd., Aurora, OH 44202.

³ Polyethylene bags, Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.

⁴ Glass-fiber filter paper (GF/A), Whatman International, Maidstone, UK.

⁵ Broomrape seed provided by Dr. Y. Kleifeld, Newe Ya'ar Research Center, Ramat Ishay 30095, Israel.

⁶ Glass-fiber filter paper (GFFP), Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.

⁷ GR-24 provided by Dr. Riopel, University of Virginia, Charlottesville, VA.

⁸ Whatman No. 1 filter paper, Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.

⁹ Murashige and Skoog basal salt mixture, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.

¹⁰ Sucrose, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.

¹¹ Myo-inositol, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.

¹² Pyridoxine, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.

¹³ Thiamine, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.

¹⁴ Nicotinic acid, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.

¹⁵ Agar, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.

¹⁶ Naphthylacetic acid, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.

¹⁷ Benzyl adenine, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.

¹⁸ Udy cyclone mill, Udy Corp., 201 Rome Ct., Ft. Collins, CO 80524.

¹⁹ Fisons Instruments C-H-N-S analyzer, 55 Cherry Hill Dr., Beverly, MA 01915.

²⁰ Whatman cellulose extraction thimbles (25 by 80 mm), Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.

²¹ Soxhlet extraction tubes, Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.

²² Norvaline, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.

²³ C18 Sep-Pak cartridges, Waters Corp., 34 Maple St., Milford, MA 01757.

²⁴ 0.2 µm nylon syringe filter, Gelman Sciences, 600 S Wagner Rd., Ann Arbor, MI

48103-9019.

- ²⁵ Wheaton glass ampules, Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.
- ²⁶ Thermatic 60 autoclave, Castle Sybron, 1777 E. Henrietta Rd., Rochester, NY 14602.
- ²⁷ Savant concentrator/evaporator, Models SVC-100H/10302-B, Savant Instruments, Inc., 100 Colin Dr., Holbrook, NY 11741.
- ²⁸ α -aminobutyric acid, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.
- ²⁹ MilliQ water, Millipore, 80 Ashby Rd., Bedford, MA 01730.
- ³⁰ Teflon syringe filter, Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.
- ³¹ Pyrex® culture tubes, Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.
- ³² Triethylamine, Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.
- ³³ Phenylisothiocyanate, Pierce Chemical Co., PO Box 117, Rockford, IL 61105.
- ³⁴ Acetonitrile, Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.
- ³⁵ Individual amino acids, Sigma Chemical Co., PO Box 14508, St. Louis, MO 63178.
- ³⁶ Protein standard H, Pierce Chemical Co., PO Box 117, Rockford, IL 61105.
- ³⁷ Perkin Elmer Series 200 LC pump, 761 Main Ave., Mail Stn. 10, Norwalk, CT 06859.
- ³⁸ Beckman Instruments Inc. model 165 variable wavelength detector, P.O. Box 3100, Fullerton, CA 92834-3100.
- ³⁹ Thermo Separation Products Spectra System model AS3000 auto-sampler, 355 River Oaks Pkwy., San Jose, CA 95134.
- ⁴⁰ Pico-Tag Free Amino Acid analysis column, Waters Corp., 34 Maple St., Milford, MA 01757.
- ⁴¹ Metal-block column heater, Scientific Systems Inc., 349 N Science Park Rd., State College, PA 16403
- ⁴² PE Nelson Turbochrome 4 chromatographic data system, 761 Main Ave., Mail Stn. 10, Norwalk, CT 06859.

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Table 3.1. Gradient table for analysis of phenylthiocarbamyl derivatives of amino acids using a Perkin Elmer series 200 quarternary pump.^a

Step	Time	Flow	%A ^b	%B ^c	Curve
0	5.0	1.0	100	0	0
1	1.0	1.0	100	0	0
2	5.0	0.6	95	5	1
3	15.5	1.0	54	46	1
4	7.5	0.6	0	100	1
5	2.0	1.2	0	100	0
6	6.0	1.4	0	100	0
7	2.0	1.4	100	0	1
8	2.0	1.4	100	0	0
9	2.0	1.2	100	0	0

^a Detector: Perkin Elmer Diode Array Model 235 C.

Column Oven: Perkin Elmer Series 200 Column Oven.

Autosampler: Perkin Elmer Series 200 Autosampler equipped with peltier accessory.

Pump: Perkin Elmer Series 200 Quarternary gradient LC Pump.

Data System: Perkin Elmer Nelson Turbochrome X.

^bEluent A: 0.14 M sodium acetate, 0.05% (by vol) triethylamine, pH 6.4 containing 6% (by vol) acetonitrile

^cEluent B: 60% (by vol) acetonitrile in water.

Table 3.2. Elemental composition of tissues of carrot and broomrape. Subsamples of 20 mg of each tissue were analyzed.

Tissue	Total C	Total N	Total S
	% ^a		
Carrot leaf (nonparasitized)	40.6 ± 0.2	4.3 ± 0.0	0.5 ± 0.0
Carrot root (nonparasitized)	33.4 ± 0.5	3.8 ± 0.1	0.4 ± 0.0
Carrot leaf parasitized)	42.0 ± 0.1	4.2 ± 0.0	0.5 ± 0.0
Carrot root (parasitized)	39.4 ± 0.1	2.8 ± 0.0	0.3 ± 0.0
<i>Broomrape</i> dry seed	52.1 ± 0.9	1.9 ± 0.1	0.2 ± 0.0
Broomrape germinated seed	55.2 ± 0.2	1.7 ± 0.0	0.2 ± 0.0
Broomrape tubercle	42.6 ± 0.2	2.8 ± 0.0	0.6 ± 0.00
Broomrape shoot	43.2 ± 0.21	2.1 ± 0.0	0.2 ± 0.0
Broomrape callus	45.3 ± 0.1	3.0 ± 0.0	0.5 ± 0.0

^aValues expressed on a dry weight basis. Data are means and standard deviations of duplicate analyses.

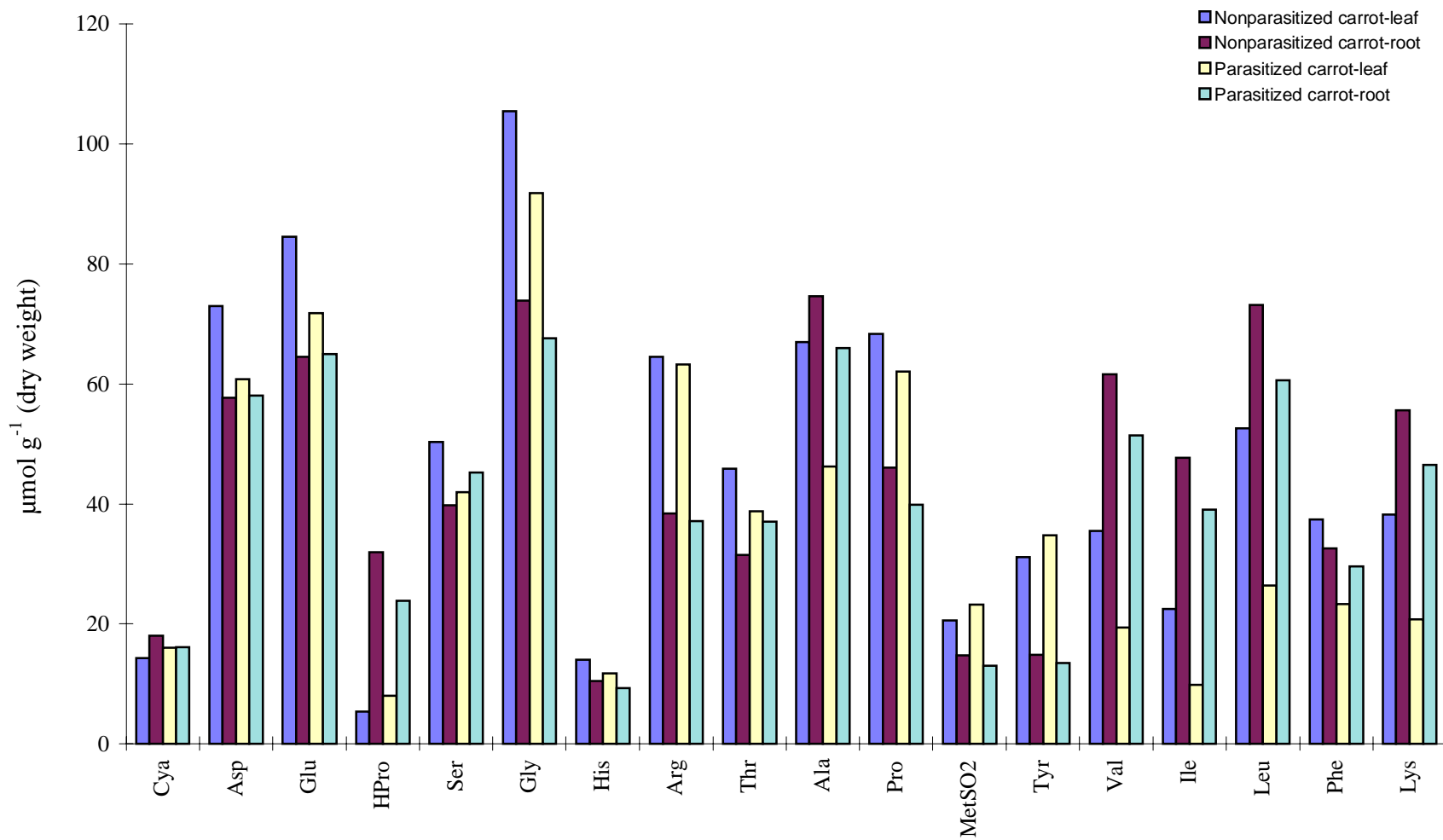


Figure 3.1. Amino acid composition of leaves and roots of nonparasitized and broomrape-parasitized carrot plants (obtained by hydrolysis with 6 N HCl). Note: Asp = Asp + Asn and Glu = Glu + Gln.

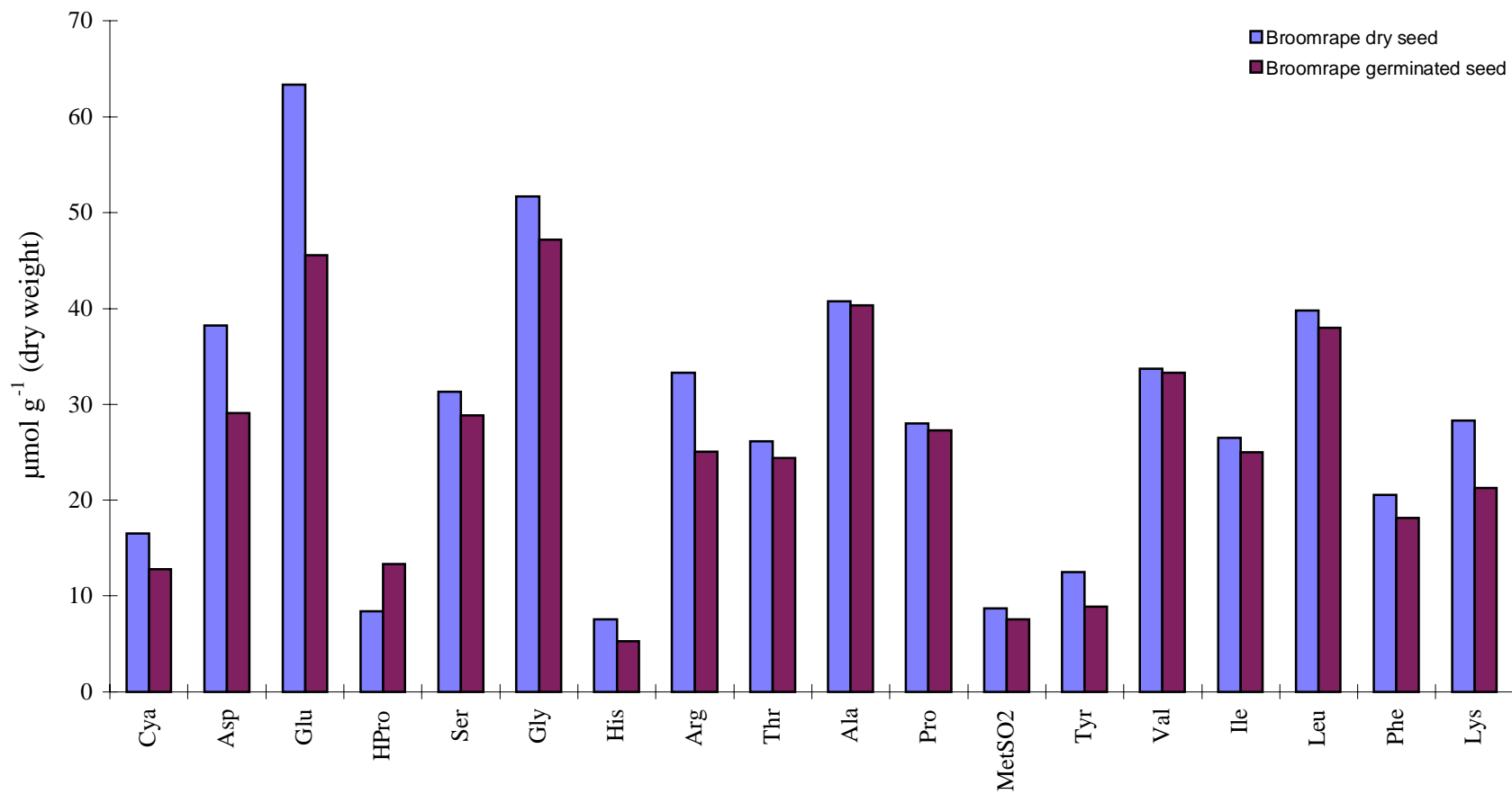


Figure 3.2. Amino acid composition of dry and germinated broomrape seeds (obtained by hydrolysis with 6 N HCl). Note: Asp = Asp + Asn and Glu = Glu + Gln.

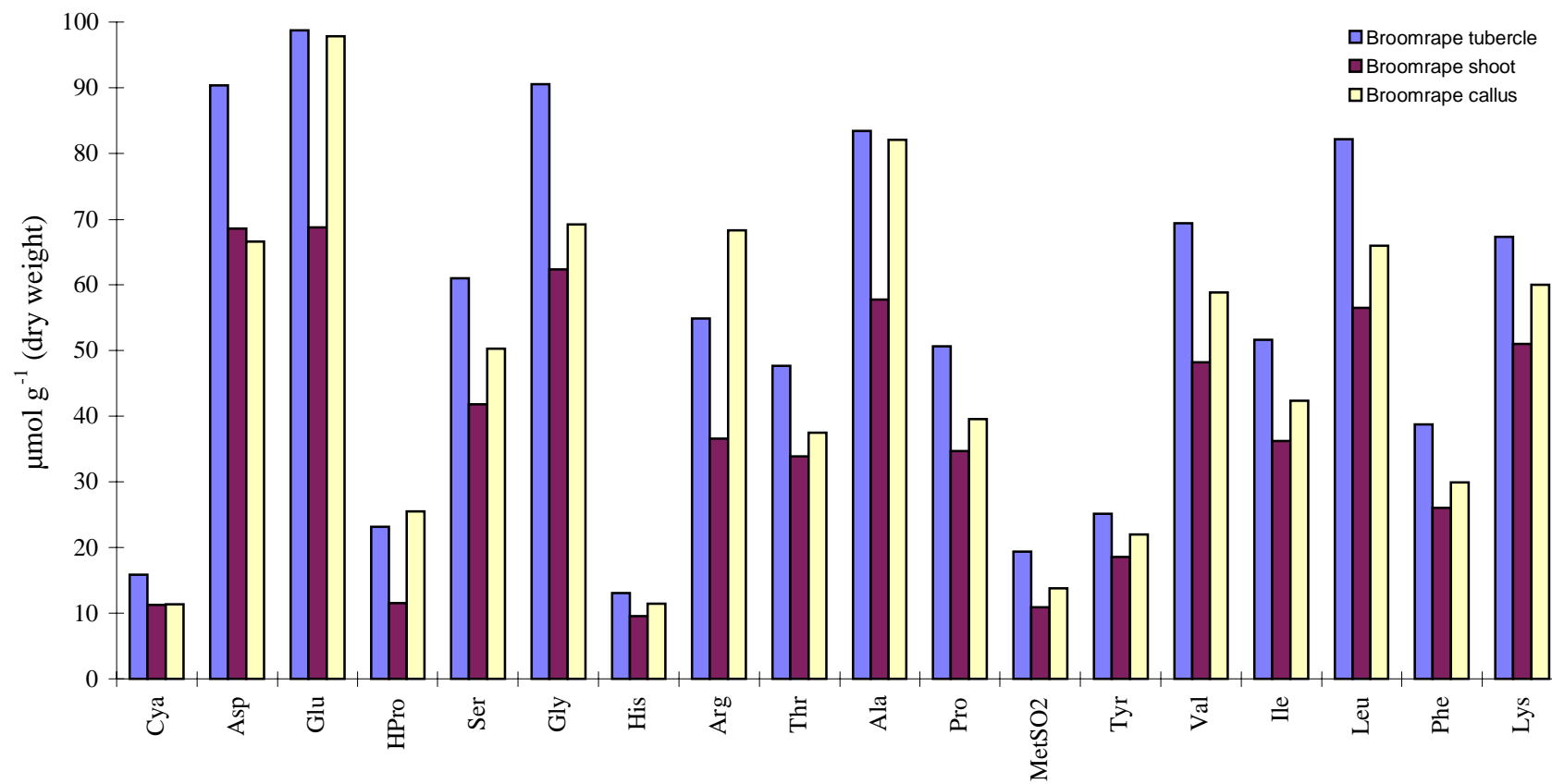


Figure 3.3. Amino acid composition of broomrape tubercle, shoot, and callus (obtained by hydrolysis with 6 N HCl). Note: Asp = Asp + Asn and Glu = Glu + Gln.

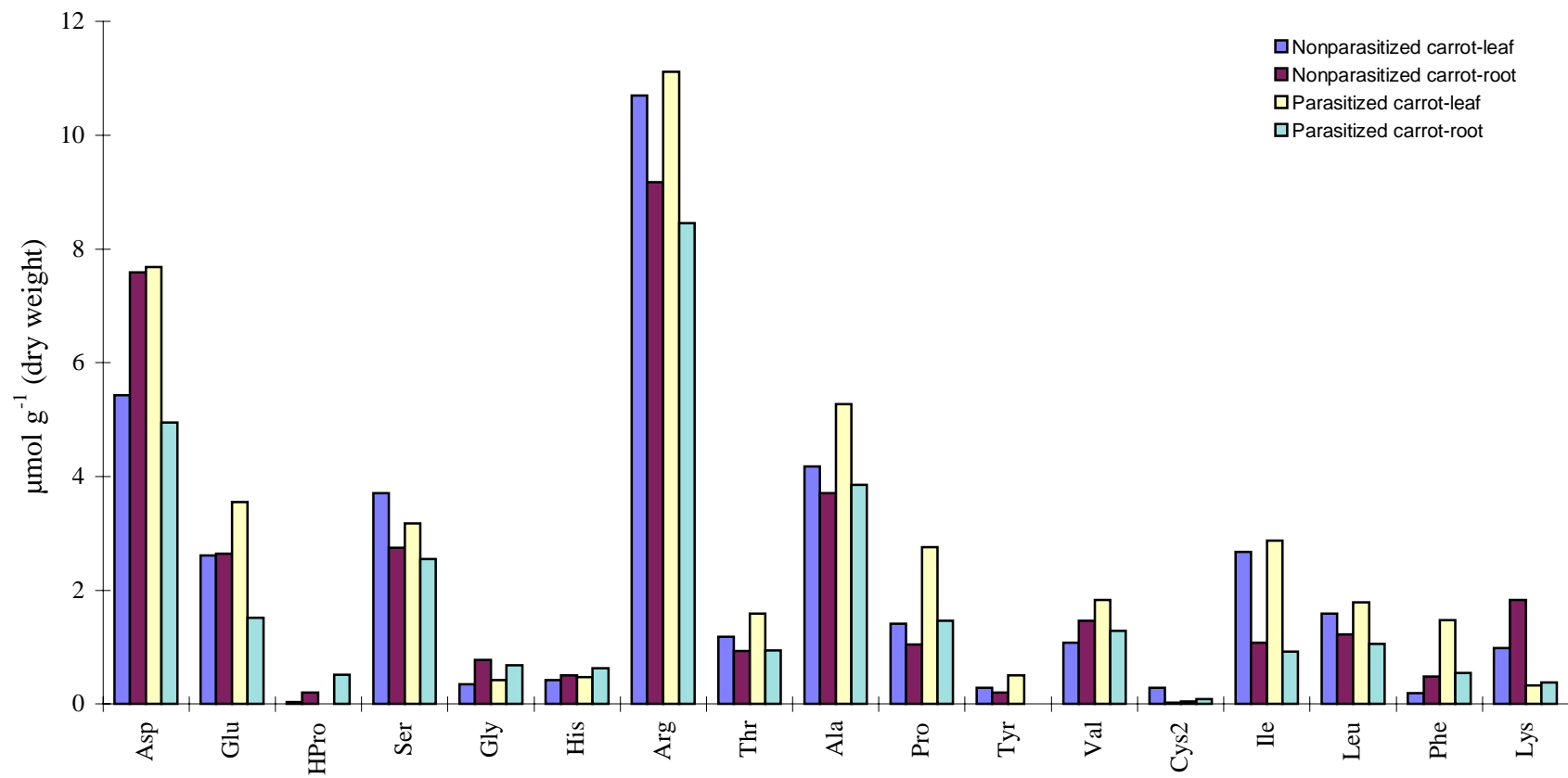


Figure 3.4. Free amino acid composition of leaves and roots of nonparasitized and broomrape-parasitized carrot plants (obtained by Soxhlet extraction with 35% (by vol) aqueous ethanol).

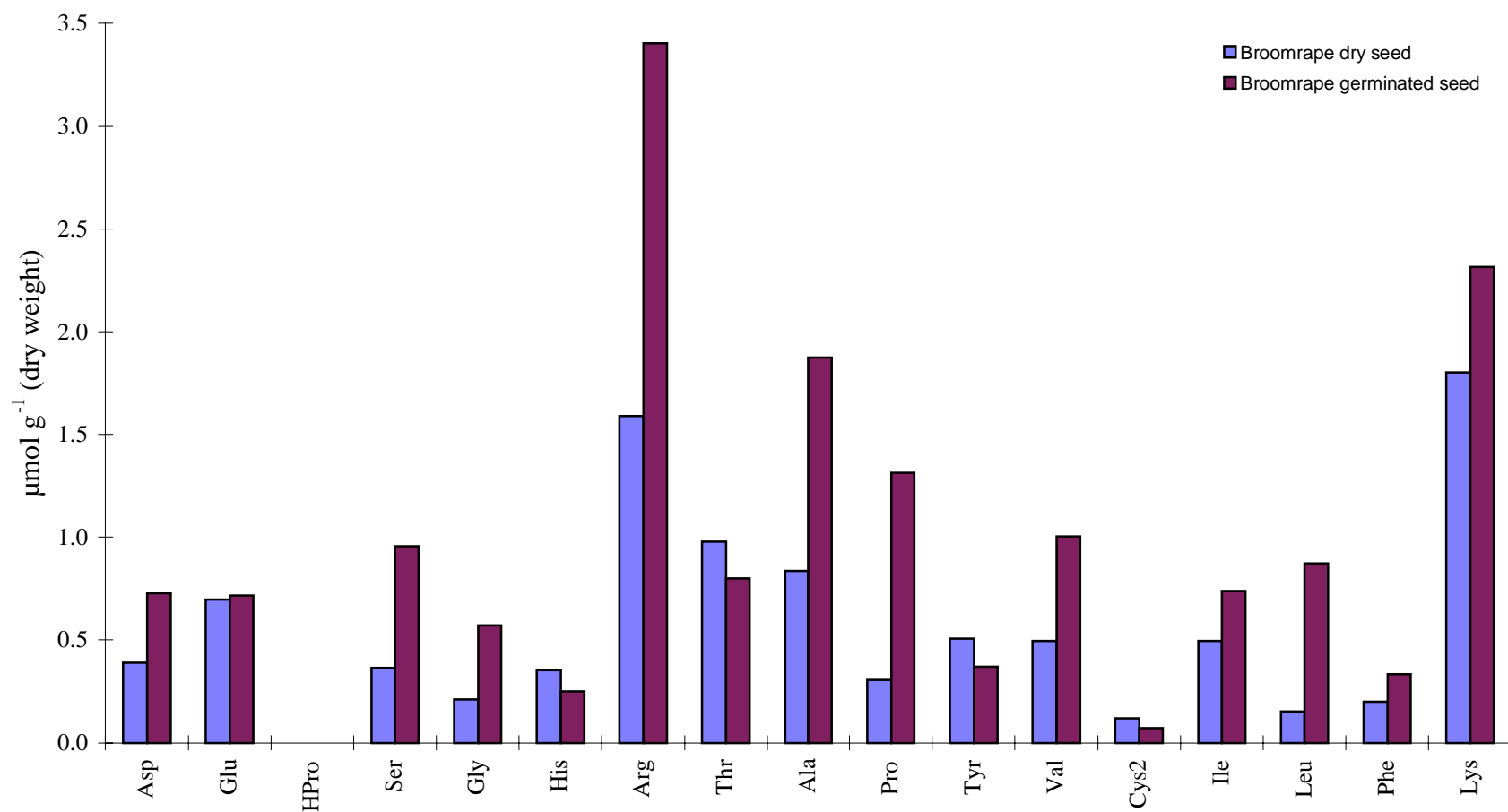


Figure 3.5. Free amino acid composition of dry and germinated broomrape seeds (obtained by Soxhlet extraction with 35% (by vol) aqueous ethanol).

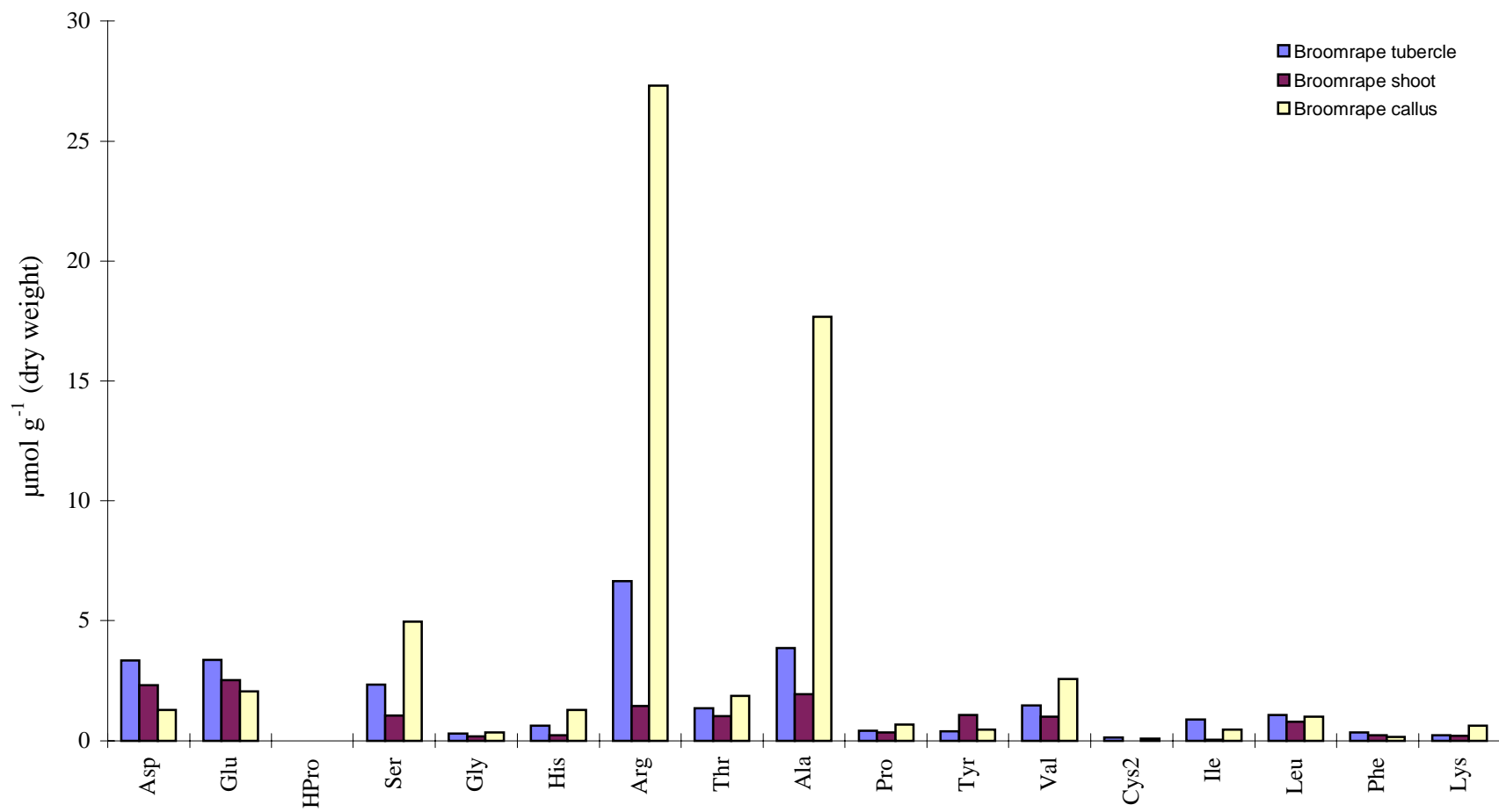


Figure 3.6. Free amino acid composition of tubercle, shoot, and callus of broomrape (obtained by Soxhlet extraction with 35% (by vol) aqueous ethanol).

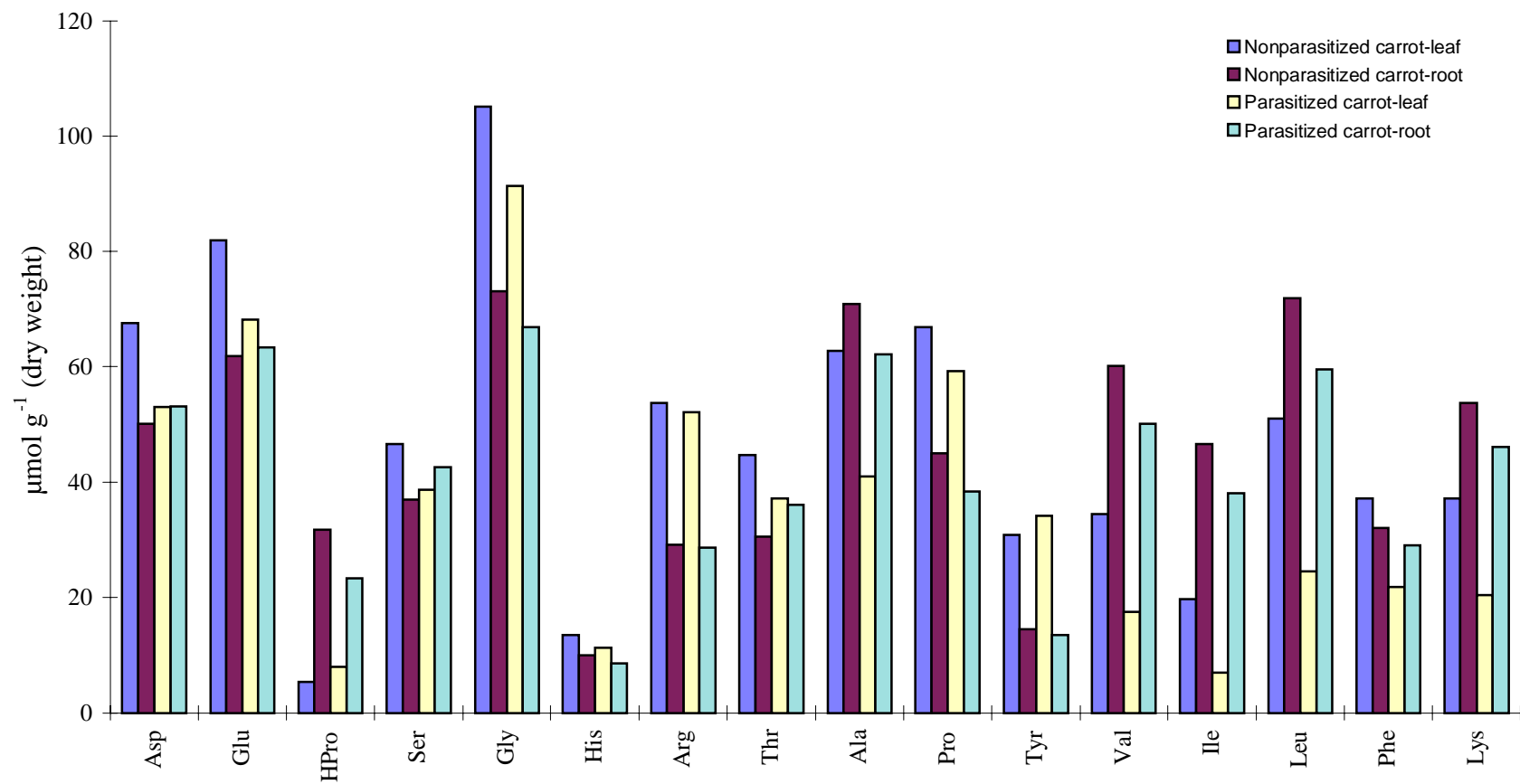


Figure 3.7. Protein amino acid composition of leaves and roots of nonparasitized and broomrape-parasitized carrot plants (obtained by subtracting free amino acid concentrations from total amino acid concentrations).

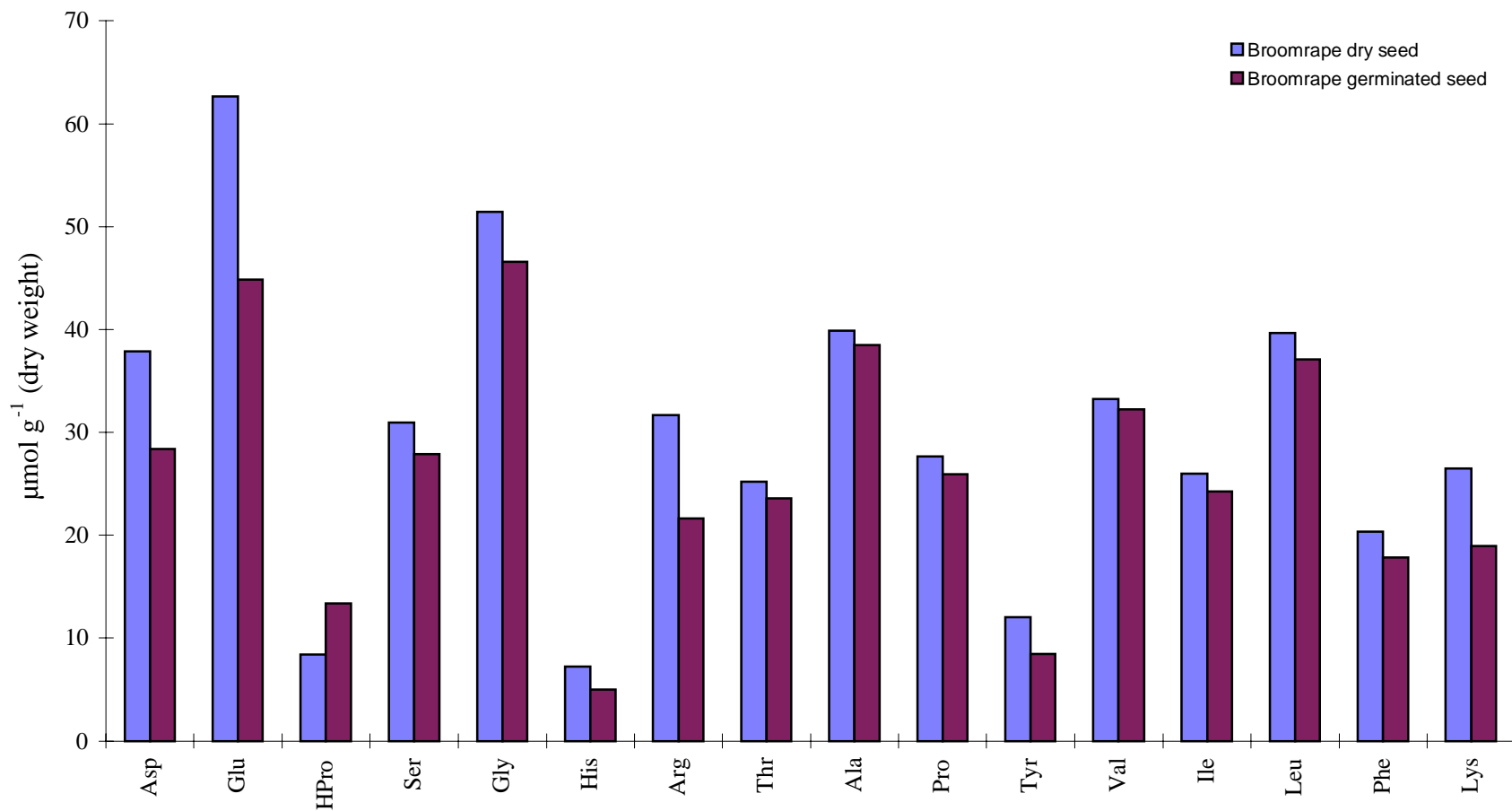


Figure 3.8. Protein amino acid composition of dry and germinated broomrape seeds (obtained by subtracting free amino acid concentrations from total amino acid concentrations).

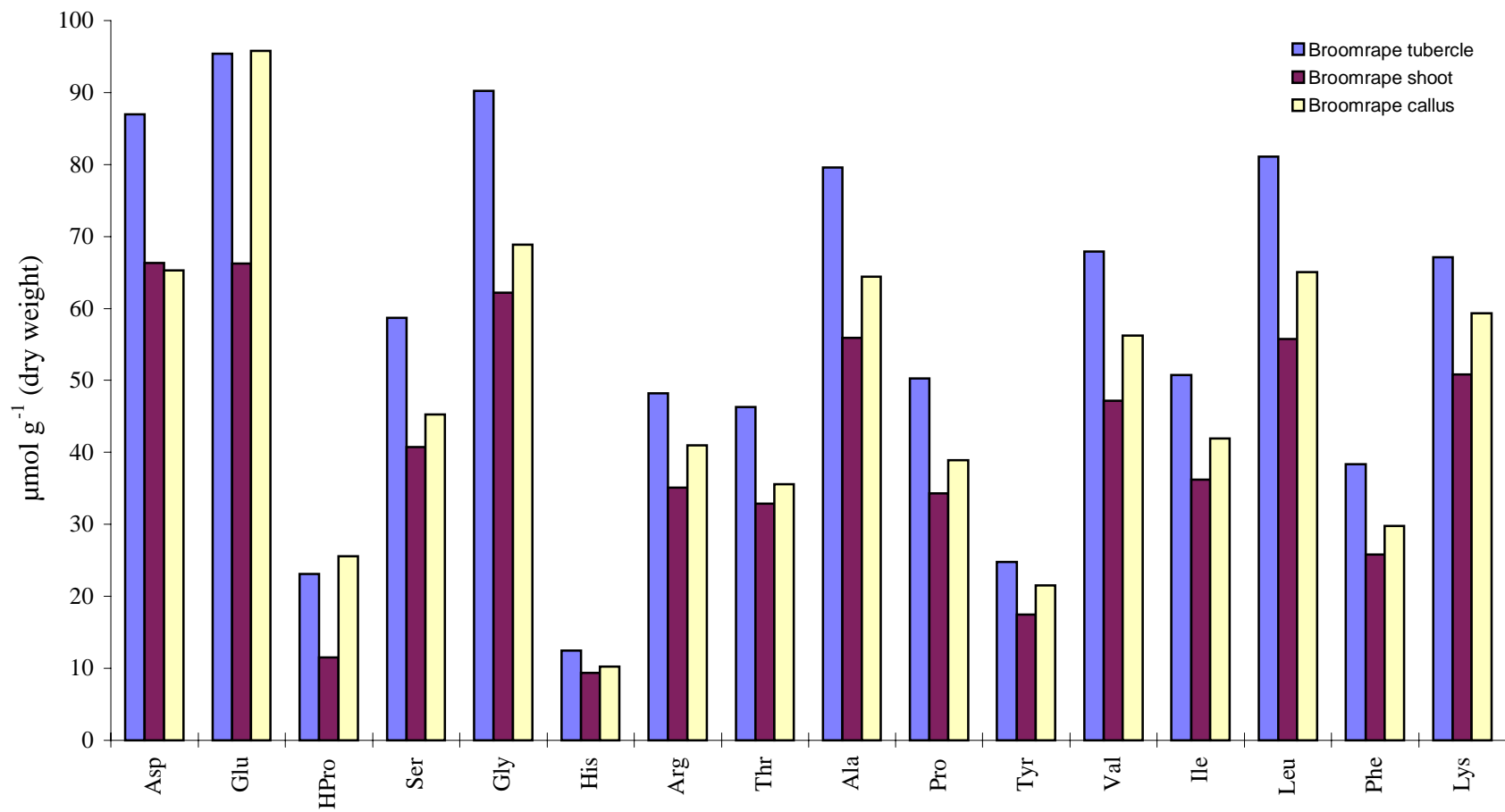


Figure 3.9. Protein amino acid composition of tubercle, shoot, and callus of broomrape (obtained by subtracting free amino acid concentrations from total amino acid concentrations).

4.0 INFLUENCE OF GLYPHOSATE ON AMINO ACID COMPOSITION OF EGYPTIAN BROOMRAPE AND TWO HOSTS

4.1 INTRODUCTION

Egyptian broomrape (*Orobanche aegyptiaca* Pers.) is a parasite that attacks the roots of many dicotyledonous crops. It draws carbon, nutrients, and water through haustoria, which connect the parasite with the host's vascular system. Broomrape infestations cause reductions in crop yield, adversely affect crop quality, and result in loss of cultivated land due to reduced crop alternatives. Commonly affected host crops include legumes such as common vetch (*Vicia sativa* L.) and broad bean (*Vicia faba* L.), cruciferous crops such as oilseed rape (*Brassica napus* L.), and several members of the families Apiaceae, Asteraceae, and Solanaceae. Management of broomrape is difficult because of its close association with the host for all of its life cycle.

Glyphosate [N-(phosphonomethyl)glycine] is a systemic, nonselective, and foliar-applied herbicide. It is readily translocated to underground plant parts, immature leaves, and meristems. Glyphosate acts by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase that catalyzes the production of the aromatic amino acids, phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp). Since discovery of its herbicidal properties (Baird et al. 1971) and commercial introduction in 1974, glyphosate has been used extensively in both cultivated and noncrop lands. It is generally applied as a preplant treatment or after harvest in cropping areas for broad spectrum weed control. In spite of its nonselectivity, some degree of selectivity in certain crops, some of which are broomrape hosts, has been reported (reviewed by Foy et al. 1989; Parker and Riches 1993).

Most success with glyphosate against broomrape has been achieved in broad bean. Kasasian (1973) first reported the selective control of broomrape (*O. crenata*) in broad bean with glyphosate. Rates of 0.2 to 0.3 kg ha⁻¹ gave good selective control when sprayed 6 wk after sowing. After application to host foliage, glyphosate is thought to

translocate through the host phloem to broomrape attachments on the host roots and exert its toxic effect. Common vetch is an important legume forage crop in the Middle East. It is highly susceptible to attack by broomrape. A variety of common vetch, 'Yovel', has been reported to be tolerant to glyphosate at low rates (Kleifeld 1996).

Although glyphosate has provided good control of broomrape in certain crop situations, the margin of crop safety and applicator's error is very narrow due to its toxicity to the host crop itself. The problem of herbicide toxicity to the host plant can be avoided in crops that are genetically engineered for glyphosate resistance. The possibility of herbicide-resistant crops for broomrape control was first addressed by Foy et al. (1989) and described in detail by Gressel et al. (1994). Joel (1992) reported the first utilization of herbicide-resistant crops for broomrape control, demonstrating complete suppression of the parasite by application of chlorsulfuron to parasitized transgenic chlorsulfuron (2-chloro-*N*-[[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide)-resistant tobacco (*Nicotiana tabacum* L.).

Recently, four examples of genetically engineered resistance, three target-site and one metabolic, were tested for use in controlling broomrape (Joel et al. 1995). Excellent control of broomrape was obtained with chlorsulfuron in a transgenic tobacco line containing a modified acetolactate synthase enzyme resistant to chlorsulfuron, with glyphosate in oilseed rape plants containing a modified enolphosphate-shikimate phosphate synthase resistant to glyphosate, and with asulam (methyl[(4-aminophenyl)sulfonyl]carbamate) in tobacco plants having a modified dihydropteroate synthase resistant to asulam. A variety of tomato (*Lycopersicon esculentum* Mill.) engineered for resistance to glufosinate (2-amino-4-(hydroxymethylphosphinyl)butanoic acid), an inhibitor of GS, was infested with broomrape in spite of application of glufosinate. This was expected because the transgenic line of tomato metabolized the herbicide. In all of the four cases, control plants were severely infested with broomrape. These genetically engineered herbicide-resistant crops have great potential for use as alternative crops in broomrape-infested areas.

Broomrape has two potential sources of amino acids. First, the haustorium of broomrape serves as a conduit for translocation of amino acids from the host plant to the

parasite. Second, broomrape may be able to synthesize some amino acids by itself and draw the rest from the host. It is however, not clear what the relative importance is of these two modes of acquiring amino acids by broomrape.

Therefore, it is interesting to examine the effect of glyphosate on the host-broomrape interaction to help delineate the role of broomrape amino acid synthesis by selectively inhibiting its EPSP synthase [host's (common vetch or glyphosate-resistant oilseed rape) EPSP synthase should be tolerant to glyphosate] and to assess the impact of sublethal rates of glyphosate on amino acid composition of these hosts.

Broomrapes have been found to lack or possess low activity of nitrogen assimilating enzymes. Lee and Stewart (1978) were unable to detect nitrate reductase activity in broomrape, which indicates that the parasite is unable to utilize nitrogen in the form of nitrate. Low GS activity was found in broomrape and another holoparasite, *Lathraea* (McNally and Stewart 1987; McNally et al. 1983a, 1983b, 1984). Additionally, in *Lathraea*, nitrate reduction has been shown to be incomplete or to proceed at very low rates (Thalouarn et al. 1987, 1988, 1990).

Absence or low activity of nitrogen assimilating enzymes in broomrape may indicate that the parasite must have access to organic nitrogen in the host plant. Translocation of amino acids and amides has been shown for broomrape (Aber et al. 1983) and other angiospermous parasites (Fer 1979; McNally et al. 1983a; Renaudin and Larher 1981; Thalouarn et al 1986). Broomrape seeds were able to absorb and metabolize radioactive leucine (Leu) both during conditioning and germination, and a part of the Leu was metabolized (Bar Nun and Mayer 1993). Amino acid analyses of broomrape and its hosts, common vetch and oilseed rape, would help better understand the nitrogen status of broomrape in relation to its hosts and to assess the impact of broomrape parasitism on hosts' amino acid composition.

The objectives of this research were to examine the mode of acquisition of amino acids by broomrape from its hosts, common vetch and oilseed rape, and to better understand the impact of broomrape parasitism on amino acid composition of the two host crops.

4.2 MATERIALS AND METHODS

Plant tissues from the ‘Dose Response’ experiments described in the ‘Materials and Methods’ section of Chapter 2 were used for the amino acid analyses.

Common vetch¹ and oilseed rape² plants that were parasitized by broomrape³ were treated with glyphosate⁴ at three different rates: 0.09, 0.18, and 0.36 kg ae ha⁻¹ on common vetch, and 0.25, 0.5, and 0.75 kg ae ha⁻¹ on oilseed rape. Untreated parasitized plants of both species were used as the respective controls. All herbicide treatments were applied 5 wk after planting. Procedures used for planting, parasitizing host plants by broomrape, and herbicide treatments have been described in the ‘Materials and Methods’ section of Chapter 2.

One week after the herbicide treatment, host leaves and roots, and attachments of broomrape were harvested for both common vetch and oilseed rape. Similar tissues from all the replications within each treatment were pooled, lyophilized, and ground to pass a 0.25-mm sieve. Single subsamples of tissues were used for amino acid analyses and results are expressed on an “as is” dry weight basis. Leaf and root tissues of nonparasitized oilseed rape and common vetch plants (grown similarly as the parasitized plants, but not infected with broomrape) were also analyzed. Amino acids were analyzed as phenylthiocarbonyl derivatives using a modification of the procedure of Bidlingmeyer et al. (1984). The procedure has been described in the ‘Materials and Methods’ section of Chapter 3. During acid hydrolysis asparagine and glutamine were converted to aspartate (Asp) and glutamate (Glu), respectively. Thus, asparagine + aspartate and glutamine + glutamate were determined together. Cysteine (Cys and Cys2), Methionine (Met), and Trp, which are degraded to varying extents by acid hydrolysis, were not analyzed.

Although there was some variation in the extent of influence of glyphosate on amino acid concentrations in plant tissues analyzed between repeated experiments, the trends were the same. Therefore, data from only one experiment are presented.

4.3 RESULTS AND DISCUSSION

4.3.1 Dose Response

The effect of glyphosate on the growth of common vetch, oilseed rape, and broomrape has been discussed in the 'Results and Discussion' section of chapter 2. Glyphosate decreased the growth of common vetch and oilseed rape, but both the host crops recovered from the herbicide treatments. The growth of broomrape in both host crops was decreased by glyphosate.

4.3.2 Influence of Glyphosate on Amino Acid Composition of Broomrape and Host Crops

Glyphosate did not uniformly alter amino acid concentrations in the leaves of common vetch (data not shown). Different rates could have a different effect on the amino acid composition. On the other hand, glyphosate when applied at 0.18 and 0.36 kg ha⁻¹ provided increased concentrations of amino acids in roots of common vetch (Figure 4.1). The increase in aspartate (Asp), a major transport form of organic nitrogen in plants, by glyphosate at the higher rates was particularly noteworthy. This increase in Asp levels may reflect the general increase in the amino acid concentrations and the associated demand for organic nitrogen in the common vetch plants.

Glyphosate, at 0.18 and 0.36 kg ha⁻¹, caused an increase in the amino acid concentrations, except arginine (Arg), in broomrape attachments in common vetch (Figure 4.2). It is to be noted that glyphosate decreased growth of broomrape in common vetch (chapter 2).

Glyphosate, at all three rates, 0.25, 0.5, and 0.75 kg ha⁻¹ generally increased the amino acid concentrations in the leaves of oilseed rape (Figure 4.3) compared to those of the leaves of untreated plants. The extent of this increase with the two highest rates of

glyphosate, 0.5 and 0.75 kg ha⁻¹, was equal and higher than that with 0.25 kg ha⁻¹. The aromatic amino acids, Phe and Tyr, did not differ from this pattern.

As with the leaf, the three rates of glyphosate caused a general increase in the concentration of amino acids in the roots of oilseed rape (Figure 4.4). The amounts of amino acids in roots were lower than those of oilseed rape leaves. The increase in amino acid concentrations of the leaves and roots of oilseed rape by glyphosate may be considered as a response to stress. Increase in protein turnover rates is known to occur under stressed conditions.

The amino acid concentrations of broomrape attachments on oilseed rape were increased by glyphosate when applied at 0.25 kg ha⁻¹ (Figure 4.5). The pattern of increase did not differ appreciably for Tyr and Phe. This increase in aromatic amino acid concentrations in broomrape is an interesting development because broomrape growth was significantly suppressed by glyphosate (chapter 2). It may be recalled that glyphosate inhibits the enzyme, EPSP synthase, that catalyzes production of the aromatic amino acids. It is possible that broomrape has a form of EPSP synthase, but related research is lacking. EPSP synthase activity primarily is thought to occur in the chloroplast. Broomrapes may have lost most of their chloroplastic genome during their evolution. Beechdrops [*Epifagus virginiana* (L.) Bart.] is a nonphotosynthetic parasite on the roots of beech trees and belongs to the same family of broomrapes, Orobanchaceae. It has been reported to have lost most of its chloroplastic genome except for a few housekeeping and nonbioenergetic genes (Wolfe et al. 1992). It appears that glyphosate has a profound secondary effect on broomrape. Glyphosate may have adversely affected the starch-sucrose balance in the parasite. Tissue of broomrape was unavailable for analysis due to complete suppression of the parasite at 0.5 and 0.75 kg rate of glyphosate.

The increase in amino acid concentrations of both the hosts and the parasite can be better explained by analysis of the free amino acid pools. Cooley and Foy (1992) have reported increased levels of free amino acid pools in inflated duckweed (*Lemna gibba* L.) after glyphosate treatment. The increase in amino acid concentrations, including the aromatics, in response to glyphosate reported here may appear high on a concentration basis (per unit tissue dry wt), while the total pools may have been decreased.

4.3.3 Influence of Broomrape Parasitism on Amino Acid Composition of Host Crops

Amino acid concentrations of the leaves of parasitized common vetch plants were similar to those of leaves of nonparasitized plants, except Asp, Glu, glycine (Gly), and alanine (Ala) (Figure 4.6). These amino acids are among the major transport forms of organic nitrogen in plants. The concentrations of amino acids in roots of parasitized and nonparasitized common vetch plants were similar (Figure 4.6). The amino acid profile of broomrape attachments was comparable to that of both parasitized and nonparasitized roots of common vetch, except Asp, Glu, Gly, Arg, Ala, and Leu, which were higher in the broomrape attachments (Figure 4.6). Increased levels of Asp, Glu, Gly, Arg, and Leu were observed in broomrape attachments growing on roots of oilseed rape.

The amino acid profiles of tissues of oilseed rape are shown in Figure 4.7. In oilseed rape, leaves of nonparasitized plants had higher amino acid concentrations than those of leaves of parasitized plants. Roots of parasitized oilseed rape plants had higher amino acid concentrations than those of nonparasitized plants. This difference may be due to the higher demand for organic N compounds in the parasitized roots due to broomrape attachments. It also reflects the contribution of the broomrape attachments to the composition of the parasitized root samples. Broomrape attachments had different amino acid concentrations from those of leaves and roots of oilseed rape. Levels of individual amino acids such as Asp, Glu, Gly, Arg, and Leu were much higher in broomrape than host roots. In related studies (data not shown), Arg was uniquely elevated in broomrape attachments compared to host (carrot - *Daucus carota* L.) roots. Other amino acid levels of broomrape were identical to those of roots of oilseed rape.

Amino acid concentrations of broomrape attachments with respect to the parasitized and nonparasitized roots of common vetch and oilseed rape indicate that broomrape obtains a large part of its amino acids requirement from the host roots. It also seems to accumulate certain amino acids such as Asp, Glu, Arg, and Leu, which suggests either preferential uptake of amino acids from the host or selective synthesis by the

attachments. Investigations on profiles of enzymes involved in amino acid synthesis in broomrape will provide a better insight into N regulation in the parasite.

4.4 SOURCES OF MATERIALS

¹ Common vetch (var'Yovel') seed provided by Dr. Y. Kleifeld, Newe Ya'ar Research Center, Ramat Ishay 30095, Israel.

² Oilseed rape seed, Monsanto Canada, Inc., 5827-181 Street, Edmonton AB, Canada T6M 1V7.

³ Broomrape seed provided by Dr. Y. Kleifeld, Newe Ya'ar Research Center, Ramat Ishay 30095, Israel.

⁴ Glyphosate (Roundup®), Monsanto Co., St. Louis, MO 63167.

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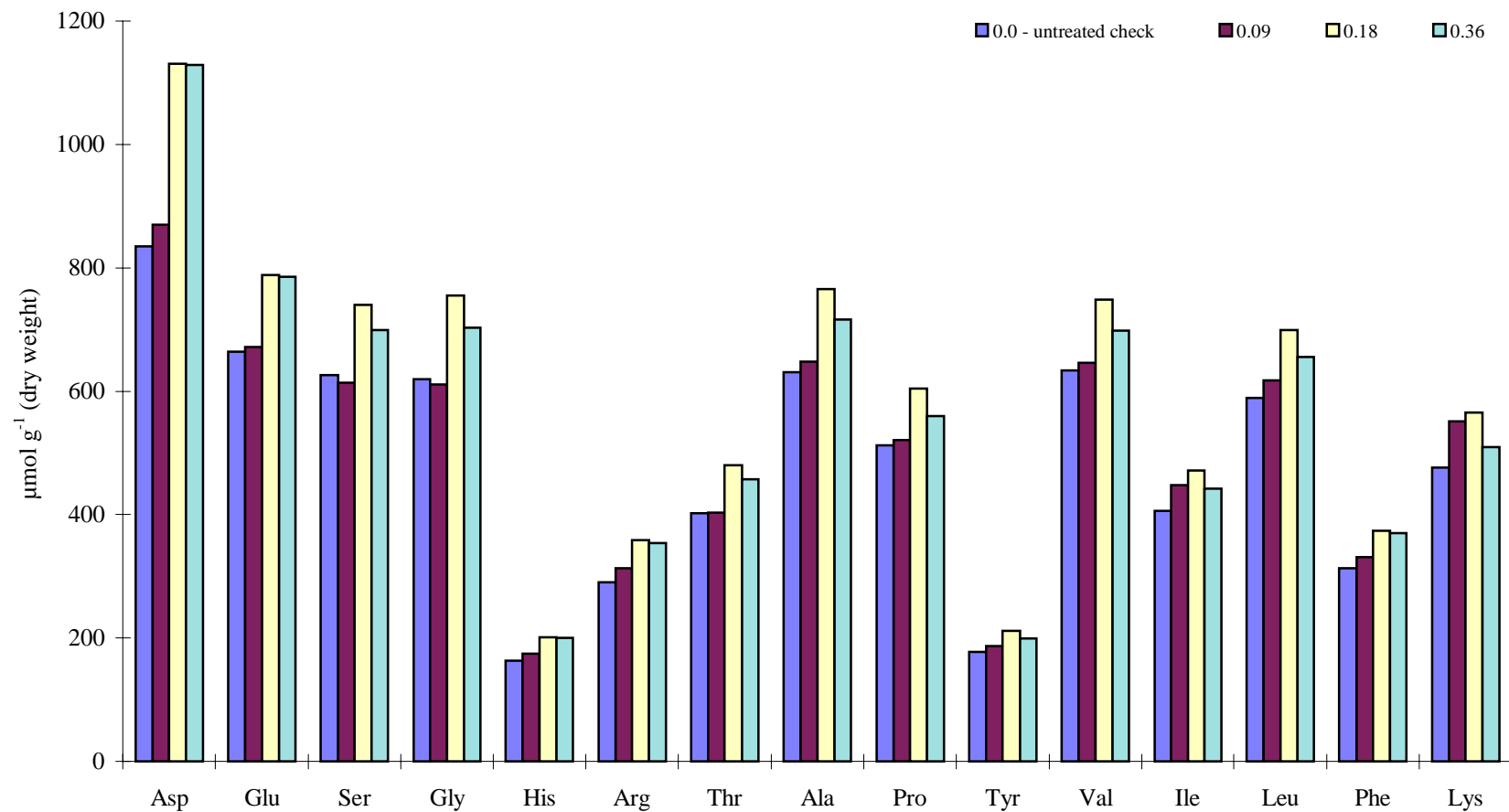


Figure 4.1. Amino acid composition of hydrolyzates of roots of broomrape-parasitized common vetch treated with glyphosate. Numbers in the legend indicate the rate of glyphosate in kg ha^{-1} .

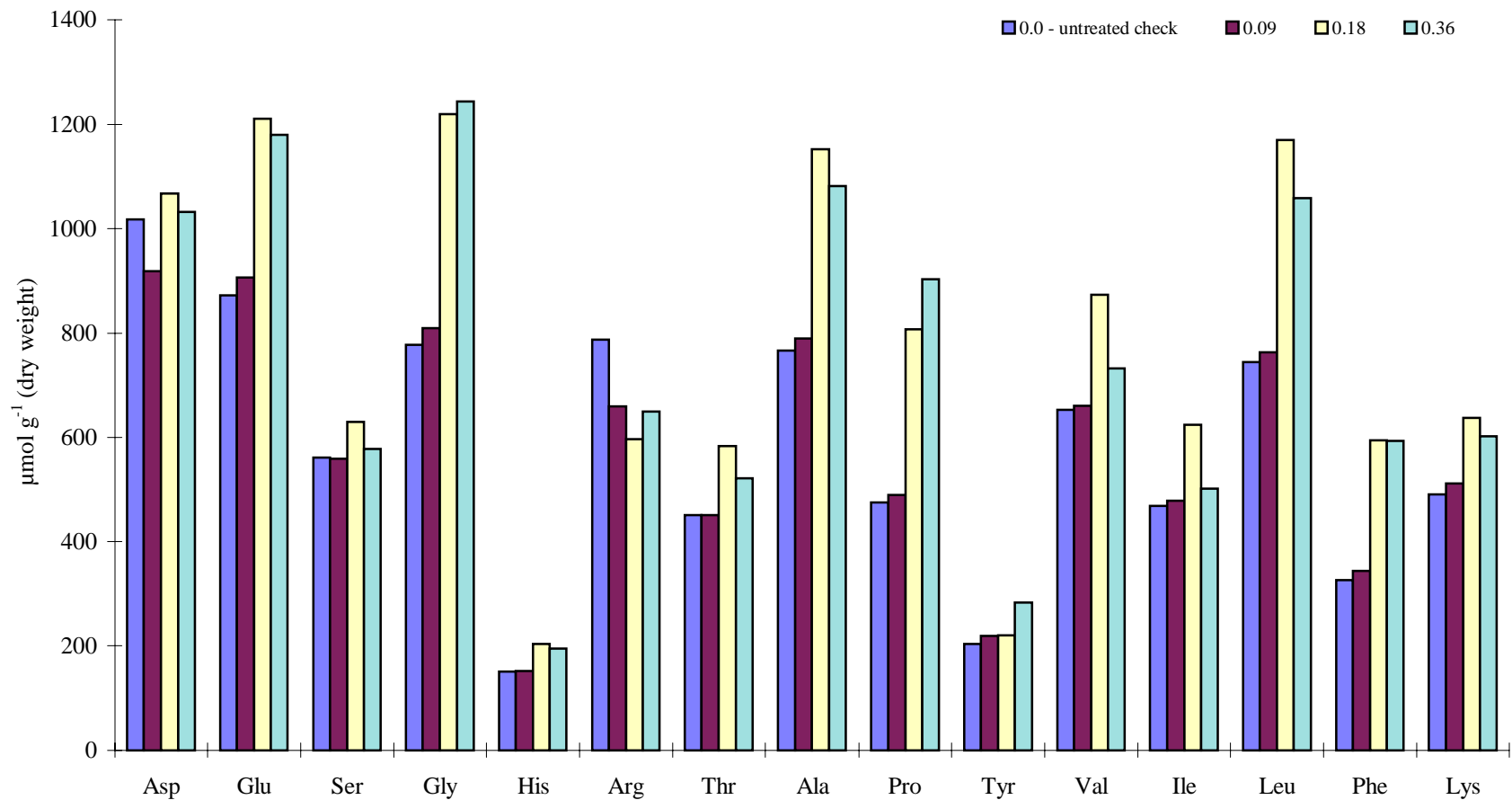


Figure 4.2. Amino acid composition of hydrolyzates of broomrape attachments on common vetch treated with glyphosate. Numbers in the legend indicate the rate of glyphosate in kg ha^{-1} .

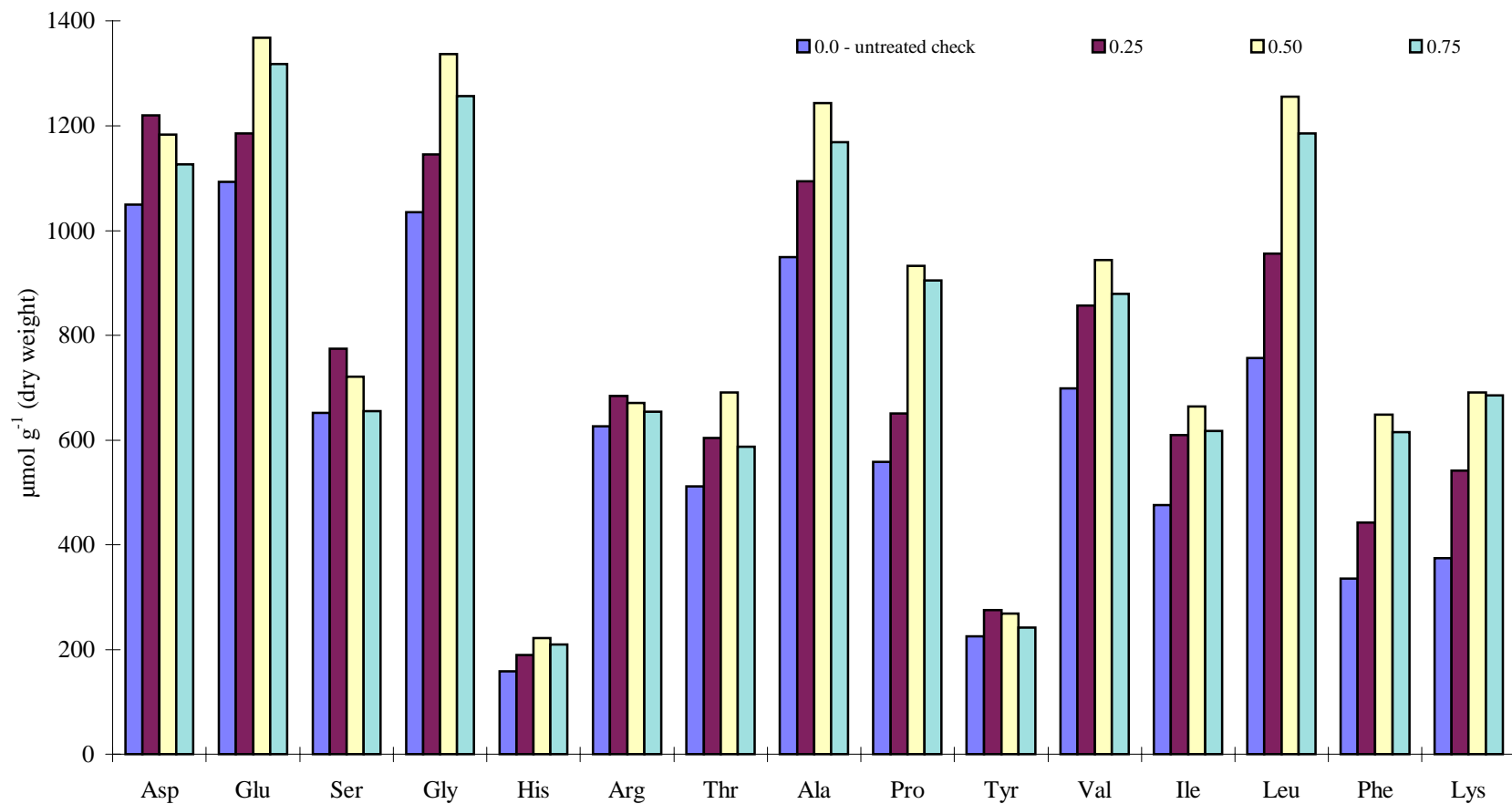


Figure 4.3. Amino acid composition of hydrolyzates of leaves of broomrape-parasitized oilseed rape treated with glyphosate. Numbers in the legend indicate the rate of glyphosate in kg ha^{-1} .

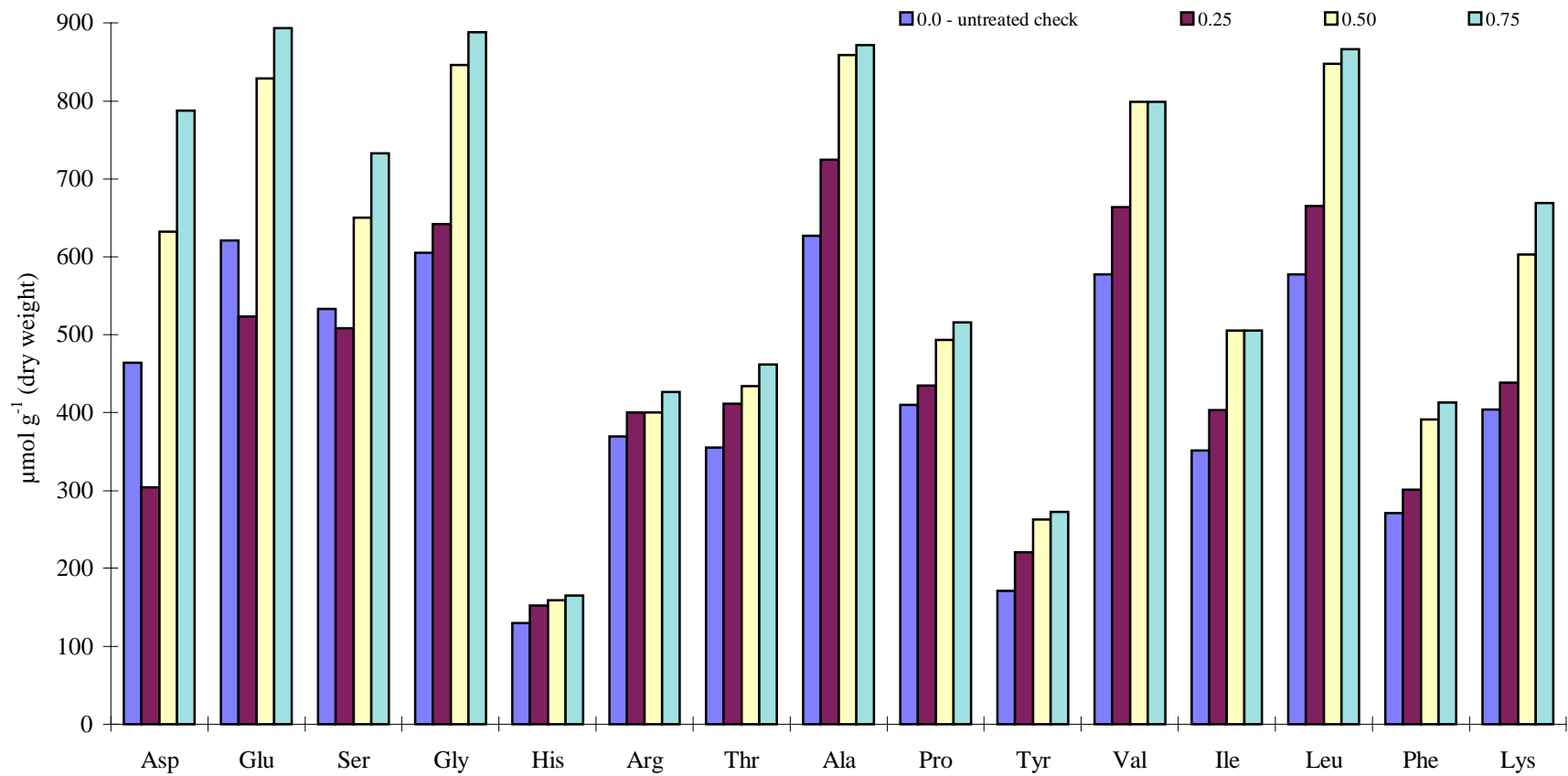


Figure 4.4. Amino acid composition of hydrolyzates of roots of broomrape-parasitized oilseed rape treated with glyphosate. Numbers in the legend indicate the rate of glyphosate in kg ha^{-1} .

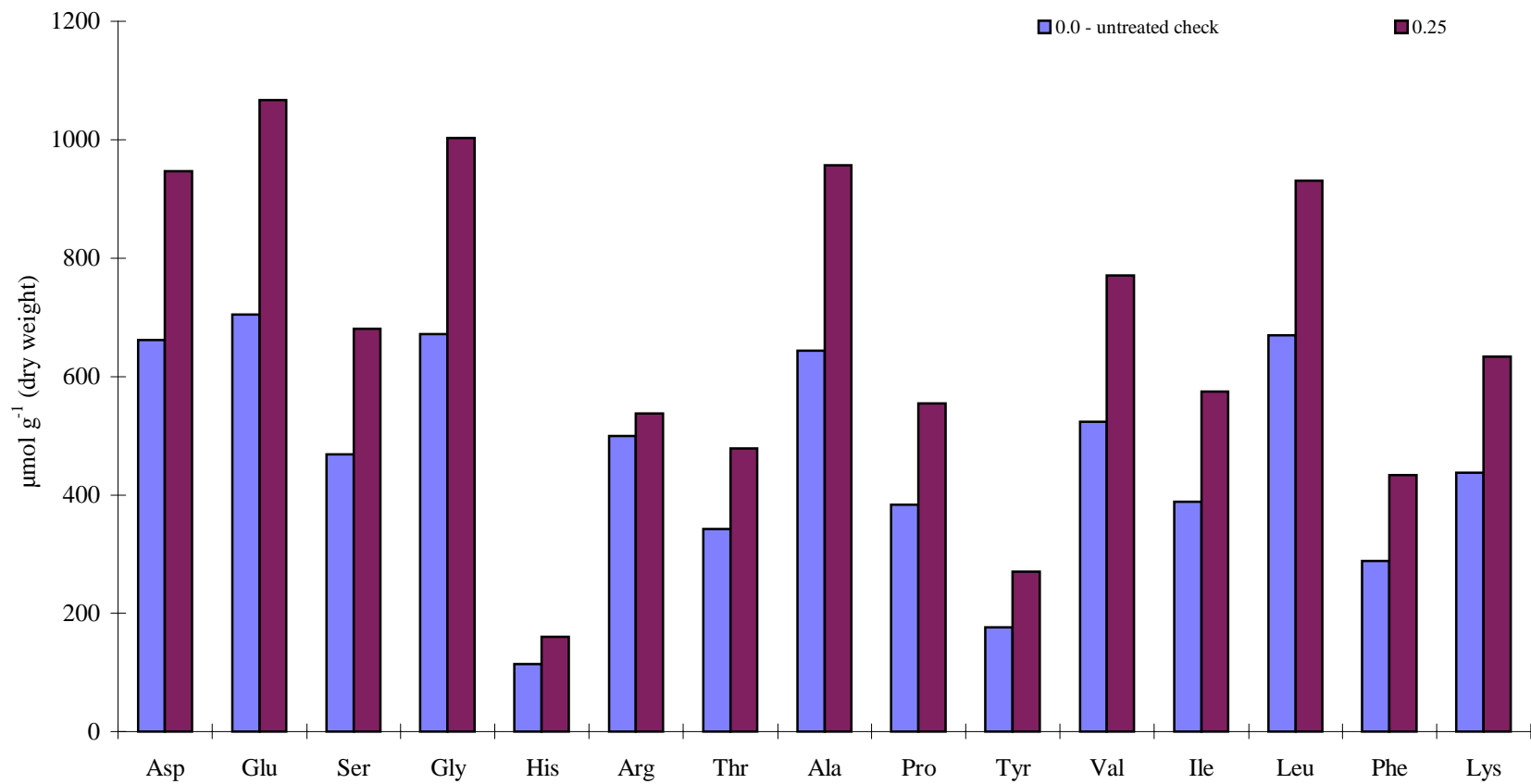


Figure 4.5. Amino acid composition of hydrolyzates of broomrape attachments on oilseed rape treated with glyphosate. Numbers in the legend indicate the rate of glyphosate in kg ha⁻¹.

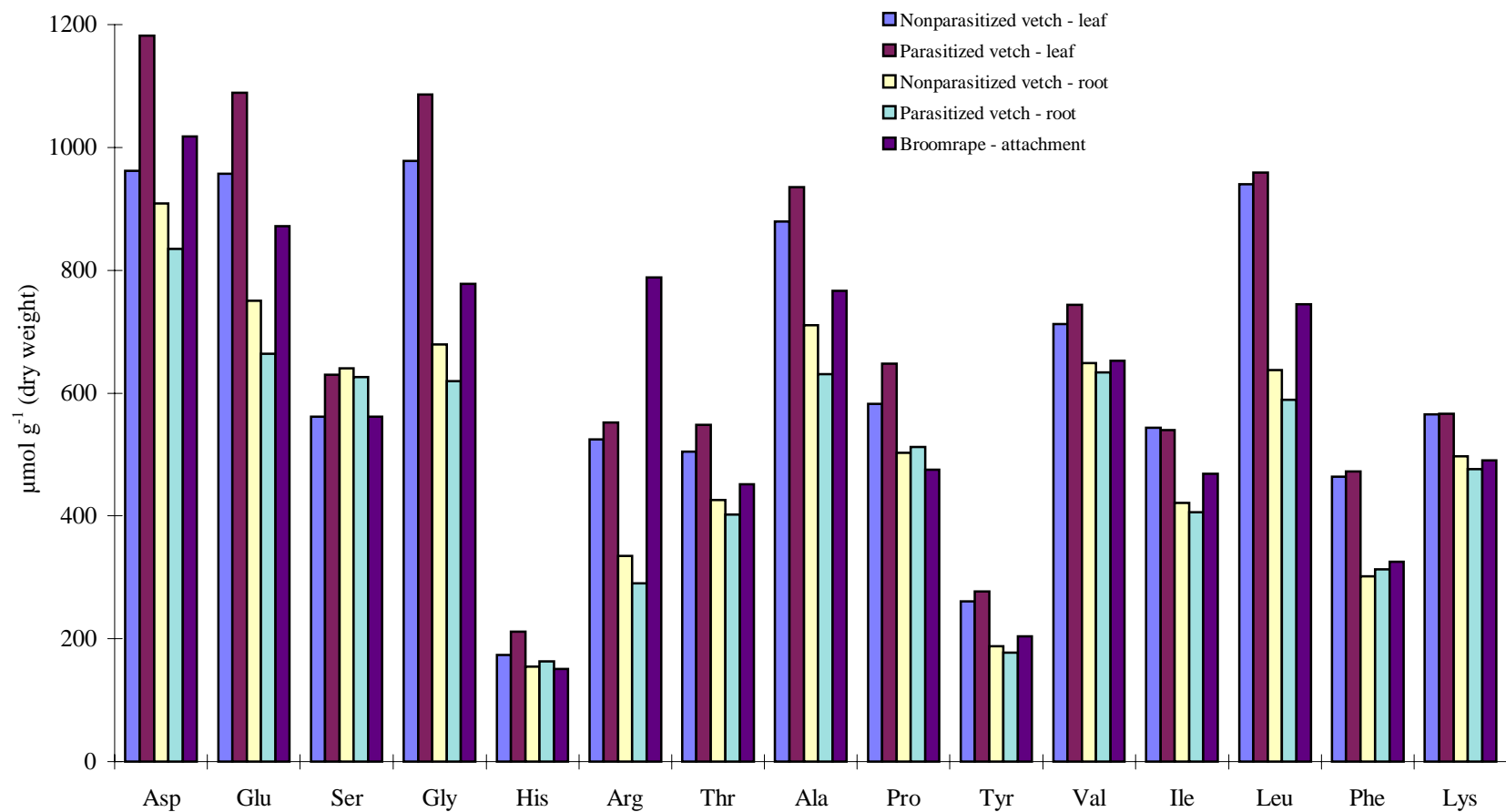


Figure 4.6. Amino acid composition of hydrolyzates of tissues of nonparasitized and broomrape-parasitized common vetch, and broomrape attachments.

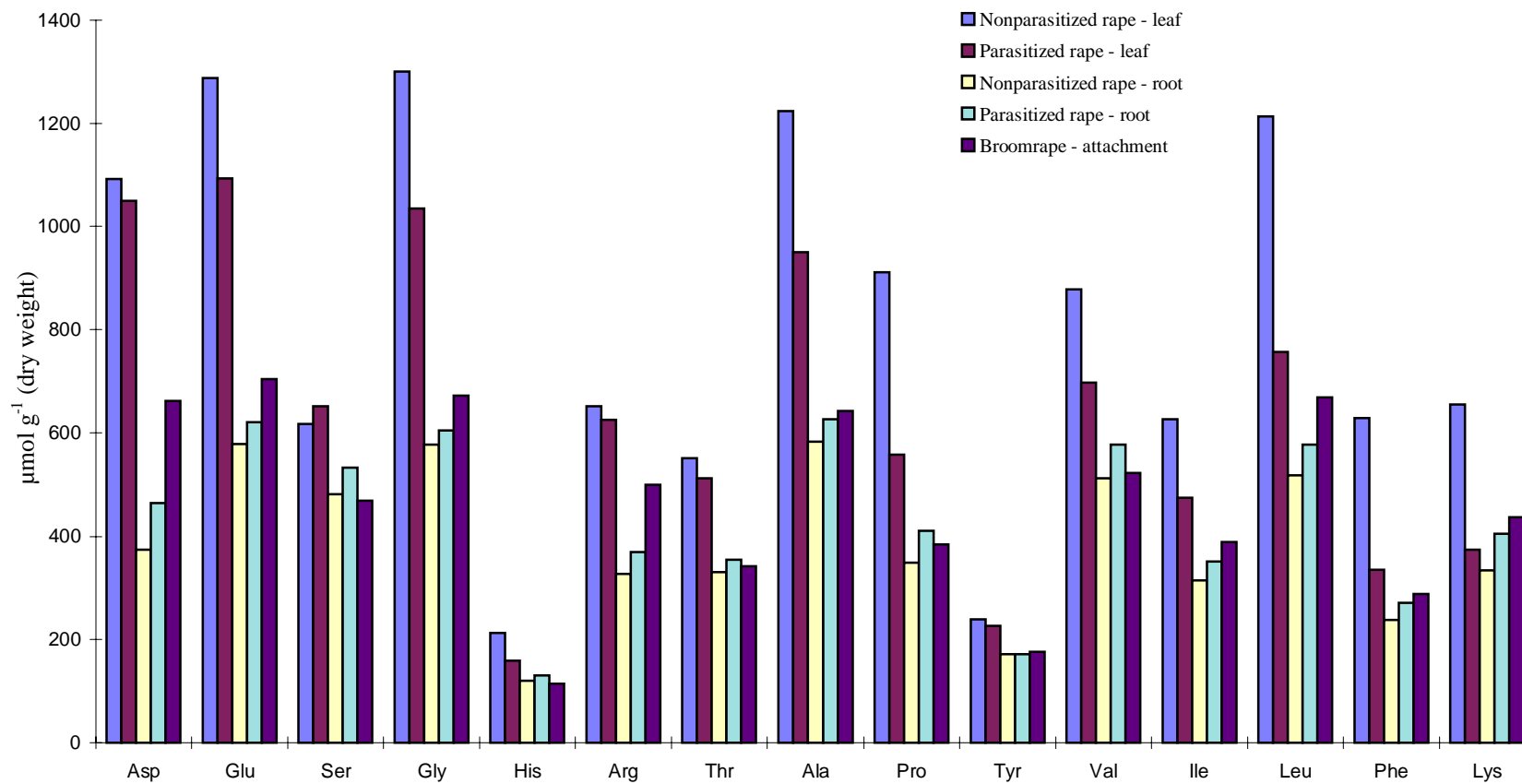


Figure 4.7. Amino acid composition of hydrolyzates of tissues of nonparasitized and broomrape-parasitized oilseed rape, and broomrape attachments.

5. INFLUENCE OF OSMOTIC POTENTIAL AND TEMPERATURE ON THE GERMINATION OF BROOMRAPES

5.1 INTRODUCTION

Broomrapes (*Orobanche* spp.) are phanerogamic holoparasites that attack the roots of many dicotyledonous crops. They lack chlorophyll (Baccarini and Melandri 1967; Saghir et al. 1973) and obtain carbon, nutrients, and water from their hosts through haustorial connections.

Before germination, broomrape seeds must undergo conditioning under suitable temperature and moisture conditions. The conditioning phase may range from five to several days, depending on the species. The requirement of conditioning of the seeds is not completely understood. Following the conditioning phase, the seeds produce a 'germ tube' or radicle in response to a chemical stimulant from the host root. Several factors influence germination of broomrapes in the soil including temperature, moisture, pH, nutrients, osmotic potential, soil type, and stimulants produced by host plants. This chapter deals with the investigation of the role of osmotic potential and temperature in the germination of broomrapes.

Reports of inhibitory effects of nitrogen on the growth of broomrapes, including germination have been common in the literature for many years (reviewed by Parker and Riches 1993; Sauerborn 1991). Osmotic stress has been implicated as a possible reason for inhibition of broomrape germination by nitrogen (Ernst 1986). Wegmann (1986) observed that broomrape has a lower osmotic potential compared to the host caused by mannitol synthesis, hence its ability to retain water and nutrients. Germination tests at different osmotic potentials demonstrated an adaptation of the germ tube of *O. ramosa* L. to dry habitats (Linke 1987). To date, there has been no attempt to correlate osmotic potential with nitrogen-induced inhibition of broomrape germination.

Optimum temperatures for conditioning and germination are different among broomrape species. Studies on the effect of temperature on germination of *O. aegyptiaca*

Pers., *O. crenata* Forsk., and *O. cumana* Wallr. indicated that every species had a specific optimum temperature range for germination and development which generally reflected its geographical distribution (Sauerborn 1991). Kasasian (1973) showed that optimum temperatures for both conditioning and germination were about 18 C for *O. crenata* and about 23 C for *O. ramosa*. Similarly, Weldeghiorghis and Murdoch (1996) reported an optimal temperature of 18 C for *O. crenata* germination. van Hezewijk et al. (1991, 1993) reported an optimum conditioning temperature of 15 to 20 C for *O. crenata*. Although temperature is known to influence germination in broomrape, its effect on subsequent development of the parasitic seedling has not been studied.

The objectives of this research were to examine osmotic potential as a mechanism for inhibition of germination and subsequent development of broomrapes, and to determine the effect of temperature on the early development of broomrapes.

5.2 MATERIALS AND METHODS

5.2.1 Broomrape Seeds

All broomrape species seed stocks¹ were stored in the dark at 25 C before the start of the experiments. Seeds of *O. aegyptiaca*, *O. cernua*, *O. crenata*, and *O. ramosa* were surface cleaned and conditioned on glass-fiber filter paper disks as outlined in Chapter 3.

5.2.2 Osmotic Potential

Various levels of osmotic potential (0 to -0.8 MPa) were induced on conditioned broomrape seeds with analytical grade polyethylene glycol 8000² (PEG). Osmotic potential of PEG solutions was measured with a vapor pressure osmometer³. A 10 µl sample of the test solution was added to a small, solute-free paper disc which was then inserted into a sample chamber and sealed. A thermocouple hygrometer was incorporated integrally within the

chamber. The sensitive temperature sensor operates on the basis of a unique thermal energy balancing principle to measure the dew point temperature depression within the chamber. This parameter, in itself a colligative property of the solution, was an explicit function of solution vapor pressure. Vapor pressure osmometer readings, expressed in mosmol kg⁻¹ of solvent, were predicted according to equations developed by Michel (1983). Mosmol kg⁻¹ units were converted to MPa using a conversion factor. PEG solutions were replaced every 2 d with fresh solutions to maintain precise osmotic potentials.

5.2.3 Temperature

Eight different temperature treatments ranging from 16 to 32 C were induced on conditioned broomrape seeds with a two dimensional temperature gradient plate similar to that described by Murdoch et al. (1989). Fluctuations in temperature were minimum during the course of the experiment, with the variation limited to ± 0.1 C about each treatment. Temperature was measured every other day with a thermocouple thermometer⁴.

All the treatments, in both the osmotic potential and temperature experiments, contained a synthetic germination stimulant, GR-24, a strigol analog, at 10 mg L⁻¹. Germination and radicle length measurements were taken 8 d after incubation. A seed was considered to have germinated with the protrusion of the radicle. Radicle length was measured using a micro-slide with a resolution of 0.1 mm. For each replication, radicle length was the mean of 10 seedlings selected at random. All experiments were conducted in a randomized complete block design with four replications. All data were subjected to ANOVA using SAS statistical software (SAS 1989). Fisher's Protected LSD procedure ($p = 0.05$) was used to compare the treatment means. Results were similar between repeated experiments, hence data from combined experiments are presented.

5.3 RESULTS AND DISCUSSION

5.3.1 Osmotic Potential

The osmotic potential of the surrounding solution significantly affected the germination percentage of all species tested except *O. crenata* (Table 5.1). In general, germination percentage was highest for all species when osmotic potential was 0 to -0.2 MPa and gradually declined as osmotic potential decreased to -0.8 MPa (Table 5.1). Similarly, radicle length of all four species was significantly influenced by osmotic potential. The longest radicles were recorded at -0.15 MPa for all species and length decreased as osmotic potential either increased to 0 or decreased past -0.2 MPa.

Nitrogen-containing nutrients, NH_4NO_3 , and NH_4Cl inhibited the germination and radicle elongation of broomrapes such as *O. aegyptiaca* and *O. ramosa* (Westwood 1995). Osmotic potential of the nutrient solutions was measured to relate the influence of nutrient concentration to osmotic effects. Osmotic potentials of all three nutrient solutions were similar at 5 mM (-0.13 MPa), 10mM (-0.14 MPa), and 25 mM (-0.17 to 0.19 MPa) (Table 5.2).

Osmotic stress was proposed to be a component of nitrogen-induced inhibition of broomrape germination (Abu-Irmaileh 1981). The above results agree with the observations of Linke (1987) that osmotic stress reduces broomrape germination. However, even though 25 mM concentrations of NH_4NO_3 and NH_4Cl were highly inhibitory to radicle development of broomrapes (Westwood 1995), the osmotic potentials of these solutions were close to -0.15 MPa, which was the optimum value for both germination and radicle elongation. Thus, there appears to be no correlation between osmotic potential, *per se*, and ammonium-induced inhibition. The inhibitory effect of nitrogen on broomrape growth could be due to a nonphysical effect such as a biochemical or physiological response. Further research is required to confirm this hypothesis.

5.3.2 Temperature

Temperature significantly influenced germination percentage of all four species. Optimal temperatures for germination of *O. aegyptiaca*, *O. cernua*, *O. crenata*, and *O. ramosa* were approximately 25, 26, 20, and 28 C, respectively (Figure 5.1). The radicle length of all species was also significantly affected by temperature. All species showed greatest radicle elongation at 23 to 25 C except *O. ramosa* which had a broader optimal range extending up to 28 C. Radicle elongation of all species was dramatically reduced as temperature increased to 32 C.

The different optimum temperatures for germination and radicle elongation reflect fundamental differences between these two aspects of development. Although different temperature requirements have been described for germination of broomrape species (Sauerborn 1991), the effect of temperature on radicle growth has not been addressed. Unlike germination, the optimal temperature for radicle growth was the same for all species (between 23 and 25 C). This suggests that radicle elongation is determined by the rate of metabolic activity and is similar for all species. These findings emphasize the uniqueness and sensitivity of the germination trigger. A better understanding of the influence of temperature on broomrape development after germination may lead to improved timing of control measures.

5.4 SOURCES OF MATERIALS

¹ Broomrape seeds provided by Dr. Y. Kleifeld, Newe Ya'ar Research Center, Ramat Ishay 30095, Israel.

² Polyethylene glycol 8000, Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219.

³ Vapor pressure osmometer, Model 5500, Wescor Inc., Logan, UT.

⁴ Thermocouple thermometer, Model TH-65, Wescor, Inc., Logan, UT.

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Table 5.1. Effect of osmotic potential on germination percentage and radicle length of four broomrape species. Osmotic conditions were generated with PEG and applied to preconditioned seeds.

Osmotic potential (-MPa)	<i>O. aegyptiaca</i>	<i>O. cernua</i>	<i>O. crenata</i>	<i>O. ramosa</i>
	Germination %			
0	80	22	37	0
0.15	60	50	38	48
0.2	67	46	36	46
0.3	46	47	36	26
0.4	49	41	37	33
0.5	47	29	32	27
0.6	47	20	24	23
0.8	0	10	21	5
lsd (0.05)	8	16	NS ^a	11
	Radicle length (mm)			
0	0.38	0.2	0.2	0
0.15	0.55	0.33	0.4	0.28
0.2	0.33	0.2	0.18	0.23
0.3	0.2	0.2	0.1	0.15
0.4	0.23	0.23	0.1	0.15
0.5	0.18	0.15	0.1	0.18
0.6	0.23	0.13	0.1	0.18
0.8	0	0.1	0.1	0.05
lsd (0.05)	0.13	0.11	0.1	0.1

^aIndicates no significant difference between treatment means within each column according to Fisher's Protected LSD ($p = 0.05$).

Table 5.2. Osmotic potential of nitrogen-containing nutrient solutions.

Concentration	KNO ₃	NH ₄ NO ₃	NH ₄ Cl
mM		-MPa	
5	0.13	0.13	0.13
10	0.14	0.14	0.14
25	0.19	0.17	0.18

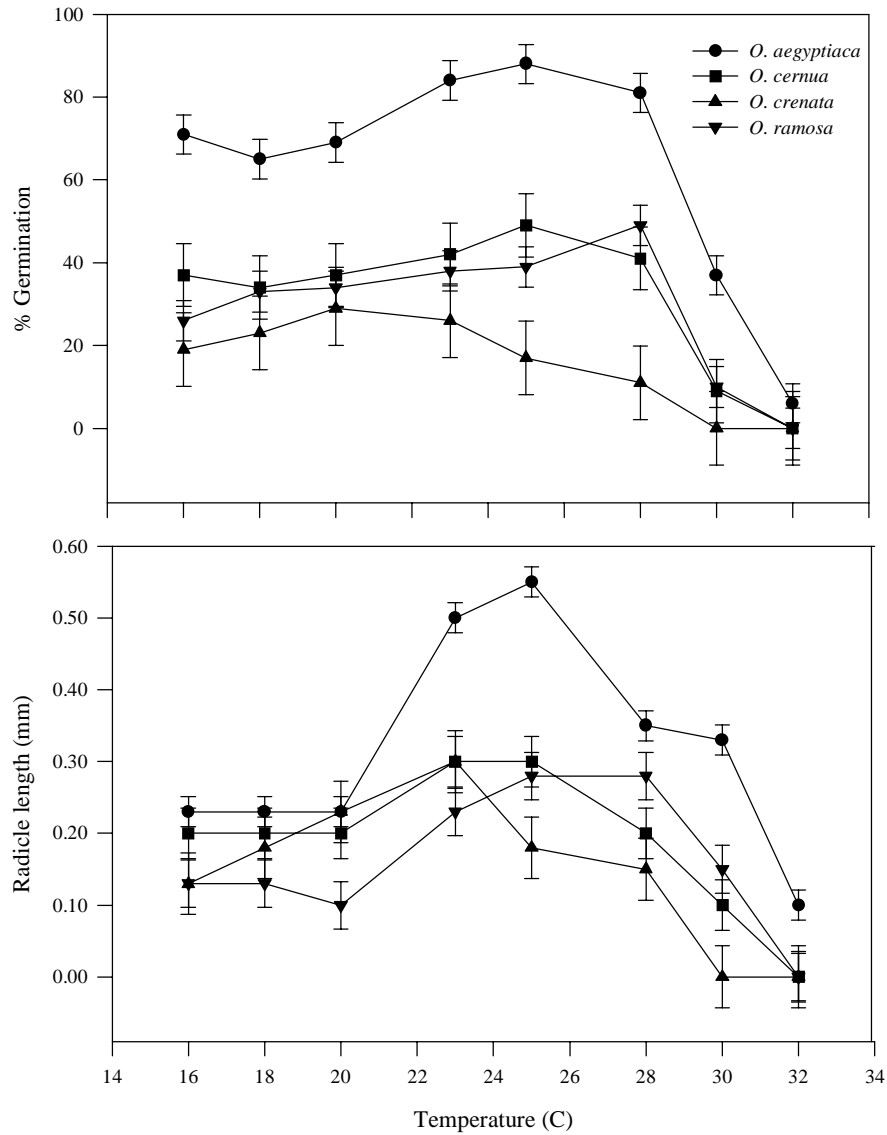


Figure 5.1. Effect of temperature on germination and radicle length of four broomrape species. Vertical bars represent Fisher's Protected LSD ($p = 0.05$) within each species within % germination and radicle length.

6. SUMMARY AND CONCLUSIONS

The objectives of this research were a) to determine the use of glyphosate in controlling broomrape in common vetch (*Vicia sativa* L.) that is tolerant to low rates of glyphosate, and to compare this response with broomrape control in oilseed rape (*Brassica napus* L.) that has been engineered for glyphosate resistance, b) to determine the nitrogen status of broomrapes and their hosts by analyzing their respective amino acid profiles, c) to examine the mode of acquisition of amino acids by broomrape from two selected hosts, common vetch and oilseed rape, and to better understand the impact of broomrape parasitism on amino acid composition of these host crops, d) to examine osmotic potential as a mechanism for inhibition of germination and subsequent development of broomrapes, and e) to determine the effect of temperature on the early development of broomrapes.

Common vetch and oilseed rape plants were parasitized with Egyptian broomrape (*Orobanche aegyptiaca* Pers.) and treated with a commercial formulation of glyphosate [N-(phosphonomethyl) glycine]. Effect of glyphosate on growth of hosts and the parasite was evaluated 1 wk after herbicide treatment. Absorption, translocation, and metabolism studies were also conducted using ^{14}C -glyphosate. Tissues of carrot (*Daucus carota* L.) and Egyptian broomrape were collected from plants grown under laboratory conditions and amino acid compositions were analyzed. Glyphosate-treated and untreated tissues of common vetch, oilseed rape, and broomrape were analyzed for amino acid composition. *In vitro* studies were conducted to evaluate influences of osmotic potential and temperature on germination and radicle development of broomrapes.

Glyphosate, a foliar-applied, nonselective, and systemic herbicide, when applied at 0.09, 0.18, and 0.36 kg ha⁻¹, affected growth of common vetch to some extent. Application of glyphosate significantly reduced the growth and the number of live Egyptian broomrape attachments in common vetch. Almost 27% of translocated ^{14}C -glyphosate, applied to leaves of parasitized common vetch plants, accumulated in broomrape tubercles. X-ray autoradiographic studies revealed similar results. TLC analysis of extracted ^{14}C compounds from common vetch tissues, after treatment with ^{14}C -glyphosate, indicated 11 to 27% metabolism of ^{14}C -glyphosate, depending on plant tissue type.

Glyphosate, when applied at 0.25, 0.5, 0.75 kg ha⁻¹ to parasitized oilseed rape plants, reduced growth of the host, but inhibition of growth was temporary. Glyphosate, at all three rates, significantly affected the growth of broomrape in oilseed rape and reduced the number of live attachments. The reduction in number of live attachments rather than fresh weight of attachments may be a better measure for estimating efficacy of glyphosate or other herbicides for control of broomrape because surviving attachments may be expected to adversely affect the host crop. Nearly, 33% of translocated ¹⁴C-glyphosate accumulated in broomrape tubercles in oilseed rape. No metabolism of glyphosate was evident in oilseed rape.

Thus, in both common vetch and oilseed rape, broomrape parasitism can be reduced by application of glyphosate. Broomrape parasitism caused a redistribution of translocated glyphosate, especially in the root. The accumulation of translocated radioactivity in broomrape attachments was more than 25% in both common vetch and oilseed rape. Broomrape acts as a sink and competes directly with other actively growing parts of the host plant for photoassimilates, similar to the translocation pattern of ¹⁴C-glyphosate observed here.

Elemental analysis of the carrot and broomrape tissues revealed differences in the C, N, and S composition of the tissues. Although total N (expressed as % on dry weight basis) was similar in leaves of nonparasitized and parasitized carrot plants, amino acid concentrations were lower in leaves of parasitized plants compared to those in leaves of nonparasitized plants. Apparently, more N has been incorporated into compounds other than amino acids following parasitization. Also, broomrape parasitism may have altered the functioning of certain enzymes which may have caused metabolism of amino acids and subsequent incorporation of N into other compounds such as alkaloids, ureides, nitrogenous bases of DNA and RNA, and precursors of chlorophyll.

A particularly noteworthy observation is that tubercles had similar or higher concentrations of amino acids compared to leaves of nonparasitized carrot plants. It is possible that broomrape parasitism has altered the activity of certain enzymes in carrot plants by inactivation, inhibition, or reduction of enzyme protein. Amino acids such as glutamate, aspartate, and asparagine are translocated through the xylem and phloem to different parts of the plant serving as sources of organic N. Disruption of enzyme activity in carrot plants by

broomrape parasitism may adversely affect translocation of amino acids in carrot and further metabolism of amino acids. Effect of broomrape on host plants differs from other interactions such as between plants and mycorrhizae. Mycorrhizae are symbiotic and mutualistic associations between nonpathogenic or weakly pathogenic fungi and primarily cortical and epidermal cells of roots. The fungi improve the mineral- and water-absorbing capacity of roots and receive organic nutrient from the plant in return.

Glutamate content was higher in the dry compared to the germinated seeds. This amino acid, which contributes carbon skeletons for the synthesis of several other amino acids, may have been metabolized during germination. The tubercle, shoot, and callus had higher amino acid content compared to the dry and germinated seeds, indicating a higher rate of metabolic activity in the former compared to the latter. The amino acid composition of the callus was comparable to that of the tubercle, in spite of the absence of a host. Profiles of enzymes involved in amino acid synthesis in the callus will provide more information on its biosynthetic capability and N regulation of broomrape.

Glyphosate, applied at 0.18 and 0.36 kg ha⁻¹, to common vetch that was parasitized by Egyptian broomrape, provided increased concentrations of amino acids in roots of common vetch as well as broomrape attachments in common vetch. The increase in aspartate, a major transport form of organic nitrogen in plants, by glyphosate at the higher rates was particularly noteworthy. This increase in aspartate levels may reflect the general increase in the amino acid concentrations and the associated demand for organic nitrogen in the common vetch plants. Glyphosate, at all three rates, 0.25, 0.5, and 0.75 kg ha⁻¹, generally increased the amino acid concentrations in the leaves and roots of parasitized oilseed rape compared to those of the leaves and roots of untreated plants, respectively. The concentrations of the aromatic amino acids, Phe and Tyr, in the leaves and roots of glyphosate-treated common vetch did not differ from this pattern. The amino acid concentrations of broomrape attachments on roots of oilseed rape were increased by glyphosate when applied at 0.25 kg ha⁻¹. The pattern of increase did not differ appreciably for Tyr and Phe. This increase in aromatic amino acid concentrations in broomrape is an interesting development because broomrape growth was significantly suppressed by glyphosate. It may be recalled that glyphosate inhibits the enzyme, EPSP synthase, that catalyzes production of the aromatic

amino acids. It is possible that broomrape has EPSP synthase, but related research is lacking. Thus, it appears that glyphosate has a profound secondary effect on broomrape.

Amino acid concentrations of the leaves and roots of Egyptian broomrape-parasitized common vetch plants were similar to those of leaves and roots of nonparasitized plants, respectively, except aspartate, glutamate, glycine, and alanine. These amino acids are among the major transport forms of organic nitrogen in plants. In oilseed rape, leaves of nonparasitized plants had higher amino acid concentrations than those of leaves of parasitized plants. Roots of parasitized oilseed rape plants had higher amino acid concentrations than those of nonparasitized plants. This difference may be due to the higher demand for organic N compounds in the parasitized roots due to broomrape attachments. The amino acid composition of broomrape attachments was comparable to that of both parasitized and nonparasitized roots of common vetch and oilseed rape, except aspartate, glutamate, glycine, arginine, alanine, and leucine, which were higher in the broomrape attachments.

Amino acid concentrations of broomrape attachments with respect to the parasitized and nonparasitized roots of common vetch and oilseed rape indicate that broomrape obtains a large part of its amino acids requirement from the host roots. It also seems to accumulate certain amino acids such as Asp, Glu, Arg, and Leu, which suggests either preferential uptake of amino acids from the host or selective synthesis by the attachments. Investigations on profiles of enzymes involved in amino acid synthesis in broomrape will provide a better insight into N metabolism in the parasite.

Osmotic potential significantly affected the germination and radicle length of broomrapes. Osmotic potential of ammonium-containing nutrients, at concentrations shown to inhibit germination and radicle elongation of broomrapes, was within the optimal range for broomrape germination and radicle development. There appears to be no correlation between osmotic potential and ammonium-induced inhibition. The inhibitory effect of nitrogen on broomrape growth could be due to a nonphysical effect such as a biochemical or physiological response. Further research is required to confirm this hypothesis.

Temperature significantly influenced germination and radicle elongation of broomrapes. The different optimum temperatures for germination and radicle elongation

within each broomrape species reflect fundamental differences between these two aspects of development. Unlike germination, the optimal temperature for radicle growth was the same for all species (between 23 and 25 C). This suggests that radicle elongation is determined by the rate of metabolic activity and is similar for all species. A better understanding of the influence of temperature on broomrape development after germination may lead to improved timing of control measures.