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Study of resistance to *Orobanche ramosa* in host (oilseed rape and carrot) and non-host (maize) plants

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Abstract

Orobanche ramosa is a parasitic Angiosperm responsible for severe yield losses in several economical crops. It is a serious threat in oilseed rape in France and Morocco and is appearing in carrot crops in Morocco. In this study, several varieties of oilseed rape and carrot were screened in order to identify resistant cultivars and to characterize the resistance mechanisms involved. All the 15 oilseed rape varieties tested were susceptible. In carrot, the varieties 'Colmar à coeur rouge' and 'Nantaise demi-longue' were susceptible, whereas 'Palaiseau' and 'Buror' were resistant. In the susceptible 'Colmar à coeur rouge' carrot no defence reactions were found and the development of the parasite inhibited carrot tap root formation. In the resistant carrot varieties, the parasite germinated, became attached to the host root but became necrotic before emergence. In 'Buror' carrot, formation of a mechanical barrier was associated with the restriction to the cortex of the parasite. In maize cv. 'Vigni', a non-host of *O. ramosa,* thickening of xylem vessels, cell divisions in the central cylinder and formation of an encapsulation layer were observed in association with restricted development of *Orobanche* haustoria.

Introduction

Broomrapes (*Orobanche* spp.) are holoparasitic plants lacking chlorophyll and photosynthetic function. They cause severe damage to a wide range of dicotyledonous crops (Foy et al., 1989; Parker and Riches, 1993). Several species cause dramatic damages to crops in the Mediterranean region (Saghir, 1986). Among them, *Orobanche ramosa* causes severe yield losses in Solanaceae such as tomato and tobacco, in Brassicaceae such as *Brassica napus* var. oleifera (oilseed rape) and in Apiaceae such as *Daucus carota* (carrot) (Parker and Riches, 1993; Gibot-Leclerc et al., 2001).

Several means, including chemical, biological and cultural methods, have been used for broomrape control (Foy et al., 1989; Sauerborn et al., 1989; Castejon-Munoz et al., 1993; Parker and Riches, 1993). Rotation with trap crops (plant that stimulate parasite seed germination but do not allow normal attachment and parasite development) can also be used in an attempt to decrease the broomrape seed bank in soil (Foy et al., 1989; Parker and Riches, 1993). However, no method appeared to be economic and effective to control the parasite. Thus, breeding for broomrape resistance could be the most likely control method.

Broomrape germination requires a moist period (conditioning) and a chemical stimulus from host roots. After germination, the procaulome of the broomrape seedling reaches a host root and attaches to its surface. After attachment to the host, the tip of the *Orobanche* procaulome forms a haustorium which penetrates the host root and establishes connections with the vascular system of the host, from which the parasites obtains the water and nutrients needed for growth (Parker and Riches, 1993).

Several mechanisms have been proposed for resistance to broomrape. Among them are lignification, vessel occlusion and cell wall deposition reported in *Helianthus* attacked by *O. cumana* (Dörr et al., 1994; Labrousse et al., 2001). Induction of phytoalexin synthesis has been indicated in *Helianthus* parasitized by *O. cernua* (Jorrin et al., 1996). Accumulation of phenolic compounds occurred in *Vicia* attacked by *O. aegyptiaca* (Goldwasser et al., 1999).

The aim of the present study was to identify resistant varieties of oilseed rape and carrot and to investigate the mechanisms involved in the resistance of the host varieties and in a non-host plant (maize).

Materials and methods

Plant material

Orobanche ramosa seeds were collected in Sudan in 1995. Seeds of different varieties of oilseed rape (CWH17, Cando, Ténor, Carolus, Zénith, Alamo, Capitol, Lipton, Tradition, Canary, RPC801 and Parapluie) were obtained from CETIOM (Centre Technique Interprofessionnel des Oléagineux Métropolitain, Paris, France). Seeds of other varieties (Lutia, Tratia and Kabel) were obtained from SONACOS (Société Nationale de Commercialisation des Semences, Kénitra, Morocco). Vigni maize was obtained from Caussade Semences (France). The carrot varieties Colmar à coeur rouge, Nantaise demi-longue, Palaiseau and Buror were obtained from Clause (France).

Screening under greenhouse conditions

Ten milligram of *O. ramosa* seeds were mixed in a 2 : 1 sand : soil mixture in 1.3 liter pots which were watered and put in the dark for 1 week at 25–20 °C (day–night) to allow broomrape conditioning. In order to have host seedlings present at the end of the broomrape conditioning period, carrot seeds were sown at the same time as the parasite seeds and oilseed rape seeds were sown 3 days after the broomrape seeds. The plants were grown under a photoperiod of 16 h (300 μmol m^{-2} s^{-1} PAR). At different times after sowing, host plants were uprooted and roots were observed under a binocular microscope, after gentle washing in water, to determine the number and development stage of broomrape attachments. Each measurement was performed on three pots. The different stages of broomrape development are those described by Labrousse et al. (2001): stage 1: early broomrape attachment to the host root; stage 2: tubercle formation; stage 3: tubercle with adventitious roots; stage 4: underground shoot growth; stage 5: emergence of parasite stems.

Hydroponic co-culture

To carry out histological studies, carrot and maize were grown in hydroponic co-culture. Carrot or maize seeds were sterilized in hydrogen peroxide (0.3%) for 15 h and rinsed in sterile distilled water before putting them in Petri dishes on glass fiber filter in the dark at 25 °C. Carrot and maize seedlings were transferred to other Petri dishes, respectively, 7 and 4 days after sowing. Roots of the host seedlings were spread in the bottom of the Petri dishes, while host shoots were allowed to emerge out of the dishes through a hole made in the border. Roots were covered with a glass fiber filter paper and a 1 cm thick layer of rock wool. Then Petri dishes were placed vertically in a plastic tray, partly immersed in a sterile half-strength Coïc nutrient medium (Coïc and Lesaint, 1975) and covered with black polyethylene sheet. Experiments were performed at 21 °C with a photoperiod of 16 h (300 μmol m^{-2} s^{-1} PAR). Seeds of *O. ramosa* were conditioned for 7 days after sterilization, and treated with GR24 1 μg ml^{-1} (Zehhar et al., 2002). Three days later, when the seeds started to germinate, they were placed near the roots of 4-week-old carrot plants and of 2-week-old maize plants.

Histological studies

Broomrape attachments on maize or carrot roots were fixed in 2.5% glutaraldehyde in Sörensen phosphate buffer 0.1 M (pH 7.2) for one night at 4 °C. After dehydration in an ethanol graded series, they were embedded in Epon 812. Semithin sections (1–2 μm) were stained with toluidine blue O and observed with a light microscope.

Results

Screening for resistance

Oilseed rape

Fifteen oilseed rape varieties were screened in order to test their susceptibility or resistance to broomrape (Figure 1). Observations of host root systems were

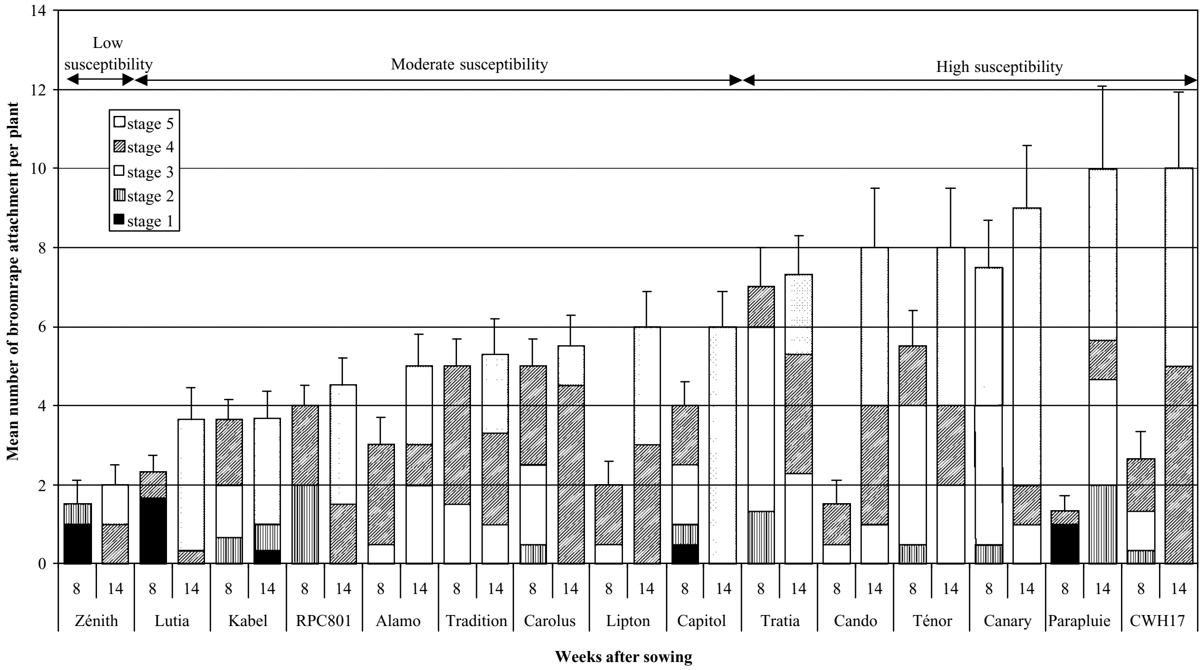

Figure 1. Development of *O. ramosa* on 15 varieties of oilseed rape. Results represent mean numbers of broomrape attachment ±SE of three experiments. The different development stages of broomrape are also indicated. Stage 1: early broomrape attachment to the host root; stage 2: tubercle formation; stage 3: tubercle with adventitious roots; stage 4: underground shoot growth; stage 5: emergence.

made 8 and 14 weeks after broomrape sowing. All varieties were susceptible to broomrape. In all cases, the parasite attached to the host's roots, grew and realized its cycle of development (until seed production). Fourteen weeks after sowing, broomrapes emerged on all the varieties tested; however, 'Zénith' oilseed rape presented the smallest number of broomrape attachment (Figure 1).

Carrot

Four carrot varieties were tested. The varieties 'Colmar à coeur rouge' and 'Nantaise demi-longue' were susceptible to broomrape. In these two varieties, some of the attached parasite developed and emerged. Death of parasite infections probably occurs in these two varieties since the number of attached broomrapes decreases from the 5th to the 15th week after sowing. On the variety 'Colmar à cœur rouge' broomrape development was more rapid than on the variety 'Nantaise demi-longue' (Figure 2).

The variety 'Palaiseau' showed some resistance to broomrape. The number of broomrape attachments at stage 1 (early broomrape attachment to the host root) was highest 9 weeks after sowing, but only a small number of stages 2 were observed. Subsequently, less than one broomrape per plant emerged and these became necrotic before flowering (Figure 2).

The variety 'Buror' allowed the parasite to develop only until stage 3 (production of adventitious roots on the tubercle), so that no broomrape shoots emerged (Figure 2). Thus, the variety 'Buror' could be considered as the most resistant, even though it presents, 5 weeks after sowing, a number of attachments higher than that measured in the three other varieties. Indeed, many dead *Orobanche* seedlings were observed from the 9th week after sowing (23% and 46% after 9 and 11 weeks, respectively), until, 15 weeks after sowing, less than one attachment was observed.

Impact of broomrape on a susceptible carrot variety

Impact of *O. ramosa* on carrot 'Colmar à coeur rouge', as observed after 15 weeks of hydroponic co-culture, was dramatic. Indeed, the leaves of infected carrot were much less developed than those of the control (Figure 3A) and broomrape prevented the formation of the host tap root (Figure 3B).

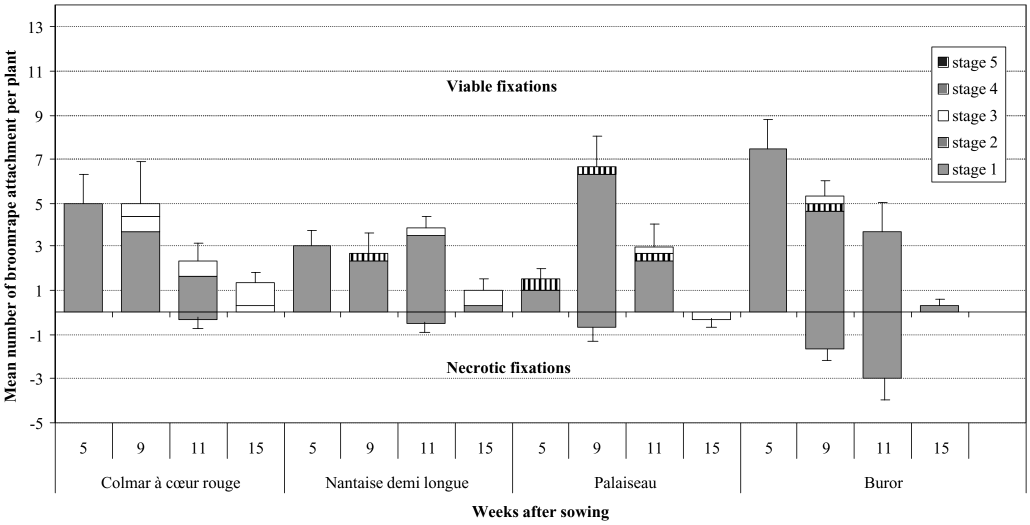

Figure 2. Development of *O. ramosa* on four varieties of carrot. Results represent mean numbers of viable and necrotic broomrapes ±SE of three experiments. The different development stages of broomrape are also indicated. Stage 1: early broomrape attachment to the host root; stage 2: tubercle formation; stage 3: tubercle with adventitious roots; stage 4: underground shoot growth; stage 5: emergence.

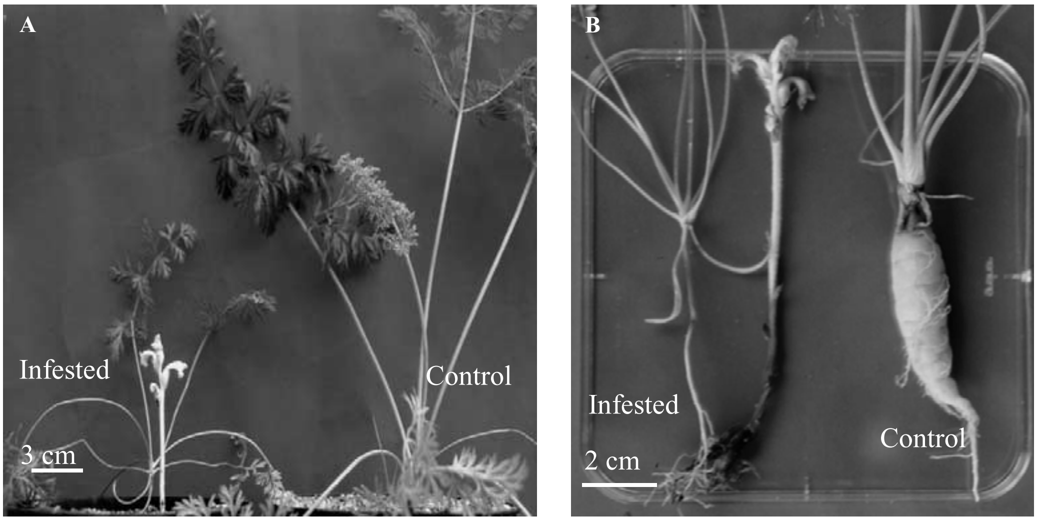

Figure 3. Impact of *O. ramosa* on shoot (A) and root development (B) of the susceptible carrot ‘Colmar à coeur rouge’, after 15 weeks of hydroponic co-culture.

Histological aspects of broomrape–carrot relationships

The interaction between carrot roots and broomrape was studied by examination of the structure of infected tissue. In the susceptible carrot ‘Colmar à coeur rouge’, semithin longitudinal sections of broomrape at stage 2 (tubercle formation) showed that the parasite had penetrated the host root up to the central cylinder and was already connected to host xylem vessels through xylem elements (Figure 4A). No defence reactions were detected in the host tissues.

In variety ‘Palaiseau’ some broomrape xylem elements came in contact with the host xylem vessels, when the parasite had reached the stage 3 of its development. However, many of the parasite xylem elements

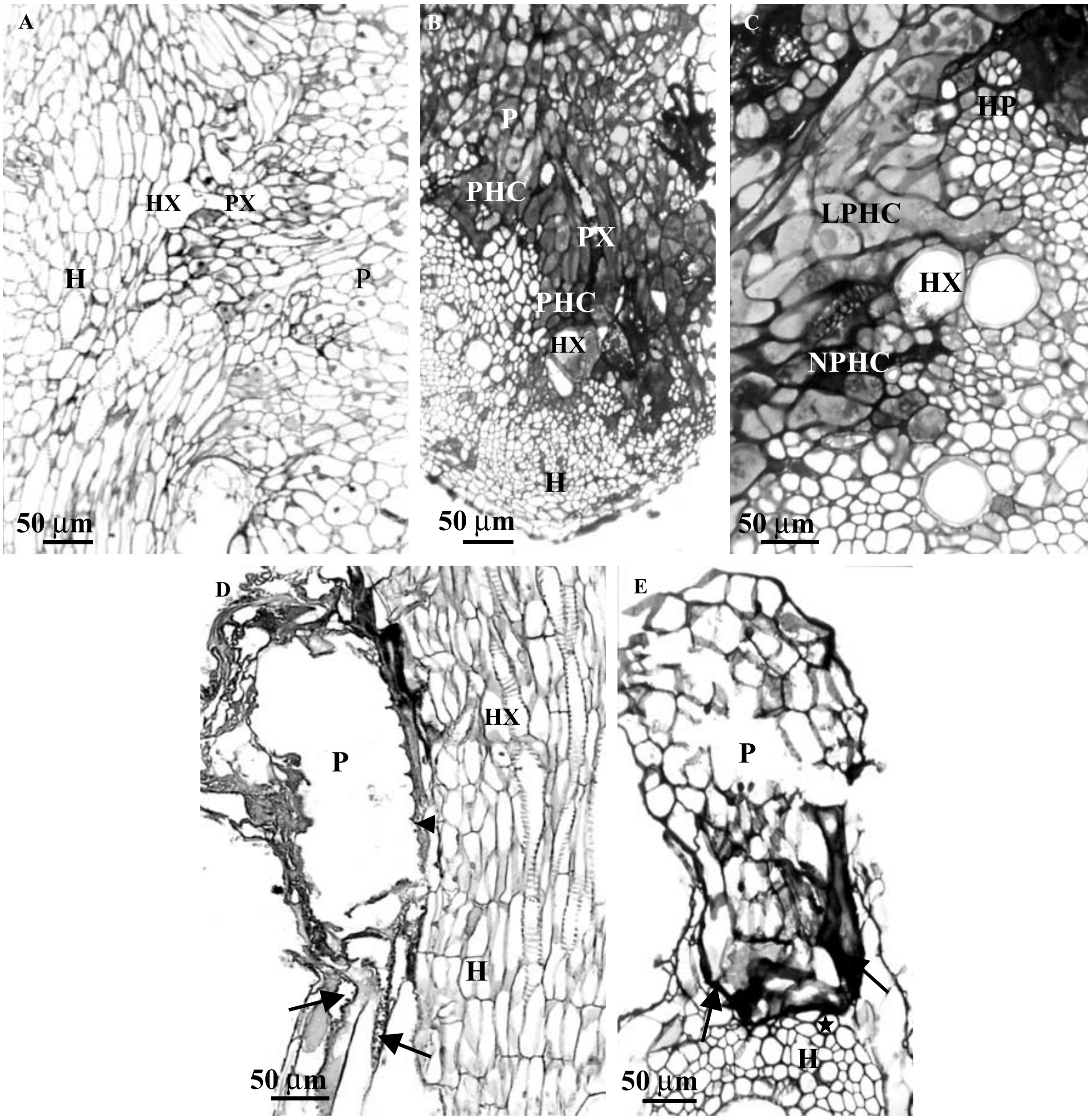

Figure 4. (A) Longitudinal section of susceptible carrot 'Colmar à coeur rouge' root (H) supporting broomrape (P) at stage 2. A parasite xylem element (PX) is connected to the host xylem vessel (HX). (B) Transverse section of resistant carrot 'Palaiseau' root (H) infested by broomrape (P) at stage 3. An unfunctional parasite xylem element (PX) is connected to a host vessel (HX) which exhibits a thickened cell wall. Other intrusive haustorial cells of the parasite (PHC) establish contact with xylem cells (HX) or phloem cells (HP) of the host. Some of these intrusive haustorial cells are living, other are already necrosed (see Figure 3C). (C) Enlargement of Figure 3B, showing intrusive haustorial cells of the parasite (PHC) present in the host xylem (HX) and host phloem (HP). Some intrusive haustorial cells are necrosed (NPHC); other are still living (LPHC), but are impregnated with compounds which stained with toluidine blue O. (D) Longitudinal section of resistant carrot 'Buror' root (H) parasitized by broomrape (P). The parasite is halted at the inner cortical parenchyma by a layer of crushed and lignified host cells containing globular structure (arrow). (E) Transverse section of 'Vigni' maize root (H) parasitized by broomrape (P) at stage 1. An encapsulation layer (mechanical barrier) stopped the penetrating haustorium of the parasite (arrow). A thickening of cell wall of some endodermis cells (⋆) is also observed near the parasite/host interface.

were crushed and their cell walls impregnated with deep colored material. There was thickening and lignification of the host xylem vessels (Figure 4B) and complete host vessel occlusion were observed. However, many new parasite cells invaded xylem and phloem of the host. Some of these intrusive cells, which came in contact with xylem and phloem host cells, died. Other intrusive cells were still alive, but were impregnated with compound(s) which stained with toluidine blue O (Figure 4C). These intrusive cells of *Orobanche* did not establish functional connection with host conducting tissues (xylem and phloem).

In the most resistant carrot, 'Buror', broomrape attachments were usually blocked at stage 1. Indeed, parasite penetration stopped at the host inner cortical parenchyma by lignification of host cells (1–5 layers which are crushed at the parasite/host interface). These cells contain globular structures which stained with toluidine blue O (Figure 4D). As a consequence of this mechanical barrier, the parasite cells died.

Histological aspects of broomrape–maize relationships

In a non-host of *O. ramosa, Zea mays* variety 'Vigni', root exudates stimulated broomrape seed germination up to 70% and most of the broomrape seedlings attached to maize roots. However the cycle of development of the parasite stopped at stage 1 as observed in hydroponic co-culture (data not shown). In most cases, the broomrape penetrated the host root but was stopped at the endodermis by the formation of an encapsulation layer (as defined by Dörr et al., 1994) (Figure 4E). A thickening of endodermis cell walls near the penetrating parasite was also observed. The encapsulation layer clearly indicates the host/parasite interface, but it was difficult to determine the relative contribution of the encapsulation layer and endodermis in preventing the parasite penetration. In a few cases, broomrape reached the central cylinder but thickening of the cell walls of the host xylem vessels near the haustorial cells and host cell divisions were observed (data not shown).

Discussion

Oilseed rape

To our knowledge, no other study has been carried out to search for oilseed rape and carrot varieties resistant to *O. ramosa* or to study resistance mechanisms. In pot experiments all the oilseed rape varieties were susceptible to broomrape. Fourteen weeks after sowing, in all the varieties screened, at least one emerged living broomrape could be observed (Figure 1). It should be noted that a gradation in susceptibility exists within the 15 oilseed rape varieties. The 'Zenith' variety presents a low level of susceptibility with two broomrape attachments only. Eight varieties with a maximum number of six broomrape attachments exhibit a moderate susceptibility level. In this group, 'Capitol' allowed the most rapid development of *O. ramosa* since, 14 weeks after sowing, all the attached parasites were at stage 5. Finally, six oilseed rape varieties were highly susceptible to *O. ramosa* and supported between 6 and 10 parasite attachments 14 weeks after sowing.

Further searches for resistance in other oilseed rape varieties and among wild relatives of this crop could provide new sources of resistance.

Carrot

Screening performed with four carrot varieties revealed that *O. ramosa* reached the later stage of development less frequently than on oilseed rape (Figures 1 and 2). As a general rule, carrot is therefore less susceptible to *O. ramosa* than oilseed rape. 'Colmar à cœur rouge' and 'Nantaise demi longue' showed low susceptibility to broomrape since there were some dead broomrapes, but also a low number of parasite attachments and emergences (Figure 2). But *O. ramosa* can have a dramatic effect on the crop. Thus, in 'Colmar à coeur rouge' carrot, the most susceptible variety, *O. ramosa* reduced growth of host leaves (Figure 3A) and totally inhibited tap root formation (Figure 3B). The strong decrease of the number of broomrape attachments from the 9th to the 15th week was probably due to death of most of the young parasites as the result of strong competition for nutrients between the parasites themselves. The strong impact of only one parasite reaching the flowering stage on carrot (Figure 3) is a good illustration of this competition for nutrients between developing parasites. Some other *Orobanche* species also strongly reduce growth of their hosts; this is the case, for example, of *O. cumana* growing on susceptible sunflower (Labrousse et al., 2001).

'Buror' and 'Palaiseau' carrot were resistant to broomrape. The carrot varieties stimulated the parasite seed germination and allowed its earlier stages of development (mainly stage 1). However, the number of viable stages 1 started to decrease from the 5th and 9th weeks after sowing respectively for ‘Buror’ and ‘Palaiseau’. However, histological studies clearly show that resistance mechanisms involved in the two carrot varieties are different (Figure 4B and D).

In ’Palaiseau’ carrot, the parasite penetrated the host root, reached the central cylinder and developed xylem elements, which connected to the host xylem, but then they died and were crushed. There was a thickening of the host xylem vessel cell walls and, sometimes, a complete occlusion of these vessels by phenolic compounds, gel or gum-like substance at the interface host xylem/parasite xylem. Thus, the xylem connections were not functional. Some other parasite haustorial cells invade xylem and phloem of the host in order to establish other types of contact with the host (Figure 4B). These intrusive haustorial cells growing between host cells became colored or died (Figure 4C). Goldwasser et al. (2000) detected a reddish brown secretion between *O. aegyptiaca* and its resistant host *Vicia atropurpurea* variety ‘Popany’. Arnaud et al. (1999) also found a strongly colored material in areas of the central cylinder surrounding the haustorium of *Striga hermonthica* infesting the resistant host *Sorghum bicolor* var. Framida. This coloration was only restricted to crushed material at the host/parasite interface. Vessel occlusion is a general feature observed in many other parasite/resistant host interactions. Such occlusions were observed in the LR1 resistant sunflower genotype (Labrousse et al., 2001) and in *Vicia sativa* infested by *O. crenata* (Perez de Luque et al., 2001).

In Buror carrot, histological studies revealed that the death of broomrape is due to its halt, in most cases, at the inner cortical parenchyma by several layers of dead cells often containing globular structures which strongly colored with toluidine blue O (Figure 4D). This dead cell layer acts as a mechanical barrier to further parasite growth and impedes nutrient transfer to the parasite. Other studies have reported the formation of a mechanical barrier, which is responsible for the inability of *O. cernua* (Panchenko and Antonova, 1974), *O. cumana* (Dörr et al., 1994) and *O. aegyptiaca* (Goldwasser et al., 2000) to develop on their respective hosts.

Maize

In ‘Vigni’ maize, broomrape development did not exceed the first stage of development (stage 1) because an encapsulation layer halted the parasite in the cortex (Figure 4E). It is possible that the endodermis also plays a role by preventing haustorial penetration in the central cylinder since there was a thickening of the wall of some of its cell. In maize, like in ‘Buror’, resistance is due to the formation of a mechanical barrier. Similar responses were also observed when other parasitic angiosperm species attack non-host plants, for example, the case of *Vicia faba* parasitized by *Alecta vogelii* (Visser et al., 1990) (host cell division and formation of an encapsulation layer). Hood et al. (1998) working with sorghum, a host of *Striga asiatica,* and several non-host species reported that attachment and early stages of haustorial development occurred on roots of non-host species in a similar manner to that observed on roots of host species, suggesting thus that the later stages of haustoria formation may be important in determining host specificity. Our results support this conclusion.

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References

Arnaud MC, Veronesi C and Thalouarn P (1999) Physiology and histology of resistance to *Striga hermonthica* in *Sorghum bicolor* var. Framida. Australian Journal of Plant Physiology 26: 63–70

Castejon-Munoz M, Romero-Munoz F and Garcia-Torres L (1993) Effect of planting date on broomrape (*Orobanche cernua* Loefl.) infections in sunflower (*Helianthus annuus* L.). Weed Research 33: 171–176

Coïc Y and Lesaint C (1975) La nutrition minérale et en eau des plantes en horticulture avancée. La documentation technique de la SCPA. 23: 1–22

Dörr I, Staak A and Kollmann R (1994) Resistance of *Helianthus* to *Orobanche* – histological and cytological studies. In: Pieterse AH, Verkleij JAC and ter Borg SJ (eds) Biology and Management of *Orobanche*. Proceedings of the Third International Workshop on *Orobanche* and Related Striga Research (pp 276–289) Amsterdam, the Netherlands

Foy CL, Jain R and Jacobson R (1989) Recent approaches for chemical control of broomrape (*Orobanche* spp.). Reviews of Weed Science 4: 123–152

Gibot-Leclerc S, Tuquet C, Corbineau F, Arjaure G and Sallé G (2001) New insights on *Orobanche ramosa* L. parasiting oilseed rape in wester part of France. In: Fer A, Thalouarn P, Joel DM, Musselman LJ, Parker C and Verkleij JAC (eds) Proceedings of the 7th International Parasitic Weed Symposium (p 45) Nantes, France

Goldwasser Y, Hershenhorn J, Plakhine D, Kleifeld Y and Rubin B (1999) Biochemical factors involved in vetch resistance to *Orobanche aegyptiaca.* Physiological and Molecular Plant Pathology 54: 87–96

Goldwasser Y, Plakhine D, Kleifeld Y, Zamski E and Rubins B (2000) The differential susceptibility of vetch (*Vicia* spp.) to *Orobanche aegyptiaca*: Anatomical studies. Annals of Botany 85: 257–262

Hood ME, Condon JM, Timko MP and Riopel JL (1998) Primary haustorial development of *Striga asiatica* on host and non-host species. Phytopathology 88: 70–75

Jorrin J, De Ruck E, Serghini K, Perez De Luque A and Munoz-Garcia J (1996) Biochemical aspects of the parasitism of sunflower by *Orobanche*. In: Moreno MT, Cubero JI, Berner D, Joel D, Musselman LJ and Parker C (eds) Proceedings of the Sixth International Parasitic Weed Symposium (pp 551–558) Cordoba, Espagne

Labrousse P, Arnaud MC, Serieys H, Berville A and Thalouarn P (2001) Several mechanisms are involved in resistance of *Helianthus* to *O. cumana* Wallr. Annals of Botany 88: 859–868

Panchenko AYA and Antonova TS (1974) Characteristics of the protective reaction of varieties of sunflower resistant to penetration by broomrape. Sel'skokhozyaitvennaya Biologiya (USSR) 9: 554–558

Parker C and Riches CR (1993) *Orobanche* species: The broomrapes. In: Parasitic Weeds of the World. Biology and Control (pp 111–164) Wallingford, UK, CAB International eds

Perez De-Luque A, Cubero JI, Rubiales D and Joel DM (2001) Histology of incompatible interactions between *Orobanche crenata* and some host legumes. In: Fer A, Thalouarn P, Joel DM, Musselman LJ, Parker C and Verkleij JAC (eds) Proceedings of the 7th International Parasitic Weed Symposium (pp 174–177) Nantes, France

Saghir AR (1986) Dormancy and germination of *Orobanche* seeds in relation to control methods. In: ter Borg SJ (ed.) Proceedings of a Workshop on Biology and Control of *Orobanche* (pp 25–34) LH/VPO, Wageningen, the Netherlands

Sauerborn J, Linke KH, Saxena MC and Koch W (1989) Solarization; a physical control method for weeds and parasitic plants (*Orobanche* spp.) in Mediterranean agriculture. Weed Research 29: 391–397

Visser JH, Dörr I and Kollmann R (1990) Compatibility of *Alectra vogelii* with different leguminous host species. Journal of Plant Physiology 135: 737–745

Zehhar N, Igouff M, Bouya D and Fer A (2002) Possible involvement of gibberellins and ethylene in *Orobanche ramosa* germination. Weed Research (in Press)