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Innovations in parasitic weeds management in legume crops

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Abstract – A number of parasitic weeds pose severe constraints to major grain and forage legumes. The most economically damaging on temperate legumes are the broomrapes, particularly *Orobanche crenata*. But also other broomrape species such as *O. foetida*, *O. minor* and *Phelipanche aegyptiaca* can be of local importance. Other parasitic weeds such as *Striga gesnerioides* and *Alectra vogelii* cause considerable yield reduction of legume crops throughout semi-arid areas of sub-Saharan Africa. Dodders, particularly *Cuscuta campestris* can be damaging in some crops.

Preventing the movement of parasitic weed seeds into un-infested areas is a crucial component of control. Once a field is infested with parasitic weeds, controlling its seed production is very difficult. The only effective way to cope with parasitic weeds is through an integrated approach. Seedbank demise can be achieved by fumigation or solarization, however, this is not economically feasible in low-value and low input legume crops. A number of cultural practices, from delayed sowing, hand weeding, no-tillage, nitrogen fertilization, intercropping or rotations can contribute to seed bank demise. Other strategies such as suicidal germination, activation of systemic acquired resistance, biocontrol or target site herbicide resistance might be promising solutions that are being explored, but are not yet ready for direct application. The only methods currently available to farmers are the used of resistant varieties and chemical control, although both have their limitations. Chemical control with systemic herbicides such as glyphosate or imidazolinones at low rates is possible.

Advances in modelling and the availability of new technologies are allowing the development of precision agriculture or site-specific farming. The most economical and environmentally friendly control option is the use of resistant crop varieties, however, breeding for resistance is a difficult task considering the scarce and complex nature of resistance in most crops. These
5 strategies for parasitic weed management in legume crops will be presented and critically discussed.

Parasitic weeds / control / breeding / intercropping / germination /biological control /
10 chemical control / *Alectra* / *Cuscuta* / *Orobancha* / *Striga* / broomrape / dodder/

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1. INTRODUCTION

20 About 4000 flowering plant species have adapted to parasitize other plants. Unfortunately for farmers, a small number of these species have become weeds, posing severe constraints to major crops including grain and forage legumes (Rubiales and Heide-Jørgensen, 2011). The most economically damaging on legumes are the broomrapes (*Orobanche* and *Phelipanche*). Other parasitic weeds such as *Striga gesnerioides* (Willd.) Vatke, *Alectra voguelii* Benth. and the dodders (*Cuscuta* spp.) can be also damaging on some legumes (Joel et al., 2007; Parker, 2009) (Fig. 1).

O. crenata Forsk. (crenate broomrape) is an important pest of most grain and forage legumes being widely distributed in the Mediterranean basin and Middle East (Rubiales et al., 2009b) (Fig. 2). *O. foetida* Poir. in contrast is of importance only on faba bean (*Vicia faba* L.) in Beja region of Tunisia (Kharrat et al., 1992) although it has recently also been found in Morocco infecting common vetch (*V. sativa* L.) (Rubiales et al., 2005b). *O. minor* Sm. is widely distributed, but of economic importance on clover (*Trifolium* sp.) only (Eizenberg et al., 2005). *P. aegyptiaca* (Pers.) Pomel (syn. *O. aegyptiaca* Pers.) is an important pest of

legumes but also of many vegetable crops in the Middle East and Asia (Parker, 2009). *S. gesnerioides* and *A. vogelii* cause considerable yield reduction of grain legume crops, particularly cowpea (*Vigna unguiculata* (L.) Walp), throughout semi-arid areas of sub-Saharan Africa (Parker and Riches, 1993). Dodders (*Cuscuta* spp.) are widely distributed, being a threat to alfalfa (*Medicago sativa* L.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medik.) in certain locations. The most important species is *C. campestris* Yunck (Fig. 3).

There is no single technology to control these parasitic weeds (Joel et al., 2007; Parker, 2009; Rubiales et al., 2009b). Large areas of new territory are at risk of invasion if care is not taken to limit the introduction of parasitic weed seeds and to educate farmers and others to be on alert for new infestations (Mohamed et al., 2006; Grenz and Sauerborn, 2007). The only way to cope with parasitic weeds is through an integrated approach, employing a variety of measures in a concerted manner, starting with containment and sanitation, direct and indirect measures to prevent the damage caused by the parasites, and finally eradicating the parasite seedbank in soil.

2. Cultural practises

Preventing the movement of parasitic weed from infested into un-infested areas is a crucial component of control. Both sanitation and quarantine are required in order to prevent the dispersal of seeds. The minute seeds can easily be transferred from one field to another by cultivation, and also by water, wind and animals. However, the most significant seed transfer agents are people, transportation vehicles and farming machines, which easily transfer seeds and contaminated soil. Once a field is infested with parasitic weeds, controlling its seed production is very difficult. Extermination of seeds before their spread to new fields and regions is a crucial component in parasitic weed prevention program (Panetta and Lawes,

2005). Quaternary ammonium salts have been found effective in seed eradication with complete seed kill achieved under commercial conditions at 0.5% a.i. of the disinfectant didecyl dimethyl ammonium bromide.

Early plantings of cool season legumes are more severely infected by parasitic weeds.

5 Delayed sowing is the best-documented traditional method for *O. crenata* avoidance (Rubiales et al., 2003b; Pérez-de-Luque et al., 2004b; Grenz et al., 2005). It also reduces *S. gesnerioides* (Touré et al., 1996) and dodder infection (Mishra et al., 2007). However, delaying sowing date implies shortening grain filling, which is detrimental for yield. Thus, options are needed that combine the yield benefit of early sowing with a decreased parasitic
10 weed infestation.

Hand weeding can only be recommended in cases of limited infestation to prevent any further increase in the parasite population and to reduce the seed bank in the soil. However, even when hand weeding is still commonly used in some countries where no other feasible means of control are available and the wages for labour are cheap, it is only practical in
15 preventing build-up of parasite seeds in slightly infested soils.

Solarization utilizes the sunlight in the summer to produce high temperatures under clear polyethylene mulch that covers the soil for several weeks. Mulching wet soil with transparent polyethylene sheets for a period of 4-8 weeks during the warmest season proved to be effective for a number of vegetables but also for faba beans and lentil (Sauerborn et al.,
20 1989a; Mauromicale et al., 2001).

Cultivation of the soil can strongly affect the parasitic weed seed bank. Minimum tillage can contribute to parasitic weed control by reducing the amount of viable seeds incorporated into the soil (Ghersa and Martínez-Ghersa, 2000). No-tillage has been reported to considerably reduce *O. crenata* infection on faba bean (López-Bellido et al., 2009). On the
25 other hand deep-ploughing has been recommended to bring the seeds into a depth, where they

cannot germinate due to the lack of oxygen (Van Delft et al., 2000) but expectations have not been met sufficiently perhaps due to problems with the costs and practicability.

Nitrogen compounds and manure fertilization has potential for control of broomrape species. Nitrogen in ammonium form affects negatively root parasitic weed germination (Van Hezewijk and Verkleij, 1996) and/or elongation of the seedling radicle (Westwood and Foy, 1999). In addition, manure fertilization augments the killing effect of solarization on *O. crenata* seeds (Haidar and Sidahmed, 2000).

Intercropping is already used in Africa as a low-cost method of controlling *S. hermonthica* on cereals (Oswald et al., 2002). It has recently been shown that intercrops with oat (*Avena sativa* L.) or with fenugreek (*Trigonella foenum-graecum* L.) or berseem clover (*Trifolium alexandrinum* L.) can reduce *O. crenata* infection on legumes being allelopathy a major component for the reduction (Fernández-Aparicio et al., 2007; 2008b; 2008c; 2010a) (Fig. 4). The finding that germination of seeds exposed to synthetic germination stimulants is inhibited in presence of oat or fenugreek roots suggest that oat roots might be exuding substances that inhibit *O. crenata* seed germination. This has been confirmed in a subsequent work, and trigoxazonane identified from fenugreek (Evidente et al., 2007) or benzoxazolinones (Fernández-Aparicio, unpublished) from oat root exudates that might be responsible for inhibition of *O. crenata* seed germination. Considerable genetic variation in allelopathic activity has been found within crops (Baghestani et al., 1999) which may allow for selection of more allelopathic cultivars.

Rotation may have direct and indirect impacts on parasitic weed in infested areas. While trap- and catch-crops in rotation may reduce to some extent the parasite seed bank in soil, as discussed above, other rotation crops may have allelopathic effects on parasitic weed seeds. Decreasing host cropping frequency cannot, by itself, solve the parasitic weed problem. A

nine-course rotation would be required to prevent *O. crenata* seedbank increases (Grenz et al., 2005).

The use of trap crops offers the advantage of stimulating parasitic weed seed germination without being parasitized, contributing to seed bank depletion. However, given the high amount of seeds in infested soils, it cannot be expected that the application of trap crops or germination stimulants will eliminate the seed bank in the soil immediately. It is a feature of parasitic weeds that their numerous tiny seeds stay viable for along time and never do germinate all at the same time. The application of trap crops therefore should be included in the regularly rotation and fallow management for the infested fields. For each species suitable trap crops need to be identified, as they show host specificity (Fernández-Aparicio et al., 2009a). For instance, pea is only infected by *O. crenata* but pea root exudates stimulate germination of *P. aegyptiaca*, *O. foetida* and *O. minor* (Fernández-Aparicio et al., 2008d) that are known to infect other legumes. Pea could be efficiently used as trap crop for these species inducing suicidal broomrape germination. Other examples of potential trap crops are cereals such as durum wheat (*T. turgidum* L.) and oat that stimulated high germination of *O. minor* and *P. aegyptiaca* (Lins et al., 2006; Fernández-Aparicio et al., 2009a) or sorghum (*Sorghum bicolor* (L.) Moench) that strongly stimulated *S. gesnerioides* (Berner and Williams, 1998). The use of cereals could be a suitable solution due to the high frequency in which they are cultivated and its importance for human consumption. For that reason, breeding on cereal germplasm for the maximum inductor effect on broomrape seeds could be of great interest. In fact clear genotypic effects have been reported in sorghum in induction of *S. gesnerioides* germination (Berner and Williams, 1998). Growing the selected varieties with high inductor potential for suicidal broomrape germination in rotations introduced in infected soils could shorten the time necessary between host crops.

Catch crops in contrast to trap crops are plants which are heavily parasitized. Harvesting or destroying them after the appearance of the parasite would dramatically reduce the seed potential in the soil. The ideal solution would be a catch crop of agronomical interest by itself.

5 There are claims of reduction in infestation in rotations with rice due to water flooding (Sauerborn and Saxena 1986) however this has not been substantiated. It has also been evidenced that soil irrigation during summer does not change broomrape seed germination (López-Granados and García-Torres 1996) what is in agreement with the high levels of infection in faba bean in irrigated areas such as the Nile Valley, quite often in
10 rotations with rice.

3. CHEMICAL CONTROL

3.1 Herbicides

 The intimate connection between host and parasite also hinders efficient control by
15 herbicides. Volatile compounds such as methyl bromide, ethylene dibromide, metham-sodium or formalin are effective for the control of broomrape (Foy et al., 1989) but the high cost seldom justifies their use in legumes. The herbicides that are currently in use for parasitic weed control are glyphosate, imidazolinones or sulfonylureas (Joel et al., 2007). Glyphosate disrupts the biosynthesis of aromatic amino acids inhibiting the key enzyme 5-
20 enolpyruvylshikimate-3-phosphate synthase (EPSPS), whereas imidazolinones and sulfonylurea herbicides inhibit acetolactate synthase (ALS) (Schloss, 1995). All of them are systemic herbicides absorbed through foliage and roots of plants with rapid translocation to the attached parasite, which acts as a strong sink (Colquhoun et al., 2006).

 Chemical control of broomrape by foliar applications of glyphosate at low rates is
25 recommended for faba bean and vetches (Jacobsohn and Kelman 1980; Mesa-García and

García-Torres 1985; Sauerborn et al. 1989b) but problems of lack of effectiveness or phytotoxicity can arise when it is not applied properly. Selective control has also been reported in lentils (Arjona-Berral and García-Torres, 1983) at lower rates, but not in peas that are very sensitive to glyphosate. However, tolerance to glyphosate exists in pea germplasm
5 that could be exploited in breeding (Sillero et al., 2001). Further, pea and other legumes could be genetically engineered with the glyphosate-resistance gene.

Pea and lentil tolerate better pre- and post-emergence treatment of other herbicides suitable for broomrape control such as imidazolinones (Jurado-Expósito et al., 1986). However, no complete control is provided and treatment is less effective in the earlier sowing
10 dates (Rubiales et al., 2003c) probably due to the dissipation of the herbicide from the sowing date till the time of broomrape establishment. Faba bean tolerates pre-emergence treatments of imazapyr (12.5-25 g/ha) and imazethapyr (75-100 g/ha) and post-emergence treatments of imazapyr (2.5-5 g/ha), imazethapyr (20-40 g/ha) and imazaquin (40-60 g/ha) (García-Torres and López-Granados, 1991). Lentil tolerates pre-emergence treatments of imazapyr (25 g/ha)
15 and imazethapyr (75 g/ha) and postemergence treatments of imazaquin (7.5 ml/ha) (Arjona-Berral et al., 1988; Jurado-Expósito et al., 1997) and imazapic (3 g/ha) (Bayaa et al., 2000). Two post-emergence applications of imazapic are recommended, with the first application recommended when lentil has 5 to 7 true leaves, when broomrape usually starts to develop attachments on lentil roots, followed by a second application 2 to 3 weeks later. Lentil may
20 need a third application of 2 g/ha when late rain comes and extends the growth period, or when lentil is supplementary irrigated (Bayaa et al., 2000). Phytotoxicity might be higher in the presence of water, low temperature and heat stresses and vary with the lentil cultivar used (Hanson and Hill, 2001). An additional problem of these imidazolinone herbicides for broomrape control is that they are not registered in every country, and that doses and timing
25 for application need to be adjusted case by case. Also, traditional imidazolinones are being

replaced in some countries by imazamox that has less residual in the soil, so doses and timing of application need re-adjustment.

Knowledge of the phenology of parasitic weeds is essential for their effective chemical control. Application must be repeated in time interval of 2 to 4 weeks, because
5 *Orobanche* seeds may germinate throughout the season, and may therefore re-establish on newly developed hosts roots. Models for broomrape development can be helpful for precise chemical control. Such models, which exist for *O. minor* infecting red clover (Eizenberg et al., 2005) and as a subroutine of the Manschadi et al. (2001) model, can help to anticipate the occurrence of parasite development stages susceptible to e.g. herbicide application. Advances
10 in modelling and the availability of new technologies are allowing the development of precision agriculture or site-specific farming. Parasitic weeds are usually distributed in patches within the field (González-Andújar et al., 2001), so precision agriculture techniques like discrimination by near-infrared reflectance spectroscopy (Jurado-Expósito et al., 2003) could be adapted to detect broomrape infected areas of the crop, for example attending to
15 differences in transpiration within infected and no infected plants.

Soil applications of sulfonylurea herbicides have proven effective to control broomrape in tomato (*Solanum lycopersicum* L.) and in potato (*S. tuberosum* L.) but the herbicide should be incorporated with irrigation. However, most cool season legume fields are not equipped with irrigation systems, because they are low input crops. Furthermore, legumes
20 are not tolerant to sulfonylurea herbicides and probably will be injured when applied. For such reasons, the only alternative should be pre-planting application, or post planting incorporated with rainfall.

Seed treatments with imidazolinones have proven to be effective controlling *O. crenata* in faba bean and lentil (Jurado-Expósito et al., 1997) and *S. gesnerioides* and *A. vogelii*
25 in cowpea (Berner et al., 1994). The herbicide is incorporated as a coating on the seeds

and distributed with them at planting. This replaces a pre-emergence treatment and saves mechanical application costs. In addition, reduces the herbicide rate required by 2-3 folds, being more environmental friendly. However, under favourable environmental conditions for broomrape attack, the treatment must be supplemented to obtain high broomrape control.

5 The efficient management of dodders starts with the use of dodder-free seed and the spraying of the first affected patches with a contact herbicide to prevent spread. Infested equipment should be cleaned before and after use, and the movement of domestic animals from infested to dodder-free areas should be limited. The application of pendimethalin at 0.7-1.0 kg/ha as pre-emergence or early post emergence is effective against *Cuscuta* in lentil. In 10 chickpea *Cuscuta* is selectively controlled by pre-emergence application of propyzamide (0.5-0.75 kg/ha), pronamide (2.5 kg/ha) or pendimethalin (1.0 kg/ha) or by pre-sowing application of Weedazol (Aminotriazol+ammonium thiocyanate) at 1.2-2.5 Kg/ha. In faba bean *Cuscuta* can be controlled with imazethapyr at 75 g/ha when applied pre-emergence or 20 g/ha post-emergence (Khallida et al., 1993). In alfalfa *Cuscuta* can be controlled by pre-emergence 15 application of imazethapyr at 0.1-0.15 Kg/ha (Cudney and Lanini, 2000) or pendimethalin (1.0 kg/ha) (Dawson, 1990; Orloff et al., 1989) or by post-emergence treatment with low doses of glyphosate (0.075-0.15 Kg/ha) (Dawson, 1989).

Nanotechnology applications are already being explored and used in medicine and pharmacology, but interest for use in crop protection is just starting. Nanoparticles can be 20 used as smart delivery systems for targeting and uploading substances at specific areas within whole plants (González-Melendi et al., 2008; Corredor et al., 2009). The development of nanocapsules for controlled release and systemic application of herbicides will greatly increase the possibilities of their utilisation against parasitic weed, for example by allowing the use of herbicides with different modes of action such as contact herbicides. The 25 nanoparticles would carry the active substance and specific property and/or external

modification of these nanoparticles would allow their accumulation and/or guidance into broomrape specific areas in which the chemical charge would be unloaded. Nanoencapsulation of herbicides could be used to solve problems regarding phytotoxicity on the crop. Lower doses of herbicides would be needed because they will not be degraded by the crop, and they will accumulate preferentially in the parasitic weed due to the sink effect (Pérez-de-Luque and Rubiales, 2009).

3.2. Suicidal germination

Orobanche, *Phelipanche*, *Striga* and *Alectra* seeds require chemical stimulation from the host root to germinate. This suggests that the artificial use of suitable chemicals may reduce the parasitic weed seed bank by stimulating seeds germination in the absence of a suitable host, which should lead to their demise due to their inability to survive without nutritional supply by a host. Attempts have been made with the application to the soil of synthetic strigolactones, such as GR24 (Johnson et al. 1976) or Nijmegen-1 (Zwanenburg and Thuring, 1997). However, the application in the field has not yet been successful, due to the instability of the compound, particularly in alkaline soil, and probably also to the lack of proper field application formulation for these compounds.

Several classes of plant secondary metabolites are known to induce seed germination of root parasitic weeds (Yoneyama et al., 2008). More than ten strigol-related compounds, collectively called strigolactones, have been identified as germination stimulants for root parasitic weeds (Yoneyama et al., 2009). Orobanchol, orobanchyl acetate, and 5-deoxystrigol are widely distributed in the Fabaceae (Yoneyama et al., 2008). Fabacyl acetate has recently been identified from root exudates of pea (Xie et al., 2009). Strigolactones play a major role in host specificity (Fernández-Aparicio et al., 2011). In addition to these, two new compounds (peagol and peagoldione) have been identified in pea root exudates showing a selective

stimulation of *Orobanch*e seed germination (Evidente et al., 2009). Successively, three polyphenols, named peapolyphenols A-C, together with a polyphenol and a chalcone were isolated from same root exudates. Interestingly, only peapolyphenol A, 1,3,3-substituted propanone and 1,3-disubstituted propenone had specific stimulatory activity on *O. foetida*, not stimulating any other *Orobanch*e or *Phelipanche* species tested (Evidente et al., 2010). Also, a triterpene and a sterol, identified as syasapogenol B and *trans*-22-dehydrocampesterol, have recently been isolated from common vetch root exudates. Soyasapogenol B was very specific stimulating germination of *O. minor* seeds only, whereas *trans*-22-dehydrocampesterol stimulated *P. aegyptiaca*, *O. crenata*, *O. foetida* and *O. minor* (Evidente et al., 2011). Stimulatory activity of these metabolites on parasitic weed germination could be exploited in suicidal germination control strategies by synthesizing and directly applying them to the field. Also, their production in plants could be increased by breeding or by genetic transformation. Soyasapogenol B increased has already been achieved in transgenic *M. truncatula* (Confalonieri et al., 2009).

Ethylene stimulates the germination of *Striga* and *Orobanch*e seeds (Berner et al., 1999; Zehar et al., 2002). Other compounds of natural origin, such as fungal metabolites (Fernández-Aparicio et al., 2008a), fungal phytotoxins, natural amino acids (Vurro et al., 2009), or plant or algae extracts (Economou et al., 2007) have also been suggested for use in parasitic weed management, being able to inhibit seed germination or seedling elongation, or, conversely, stimulate suicidal seed germination in the absence of the host.

3.3. Activators of systemic acquired resistance

Systemic acquired resistance (SAR) has been reported in a number of crops against different broomrape species by application of chemical agents (Sauerborn et al., 2002; Gonsior et al., 2004; Pérez-de-Luque et al., 2004a; Kusumoto et al., 2007). A significant

reduction of *O. crenata* infection in faba bean and pea was achieved under field conditions by foliar application of BTH (1,2,3-benzothiadiazole-7-carbothioic acid S-methyl ester) (Pérez-de-Luque et al., 2004c). *O. minor* control has also been reported on red clover grown in plastic chambers after application of salicylic acid and BTH by inhibition elongation of *O.*
5 *minor* radicles and the activation of defence responses in the host root including lignification of the endodermis (Kusumoto et al., 2007). Pérez-de-Luque (2004a) described highly varying success on broomrape-control after different BTH and salicylic acid modes of application on pea in growth chamber experiments. This shows that more host-parasite specific application techniques or perhaps other resistance inducing products are necessary to increase efficacy
10 and reduce negative effects.

4. BIOLOGICAL CONTROL

Numerous microorganisms that might be useful for biocontrol of parasitic weeds have been isolated and reported (Amsellem et al., 2001; Boari and Vurro, 2004; Sauerborn et al.,
15 2007; Zermane et al., 2007). The fungal isolates reported to be pathogenic to parasitic weeds are for the most part *Fusarium* spp., prevailing especially strains of *F. oxysporum* Schlecht.: Fr.. Advantages of *Fusarium* spp. relate to their host specificity and longevity in soil. However, to date only *F. oxysporum* f. sp. *orthoceras* (Müller-Stöver et al., 2004) and *F. solani* (Mart.) Sacc. (Dor and Hershenhorn, 2009) are under investigation as potential
20 candidates for broomrape control. *Ulocladium atrum* (Preuss) Sacc. and *U. botrytis* Preuss have been found pathogenic towards *O. crenata* (Linke et al., 1992; Müller-Stöver and Kroschel, 2005). *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar isolated from faba bean roots has been found to inhibit germination of *O. crenata* seeds due to the production of the macrocyclic trichothecene, verrucarín A (El-Kassas et al., 2005). Preliminary results
25 demonstrated control of infection of faba bean by *O. crenata* by the addition of spores of *M.*

verrucaria to soil, raising the possibility that this approach might be applicable in the field. Andolfi et al. (2005) isolated seven macrocyclic trichothecenes, namely, verrucarins A, B, M, and L acetate, roridin A, isotrichoverrin B, and trichoverrol B from *M. verrucaria* and neosolaniol monoacetate from *F. compactum* was, a trichothecene, all being potent inhibitors of *P. ramosa* seed germination. Roridin A was considered the metabolite with highest potential as herbicide as at 1micromolar concentration it preserves a high phytotoxic activity lacking zootoxicity (Andolfi et al., 2005).

Some compatible *Rhizobium* strains have been reported to decrease *O. crenata* infections in pea by activation of oxidative process, LOX pathway and production of possible toxic compounds, including phenolics and pisatin, inhibiting germination of *O. crenata* seeds and causing a browning reaction in germinated seeds (Mabrouk et al., 2007). Colonisation by the nitrogen-fixing bacterium *Azospirillum brasilense* has also been reported to inhibit germination and radicle growth of *P. aegyptiaca* (Dadon et al., 2004). Also colonization by Arbuscular mycorrhizal fungi can provide protection against parasitic weeds as reported in cowpea reducing germination of *S. gesnerioides* (Lendzemo et al., 2009) or in pea reducing germination of various *Orobanch*e and *Phelipanche* species (Fernández-Aparicio et al., 2010b).

Most of the insects reported to occur on root parasitic weed species are polyphagous without any host specificity and thus, damage to these parasitic weeds is limited (Klein and Kroschel, 2002). However, for biological control only oligo- and mono-phagous herbivorous insects are of interest. *Smicronyx* spp. has potential for *Striga* seed reduction (Kroschel et al., 1999). *Phytomyza orobanchia* Kalt. is a fly monophagous on broomrape. The feeding of the larvae within the capsules markedly diminishes seed multiplication of the parasite (Klein and Kroschel, 2002). *P. orobanchia* is widely distributed in the broomrape infected area, eating a substantial number of seeds (Rubiales et al., 2001). This natural infestation is however

insufficient to reduce broomrape in areas with heavy broomrape infestations. Nevertheless, bio-control with *P. orobanchia* can be helpful to slow down further dissemination and infestation in weakly infested areas, and can be part of an integrated control approach to reduce the seed bank in heavily infested soils. This effect could be substantially increased by massive propagation and inundative release of this insect (Klein and Kroschel, 2002).

Further success of mycoherbicides in agricultural applications will depend largely on the development of an appropriate formulation which allows storage, handling and a successful application of the fungal propagules (Shabana et al., 2003; Sauerborn et al., 2007). Effectiveness of biological control could be increased by the combination of two or more pathogens (Charudattan, 2001). For instance, *F. oxysporum* f. sp. *orthoceras* and *F. Solani* gave a low control *O. cumana* in sunflower (*Helianthus annuus* L.) when used individually, but when applied as a mixture control improved (Dor et al., 2003). Integration of SAR inducers with *F. oxysporum* f.sp. *orthoceras* also resulted in highly reliable control of *O. cumana* (Müller-Stöver et al., 2005). The engineering of hypervirulence genes into weed-specific pathogens is receiving increasing attention (Gressel et al., 2004).

Another potential strategy would be to change the end uses, so that broomrape would be a desirable product in its own right. In the Puglia region of Italy *O. crenata* is considered a tasty vegetable very appreciated by consumers. There are several recipes for cooking broomrape that are offered in restaurants (Rubiales, 1999). Alternative uses might include the pharmaceutical and cosmetic industries (Andary, 1993).

5. RESISTANCE BREEDING

5.1 Breeding for resistance to the parasites

Breeding for disease resistance is straight-forward when a good source of resistance is available and an efficient, easily controlled and practical screening procedure exists to provide good selection pressure. Unfortunately, this is not the case in legumes against broomrape, (Rubiales 2003; Rubiales et al., 2006). The only instance in which broomrape resistance of simple inheritance has been identified and widely exploited in breeding is in sunflower against *O. cumana* Wallr. This has been particularly important allowing a rapid progress in sunflower breeding against *O. cumana* (Fernández-Martínez et al., 2008). *O. crenata* resistance identified in legumes so far is of polygenic nature (Román et al., 2002a; Valderrama et al., 2004; Fondevilla et al., 2010). Faba bean breeding for *O. crenata* resistance was founded using the Egyptian line F402 as source of resistance (Nassib et al., 1982). Similarly, resistance against *O. foetida* has also been identified in faba bean germplasm. Some lines selected against *O. crenata* have also shown a high level of resistance to *O. foetida*, with a high yield potential (Abbes et al., 2007).

Little resistance is available within pea germplasm against *O. crenata* (Rubiales et al., 2003c), but promising sources of resistance have been identified in wild relatives within the genus *Pisum* (Rubiales et al., 2005a; Pérez-de-Luque et al., 2005a), which have successfully been hybridised with cultivated pea (Rubiales et al., 2009a). Resistance is also very limited in grass pea (*Lathyrus sativus* L.) and chickling vetch (*L. cicera* L.) (Fernández-Aparicio et al., 2009b; Fernández-Aparicio and Rubiales, 2010), but is available in related *Lathyrus* species (Sillero et al., 2005). Resistance in lentils has only recently been reported (Fernández-Aparicio et al., 2008e; 2009c). However, resistance is frequent in common vetch and chickpea germplasm and cultivars (Gil et al., 1987; Rubiales et al., 2003a; Fernández-Aparicio et al., 2008f), as well as in their wild relatives (Rubiales et al., 2004, 2005a; Sillero et al., 2005). Resistance to *P. aegyptiaca* has been found in purple vetch (*V. atropurpurea* Desf.) (Goldwasser et al., 1997) (Fig. 7).

Cowpea breeding for resistance to *S. gesnerioides* in West Africa was founded using the Botswana landrace B301 as a source of resistance. Following extensive field evaluation a number of new resistant cultivars have been released. Different dominant genes for resistance have been identified in lines resistant to different *S. gesnerioides* strains identified in West Africa so these have been used as complimentary parents when breeding for all strains (Singh, 2002; Timko et al., 2007). Resistance to *Alectra* was discovered after screening more than 650 cowpea accessions. Landraces B301 and B359 appeared to be particularly useful (Riches, 1987, 1989). It is important to notice that landrace B301 is resistant to both *S. gesnerioides* and *A. voguelii*, being resistance to each weed conferred by independent non-allelic genes (Atokple et al., 1993).

5.2 Pathogenic variation

Knowledge on the existence of host specialisation and parasite races is vital for any breeding programme. There is no clear evidence for the existence of races of *O. crenata*. Molecular analysis suggest that most of the intraespecific variation in *O. crenata* is among individuals and not among hosts nor between regions (Román et al., 2002a) what supports the lack of physiological races and the high flow among broomrape populations. Only several levels of host driven differentiation have been described in *O. minor* growing on *Trifolium pratense* and *Daucus carota* ssp. *gumminfer* (Thorogood et al., 2009) or *O. foetida* on chickpea and faba bean (Román et al., 2007). Races have not been described in any of these species, and the lack of selection pressure by the host due to the lack of highly resistant cultivars to any of these species. It would appear at least possible that new parasitic biotypes could originate, however, as *O. crenata* populations are very heterogeneous (Román et al., 2001, 2002b). There is the risk that diverse *O. crenata* populations could be selected for virulence when challenged by the widespread use of highly resistant cultivars. In fact a new

race of *O. crenata* has been suggested in Israel attacking resistant vetches (Joel, 2000). This putative race seems to have been selected by the frequent culture of the resistant vetch cultivar in the area.

There is considerable variation in host specificity between isolates of *S. gesnerioides*, and different host species vary in their susceptibility to different parasite isolates. Mohamed et al. (2001) described eight *Striga* host-specific strains called *Euphorbia*, *Ipomoea*, *Indigofera*, *Jaquemontia*, *Merremia*, *Nicotiana*, *Tephrosia* and *Vigna* strains. The *Vigna* strain is by far the most important, causing considerable damage to cowpea. It was initially proposed that there are five distinct races of *S. gesnerioides* in West and Central Africa, based on their ability to differentially parasitize different cowpea lines (Lane et al., 1997). At least seven distinct races were recognized in a later study with genome profiling with molecular markers and host differential resistance response (Botanga and Timko, 2006). Because of the autogamous nature of *S. gesnerioides*, variations among isolates become genetically fixed, and geographic distributions of morphotypes, strains, and races frequently overlap (Parker and Riches, 1993).

Although less systematic research has been completed with *A. vogelii*, it is clear that there is also variability in the virulence of populations of this parasite in different areas of Africa (Riches et al. 1992). *A. vogelii* also has distinct races that differentially parasitize cowpea (Polniaszek et al., 1991).

5.3 Resistance mechanisms

Resistance against root parasitic weeds is a multi component event, being the result of a battery of avoidance factors and/or resistance mechanisms acting at different levels of the infection process (Joel et al., 2007; Pérez-de-Luque et al., 2008; 2009; Rubiales et al., 2009a).

Avoidance due to precocity is known in some legumes, early flowering genotypes having an

advantage limiting *O. crenata* infection (Rubiales et al., 2005a; Fernández-Aparicio et al., 2009b) (Fig. 5) what can be explained by an earlier pod-setting and maturity, which would restrict the dry-matter partitioning into parasites (Grenz et al., 2005).

Some pre-attachment mechanisms of resistance could be acting prior to parasite
5 contact with the host root. One of them is low induction of seed germination. It has been
successfully used in sorghum breeding for resistance to *S. hermonthica* (Ejeta, 2007) but has
not been identified in legumes against *S. gesnerioides*. Resistance to *O. crenata* associated to
low induction of parasite seeds germination has been reported in several legumes (Pérez-de-
Luque et al., 2005a; Rubiales et al., 2003a; 2004; Sillero et al., 2005). Another pre-attachment
10 mechanism could be related to the chemotropism responsible for the correct guidance of the
broomrape seedling towards the host root. A wrong orientation of germinated *O. crenata*
seeds within the potentially infective distance has been observed in pea (Pérez-de-Luque et
al., 2005a) (Fig. 6) probably due to changes in the concentration of compounds exuded from
host roots that are responsible for the chemotropic response of the seedlings (Whitney and
15 Carsten, 1981). Pre-haustorial mechanisms of resistance consisting in physical barriers such
as protein crosslinking, callose depositions and suberisation reinforcing cortical cell walls
(Pérez-de-Luque et al., 2006a; 2007) or in lignification of endodermal cells (Pérez-de-Luque
et al., 2005b; 2007) have also been identified in legumes . In addition, chemical barriers in the
form of accumulation and excretion of phytoalexins into the apoplast have been identified in
20 the central cylinder of *M. truncatula* Gaertn. (Lozano-Baena et al., 2007).

Post-haustorial resistance mechanisms, where attached parasites fail to develop have
also been described in legumes. These can be physical response consisting on sealing of host
vessels by gel or gum-like substances and blocking the flux of water and nutrients from the
host to the parasite (Pérez-de-Luque et al., 2005b; 2006b) or chemical, consisting on delivery
25 of toxic compounds such as phenolics into the host vascular system causing death of *O.*

crenata tubercles on chickpea (Pérez-de-Luque et al., 2006c) and *M. truncatula* (Lozano-Baena et al., 2007) or of *P. aegyptiaca* in purple vetch (Goldwasser et al., 1999) (Fig. 7).

A rapid necrosis of the host cells around the point of infection, leading to the death of the parasite has also been identified in cowpea against *S. gesnerioides* (Lane et al., 1993).

5 Another resistance mechanism was observed in other cowpea cultivars consisting in growth arrest of *S. gesnerioides* tubercles. Although tubercles began to develop on the host root surface, they did not enlarge, remaining less than 0.5 mm in diameter or their cotyledons failed to expand (Lane and Bailey, 1992; Lane et al., 1993).

Resistance to *Cuscuta* has only recently been reported in chickpea characterized by the
10 failure of the prehaustorium to penetrate the chickpea stem (Goldwasser et al., 2009).

5.4 Potential use of molecular markers and biotechnologies in resistance breeding

The development of marker-assisted selection (MAS) techniques for broad-based polygenic resistance is a particularly promising approach since *Orobancha* resistance tests are
15 difficult, expensive and sometimes unreliable.

In pea, quantitative trait loci (QTLs) conferring resistance to *O. crenata* have been identified (Valderrama et al., 2004; Fondevilla et al., 2010). However, QTLs identified explained a low portion of the observed variation (altogether 21%). An *in vitro* screening method assaying different phases of the parasite cycle using a Petri dish technique, enabled
20 identification of QTLs governing specific mechanisms of resistance, explaining a higher proportion of the variation (38–59%, depending on the trait) (Fondevilla et al., 2010).

In faba bean, three QTLs linked to resistance were identified in a map developed using an F2 population segregating for the trait (Román et al., 2002b). The QTLs explained a high percentage of the phenotypic variation of the trait (74%), mainly because of one of the QTL

Oc1, which explained more than 37% of the character. Unfortunately, when a RIL population derived from this same cross was evaluated in other seasons and locations, this QTL vanished (Díaz-Ruiz et al., 2010), showing that it was not a stable QTL and should therefore be excluded for MAS. Two additional QTLs for resistance against *O. foetida* were identified using the same RIL, but both instable across environments and explained very little phenotypic variation (7-9%) (Díaz-Ruiz et al., 2009). The accuracy of phenotypic evaluation is of the utmost importance for the accuracy of QTL mapping. These screenings for broomrape resistance were performed under field conditions with not sufficient control of crucial environmental factors and of homogeneity of inoculum in the soil. Also, assessments were based on final number of emerged broomrapes per plant in the infested fields, what is likely to be influence by many confounding factors (Fernández-Aparicio et al., 2009b) such as earliness or plant vigour (Fondevilla et al., 2010). These can be avoidance to the contact by reduced root biomass or root architecture, low induction of broomrape seed germination, resistance to penetration, and/or hampered development of established tubercles (Rubiales et al., 2006; Pérez-de-Luque et al., 2010). To overcome the disadvantages of screenings under field conditions, phenotyping should be performed using mini-rhizotrons, allowing the dissection of the resistance into specific mechanisms acting in different stages of the infection process, increasing the accuracy of the assessment, as done in pea (Fondevilla et al., 2010).

The genetic basis of resistance to *S. gesnerioides* and *A. vogelii* parasitism has been examined by a few laboratories. Single genes conferring resistance to *S. gesnerioides* (named *Rsg* genes: Touré et al., 1997; Ouédraogo et al., 2001; Li et al., 2009) or to *A. vogelii* (named *Rav* genes: Atokple et al., 1993; 1995) have been reported. Molecular markers linked to *Rsg1-1*, *Rsg2-1*, *Rsg3-1*, *Rsg4-3* and *Rsg994-1* genes for *S. gesnerioides* resistance have been identified and several sequence-confirmed amplified regions (SCARs) have been developed

for use in marker-assisted selection (Ouédraogo et al., 2002; Li et al., 2009). Markers linked to *A. vogelii* resistances are being sought (Kouakou et al., 2009)

Although QTLs for resistance to *O. crenata* have been identified in pea and faba bean, they cannot yet be used in MAS. Saturation of genomic regions associated with broomrape resistance is needed to facilitate the identification of the most tightly linked markers that could be used to transfer the genes/QTLs responsible to the broomrape. The distances between the flanking markers and QTLs are still high and numerous recombinants between the QTL flanking markers and resistance are expected. Therefore, before using the available QTLs in MAS, the genomic regions containing the QTLs should be further saturated in order to refine the position of the QTLs and to identify molecular markers more closely linked to the resistance genes. The identification of the genes governing resistance included in the QTLs would allow the development of primers for these genes. These primers would be really useful for MAS, as they would be specific and recombination between the markers and resistance would be absent.

Novel strategies for the control of plant pathogens include selective silencing of target genes by small interfering RNAs (siRNA) (Prins et al., 2008). A key component to success with RNAi technology is identifying the best parasite genes to silence (Yoder et al., 2009). Sequence information obtained from different parasite species can be used to clone the homologous gene from a particular pest or can be directly transformed into crop plants.

5.5 Lessons to learn from model legumes

The use of model plants is valuable to identify new resistance genes that can be directly transferred or reintroduced under the control of a more active promoter *via* genetic transformation to improve resistance in crops. Following the example of other plant pathogen-interaction, the use of model plants such as *Arabidopsis thaliana* (L.) Heynh., *Medicago*

truncatula, *Lotus japonicus* L. and rice (*Oryza sativa* L.), may improve our understanding of the plant-parasitic plant interaction. *A. thaliana* and *M. truncatula* are suitable hosts of some *Orobanch*e species (Westwood, 2000; Rodríguez-Conde et al., 2004; Fernández-Aparicio et al., 2008d), while rice may be used to study *Striga* (Gurney et al., 2006). *L. japonicus* can be infected by *P. aegyptiaca*, but shows incompatible interaction against *O. minor* and *S. gesnerioides* (Kubo et al., 2008). Further studies should thus take advantage of these models to get insight more rapidly into the molecular bases of plant resistance, which should improve the efficiency of both MAS and transgenic approaches for crop improvement toward resistance to parasitic plants. The powerful “omics” tools implemented for *M. truncatula* makes this species highly suitable as a model for these studies (Dita et al., 2006; 2009; Die et al., 2007; Rispail et al., 2007; 2010; Castillejo et al., 2009).

M. truncatula is resistant to *O. crenata*, with rather insufficient variation in resistance among accessions hampering its use for genetic studies and presenting a non-host rather than host resistance response (Fernández-Aparicio et al., 2008d; Lozano-Baena et al. 2007). However, other *Orobanch*e species different from *O. crenata*, might infect *M. truncatula*. Significant variation in the level of resistance/susceptibility to *O. aegyptiaca*, *O. foetida*, *P. nana* (Reut.) Soják and *P. ramosa* (L.) Pomel is available in *M. truncatula* germplasm (Fernández-Aparicio et al., 2008d). Genomic resources developed in *M. truncatula* (Young and Udvardi, 2009) have the potential to accelerate practical advances in crop legumes.

Gene expression studies provide another source of candidate genes for parasitic weed resistance. They allow increasing the knowledge on the molecular basis of the resistance and the mechanisms underlying the host-parasite interaction. To improve our understanding of the *M. truncatula*-*O. crenata*, a suppression subtractive hybridization (SSH) library was created allowing the identification of candidate genes for *O. crenata* defence (Die et al., 2007). In addition, a microarray analysis of the *M. truncatula* genes regulated in response to *O. crenata*

has been performed on a M16kOLI1 microarray platform (Dita et al., 2009). These analyses revealed the activation of both the salicylic acid (SA) and jasmonate defence-pathways. Hybridisation of *M. truncatula* microarray chips with RNA from related species such as pea is possible (Fondevilla et al., 2011). Thus, the same microarray platform may eventually be used
5 directly with RNA from pea allowing a more comprehensive understanding of pea molecular response to broomrape.

Differential expression studies in cowpea suggested that *PR5* expression may be a useful marker of *S. gesnerioides* infection, and that salicylic acid signalling appears to play a role in the cowpea–*S. gesnerioides* interaction (Li et al., 2009). Resistance to *S. gesnerioides*
10 has been knocked down by virus-induced gene silencing (Li and Timko, 2009). The differential display approach has also been applied to *Cuscuta*-infected alfalfa, showing the induction of PR genes such as PPRG2, a PR-10 homologue (Borsics and Lados, 2002), and a calmodulin-related protein, PPRG1, indicating that defence signalling pathways to dodder is linked to calcium (Borsics and Lados, 2001).

A proteomic approach was also applied to compare the proteome of two pea genotypes differing in their sensitivity to *O. crenata* at different stages of the infection (Castillejo et al., 2004). However, only 25% of the proteins differentially regulated were identified by mass spectrometry, hampered by the low number of pea sequences available in databases. The use of *M. truncatula* allowed the identification of more proteins involved (Castillejo et al., 2009).
20

5.6 Breeding for resistance to the herbicides

Herbicide-treated crops with target-site resistances would allow control of parasitic weeds (Gressel, 2009). The herbicide would pass through the plant and flow into the hidden parasite; it is essential for this mode of action that the host plant does not metabolise the
25 herbicide. In target site resistance, the parasite takes the herbicide together with the nutrients

from the protected host and thereby accumulates toxic levels. This hypothesis has been already borne out by using transgenic crops with target-site resistance for the acetolactate synthase (ALS), enolphosphate shikimate phosphate (EPSP) synthase, and dihydropteroate synthase-inhibiting herbicides allowing control of *Striga* or *Orobancha* (Gressel, 2009). This is therefore, a promising solution for controlling parasitic weeds in legumes. Resistance could be transferred into legumes following existing protocols, allowing an efficient broomrape control. However apart from the difficulties of transforming legume plants other drawbacks such as high cost, lengthy development time and high economic risk, limit the use of this solution (Duke and Cerdeira, 2005).

Imidazolinone-tolerant plants with altered ALS genes and enzymes have been discovered in many crops through mutagenesis and selection. This allowed developing by conventional breeding methods of imidazolinone-tolerant maize, wheat, sorghum, rice, oilseed (*Brassica napus* L.) and sunflower (Tan et al., 2004). Imidazolinone-tolerant mutations have also been discovered in other crops and it is technically possible to identify them in legume crops and develop imidazolinone-tolerance cultivars by breeding. A number of lentil cultivars are being introduced to the market under the trademark “CLEARFIELD® lentils” (BASF Agsolutions, 2008) that are not genetically modified and that tolerate higher doses of imidazolinone herbicides.

However, ALS-inhibitor resistance can rapidly evolve in weeds as a consequence of repeated application of herbicides with this site of action. In fact, resistance to ALS inhibitor herbicides such as chlorsulfuron and sulfometuron-methyl has already been reported in *C. campestris* (Rubin, 1994). Herbicide mixtures are recommended to manage herbicide resistance.

7. CONCLUSIONS

In this review, we discuss the most advanced methods for parasitic weed control in legumes. Some of these methods are ready for commercial use by farmers (chemical control), some are in the final stages of development toward commercialization (resistant varieties and sanitation), and some need further development and improvement before commercial implementation (biological control).

Altogether the available control methods have not proven to be as effective, economical and applicable as desired. Although several potential control measures were developed in the past decades for some crops, any approach applied alone is often only partially effective and sometimes inconsistent and affected by environmental conditions.

The only way to cope with the parasitic weeds is through an integrated approach, employing a variety of measures in a concerted manner, starting with containment and sanitation, direct and indirect measures to prevent the damage caused by the parasites, and finally eradicating the parasite seedbank in soil. Much research is needed in order to develop new means for parasitic weed control. Basic research should provide new targets for control within the life cycle of the parasites and amongst their metabolic activities. The genomic research of parasitic weeds, which has already started, is likely to help in an overall understanding of some key aspects of parasitism.

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REFERENCES

- Abbes Z., Kharrat M., Delavault P., Simier P., Chaibi W. (2007) Field evaluation of the resistance of some faba bean (*Vicia faba* L.) genotypes to the parasitic weed *Orobanche foetida* Poiret. Crop Prot. 26, 1777-1784.
- Amsellem Z., Barghouthi S., Cohen B., Goldwasser I., Gressel J., Hornok L., Kerenyi Z., Kleifeld I., Klein O., Kroschel J., Sauerborn J., Müller-Stöver D., Thomas H., Vurro M., Zonno M.Ch. (2001) Recent advances in the biocontrol of *Orobanche* (broomrape) species. Biocontrol 46, 211-228.
- Andary C. (1993) Novel derivative of caffeic acid, oraposide, cosmetic or pharmaceutical, particularly dermatological, composition containing it. European Patent EP0576420.
- 10 Andolfi A., Boari A., Evidente A., Vurro M (2005) Metabolites inhibiting germination of *Orobanche ramosa* seeds produced by *Myrothecium verrucaria* and *Fusarium compactum*. J. Agric. Food Chem. 53, 1598-1603.
- Arjona-Berral A., García-Torres L. (1983) Broomrape control in lentils with glyphosate. Lens Newsl. 10, 20-22.
- 15 Arjona-Berral A., Mesa-García J., García-Torres L. (1988) Herbicide control of broomrapes in peas and lentils. FAO Plant Prot. Bull. 36, 175-78.
- Atokple I.D.K., Singh B.B., Emechebe A.M. (1993) Independent inheritance of *Striga* and *Alectra* resistance in cowpea genotype B301. Crop Sci. 33, 714-15.
- Atokple I.D.K., Singh B.B., Emechebe A.M. (1995) Genetics of resistance to *Striga* and
20 *Alectra* in cowpea. J. Hered. 86, 45-49.
- Baghestani A., Lemieux C., Leroux G.D., Baziramakenga R., Simard R.R. (1999) Determination of allelochemicals in spring cereal cultivars of different competitiveness. Weed Sci. 47, 498-504.

<http://www.ag solutions.ca/basf/agprocan/ag solutions/WebASClearfield.nsf/mainLentils.htm>

Bayaa B., El-Hosseini N., Erskine W. (2000) Attractive but deadly. ICARDA Caravan 12, 16.

- 5 Berner D.K., Awad A.E., Aigbokhan E.I. (1994) Potential of imazaquin seed treatment for control of *Striga gesnerioides* and *Alectra vogelii* in cowpea (*Vigna unguiculata*). Plant Dis. 78, 18-23.

Berner D.K., Schaad N.W., Volksch B. (1999) Use of ethylene-producing bacteria for stimulating of *Striga* spp. seed germination. Biol. Control 15, 274-282.

- 10 Berner D.K., Williams O.A. (1998) Germination stimulation of *Striga gesnerioides* seeds by hosts and nonhosts. Plant Dis. 82, 1242-1247.

Boari A., Vurro M. (2004) Evaluation of *Fusarium* spp. and other fungi as biological control agents of broomrape (*Orobancha ramosa*). Biol. Control 30, 212-219.

- 15 Borsics T., Lados M. (2001) cDNA cloning of a mechanical/abiotic stress-inducible calmodulin-related gene from dodder-infected alfalfa. Plant Cell and Environment 24, 649-656.

Borsics T., Lados M. (2002) Dodder infection induces the expression of a pathogenesis-related gene of the family PR-10 in alfalfa. J. Exp. Bot. 53, 1831-1832.

- 20 Botanga C.J., Timko M.P. (2006) Phenetic relationships among different races of *Striga gesnerioides* (Willd.) Vatke from West Africa. Genome 49, 1351-1365.

Castillejo M.A., Amiour N., Dumas-Gaudot E., Rubiales D., Jorrín J.V. (2004) A proteomic approach to studying plant response to crenate broomrape (*Orobancha crenata*) in pea (*Pisum sativum*). Phytochem. 65, 1817-1828.

- Castillejo M.A., Maldonado A.M., Dumas-Gaudot E., Fernández-Aparicio M., Susin R., Rubiales D., Jorrín J. (2009) Differential expression proteomics to investigate responses and resistance to *Orobanche crenata* in *Medicago truncatula*. BMC Genomics 10, 294.
- 5 Charudattan R. (2001) Biological control of weeds by means of plant pathogens: Significance for integrated weed management in modern agro-ecology. BioControl 46, 229-260.
- Colquhoun J.B., Eizenberg H., Mallory Smith C.A. (2006) Herbicide placement site affects small broomrape (*Orobanche minor*) control in red clover (*Trifolium pratense*). Weed Technol. 20, 356-360.
- 10 Confalonieri M., Cammareri M., Biazzi E., Pecchia P., Fevereiro M.P.S., Balestrazzi A., Tava A., Conicella C. (2009) Enhanced triterpene saponin biosynthesis and root nodulation in transgenic barrel medic (*Medicago truncatula* Gaertn.) expressing a novel β -amyrin synthase (*AsOXAI*) gene. Plant Biotechnol. J. 7, 172-182.
- Corredor E., Testillano P.S., Coronado M.J., González-Melendi P., Fernández-Pacheco R., 15 Marquina C., Ibarra R., de-la-Fuente J.M., Rubiales D., Pérez-de-Luque A., Risueno M.C. (2009) Nanoparticle penetration and transport in living pumpkin plants: in situ subcellular identification. BMC Plant Biology 9, 45.
- Cudney D.W., Lanini W.T. (2000) Dodder. In: Maloy O.C., Murray T.D. (Eds.), Encyclopedia of Plant Pathology – Volume I, John Wiley & Sons, Inc., NY. pp. 376- 20 379.
- Dadon T., Nun N.B., Mayer A.M. (2004) A factor from *Azospirillum brasilense* inhibits germination and radicle growth of *Orobanche aegyptiaca*. Isr. J. Plant Sci. 52, 83-86.
- Dawson J.H. (1989) Dodder (*Cuscuta* spp.) control in established alfalfa (*Medicago sativa*) with glyphosate and SC-0224. Weed Technol. 3, 552-559.

Dawson J.H. (1990) Dodder (*Cuscuta* spp.) control with dinitroaniline herbicides in alfalfa (*Medicago sativa*). Weed Technol. 4, 341-348.

Díaz-Ruiz R., Torres A.M., Satovic Z., Gutiérrez M.V., Cubero J.I., Román B. (2010) Validation of QTLs for *Orobanche crenata* resistance in faba bean (*Vicia faba* L.) across environments and generations. Theor. Appl. Genet. 120, 909-919.

Díaz-Ruiz R., Torres A., Gutiérrez M.V., Rubiales D., Cubero J.I., Kharrat M., Satovic Z., Román B. (2009) Mapping of quantitative trait loci controlling *Orobanche foetida* Poir. resistance in faba bean (*Vicia faba* L.). Afr. J. Biotechnol. 8, 2718-2724.

Die J.V., Dita M.A., Krajinski F., González-Verdejo C.I., Rubiales D., Moreno M.T., *et al*, (2007) Identification by suppression subtractive hybridization and expression analysis of *Medicago truncatula* putative defence genes in response to *Orobanche crenata* parasitization. Physiol. Mol. Plant Pathol. 70, 49-59.

Dita M.A., Die J.V., Román B., Krajinski F., Küster H., Moreno M.T., Cubero J.I., Rubiales D. (2009) Gene expression profiling of *Medicago truncatula* roots in response to the parasitic plant *Orobanche crenata*. Weed Res. 49, 66-80.

Dita M.A., Rispail N., Prats E., Rubiales D., Singh K.B. (2006) Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. Euphytica 147, 1-24.

Dor E., Hershenhorn J. (2009) Evaluation of the pathogenicity of microorganisms isolated from Egyptian broomrape (*Orobanche aegyptiaca*) in Israel. Weed Biol. Manag. 9, 200-208.

Dor E., Vurro M., Hershenhorn J. (2003) The efficacy of a mixture of fungi to control Egyptian and sunflower broomrape. In: COST Action 849: Parasitic plant management in sustainable agriculture. Meeting on biology and control of broomrape 30 Oct.–2 Nov. Athens, Greece.

Duke S.O., Cerdeira A.L. (2005) Potential environmental impact of herbicide-resistant crops. Coll. Bio. Rev. 2, 66-143.

- Economou G., Lyra D., Sotirakoglou K., Fasseas K., Taradilis P. (2007) Stimulating *Orobanche ramosa* seed germination with an *Ascophyllum nodosum* extract. *Phytoparasitica* 35, 367-375.
- Eizenberg H., Colquhoun J.B., Mallory-Smith C.A. (2005) A predictive degree-days model for small broomrape (*Orobanche minor*) parasitism in red clover in Oregon. *Weed Sci.* 53, 37-40.
- 5 Ejeta G. (2007) Breeding for *Striga* resistance in sorghum: Exploitation of an intricate host-parasite biology. *Crop Sci.* 47, 216-227.
- El-Kassas R., Karam El-Din Z., Beale M.H., Ward J.L., Strange R.N. (2005) Bioassay-led isolation of *Myrothecium verrucaria* and verrucarins A and B as germination inhibitors of
- 10 *Orobanche crenata*. *Weed Res.* 45, 212-219.
- Evidente A., Cimmino A., Fernández-Aparicio M., Andolfi A., Rubiales D., Motta A. (2010) Polyphenols, including the new Peapolyphenols A-C, from pea root exudates stimulate *Orobanche foetida* seed germination. *J. Agric. Food Chem.* 58, 2902-2907.
- Evidente A., Cimmino A., Fernández-Aparicio M., Rubiales D., Andolfi A., Melck D. (2011)
- 15 Soyasapogenol B and *trans*-22-dehydrocampesterol from Common Vetch (*Vicia sativa* L.) Root Exudates Stimulate Broomrape Seed Germination. *Pest Manag. Sci.*, in press.
- Evidente A., Fernández-Aparicio M., Andolfi A., Rubiales D., Motta A. (2007) Trigoxazonane, a monosubstituted trioxazonane by *Trigonella foenum-graecum* root
- 20 exudate, inhibiting agent of *Orobanche crenata* seed germination. *Phytochem.* 68, 2487-2492.
- Evidente A., Fernández-Aparicio M., Cimmino A., Rubiales D., Andolfi A., Motta A. (2009) Peagol and peagoldione, two new strigolactone-like metabolites isolated from pea root exudates. *Tetrahedron Lett.* 50, 6955-6958.

- Fernández-Aparicio M., Andolfi A., Cimmino A., Rubiales D., Evidente A. (2008a) Stimulation of seed germination of *Orobanch* species by ophiobolin A and fusicoccins derivatives. J. Agric. Food Chem. 56, 8343-8347.
- Fernández-Aparicio M., Andolfi A., Evidente A., Pérez-de-Luque A., Rubiales D. (2008b)
- 5 Fenugreek root exudates show species-specific stimulation of *Orobanch* seed germination. Weed Res. 48, 163-168.
- Fernández-Aparicio M., Emeran A.A., Rubiales D. (2008c) Control of *Orobanch crenata* in legumes intercropped with fenugreek (*Trigonella foenum-graecum*). Crop Prot. 27, 653-659.
- 10 Fernández-Aparicio M., Emeran A.A., Rubiales D. (2010a) Inter-cropping with berseem clover (*Trifolium alexandrinum*) reduces infection by *Orobanch crenata* in legumes. Crop Prot. 29, 867-871.
- Fernández-Aparicio M., Flores F., Rubiales D. (2009a) Recognition of root exudates by seeds of broomrape (*Orobanch* and *Phelipanche*) species. Ann. Bot. 103, 423-431.
- 15 Fernández-Aparicio M., Flores F., Rubiales D. (2009b) Field response of *Lathyrus cicera* germplasm to crenate broomrape (*Orobanch crenata*). Field Crops Res. 113, 321-327.
- Fernández-Aparicio M., García-Garrido J.M., Ocampo J.A., Rubiales D. (2010b)
- 20 Colonization of field pea roots by arbuscular mycorrhizal fungi reduces *Orobanch* and *Phelipanche* species seed germination. Weed Res. 50, 262-268.
- Fernández-Aparicio M., Pérez-de-Luque A., Prats E., Rubiales D. (2008d) Variability of interactions between barrel medic (*Medicago truncatula*) genotypes and *Orobanch* species. Ann. Appl. Biol. 153, 117-126.
- Fernández-Aparicio M., Rubiales D. (2010) Characterisation of resistance to crenate
- 25 broomrape (*Orobanch crenata* Forsk.) in *Lathyrus cicera* L. Euphytica 173, 77-84.

- Fernández-Aparicio M., Sillero J.C., Pérez-de-Luque A., Rubiales D. (2008e) Identification of sources of resistance to crenate broomrape (*Orobanche crenata*) in Spanish lentil (*Lens culinaris*) germplasm. Weed Res. 48, 85-94.
- 5 Fernández-Aparicio M., Sillero J.C., Rubiales D. (2007) Intercropping with cereals reduces infection by *Orobanche crenata* in legumes. Crop Prot. 26, 1166-1172.
- Fernández-Aparicio M., Sillero J.C., Rubiales D. (2008f) Resistance to broomrape species (*Orobanche* spp.) in common vetch (*Vicia sativa* L.). Crop Prot. 28, 7-12.
- Fernández-Aparicio M., Sillero J.C., Rubiales D. (2009c). Resistance to broomrape in wild lentils (*Lens* spp.). Plant Breeding 128, 266-270.
- 10 Fernández-Aparicio M., Yoneyama K., Rubiales D. (2011) The role of strigolactones in host specificity of *Orobanche* and *Phelipanche* seed germination. Seed Sci. Res. 21, 55-61.
- Fernández-Martínez J.M., Domínguez J., Pérez-Vich B., Velasco L. (2008) Update on breeding for resistance to sunflower broomrape. Helia 31, 73-84.
- Fondevilla S., Fernández-Aparicio M., Satovic Z., Emeran A.A., Torres A.M., Moreno M.T.,
15 Rubiales D. (2010) Identification of quantitative trait loci for specific mechanisms of resistance to *Orobanche crenata* Forsk. in pea (*Pisum sativum* L.). Mol. Breed. 25, 259-272.
- Fondevilla S., Küster H., Krajinski F., Cubero J.I., Rubiales D. (2011) Identification of genes differentially expressed in a resistant reaction to *Mycosphaerella pinodes* in pea using
20 microarray technology. BMC Genomics 12, 28 doi:10.1186/1471-2164-12-28.
- Foy C.L., Jain R., Jacobsohn R. (1989) Recent approaches for chemical control of broomrape (*Orobanche* spp.) - a review. Reviews of Weed Science 4, 123-152.
- García-Torres L., López-Granados F. (1991) Control of broomrape (*Orobanche crenata* Forks) in broad bean (*Vicia faba* L.) with imidazolinones and other herbicides. Weed
25 Res. 31, 227-235.

- Ghersa C.M., Martínez-Ghersa M.A. (2000) Ecological of weed seed size and persistence in the soil under different tilling systems: Implications for weed management. *Field Crops Res.* 67, 141-148.
- 5 Gil J., Martín L.M., Cubero J.I. (1987) Genetics of resistance in *Vicia sativa* L. to *Orobanche crenata* Forsk. *Plant Breeding* 99, 134-143.
- Goldwasser Y., Hershenhorn J., Plakhine D., Kleifeld Y., Rubin B. (1999) Biochemical factors involved in vetch resistance to *Orobanche aegyptiaca*. *Physiol. Molec. Plant Pathol.* 54, 87-96.
- 10 Goldwasser Y., Kleifeld Y., Plakhine D., Rubin B. (1997) Variation in vetch (*Vicia* spp.) response to *Orobanche aegyptiaca*. *Weed Sci.* 45, 756-762.
- Goldwasser Y., Sibony M., Rubin B. (2009) Screening of chickpea (*Cicer arietinum*) genotypes for Field Dodder (*Cuscuta campestris*) resistance, in: Rubiales D., Westwood J., Uludag A. (Eds.), *Proceedings of 10th World Congress of Parasitic Plants*, 8-12 June 2009, Kusadasi, Turkey, p. 126.
- 15 Gonsior G., Buschmann H., Szinicz G., Spring O., Sauerborn J. (2004) Induced resistance – an innovative approach to manage branched broomrape (*Orobanche ramosa*) in hemp and tobacco. *Weed Sci.* 52, 1050-1053.
- González-Andújar J.L., Martínez-Cob A., López-Granados F., García-Torres L. (2001) Spatial distribution and mapping of crenate broomrape infestations in continuous
20 broad bean cropping. *Weed Sci.* 49, 773-779.
- González-Melendi, P., Fernández-Pacheco R., Coronado M.J., Corredor E., Testillano P.S., Risueño M.C., Marquina C., Ibarra M.R., Rubiales D., Pérez-de-Luque A. (2008) Nanoparticles as smart treatment delivery systems in plants: assessment of different techniques of microscopy for their visualisation in plant tissues. *Ann. Bot.* 101, 187-
25 195.

- Grenz J.H., Manschadi A.M., Uygurc F.N., Sauerborn J. (2005) Effects of environment and sowing date on the competition between faba bean (*Vicia faba*) and the parasitic weed *Orobanche crenata*. Field Crops Res. 93, 300-313.
- Grenz J.H., Sauerborn J. (2007) Mechanisms limiting the geographical range of the parasitic weed *Orobanche crenata*. Agric. Ecosyst. Environ. 122, 275-281.
- Gressel J. (2009) Crops with target-site herbicide resistance for *Orobanche* and *Striga* control. Pest Manag. Sci. 65, 560-565.
- Gressel J., Hana A., Head G., Marasas W., Obilana B., Ochanda J., Souissi T., Tzotzos G. (2004) Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions. Crop Prot. 23, 661-689.
- Gurney A.L., Slate J., Press M.C., Scholes J.D. (2006) A novel form of resistance in rice to the angiosperm parasite *Striga hermonthica*. New Phytol. 169, 199-208.
- Haidar M.A., Sidhamed M.M. (2000) Soil solarization and chicken manure for the control of *Orobanche crenata* and other weeds in Lebanon. Crop Prot. 19, 169-173.
- Hanson B.D., Hill D.C. (2001) Effects of imazethapyr and pendimethalin on lentil (*Lens culinaris*), pea (*Pisum sativum*), and a subsequent winter wheat (*Triticum aestivum*) crop. Weed Technol. 15, 190-194.
- Jacobsohn R., Kelman Y. (1980) Effectiveness of glyphosate in broomrape (*Orobanche* spp.) control in four crops (broadbeans, peas, carrots, tomatoes). Weed Sci. 28, 692-699.
- Joel D.M. (2000) A new virulent race of *Orobanche crenata*. Haustorium 37.
- Joel D.M., Hershenhorn J., Eizenberg H., Aly R., Ejeta G., Rich P.J., Ransom J.K., Sauerborn J., Rubiales D. (2007) Biology and management of weedy root parasites. Horticultural Reviews 33, 267-349.
- Johnson A.W., Rosebery G., Parker C. (1976) A novel approach to *Striga* and *Orobanche* control using synthetic germination stimulants. Weed Res. 16, 223-227.

- Jurado-Expósito M., Castejón-Muñoz M., García-Torres L. (1986) Broomrape (*Orobancha crenata*) control with imazethapyr applied to pea (*Pisum sativum*) seed. Weed Technol. 10, 774-780.
- Jurado-Expósito M., García-Torres L., Castejón-Muñoz M. (1997) Broad bean and lentil seed
5 treatments with imidazolinones for the control of broomrape (*Orobancha crenata*). J. Agric. Sci. 129, 307-314.
- Jurado-Expósito M., López-Granados F., Atenciano S., García-Torres L., González-Andújar J.L. (2003) Discrimination of weed seedlings, wheat (*Triticum aestivum*) stubble and sunflower (*Helianthus annuus*) by near-infrared reflectance spectroscopy (NIRS).
10 Crop Prot. 22, 1177-1180.
- Khallida R., Beniwal S.P.S., Fatemi Z., Saxena M.C. (1993) Imazethapyr can control dodder (*Cuscuta* spp.) infestation in faba bean. FABIS Newsl. 33, 30-32.
- Kharrat M., Halila M.H., Linke K.H., Haddar T. (1992) First report of *Orobancha foetida* Poiret on faba bean in Tunisia. FABIS Newsl. 30, 46-47.
- 15 Klein O., Kroschel J. (2002) Biological control of *Orobancha* spp. with *Phytomyza orobanchia*, a review. BioControl 47, 245-277.
- Kroschel J., Jost A., Sauerborn J. (1999) Insects for *Striga* control: Possibilities and constraints, in: Kroschel J., Mercer-Quarshie H., Sauerborn J. (Eds.), Advances in parasitic weed control at on-farm level. Vol. I. Joint Action to Control *Striga* in Africa.
20 Margraf Verlag, Weikersheim, Germany, pp. 117-132.
- Kouakou C.K., Timko M.P., Akanvou1 R., Botanga R., Skizim N., Roy-Macauley H. (2009) AFLP/SSR mapping of resistance genes to *Alectra vogelii* in cowpea (*Vigna unguiculata* L. WALP). Sciences & Nature 6, 63-70.
- Kubo M., Ueda H., Park P., Kawaguchi M., Sugimoto Y. (2009) Reactions of *Lotus japonicus*
25 ecotypes and mutants to root parasitic plants. J. Plant Physiol. 166, 353-362.

- Kusumoto D., Goldwasser Y., Xie X., Yoneyama K., Takeuchi Y., Yoneyama K. (2007) Resistance of red clover (*Trifolium pratense*) to the root parasitic plant *Orobancha minor* is activated by salicylate but not by jasmonate. *Ann. Bot.* 100, 537-544.
- Lane J.A., Bailey J.A. (1992) Resistance of cowpea and cereals to the parasitic angiosperm *Striga*. *Euphytica* 63, 85-93.
- Lane J.A., Bailey J.A., Butler R.C., Terry P.J. (1993) Resistance of cowpea [*Vigna unguiculata* (L.) Walp.] to *Striga gesnerioides* (Willd) Vatke, a parasitic angiosperm. *New Phytol.* 125, 405-412.
- Lane J.A., Child D.V., Reiss G.C., Entcheva V., Bailey J.A. (1997) Crop resistance to parasitic plants, in: Crute I.R., Holub E.B., Burdon J.J. (Eds.), *The Gene-for-Gene Relationship in Plant-Parasite Interactions*, by CAB International, Wallingford, UK, pp. 81-97.
- Lenzemo V., Kuyper T.W., Vierheilig H. (2009) *Striga* seed-germination activity of root exudates and compounds present in stems of *Striga* host and nonhost (trap crop) plants is reduced due to root colonization by arbuscular mycorrhizal fungi. *Mycorrhiza* 19, 287-294.
- Li J., Lis K.E., Timko M.P. (2009) Molecular genetics of race-specific resistance of cowpea to *Striga gesnerioides* (Willd.). *Pest Manag. Sci.* 65, 520-527.
- Li J., Lis K.E., Timko M.P. (2009) Molecular genetics of race-specific resistance of cowpea to *Striga gesnerioides* (Willd.). *Pest Manag. Sci.* 65, 520-527.
- Li J., Timko M.P. (2009) Gene-for-Gene Resistance in *Striga*-Cowpea Associations. *Science* 325, 5944.
- Linke K.H., Scheibel Ch., Saxena M.C., Sauerborn J. (1992) Fungi occurring on *Orobancha* spp. and their preliminary evaluation for *Orobancha* control. *Trop. Pest Manag.* 38, 127-130.

- Lins R.D., Colquhoun J.B., Mallory-Smith C.A. (2006) Investigation of wheat as a trap crop for control of *Orobanche minor*. Weed Res. 46, 313-318.
- López-Bellido R.J., Benítez-Vega J, López-Bellido L. (2009) No-tillage improves broomrape control with glyphosate in faba-bean. Agron. J. 101, 1394-1399.
- 5 López-Granados F., García-Torres L. (1996) Effects of environmental factors on dormancy and germination of crenate broomrape (*Orobanche crenata*). Weed Sci. 44, 284-289.
- Lozano-Baena M.D., Prats E., Moreno M.T., Rubiales D., Pérez-de-Luque A. (2007) *Medicago truncatula* as a model host for legumes - parasitic plants interactions: Two phenotypes of resistance for one defensive mechanism. Plant Physiol. 145, 437-449.
- 10 Mabrouk Y., Zourgui L., Sifi B., Delavault P., Simier P., Belhadj O. (2007) Some compatible *Rhizobium leguminosarum* strains in peas decrease infections when parasitized by *Orobanche crenata*. Weed Res. 47, 44-53.
- Manschadi A.M., Sauerborn J., Stützel H. (2001) Quantitative aspects of *Orobanche crenata* infestation in faba beans as affected by abiotic factors and parasite soil seedbank.
- 15 Weed Res. 41, 311-324.
- Mauromicale G., Restuccia G., Marchese A. (2001) Soil solarization, a non-chemical technique for controlling *Orobanche crenata* and improving yield of faba bean. Agronomie 21, 757-765.
- Mesa-García J., García-Torres L. (1985) *Orobanche crenata* Forsk control in *Vicia faba* L. with glyphosate as affected by herbicide rates and parasite growth stages. Weed Res.
- 20 25, 129-134.
- Mishra J.S., Moorthy B.T.S., Manish Bhan, Yaduraju N.T. (2007) Relative tolerance of rainy season crops to field dodder (*Cuscuta campestris*) and its management in niger (*Guizotia abyssinica*). Crop Prot. 26, 625-629.

- Mohamed K.I., Musselman L.J., Riches C. (2001) The genus *Striga* (Scrophulariaceae) in Africa. Ann. Mo. Bot. Gard. 88, 60-103.
- Mohamed K.I., Papes M., Williams R., Benz B.W., Peterson A.T. (2006) Global invasive potential of 10 parasitic witchweeds and related Orobanchaceae. Ambio 35, 281-288.
- 5 Müller-Stöver D., Buschmann H., Sauerborn J. (2005) Increasing control reliability of *Orobanche cumana* through integration of a biocontrol agent with a resistance-inducing chemical. Eur. J. Plant Pathol. 111, 193-202.
- Müller-Stöver D., Kroschel J. (2005) The potential of *Ulocladium botrytis* for biological control of *Orobanche* spp. Biol. Control 33, 301-306.
- 10 Müller-Stöver D., Thomas H., Sauerborn J., Kroschel J. (2004) Two granular formulations of *Fusarium oxysporum* f. sp. *orthoceras* to mitigate sunflower broomrape (*Orobanche cumana*). BioControl 49, 595-602.
- Nassib A.M., Ibrahim A.A., Khalil S.A. (1982) Breeding for resistance to *Orobanche*, in: Hawtin, G., Webb, C. (Eds.), Faba Bean Improvement. Martinus Nijhoff Publishers, The Netherlands, pp. 199-206.
- 15 Orloff S.B., Vargas R.N., Cudney D.W., Canevari W.M., Schmierer J. (1989) Dodder control in alfalfa. Calif. Agric. 43, 30-31.
- Oswald A., Ransom J.K., Kroschel J., Sauerborn J. (2002) Intercropping controls *Striga* in maize based farming systems. Crop Prot. 21, 367-374.
- 20 Ouédraogo J.T., Maheshwari V., Berner D., St-Pierre C.A., Belzile F., Timko M.P. (2001) Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. Theor. Appl. Genet. 102, 1029-1036.
- Ouédraogo J.T., Tignegre J.B., Timko M.P., Belzile F.J. (2002) AFLP markers linked to resistance against *Striga gesnerioides* race 1 in cowpea (*Vigna unguiculata*). Genome
- 25 45, 787-793.

- Panetta F.D., Lawes R. (2005) Evaluation of weed eradication programs: the delimitation of extent. *Divers. Distrib.* 11, 435-442.
- Parker C. (2009) Observations on the current status of *Orobanche* and *Striga* problems worldwide. *Pest Manag. Sci.* 65, 453-459.
- 5 Parker C., Riches C.R. (1993) *Parasitic Weeds of the World: Biology and Control*. CAB Int., Wallingford, UK.
- Pérez-de-Luque A., Eizenberg H., Grenz J.H., Sillero J.C., Ávila C.M., Sauerborn J., Rubiales D. (2010) Broomrape management in faba bean. *Field Crops Res.* 115, 319-328.
- Pérez-de-Luque A., Fondevilla S., Pérez-Vich B., Aly R., Thoirion S., Simier P., Castillejo
10 M.A., Fernández J.M., Jorrín J., Rubiales D., Delavault P. (2009) Understanding broomrape – host plant interaction and developing resistance. *Weed Res.* 49, 8-22.
- Pérez-de-Luque A., González-Verdejo C.I., Lozano M.D., Dita M.A., Cubero J.I., González-Melendi P., Risueño M.C., Rubiales D. (2006a) Protein cross-linking, peroxidase and β -1,3-endoglucanase involved in resistance of pea against *Orobanche crenata*. *J. Exp.*
15 *Bot.* 57, 1461-1469.
- Pérez-de-Luque A., Jorrín J., Cubero J.I., Rubiales D. (2005a) *Orobanche crenata* resistance and avoidance in pea (*Pisum* spp.) operates at different developmental stages of the parasite. *Weed Res.* 45, 379-387.
- Pérez-de-Luque A., Jorrín J.V., Rubiales D. (2004a) Broomrape (*Orobanche crenata* Forks.)
20 control in pea (*Pisum sativum* L.) by foliar application of Benzothiadiazole (BTH). *Phytoparasitica* 32, 21-29.
- Pérez-de-Luque A., Lozano M.D., Cubero J.I., González-Melendi P., Risueño M.C., Rubiales D. (2006b) Mucilage production during the incompatible interaction between *Orobanche crenata* and *Vicia sativa*. *J. Exp. Bot.* 57, 931-942.

- Pérez-de-Luque A., Lozano M.D., Moreno M.T., Testillano P.S., Rubiales D. (2007) Resistance to broomrape (*Orobanche crenata*) in faba bean (*Vicia faba*): cell wall changes associated with pre-haustorial defensive mechanisms. *Ann. Appl. Biol.* 151, 89-98.
- 5 Pérez-de-Luque A., Lozano M.D., Rubiales D. (2006c) Resistencia post-haustorial a *Orobanche crenata* en garbanzo, in: De los Mozos-Pascual M., Giménez-Alvear M.J., Rodríguez-Conde M.F., Sánchez-Vioque R. (Eds.), Nuevos retos y oportunidades de las leguminosas en el sector agroalimentario español, Consejería de Agricultura de Castilla-La Mancha, Toledo, Spain, pp. 361-368.
- 10 Pérez-de-Luque A., Moreno M.T., Rubiales D. (2008) Host plant resistance against broomrapes (*Orobanche* spp.): defence reactions and mechanisms of resistance. *Ann. Appl. Biol.* 152, 131-141.
- Pérez-de-Luque A., Rubiales D. (2009) Nanotechnology for parasitic plant control. *Pest Manag. Sci.* 65, 540-545.
- 15 Pérez-de-Luque A., Rubiales D., Cubero J.I., Press M.C., Scholes J., Yoneyama K., Takeuchi Y., Plakhine D., Joel D.M. (2005b) Interaction between *Orobanche crenata* and its host legumes: unsuccessful haustorial penetration and necrosis of the developing parasite. *Ann. Bot.* 95, 935-942.
- Pérez-de-Luque A., Sillero J.C., Cubero J.I., Rubiales D. (2004b) Effect of sowing date and
20 host resistance on the establishment and development of *Orobanche crenata* on faba bean and common vetch. *Weed Res.* 44, 282-288
- Pérez-de-Luque A., Sillero J.C., Moral A., Rubiales D. (2004c) Induction of systemic resistance in pea and faba bean to crenate broomrape (*Orobanche crenata*) by

exogenous application of benzothiadiazole, in: Use of Natural Compound for Parasitic Plant Management, COST 849 meeting, 29-30 October 2004, Naples, Italy, p. 12.

Polniaszek T.I., Parker C., Riches C.R. (1991) Variation in virulence of *Alectra vogelii* populations on cowpea. Trop. Pest Manag. 37, 152-154.

- 5 Prins M., Laimer M., Noris E., Schubert J., Wassenegger M., Tepfer M. (2008) Strategies for antiviral resistance in transgenic plants. Mol. Plant Pathol. 9, 73-83.

Riches C.R. (1987) The identification of resistance to *Alectra vogelii* Benth. (Scrophulariaceae) in cowpea, in: Weber H.Chr., Forstreuter W. (Eds.), Parasitic Flowering Plants, Phillips University, Marburg, Germany, pp. 701-708.

- 10 Riches C.R. (1989) The biology and control of *Alectra vogelii* Benth. (Scrophulariaceae) in Botswana. Ph.D. Thesis, University of Reading, Reading, U.K.

Riches C.R., Hamilton K., Parker C. (1992) Parasitism of grain legumes by *Alectra species* (Scrophulariaceae). Ann. Appl. Biol. 121, 361-370.

- Rispail N., Dita M.A., González-Verdejo C., Pérez-de-Luque A., Castillejo M.A., Prats E.,
15 Román B., Jorrín J., Rubiales D. (2007) Plant Resistance to Parasitic Plants: Current Approaches for an old foe. New Phytol. 173, 703-712.

- Rispail, N., Kaló P., Kiss G.B., Ellis T.H.N., Gallardo K., Thompson R.D., Prats E.,
Larrainzar E., Ladrera R., González E.M., Arrese-Igor C., Ferguson B.J., Gresshoff
P.M., Rubiales D. (2010) Model legumes contribute to faba bean breeding. Field Crops
20 Res. 115, 253-269.

Rodríguez-Conde M.F., Moreno M.T., Cubero J.I., Rubiales D. (2004) Characterization of the *Orobanche - Medicago truncatula* association for studying early stages of the parasite-host interaction. Weed Res. 44, 218-223.

- Román M.B., Rubiales D., Cubero J.I., Torres A.M., Zatovic S. (2001) Genetic diversity in *Orobanche crenata* populations from southern Spain. Theor. Appl. Genet. 103, 1108-1114.
- Román B., Satovic Z., Alfaro C., Moreno M.T., Kharrat M., Pérez-de-Luque A., Rubiales D. (2007). Host differentiation in *Orobanche foetida* Poir. Flora 202, 201-208.
- Román B., Satovic Z., Rubiales D., Torres A.M., Cubero J.I., Katzir N., Joel D.M. (2002a) Variation among and within populations of the parasitic weed *Orobanche crenata* from two sides of the Mediterranean revealed by ISSR markers. Phytopath. 92, 1262-1266.
- Román B., Torres A.M., Rubiales D., Cubero J.I., Satovic Z. (2002b) Mapping of quantitative trait loci controlling broomrape (*Orobanche crenata* Forsk.) resistance in faba bean (*Vicia faba* L.). Genome 45, 1057-1063.
- Rubiales D. (1999) Eating broomrape?, in: Cubero J.I., Moreno M.T., Rubiales D., Sillero J.C. (Eds.), Resistance to *Orobanche*: the state of the art, Publ. Junta Andalucía, Sevilla, Spain, pp. 195-199
- Rubiales D. (2003) Parasitic plants, wild relatives and the nature of resistance. New Phytol. 160, 459-461.
- Rubiales D., Alcántara C., Joel D.M., Pérez-de-Luque A., Sillero J.C. (2003a) Characterization of resistance in chickpea to broomrape (*Orobanche crenata*). Weed Sci. 51, 702-707.
- Rubiales D., Alcántara C., Pérez-de-Luque A., Gil J., Sillero J.C. (2003b) Infection of chickpea (*Cicer arietinum*) by crenate broomrape (*Orobanche crenata*) as influenced by sowing date and weather conditions. Agronomie 23, 359-362.
- Rubiales D., Alcántara C., Sillero J.C. (2004) Variation in resistance to crenate broomrape (*Orobanche crenata*) in species of *Cicer*. Weed Res. 44, 27-32.

- Rubiales D., Fernández-Aparicio M., Pérez-de-Luque A., Prats E., Castillejo M.A., Sillero J.C., Rispaill N., Fondevilla S. (2009a) Breeding approaches for crenate broomrape (*Orobancha crenata* Forsk.) management in pea (*Pisum sativum* L.). Pest Manag. Sci. 65, 553-559.
- 5 Rubiales D., Fernández-Aparicio M., Wegmann K., Joel D. (2009b) Revisiting strategies for reducing the seedbank of *Orobancha* and *Phelipanche* spp. Weed Res. 49, 23-33.
- Rubiales, D., Heide-Jørgensen, H.S. (2011) Parasitic Plants. In: Enciclopedia of Life Sciences (ELS), John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0021271.
- Rubiales D., Moreno M.T., Sillero J.C. (2005a). Search for resistance to crenate broomrape
10 (*Orobancha crenata*) in pea germplasm. Gen. Resour. Crop Evol. 52, 853-861.
- Rubiales D., Pérez-de-Luque A., Cubero J.I., Sillero J.C. (2003c) Crenate broomrape (*Orobancha crenata*) infection in field pea cultivars. Crop Prot. 22, 865-872
- Rubiales D., Pérez-de-Luque A., Sillero J.C., Román B., Kharrat M., Khalil S., Joel D.M., Riches C.R. (2006) Screening techniques and sources of resistance against parasitic
15 weeds in grain legumes. Euphytica 147, 187-199.
- Rubiales D., Pujadas A., Román B. (2001) Occurrence and distribution of *Phytomyza orobanchia* feeding on broomrapes (*Orobancha* spp.) in southern Spain, in: 7th International Parasitic Plant Symposium, Nantes, France, pp. 254-257.
- Rubiales D., Sadiki M., Román B. (2005b) First Report of *Orobancha foetida* on Common
20 Vetch (*Vicia sativa*) in Morocco. Plant Dis. 89, 528.
- Rubin B. (1994). Group B/2 resistant field dodder (*Cuscuta campestris*).
<http://www.weedscience.org/Case/Case.asp?ResistID=104>. Visited 31 May 2010.
- Shabana Y.M., Müller-Stöver D., Sauerborn J. (2003) Granular Pesta formulation of *Fusarium oxysporum* f. sp. *orthoceras* for biological control of sunflower broomrape:
25 efficacy and shelf-life. Biol. Control 26, 189-201.

- Sauerborn J., Buschmann H., Ghiasvand Ghiasi K., Kogel K.-H. (2002) Benzothiadiazole activates resistance in sunflower (*Helianthus annuus*) to the root-parasitic weed *Orobanche cumana*. *Phytopathol.* 92, 59-64.
- Sauerborn J., Linke K.H., Saxena M.C., Koch W. (1989a) Solarization, a physical control method for weeds and parasitic plants (*Orobanche* spp.) in Mediterranean agriculture. *Weed Res.* 29, 391-393.
- Sauerborn J., Müller-Stöver D., Hershenhorn J. (2007) The role of biological control in managing parasitic weeds. *Crop Prot.* 26, 246-254.
- Sauerborn J., Saxena M.C. (1986) A review on agronomy in relation to *Orobanche* control in faba bean (*Vicia faba* L.), in: Borg S.J.ter (Ed.) Proceedings of a workshop on biology and control of *Orobanche*, LH/VpO, Wageningen, The Netherlands, pp. 160-165.
- Sauerborn J., Saxena M.C., Meyer A. (1989b) Broomrape control in faba bean (*Vicia faba* L.) with glyphosate and imazaquin. *Weed Res.* 29, 97-102.
- Schloss J.V. (1995) Recent advances in understanding the mechanism and inhibition of acetolactate synthase, in: Setter J. (Ed.), *Herbicides Inhibiting Branch Chain Amino Acid Biosynthesis*. Springer Verlag, New York, pp. 4-11.
- Schnell H., Kunisch M., Saxena M.C., Sauerborn J. (1996) Simulation of the Seed Bank Dynamics of *Orobanche crenata* Forsk. in some Crop Rotations Common in Northern Syria. *Expl. Agric.* 32, 395-403.
- Sillero J.C., del Moral J., Cubero J.I., Rubiales D. (2001) Búsqueda de tolerancia al glifosato en guisante como estrategia de control del jopo (*Orobanche crenata*), in: De Prado R., Jorrín J.V. (Eds.), *Uso de Herbicidas en la agricultura del siglo XXI*, Publ. Servicio Publicaciones Universidad de Córdoba, Spain, pp. 677-684.
- Sillero J.C., Moreno M.T., Rubiales D. (2005) Sources of resistance to crenate broomrape in *Vicia* species. *Plant Dis.* 89, 22-27.

- Singh B.B. (2002) Breeding cowpea varieties for resistance to *Striga gesnerioides* and *Alectra vogelii*. in: Fatokun C.A., Tarawali S.A., Singh B.B., Kormawa P.M., Tamo M. (Eds.), Challenges and Opportunities for Enhancing Sustainable Cowpea Production, IITA Press, Ibadan, Nigeria, pp. 154-163.
- 5 Tan S., Evans R.R., Dahmer M.L., Singh B.K., Shaner D. (2004), Imidazolinone tolerant crops: history, current status and future. Pest Manag. Sci. 61, 246-257.
- Thorogood C.J., Rumsey F.J., Hiscock S.J. (2009) Host-specific races in the holoparasitic angiosperm *Orobanche minor*: implications for speciation in parasitic plants. Ann. Bot. 103, 1005-1014.
- 10 Timko M.P., Gowda B.S., Ouedraogo J., Ousmane B. (2007) Molecular markers for analysis of resistance to *Striga gesnerioides* in cowpea, in: Ejeta G., Gressel J. (Eds.), Integrating new technologies for *Striga* control: Towards ending the witch-hunt., World Scientific Publishing Co. Pte Ltd, Singapore pp. 115-128.
- Touré M., Olivier A., Ntare B.R., Lane J.A., St-Pierre C.A. (1997) Inheritance of resistance to
15 *Striga gesnerioides* biotypes from Mali and Niger in cowpea (*Vigna unguiculata* (L.) Walp.). Euphytica 94, 273-278.
- Touré, M., Olivier, A., Ntare, B.R., St-Pierre, C.A., 1996. The influence of sowing date and irrigation of cowpea on *Striga gesnerioides* emergence, in: Moreno, M.T., Cubero, J.I., Berner, D., Joel, D., Musselman, L.J., Parker, C. (Eds.), Advances in parasitic plant
20 research. Proceedings of the Sixth International Symposium on Parasitic Weeds. Dirección General de Investigación Agraria, Córdoba, Spain, pp. 451-455.
- Van Delft G. J., Graves J. D., Fitter A. H., Van Ast A., 2000. *Striga* seed avoidance by deep planting and no-tillage in sorghum and maize. Int. J. Pest Manag. 46, 251-256.
- Van Hezewijk M.J., Verkleij J.A.C. (1996) The effect of nitrogenous compounds on in vitro
25 germination of *Orobanche crenata* Forsk. Weed Res. 36, 395-404.

- Valderrama M.R., Román B., Satovic Z., Rubiales D., Cubero J.I., Torres A.M. (2004) Locating quantitative trait loci associated with *Orobancha crenata* resistance in pea. *Weed Res.* 44, 323-328.
- 5 Vurro M., Boari A., Evidente A., Andolfi A., Zermane N. (2009) Natural metabolites for parasitic weed management. *Pest Manag. Sci.* 65, 566-571.
- Westwood J.H. (2000) Characterization of the *Orobancha-Arabidopsis* system for studying parasite-host interactions. *Weed Sci.* 48, 742-748.
- Westwood J.H., Foy C.L. (1999) Influence of nitrogen on germination and early development of broomrape (*Orobancha* spp.). *Weed Sci.* 47, 2-7.
- 10 Whitney P.J., Carsten C. (1981) Chemotropic response of broomrape radicles to host root exudates. *Ann. Bot.* 48, 919-921.
- Xie X., Yoneyama K., Harada Y., Fusegi N., Yamada Y., Ito S., Yokota T., Takeuchi Y., Yoneyama K. (2009) Fabacyl acetate, a germination stimulant for root parasitic plants from *Pisum sativum*. *Phytochem.* 70, 211-215.
- 15 Yoder J.I., Gunathilake P., Wu B., Tomilova N., Tomilov A.A. (2009) Engineering host resistance against parasitic weeds with RNA interference. *Pest Manag. Sci.* 65, 460-466.
- Yoneyama K., Xie X., Sekimoto H., Takeuchi Y., Ogasawara S., Akiyama K., Hayashi H., Yoneyama K. (2008) Strigolactones, host recognition signals for root parasitic plants and arbuscular mycorrhizal fungi, from Fabaceae plants. *New Phytol.* 179, 484-494.
- 20 Yoneyama K., Xie X., Yoneyama K., Takeuchi Y. (2009) Strigolactones: structures and biological activities. *Pest Manag. Sci.* 65, 467-470.

Young N.D., Udvardi M. (2009) Translating *Medicago truncatula* genomics to crop legumes. Curr. Opin. Plant Biol. 12, 193-201.

Zehar N., Ingouff M., Bouya D., Fer A. (2002) Possible involvement of gibberellins and ethylene in *Orobancha ramosa* germination. Weed Res. 42, 464-469.

- 5 Zermane N., Souissi T., Kroschel J., Sikora R. (2007) Biocontrol of broomrape (*Orobancha crenata* Forsk. and *Orobancha foetida* Poir.) by *Pseudomonas fluorescens* isolate Bf7-9 from the faba bean rhizosphere. Biocontrol Sci. Techn. 17, 483-497.

Zwanenburg B., Thuring J.W.J.F. (1997) Synthesis of strigolactones and analogs: A molecular approach to the witchweed problem. Pure Appl. Chem. 69, 651-654.

Fig.1: Life cycle of parasitic weeds: on the left side dodder seeds germinating, seedlings attaching to close-by plants, climbing and growing and infecting neighbouring plants, flowering and setting seeds that replete the soil seed bank and repeat the process; on the right side, seeds of a weedy root parasite (either *Orobanche*, *Striga* or *Alectra*) germinating, attaching to the roots of host plants, emerging over the soil surface, flowering and setting seeds that replete the soil seed bank and repeat the process.

Fig. 2: Infection process of *O. crenata* on pea: A) germinating seedling contacting pea root; B) haustorium formation resulting in tubercles; C) bud formation; D) shoots starting to emerge; E) flowering plants.

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Fig. 3: Chickpea crop infected by *Orobanche crenata* (A); by *Cuscuta campestris* (B)

Fig 4: Pea:oat intercrop reducing *Orobancha crenata* infection in pea (A); detail of well developed *O. crenata* seedlings when stimulated by pea root exudates (B); restricted *O. crenata* seedling elongation by addition of L-Tryptophan (C).

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Fig 5: Effects of earliness on *Orobancha crenata* infection: (A) very early *Lathyrus* accession filling pods and escaping from *O. crenata* infection; (B) late accession heavily infected.

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Fig 6: Successful *Orobanche crenata* infection on pea in mini-rhizotrons (A) versus failed *Phelipanche aegyptiaca* attempts (B): seeds germinated, but failed to infect due to a combination of wrong orientation of radicles and arrested root penetration.

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Fig. 7: Successful (A) *versus* failed (B) penetration of *Phelipanche aegyptiaca* in roots of two different vetch genotypes (Mezquita vs Popany). Pictures taken 11 days after parasitic radicle contacted with the host root.

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