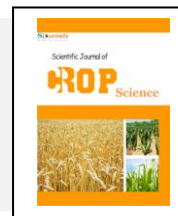


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ROP ScienceJournal homepage: www.Sjournals.com**Review article****Management of orobanche in field crops- a review****S. Habimana^{a*}, K.N.K. Murthy^a, V. Hatti^a, A. Nduwumuremyi^b**^aDepartment of Agronomy, university of agricultural sciences, GVKK- Bengaluru India.^bRwanda Agricultural Board, RAB-Rwanda.

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ARTICLE INFO

ABSTRACT

Article history,

Received 03 November 2013

Accepted 25 November 2013

Available online 29 November 2013

Keywords,

Orobanche

Broomrape

Preventive

Physical

Chemical

Agronomic

Biological

Biotechnological

Integrated control methods

Broomrape (*Orobanche* sp) is a root holoparasitic plant devoid of chlorophyll and is entirely depending on the host for its nutritional requirements. It is causing considerable yield losses (5-100 %) in the crops, especially in the drier and warmer areas of Europe, Africa and Asia where it is reported to mainly parasitize species of leguminous, oilseeds, solanaceous, cruciferous and medicinal plants. It is a serious root parasite threatening the livelihood of the farmers with its devastating effect on the some of aforementioned crops. The long-term impact of the broomrapes is even more serious, their seeds may easily spread to other fields, and can persist in soil up to 20 years, leading to an accelerated increase in the infested areas in which susceptible crops are under danger. Orobanche seed dispersal is facilitated by man, agricultural tools, crop seeds, propagules and also by animals through their excreta.

This review will discuss and summarize alternative methods viz preventive measures, physical, chemical, agronomic, biological, biotechnological and integrated methods which are needed to manage this parasitic weed. However, the main concern is that, up to date, no single cheap method of control proved to be effective, economical and complete in protection against this parasite. For that reason, an integrated approach is needed in which a variety of such techniques are combined, in order to maintain parasite populations below threshold levels of damage.

1. Introduction

Broomrapes are holoparasitic and only germinate in response to specific chemicals released by the host plant. Following germination, the seedlings attach to the host roots by the production of specialized feeding structures, described as haustoria that form a functional bridge into their hosts. Haustoria penetrate the host tissues until they reach the vascular system for uptake of water, nutrients, assimilates, and grow at the expense of the host plant's resources (Smith et al., 2001 and Joel et al., 2007). Because the infection and pathogenesis processes take place underground, damage to the crop occurs prior to the emergence of the parasite and diagnosis of infection. The particular characteristics (underground development, attachment to the host roots) of this pathogenic weed hamper the development of effective control strategies. In addition, a single broomrape plant can release more than 500,000 seeds, which are known to remain viable for decades in the soil. This provides the parasite with a great genetic adaptability to environmental changes, including host resistance, agronomical practices and herbicide treatments (Joel et al., 2007).

Due to this, the available methods of control against broomrapes have not proven as effective, economical and applicable as predicted (Alejandro et al., 2010, Joel, 2000b; Goldwasser and Kleifeld, 2004). Although several potential control measures were developed over the past few decades for some crops, any approach applied alone is often only partially effective and the results are sometimes inconsistent due to variable environmental conditions. Therefore, the only effective way to combat weedy root parasite like *Orobanche* to date is through an integrated approach, combining a variety of measures in a concerted manner.

2. Preventive measures

The strength of broomrape lies in its ability to form a bank of seeds in the soil. A management or eradication program must aim at reducing this seed bank, while minimising the production of new seeds and their dispersal to new sites. Quarantine is therefore an essential element in control or eradication programs.

The best option for winning against broomrapes is avoiding the fight. It is not possible when the fields are already infested with the seeds, but preventive measures must be taken into consideration to avoid spreading the infestation into neighbouring fields.

The main ways for broomrape seeds dispersal are through machinery and tools, and together with the host seeds; proper phytosanitary measures in and around the field are necessary to reduce the spread of *Orobanche*. Farm equipment and machinery should be cleaned prior to their use in uninfested fields. Special care must be applied to disinfection and cleaning of field machinery and harvesters, and avoid trucks going from infested to non-infested fields. Containment is a must to avoid spreading of the infestation and eradication programs should be considered. *Orobanche* shoots should be removed prior to flower opening. The collected shoots should be burnt or disposed off properly. Good extension agents could easily convince the farmers to execute such task, especially when they made aware of the tremendous production of *Orobanche* seeds per plant. One important spreading agent of various weeds, including *Orobanche*, is the uncontrolled movement of grazing animals. Grazing animals should be forbidden to enter un-infested fields after grazing infested areas (Panetta and Roger, 2005). Furthermore, farmers should use certified seed in order to insure themselves it is clean of parasite seeds.

Sanitation regarding host crop seed import and export should be implemented, mainly involving countries severely affected by broomrape infestations. Because of the tiny size of the broomrape seed, their presence is difficult to detect prior to infection of the crop. However, contamination of field soil and crop seed lots can be revealed by PCR-assays developed for diagnostic and identification of *Orobanche* species based on specific primers for unique sequences (Joel et al., 1996, 1998; Portnoy et al., 1997).

Fresh contaminated manure aggravates *Orobanche* problem. Farmers should be instructed to use fermented manure, as fermentation process kills the seeds of the parasite. Pre-plant composting fresh manure under plastic mulch in the planting rows causes *Orobanche* seeds to lose viability within six weeks, and reduces *Orobanche ramosa* infestation on many vegetables. This practice could be a useful asset in high value crops. Fermenting

manure in the farm can be easily practiced by subsistent farmer without much input and can aid sustainable farming strategy (FAO, 2008).

Strict quarantine measures, at various levels, national and international, help in preventing the introduction of the parasite into parasite-free areas. Technical inspection of imported agricultural materials should be carried out by a subject matter specialist in parasitic weeds (FAO, 2008).

3. Physical methods

Soil solarisation is the heating of soil by sunlight trapped under a mulch of black, or more usually clear, polyethylene film. The temperatures of 48-57°C kill *Orobanche* seeds that are in the imbibed state; therefore soil must be wet at the time of treatment. Seeds of *O. ramosa* can survive 35 days at 50°C in dry air, but are quickly killed by temperatures of 40°C when wet. The temperature increase achieved is primarily in the result of the elimination of evaporation, but is also partially because of the greenhouse effect created. This technique has been used successfully on cropping land in many countries around the world like Middle East with an endemic *Orobanche* problem, as a pre-planting treatment for tomato, carrot, eggplant, faba beans and lentils. Soil solarization has been proven to be the most effective methods in controlling broomrape in open crops fields (Haidar and Sidahmad, 2000 and Mauromicale et al., 2001).

In southern Italy and other Mediterranean countries, an attractive alternative method is soil solarization (Mauromicale et al., 2001, 2005), alone or in combination with other methods. This approach has attracted the interest in many warm-climate countries because of its effectiveness, simplicity, and safety for humans, plants, and the environment (DeVay and Stapleton, 1997). Solarisation entails covering wet soil with transparent polyethylene sheets during the hot season.

There are advantages to using solarization, specifically, it is a simple, non-chemical, non-hazardous method that avoids the use of any toxic materials, does not contaminate the site, and is, therefore, suited to organic farming or other low-input agricultural systems. As global environmental quality considerations grow in importance, along with an increasing human population, evolving concepts, such as soil solarization and other uses of solar energy in agriculture, will become more important (Stapleton, 2000 and Ashrafi et al., 2009).

Ashrafi et al., 2009 observed that solarization was accomplished by the application of clear polyethylene sheets to moist soil for 63 days during the hot season. The treatment increased maximum soil temperature by around 15°C, and at 5 cm below the soil surface, a temperature of more than 45 °C was reached for 34 to 60 days whereas this temperature was not reached at all in the first season and not for 20 days (second season) in unmulched soil. In solarized soil, no broomrape shoots emerged, and neither haustoria's nor underground tubercles of the parasite were found on cucumber roots. The treatment killed about 95 per cent of buried viable seed, and induced secondary dormancy in the remaining per cent. In non-solarized plots, broomrape shoots were still present at a high density, decreasing the plant growth and fruit production. Fruit yield was 33 to 88 per cent higher in the solarized as compared with the non-solarized treatment.

Solarisation is a technique of control, not eradication. Solarisation may be more effective if combined with added nitrogen fertilisers; this can dramatically improve the kill of *Orobanche* seed at greater depths. Therefore, combination of solarization (2-6 weeks) and chicken manure at all depths (0, 5, 10 cm) is an effective weed management to control *Orobanche* and suppress the infestation, and growth of other weeds in subsequent planting of cabbage. Chicken manure, however, significantly increased the yield of cabbage (Haidar and Sidahmed, 2000).

4. Chemical methods

Chemical control of broomrape has been extensively explored since the 1970s. However, this form of control is complicated by a number of factors including, (i) it is effective only as a prophylactic treatment, since in most cases we do not know the infestation level; (ii) the parasite is directly connected to the host; (iii) if the herbicide is to be applied to the parasite through the conductive tissues of its host, the host must be selective to the herbicide without reducing its phytotoxicity; (iv) the parasite can often continuously germinate throughout the season, developing new infections (Alejandro et al., 2010).

In heavy infested broomrape fields, application of sulfosulfuron at 75 g a.i. ha⁻¹ was effective in preventing the development of broomrape and reducing the seed inoculum potential in the soil by registering significantly

lowest broomrape number, spike height, spike dry weight with higher broomrape control efficiency, which also accounted for higher tomato plant height, number of branches, leaf area plant⁻¹ at harvest, higher fruit weight plant⁻¹ and fruit yield of tomato (Dinesha et al., 2012).

The herbicides that are currently in use for broomrape control are glyphosate, and herbicides belonging to the imidazolinones (Aly et al., 2001;

Eizenberg et al., 2006a) or sulfonylureas (Eizenberg et al., 2004a). Glyphosate disrupts the biosynthesis of aromatic amino acids inhibiting the key enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP). Imidazolinones and sulfonylurea herbicides inhibit acetolactate synthase (ALS), also called acetohydroxyacid synthase (AHAS), a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine and valine. 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).

Regarding field crops, three different methods of herbicide application can be considered, foliar application, soil application and seed treatments.

4.1. With foliar applications

broomrape underground development stages should be monitored because control is ineffective if the attachments are too large. On the other hand, if the herbicide is applied too early, not enough attachments will be controlled. Foliar herbicide application for broomrape control normally requires lower herbicide rates. Sequential foliar application of low rates of glyphosate could be effective for broomrape control only on a few hosts in the families Apiaceae, Fabaceae and Brassicaceae. Imazapic applied to foliage for broomrape control is effective in sunflower (*Helianthus annuus*) (Aly et al., 2001), carrot (*Daucus carota*) (Jacobsohn et al., 2001), parsley (*Petroselinum crispum*) (Goldwasser et al., 2003), faba bean, celery (*Apium graveolens*) and vetch (*Vicia* spp.). Both pre and post-crop emergence treatments with imazethapyr have effectively protected peas against broomrape damage, with consequent increases in yield (Rubiales et al., 2003). In some cases, herbicides control the parasite but are only moderately selective to the host, e.g. imazapic did not inhibit the vegetative growth of sunflower, but high rates of application of the herbicide in the initial sunflower inflorescences stage injured the reproductive production of sunflower (Aly et al., 2001).

4.2. Soil applications

of sulfonylurea herbicides effectively control broomrape in tomato (*Solanum lycopersicum*) and in potato (Eizenberg et al., 2001, 2004a, 2006b; Goldwasser et al., 2001), by killing pre-conditioned seeds or young attachments.

4.3. Seed treatments

with imidazolinones have proven to be effective for controlling *O. crenata* in faba bean (Jurado-Exposito et al., 1997). The great advantage of this method is the low cost of application. The herbicide is incorporated as a coating on the seeds and distributed with them at the time of planting. Using seed coating required high specificity between host and parasite, therefore it is not wide-spread. This replaces a pre-emergence treatment and saves mechanical application costs. In addition, the application of imidazolinones reduces the herbicide rate required by two to three folds, hence being less harmful to the environment. However, under favourable environmental conditions for broomrape attack, the treatment must be supplemented to obtain high broomrape control.

Coating sunflower seed with 2 kg a.i ha⁻¹ and soaking the seeds in 50 per cent a.i of pronamide has lowered broomrape shoot dry weight and increased the yield of sunflower from 2141(control) to 2849 kg ha⁻¹ in coated seed and from 1240 (control) to 1795 kg ha⁻¹ in soaked seeds (Jorge et al.,2003).

The sulfonylureas also have the advantage of selectivity for preventing emergence of broomrape growing on broad leaved weeds in a non-host cereal crop, 3 g ha⁻¹ metsulfuron-methyl, 15 g ha⁻¹ chlorsulfuron or 22.5 g ha⁻¹ triasulfuron gave 100 per cent control of *O. ramosa* without damage to wheat or barley crops (Matthews, 2002). This may be due both to their direct effect on *Orobancha* and to their reduction of broad leaved weed hosts.

Goldwasser et al., 2000 observed that, in greenhouse and field experiments, *O. aegyptiaca* and *O. ramosa* were controlled in potato-infested soils by split foliar applications of low rates of the herbicides imazapic and rimsulfuron. Three doses of imazapic at 4.5 g ha⁻¹ each, sprayed 2 weeks after crop emergence and re-applied at 2 weeks intervals, prevented *Orobancha* infestation.

Seed dressing with herbicides using ALS inhibitor slow release formulations of fertilizers, pesticides, and drugs are common. The principle of this technique is the development of biodegradable formulations for seed dressing with small amounts of herbicide for broomrape control. The slow-release herbicide formulations will achieve longer control of *Orobanche* with the ALS inhibitor imazapyr. The seed dressing allows imazapyr to spread throughout the crop root zone as the roots grow, prevents imazapyr from leaching away from the host rhizosphere, and requires less herbicide (Kanampiu et al., 2002).

Chemical control based on growing-degree-days (GDD); after establishment of the parasitic weed on the host roots, degeneration and death of the parasite is the main factor that determines the host resistance. In sunflower, higher temperature was correlated with degeneration and death of more *Orobanche* tubercles increasing resistance in some varieties (Eizenberg et al., 2003a).

A field study confirmed that GDD could be a predictive parameter for *O. minor* parasitism. Parasitism of *O. minor* in red clover could be predicted by growing-degree-days (GDD) under controlled conditions (Eizenberg et al. 2004). This model was validated under field conditions (Eizenberg et al. 2005). Therefore, a predictive model may be a base for developing a decision-support system for chemical control (suitable timing for precise chemical control) of the parasite (Eizenberg et al. 2003a,b).

In investigation carried out in Palestine, three herbicides; chlorsulfuron, triasulfuron and imazaquin were tested to evaluate their efficiency in controlling the tomato broomrape. The herbicides significantly reduced the broomrape parasitizing tomato plants growing in pots, irrigated open field and under greenhouse conditions as foliar spray at the concentrations (0.5-10 $\mu\text{g}\cdot\text{ml}^{-1}$) without visible injury effect on the plants. The foliar application of herbicides were able to increase the broomrape dead spikes attached to the tomato plants at the concentrations (3-5 $\mu\text{g}\cdot\text{ml}^{-1}$) without visible negative effect on tomato plants (Ibrahim et al., 2012).

In some situations, low rates of herbicide can control broomrapes when applied to the host crop. This has the attraction of allowing cropping on infested land, but rates and timing must be precise to prevent broomrape emergence. There is also the risk of developing resistance when low rates of the same herbicide are applied to a weed population in successive years.

5. Agronomic methods

5.1. Sowing date

Germination of *Orobanche crenata* tends to be very much reduced below 8°C and further development is greatly reduced at low temperatures. Delaying the planting date affects *Orobanche* more than its hosts; the delay should be two weeks only from the date optimal for sowing in an uninfested field. However, this method must be adapted for different regions and for different hosts. Earlier planting dates are beneficial in certain instances. The change of the sowing date seems not to be very promising due to uncertainty of the environmental conditions, specifically temperature and rainfall situation. Farmer's negative perception to this method is controlled by the inherent traditional soil preparation which is largely linked to the onset of rain, and to the market demand for the produce.

Abundant experimental evidence in faba bean shows that shifting sowing from October to November, December or January reduces numbers and dry weight of attached and emerged broomrapes, both *O. crenata* and *O. foetida* (Manschadi et al., 2001; Perez-de-Luque et al., 2004b; Grenz et al., 2005a). Two factors are known to reduce parasite damage in late-sown crops, (i) decreased *O. crenata* germination due to suboptimal soil temperatures and (ii) obstructed *O. crenata* development during underground stages. Since faba bean development is less susceptible to low temperatures and can be accelerated by increasing day-length, pods enter the critical phase of rapid biomass accumulation relatively earlier than parasites. As a result, more parasites and lesser pods are aborted (Manschadi et al., 2001; Grenz et al., 2005a), Perez-de-Luque et al. (2004b) observed a more pronounced effect of late sowing in dry years, which also may indicate the existence of soil moisture-driven effects.

5.2. Soil management

Management of the soil can strongly affect the seed bank, including broomrape seeds. Trench ploughing 45-50 cm deep with a mouldboard plough reduced *O. ramosa* by 80-90 per cent in tobacco fields of Eastern Europe by burying seed to depths where it is unlikely to germinate. This method would have limited usefulness here due to

the risk of drift on light soils and the need to follow up with minimum tillage for several years. Minimum tillage can contribute to broomrape control by reducing the amount of viable seeds incorporated into the soil.

Nitrogen compound application and manure fertilization also appear to be candidates for putative control of broomrapes, some studies have shown that nitrogen in ammonium form negatively affects broomrape germination and/or elongation of the seedling radical. In addition, manure fertilization augments the killing effect of solarization on *O. crenata* seeds (Haidar and Sidahmed, 2000).

5.3. Hand weeding

Weeding of *Orobanche* is mainly accomplished after the parasitic damage has already been done. It is not likely to show any yield increase in the short term. Weeding is laborious and time-consuming, and not very promising in highly infested areas. However, in combination with other methods, it can reduce the seed bank very efficiently (FAO, 2008).

5.4. Intercropping

Intercropping is a method facilitating simultaneous crop production and soil fertility building. There is a renewed interest in intercropping linked to the need for reducing nitrogen cost and soil erosion. Intercropping is already used in regions of Africa as a low-cost technology of controlling the broomrapes (Oswald et al., 2002).

Recently it has been demonstrated that intercropping with cereals or with fenugreek can reduce *O. crenata* infection on faba bean and pea due to allelopathic interactions (Fernandez-Aparicio et al., 2007, 2008). This has been confirmed in a subsequent study, in which trigoxazonane was identified in the root exudates of fenugreek which may be responsible for the inhibition of *O. crenata* seed germination (Evidente et al., 2007).

Maize and snap bean as potential trap crops on *Orobanche* soil seed bank showed better performance in stimulating germination of *Orobanche* seed bank and raised the germination by 74 and 71 per cent, respectively. Maize and Snap bean were also complementing each other under inter-cropping and soil seed bank of *O. ramosa* and *O. cernua* was depleted by 72.5 per cent per season. Yield of tomato was significantly increased due to the reduction of *Orobanche* seed bank in the 3rd season (2004) (Girma et al., 2005).

Field experiments noticed that *O. crenata* infection on faba bean and pea is reduced when these host crops are intercropped with oat. The number of *O. crenata* plants per host plant decreased as the proportion of oats increased in the intercrop. Pot and rhizotron experiments confirmed the reduction of infection in faba bean intercropped with cereals. It is suggested that inhibition of *O. crenata* seed germination by allelochemicals released by cereal roots is the mechanism for reduction of *O. crenata* infection (Monica et al., 2007).

5.5. Crop rotations

Rotation with non-host crops is usually suggested. The use of trap crops offers the advantage of preferentially stimulating broomrape suicidal germination. Flax, fenugreek and Egyptian clover are established to be successful trap crops for *O. crenata*. There are claims that a reduction in infestation has been reported in rotations with rice, due to water flooding (Sauerborn and Saxena, 1986), however, this has not been substantiated. The incorporation of resistant legumes in crop rotations may also maintain broomrape infestation at low levels (Schnell et al., 1996).

Gawahir, 2006 reported that Infestation of *Orobanche* decreased by 37 per cent at Elgeli/Sudan under the crop sequence of onion-onion onion-tomato where tomatoes yield was increased by 38 per cent. At Alafoon/Sudan crop sequence of onion-onion-onion/tomato and/or onion-alfalfa-alfalfa-tomato decreased *Orobanche* infestation by 90-95 per cent and the yield of tomato was increased by 60 per cent. Crop sequences in the two locations, in addition to increase of tomato yield, decreased the *Orobanche* incidence, branching and seed production.

Acharya et al., 2002 noticed that a local cultivar of *Brassica campestris* has been used as a catch crop in Nepal, reducing the *O. aegyptiaca* seed bank by around 33.35 per cent.

Experimental results in Tehran indicated that using trap crops namely sesame, brown indian-hemp, common flax and black-eyed pea decreased broomrape biomass by 86, 85.3, 75.2, and 74.4 per cent, respectively. Reducing broomrape biomass caused increases in the tomato yield. Meanwhile, sesame, brown Indianhemp, Egyptian clover and mungbean increased total biomass of tomato by 71.4, 67.5, 65.5, and 62.5 per cent, respectively. It was observed that these plants have a great potential to reduce broomrape damage and they can be used in rotation in broomrape infested fields (Sirwan et al., 2010).

In Tunisia, the results suggested that in fields infested with *O. foetida*, the use of bean, pea, flax and fenugreek in the crop rotation may reduce the Orobanche seed bank. Whereas in fields infested with *O. crenata*, crops such as bean, flax, alfalfa, wheat and oat used in the crop rotation may reduce the soil seed bank of this broomrape (Abbes, 2008).

5.6. Fertilization

Orobanche tends to be associated with less fertile soil conditions. High levels of nitrogen fertilizer or chicken manure showed a suppressive effect. The main effects could be due to,

- Reduction of stimulant exudation;
- Direct damage to Orobanche seeds and seedlings in the soil;
- Reduced osmotic pressure in the parasite relative to the host;
- A toxic effect of nitrogen on the parasite development;
- Alternation of host roots and shoots balance.

Urea at 276 and 207 kg N ha⁻¹, ammonium nitrate, and ammonium sulfate at 207 kg N ha⁻¹ and the goat manure at 20 and 30 t ha⁻¹ were found to be most effective in reducing parasitism of Orobanche and enhancing growth of tomato plants. Even though drastic reduction of broomrape infestation was obtained, ammonium nitrate and ammonium sulfate at 276 kg N ha⁻¹ seemed to be injurious to tomato plants. As nitrogen rates increased, the numbers and dry weights of shoot of branched broomrape decreased and the yields of tomato increased linearly except the yields obtained from the highest rate of ammonium nitrate and ammonium sulfate. This result indicated that broomrape infestation of tomato decreased with increases of soil nitrogen (Mariam and Rungit, 2004).

The mixtures of chicken manure (20 t ha⁻¹) and sulphur (0, 1, 4, 8, and 12 t ha⁻¹) at all tested rates significantly reduced the dry weight of Orobanche and increased eggplant and potato yield compared with the control (Haidar and Sidahmed, 2006).

6. Biological methods

Like all other plants, broomrapes have natural enemies which can affect their growth and can potentially be used as agents for their control. Biological control of weeds is defined as the use of natural antagonists to exert pressure on the population of their host to reduce it to levels below economic importance. Unlike chemical compounds, biological control agents have the advantage of being specific to the weed, and do not directly contribute to environmental pollution.

This technique utilizes living organisms (insects, fungi, viruses, bacteria etc.) to suppress or reduce broomrape infestation. Pathogenicity toward non-target plants is a major constraint; therefore, it is very important that host-specificity and risk assessment should be made before the release of a control organism into the environment.

The 'bioherbicide approach' employs virulent strains of pathogens (viruses, bacteria or fungi) which naturally occur on the weed and enhances their destructive activity. The infection build-up of the pathogen is manipulated to the extent of causing significant damage to the parasitic weed. Pathogens can be used as sole agents or as part of a complex integrated control strategy (Sauerborn et al., 2007).

Biological control is particularly attractive in suppressing root parasitic weeds in annual crops because the intimate physiological relationship with their host plants makes it difficult to apply conventional weed control measures. Both insects and fungi have been isolated that attack parasitic angiosperms.

Most of the insects which have been reported to occur on Orobanche species are polyphagous without any host-specificity and thus damage to these parasitic weeds is limited (Klein and Kroschel, 2002). For biological control, oligo- and monophagous herbivorous insects are of interest. The fly *Phytomyza orobanchia* (Diptera, Agromyzidae) is reported to be host-specific attacking only Orobanche species. Its distribution is related to the natural occurrence of Orobanche spp. and *Smicronyx* spp. (Coleoptera, Curculionidae). These insects prevent seed production through the development of larvae inside the seed capsules of their target hosts and thus contribute to reduce their reproductive capacity and spread. However, research with both insects has revealed that their effectiveness to prevent seed set is limited and will not be enough to lower the soil seed bank significantly (Klein and Kroschel, 2002).

A factor that may limit the effect of *P. orobanchia* and *Smicronyx* spp. is soil cultivation, especially deep ploughing. Hibernating pupae can be destroyed and/or buried and prevent insect emergence. Further limiting

factors of cultivation are pesticide applications against crop pests if these coincide with the flight periods of the two beneficial insects. Moreover, *Phytophthora* and *Smicronyx* suffer from indigenous antagonists which may have an important impact on their population levels. Because of their short lifetime and enormous seed production and the vast damage caused to the host by unemerged plants, *Orobanche* cannot be regarded ideal organisms for biological control by insects. Approximately 30 fungal genera were reported to occur on *Orobanche* spp. (Boari and Vurro, 2004).

Soil-dwelling microorganisms have been specifically sampled, since they have a number of advantages in the control of root parasites, (i) they can provide effective control by attacking the seeds and the early stages of the developing parasite; (ii) they are less sensitive to the environmental conditions compared with aerial pathogens; and (iii) they are expected to survive in the soil by producing resting structures at population levels sufficient to provide residual control of the parasitic plant. Numerous microorganisms potentially useful for the biocontrol of *Orobanche* species have been isolated and reported, but none has been subject to continuous wide-spread use (Amsellem et al., 2001; Hameed et al., 2001; Boari and Vurro, 2004; Zermane et al., 2007).

Results of surveys for fungal pathogens of *Orobanche* revealed that *Fusarium* species were the most prominent ones associated with diseased broomrapes. Of these, *F. oxysporum* was the predominant species. *Fusarium* species as soil-borne fungi possess several advantages which render them suitable for the bioherbicide approach. In the soil they are relatively protected from environmental stress of drought and heat, frequently occurring in the area of distribution of *Orobanche*. The saprophytic nature of *Fusarium* spp. allows them to be cultured in liquid as well as solid media; and particularly the formae speciales of *F. oxysporum* are highly host-specific. Because most of the damage to host crops occurs while the parasitic weed is still underground, use of soil-borne biocontrol agents such as *Fusarium* spp. can add to improving crop yield by destroying the parasite at its early developmental stages. To date about six *Fusarium* species are reported to be associated with *Orobanche* (*F. arthrosporioides*, *F. nygamai*, *F. oxysporum*, *F. oxysporum* f.sp. *orthoceras*, *F. semitectum* var. *majus*, *F. solani*) have shown significant disease development in selected species of *Orobanche* (Müller-Stöver et al., 2002; Shabana et al., 2003) when tested under controlled and/or field conditions.

Under laboratory and greenhouse conditions excellent control was repeatedly observed with *F. oxysporum* f.sp. *orthoceras* against *O. cumana* on sunflower. The total number of *O. cumana* could be reduced by about 80 per cent after soil application of a simple granular formulation of the fungus. Amsellem et al. (2001b) and Cohen et al. (2002a) observed reduction in *O. aegyptiaca* attached to tomato in greenhouse experiments using host-specific strains of *F. oxysporum* and *F. arthrosporioides*.

Although data on the efficiency of *Fusarium* spp. to control *Orobanche* in the field are rare, the results already indicate that *Fusarium* spp. in most cases do not provide the level of control desired by farmers. Thus there has been no successfully demonstrated control of this weed using potential inundative bioherbicides. The question arises, how the efficacy of the pathogens can be improved under field conditions in order to fight the target organism (Sauerborn et al., 2007).

In the novel approach strategy two or more pathogens are combined and applied before or after parasite emergence. Some applied fungal mixtures caused a significant reduction of the number of emerging *O. cumana* (Charudattan 2001), Amsellem et al. (2001) and Cohen et al. (2002) observed reduction in *O. aegyptiaca* attached to tomato in greenhouse experiments using host-specific strains of *F. Oxysporum* and *F. arthrosporioides*.

The feasibility of this approach has been demonstrated in the control of *O. cumana* in sunflower (Dor et al., 2003). A bioherbicide system was based on two fungal pathogens *F. oxysporum* f. sp. *orthoceras* and *F. solani* which had been isolated from *O. cumana* on sunflower and *O. aegyptiaca* on tomato, respectively. In pot trials, the pathogens gave a low control level when used individually but when applied as a mixture; both fungi caused a significant reduction of the number of emerged *O. cumana* and of the parasite's dry weight. The inoculum density of each fungus when applied alone was 105 colony forming units (cfu) ml⁻¹. The same inoculum level of each fungus was used with the mixture, thus resulting in an inoculum density of 2x10⁵ cfu ml⁻¹.

Another approach, which only recently has shown to provide successful control of *O. cumana*, is to integrate the resistance inducer BTH (benzothiadiazole) with the bioherbicide pathogen *F. oxysporum* f. sp. *orthoceras*. The combined treatment of the two agents resulted in highly reliable control of *O. cumana* and reduced the parasite's emergence up to 100 per cent (Müller-Stöver et al., 2005). The excellent control level in the combined treatments resulted from a lower number and reduced dry weight of *O. cumana* shoots, indicating that the combination of control strategies takes effect already in the early developmental stages of the parasite. This could either be due to an enhanced activity of the fungus against the early underground stages of the parasite or to an enhanced induced

resistance within the sunflower plant. In laboratory experiments, no enhancing effect of BTH on virulence and growth of the fungus has been observed so far.

Yet another approach which receives increasing attention is the engineering of hypervirulence genes into weed specific pathogens, e.g. genes which encode enzymes that degrade metabolites involved in crop defence mechanisms such as phytoalexins or coding for enhanced virulence by the production of fungal toxins (Gressel, 2002, 2004). To enhance virulence, two *Fusarium* species that attack *Orobanche* have been transformed with two genes of the indole-3-acetamide pathway that converts tryptophan into the plant hormone indoleacetic acid IAA, (Cohen et al., 2002b). It was shown that overproduction of IAA provided a slight increase in virulence compared to the wild type but not enough to attain a satisfactory level of control. Extreme care should be taken with such genetically modified organisms due to their possible environmental impact, and fail-safe mechanisms should be installed and tested prior to release into the environment. Some possible fail-safe mechanisms are discussed by Gressel (2001, 2002).

A synergized effect was found between *Fusarium oxysporum* f.sp. *orthoceras* and *Fusarium solani* on sunflower broomrape. Application of two fungi together to control sunflower broomrape was rusticated to the first weeks after application. Repeated applications are needed for adequate long season control. The development of fungal inocula application through drip irrigation system developed in Bari, Italy, opens new horizons in biological control methodology (Hershenhorn et al., 2006).

Myrothecium verrucaria isolated from faba bean roots has been found to inhibit germination of *O. crenata* seeds due to the production of the macrocyclic trichothecene, verrucarins A (El-Kassas et al., 2005). Preliminary results 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.

7. Biotechnological methods

A novel chemical control strategy has been developed during the past few years, systemic acquired resistance (SAR). SAR can be induced in plants by the application of chemical agents. Recently it has been shown that SAR of host plants can be used for the control of important broomrape species (Sauerborn et al., 2002; Perez-de-Luque et al., 2004a; Gonsior et al., 2004; Buschmann et al., 2005). However, research on the effects of SAR on host-*Orobanche* systems is still in its infancy. Application of the resistance inducing agent BION® (1,2,3-benzothiadiazole-7-carbothioic acid S-methyl ester, Syngenta) stimulates the production of defence mechanisms in sunflower roots, which protects against parasitism of *O. cumana* (Buschmann and Sauerborn, 2002). Recently, Fan et al. (2007) evaluated the effects of prohexadione-calcium (PHDC) on *O. cumana* seed germination and induction of sunflower resistance to this root parasitic weed, and showed retarded *O. cumana* tubercle formation and development. Despite no chemical agents were used.

Mabrouk et al. (2007a,b,c) have also shown that some *Rhizobium leguminosarum* strains decrease *O. crenata* infections in peas by inducing systemic resistance.

Resistance to broomrapes is a multi-faceted response in faba bean and legumes. Several defence mechanisms have been detected in plants resistant to broomrape attack, mainly involving cell wall reinforcement (Perez-de-Luque et al., 2005, 2006a, 2007), production of toxic compounds (Echevarria-Zomero et al., 2006; Lozano et al., 2007) and sealing of vascular tissues (Perez-de-Luque et al., 2006b). In several *Vicia* species other than *V. faba*, an early mechanism of resistance has been detected against crenate broomrape characterized by a reduction in broomrape seed germination induction (Sillero et al., 2005). However, in faba bean this low induction was considered to be rare against both *O. crenata* and *O. foetida*. Regarding faba bean, the mechanisms described to date are pre-haustorial, i.e. they are observed after attachment and penetration into the host tissues but prior to the development of the haustorium (the organ connecting both, the host and parasite vascular tissues). According to Perez-de-Luque et al. (2007), these defensive responses correspond to, (i) callose depositions in the host cell walls from the cortex and in contact with the parasite tissues, and (ii) lignification of host pericycle and endodermal cells. The second response (lignification) occurs after the obstruction of parasite intrusive cells in the host cortex has been overcome, and prevents further penetration into the central cylinder and formation of a haustorium.

In the last five decades, crop yields increased because of chemical control of weeds, especially with selective herbicides. Recently, unfortunately, herbicides have been removed from markets because of toxicity, weed resistance to the herbicides, and environmental concerns (Gressel, 2002). Developing a new herbicide by chemical

companies is difficult, time-consuming, and very expensive. Accordingly, there is a pressing need for biotech-derived crops (not only crops with engineered herbicide resistance adapted over the last few years), but also using newer technology based on genomic, proteomic, and metabolomic tools. Gressel (2002) described newer technologies that will assist in meeting the needs for herbicide-resistance crops. Notably, herbicides that are metabolized by transgenic plants, i.e., glufosinate (Basta®), which is metabolized by the bar gene in transformed plants before reaching the roots of transgenic-resistant crops, would be ineffective for parasitic weed control. Transgenic herbicide resistance may also pose food safety issues through the expression of the new gene in the crop plant. Concern may also arise regarding the possible gene transfer from transgenic crop plants to wild plants, although different ways to overcome these concerns have been proposed (Gressel, 2002). Therefore, these parameters should be taken into consideration while applying chemicals to herbicide-resistant crops.

Aviv et al. (2002) engineered a mutant ALS gene into carrot, allowing the control of broomrape by imazapyr (an imidazolinone ALS inhibitor). Several tobacco cultivars transformed with a mutant acetohydroxy acid synthase (AHAS) 3R gene (isolated from a sulfonylurea resistant Brassica napus cell line) were resistant to the herbicide chlorsulfuron (Slavov et al. 2005). The effect of chlorsulfuron on broomrape was clearly demonstrated, A very low percentage (from 0.1 to 4 %) of its active ingredient that reached the plant roots was sufficient to kill the parasite at an early developmental stage after two treatments (Slavov et al. 2005).

Parasitic weeds will rapidly evolve resistance to herbicides because of their prolific seed production. Therefore, resistance to glyphosate, asulam, chlorosulfuron, or imazapyr will eventually appear. Therefore, herbicide resistance crops should be wisely used or combined with other control methods, and new resistant crops continually developed (Radi, 2007).

8. Integrated methods

Sulfosulfuron at a rate of 40 g. a.i. ha⁻¹ applied 2-3 times in two-week intervals starting two weeks after planting followed by sprinkler irrigation of 300 m³ ha⁻¹ delays the appearance of broomrape inflorescences above ground by 3-4 weeks. Such a delay prevents the damage caused to the yield but not the continuation of the field contamination with broomrape seeds. Addition of an imidazolinone herbicide application such as imazapic or imazamox 63-70 days after planting prevents almost completely broomrape shoot emergence and seed setting of inflorescences present in the field during herbicide application (Hershshorn et al., 2006).

Mohammed et al., (2012) conducted experiment to assess the effect of combination of bacterial strains and chicken manures on broomrape on faba bean, the results displayed that among all treatments faba bean inoculated with TAL 1399 alone or in combinations with *Bacillus megatherium* var *Phosphaticum* (BMP) or *Azospirillum brasiliense* (Ab) plus chicken manure at 35 g pot⁻¹ displayed no *Orobanche* emergence (above the ground) until the end of experiment. Furthermore, crop treated with TAL 1399 plus chicken manure at 35 g pot⁻¹ was significantly higher in root, shoot and total dry weight as compared to control and other treatments.

Three isolates of *Trichoderma* species including *T. harzianum* T1, *T. harzianum* T3 and *T. viride* T2 were tested for control of *Orobanche* species in peas, faba bean and tomatoes under field conditions in Egypt. Results of field studies showed that soil treatment with these three fungal agents alone or soil treatment with fungal agents plus aerial spray of glyphosate (50 ppm) was efficient and cost-effective method in reducing infection, minimizing the number of spikes parasitic on host plants and increasing yields of peas, faba bean and tomatoes (Mokhtar et al., 2009).

Maize (*Zea mays* L.) ranks after rice and wheat as the third most important cereal in Bangladesh. It is the highest grain-yielding crop having multiple uses as every part of the plant or its product is used in one form or the other. Grain can be used for human consumption in various ways, such as corn meal, fried grain, roasted cob or popped and corn flour. Maize starch can be compared with rice and wheat nutritionally, is used in the food, chemical, textile, paper and plastic industries. Green maize plants are grown mainly as fodder and its grains are used for human consumption and as dairy and poultry feed in many areas in Bangladesh (Awal and Khan, 2004). The production areas of maize are increasing day by day due to increase in poultry and dairy farms in the country. The farmers are also interested to grow it, so the maize crop has been included in the crop diversification programme in Bangladesh (Bhuiya et al., 2005). Pea (*Pisum sativum* L.) is an important leguminous crop and has a tremendous value in agriculture as a good source of plant protein. It is used both as pulse and vegetable. Pea can play an important role in agro-economy and national health of Bangladesh. Pea seeds contain about 3-4 times as

much protein and 2-3 times as much mineral as rice. It provides an important source of protein to human diets (Martin et al., 1976). It can also be used as green fodder for animals. As legume, growing pea crops add substantial amount of atmospheric nitrogen to the soil by Biological Fixation System through Rhizobium bacteria in root nodules. Therefore, cultivation of maize and pea could mitigate carbohydrate, protein and other nutritional deficiencies in Bangladesh. Cultivation of maize and pea as sole crops require separate lands and/or, times which are critical as the arable land is gradually decreasing in Bangladesh. However, cultivation of these two crop species in intercropping mixture may save the land and time substantially. Intercropping is proved to be an excellent technique to increase total yield, higher monetary return, and greater resource utilization and fulfil the diversified needs of the farmers (Singh et al., 1986). Although some cereal/legume intercrop associations are tested elsewhere (Ofori and Stern, 1987) but no study is yet to be reported on maize/pea intercropping under the agro-climatic conditions of Bangladesh. Crop compatibility is the most essential factor for a feasible intercropping system. Thus, the success of any intercropping system depends on the proper association of crop species where competition between them for natural resources is minimum (Awal et al., 2006). Competition in intercropping can be reduced considerably through either judicious selection of crop species or by changing plant population i.e., spatial orientation of row spacing (Rahman et al., 2009). A careful selection of crop species could reduce the competition to a considerable extent (Singh, 1983). Maize is a tall stature cereal whereas pea is a short stature legume crop and in intercrop mixture they may also provide profitable return to the farmers. Therefore, the present study was undertaken to assess the suitability of pea plants as intercrop with maize stands and to find out the competition between maize and pea stands, and their growth and productivity in intercropping mixture as compared to their sole crops.

9. Conclusions

The principal conclusion to be drawn from these studies must be that no single technique provides complete control of Orobanche, and resorting to some of them is unavoidable. Physical methods are very useful to prevent the Orobanche but are tedious, time-consuming and costly. Chemical, agronomic control methods and host resistance appear to be the most appropriate measures when available and affordable. Moreover, some biological and biotechnological approaches are promising but they are too expensive and control may not be complete, by this, still need more research. It was claimed that integrated approaches combining several techniques could be more effective. However, these integrated programmes are practiced only on a small scale in a few countries because of cost and technical problems. While avoidance of dispersal of broomrape, crop resistance, and prevention measures could be effective and the most economical methods to reduce this root parasitic weed infestations in agricultural fields. Advantages of these approaches are no chemical applications and no need for additional labour or complicated management, expensive equipment, or instrumentation, low cost, environmental safety, and may deplete the parasite soil seed bank. It is important both to assess the most severely infested areas in order to target these control measures most effectively, and maintain the seed bank of less infested areas beneath a threshold level of damage.

Acknowledgements

The Author gratefully acknowledges Dr. Sunil C.M. for his assistance, helpful critical reading and comments on the manuscript.

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