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Phelipanche aegyptiaca management in tomato

J HERSHENHORN*, H EIZENBERG*, E DOR*, Y KAPULNIK† & Y GOLDWASSER‡

**Institute of Plant Protection, Agricultural Research Organization (ARO), Newe Ya'ar Research Center, Ramat Yishay, Israel, †Institute of Plant Sciences, Agricultural Research Organization (ARO), the Volcani Center, Bet Dagan, Israel, and ‡RH Smith Institute of Plant Science and Genetics in Agriculture, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel*

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Summary

Phelipanche and *Orobanche* species (broomrapes) are root holoparasitic plants that cause severe damage to economically important crops. *Phelipanche* and *Orobanche* spp. are widespread in Mediterranean areas, in Asia and in Southern and Eastern Europe, attacking dicotyledonous crops and depending entirely on their hosts for all nutritional requirements. *Phelipanche aegyptiaca*, *Phelipanche ramosa* and *Orobanche cernua* are extremely troublesome weeds on tomatoes. These noxious parasites exert their greatest damage prior to their shoot emergence and flowering; therefore, the majority of field loss may occur before diagnosis of infection. This review summarises the four main control measures for the weedy root parasites *Phelipanche* and *Orobanche* in tomato, namely chemical and biological control, resistant varieties and sanitation. Some of these methods are commercially widely used by farmers in Israel (chemical control), some are in the final stages of development towards commercialisation (resistant varieties and sanitation), and some still require further development and improvement before commercial implementation (biological control). The review presents an up-to-date summary of the available knowledge on their use for broomrape management in processing tomatoes.

Keywords: *Phelipanche, Orobanche,* management, chemical control, biological control, sanitation, breeding, resistant varieties, *Solanum lycopersicum*.

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Introduction

The weedy root parasitic plants *Striga* spp. (witchweeds) and *Phelipanche* and *Orobanche* spp. (broomrapes) pose a serious threat to world agricultural production, mainly because there are no practical methods to control them effectively (Gressel *et al*., 2004). *Phelipanche* and *Orobanche* spp. are widespread in Mediterranean climate areas, in Asia and in Southern and Eastern Europe, attacking dicotyledonous crops and depending entirely on their hosts for all nutritional requirements (Goldwasser & Kleifeld, 2004; Joel *et al*., 2007). Yield losses range from 5% to 100%, depending on host susceptibility, level of infestation and environmental conditions. Increased population pressure in Syria, Algeria, Morocco, Tunisia, Egypt, Sudan and Ethiopia has intensified production of broomrape-sensitive crops. As a result, the extent and intensity of parasitic weed infestation (by *Phelipanche* and *Orobanche* spp. and *Striga* spp.) has increased rapidly and currently threatens food production in these countries (Abang *et al*., 2007). Tomato (*Solanum lycopersicum* L.) is highly vulnerable to three broomrape species, *Phelipanche aegyptiaca* Pomel (syn. *O. aegyptiaca*), *Phelipanche ramosa* Pomel (syn. *O. ramosa*) and *O. cernua* Loefl. that are known to cause damage and reduce yields in this crop (Joel *et al*., 2007). *Phelipanche aegyptiaca* is the main limiting factor in processing-tomato production in

Correspondence: J Hershenhorn, Department of Phytopathology and Weed Research, Agricultural Research Organization, Newe Ya'ar Research Center, P.O. Box 1021, Ramat Yishay 30095, Israel. Tel: (+972) 4 9539534; Fax: (+972) 4 9836936; E-mail: josephhe@volcani.agri.gov.il

Israel and some countries of the Arabian Peninsula. Most of the fields in the northern part of Israel, the main region for processing tomato growth, are infested with *P. aegyptiaca* with small infestation patches of *P. ramosa*. In recent years, an increase in *P. aegyptiaca* infestations of tomato grown in glasshouses in the south was recorded. The annual loss because of *Phelipanche* in Israel is estimated at $5 million (unpubl. data Erlick, A). The main *broomrape* species in Tunisia include *O. crenata* Forsk., *O. foetida* Poir. and *P. ramosa*. In Egypt, *Phelipanche* significantly reduces tomato yield. Infestation in newly reclaimed land is high because of soil, manure transfer, grazing animals and the use of contaminated water for irrigation. The average yield losses from *broomrapes* alone account for about 30% of the total losses caused by all other crop management constraints. *Phelipanche ramosa* is the most prevalent and devastating species to vegetables in both irrigated and rain-fed agriculture and most destructive to tomato in Egypt, the main vegetable crop grown in the country. In Ethiopia, the area of tomato production has increased many folds in recent years. However, because of *Phelipanche* problems, some State Farms have had to give up growing tomato. In Sudan, a complete failure of production was claimed due to heavy *Phelipanche* infestation. In fact, it was reported that tomato-canning factory (Kariemeh) was closed down in the most suitable area production because farmers abandoned growing tomatoes due to *P. ramosa* infestations. In Lebanon, broomrapes affect several crops, among them potato (*Solanum tuberosum* L.), tomato, eggplant (*Solanum melongena* L.) and faba bean (*Vicia faba* L.). The fast-spreading parasites, *P. ramosa*/*P. aegyptiaca* cause heavy yield losses in potato and tomato (Abu-Irmaileh & Labrada, 2008). In Turkey, processing tomato are produced on an area of 60 000 ha in the Aegean and the South Marmara regions. In the same regions there are also 20 000 ha of fresh market tomato fields. About 30% of the fields are heavily infested with *Phelipanche* spp. and the total infested area is 75%. Yield loss due to the parasite in tomato is 25–40 t ha^{-1}. In the other regions, the area infested varies between 5% and 80%. The glasshouse area for tomato production is 20 000 ha and 27% of it is slightly infested with *Phelipanche* spp., but no significant losses in glasshouses are reported. The overall annual loss in Turkish tomato production is estimated at €200 million.

A continuous increase in *P. aegyptiaca* infestations in the north of Israel over the last three decades has forced farmers to abandon the fertile infested lands or to grow less profitable non-host crops. In other countries where land is not a limiting factor, such as Ethiopia and Sudan, farmers abandon fertile infested land and move to new non-infested land in order to escape broomrape damage (Hershenhorn *et al*., 1998c; Abu-Irmaileh & Labrada, 2008).

Phelipanche and *Orobanche* spp. exert their greatest damage prior to emergence of their flowering shoot; therefore, most of the field losses may occur before diagnosis of infection. Many different strategies have been tested for broomrape management (Joel *et al*., 2007; Rispail *et al*., 2007), such as sanitation and manual weeding (Ransom, 2000), selective herbicides (Hershenhorn *et al*., 1998a; b, c), biological control (Sauerborn *et al*., 2007), soil treatment with fumigants and solar heating (Jacobsohn *et al*., 1980; Kleifeld *et al*., 1998) and trap crops (Hershenhorn *et al*., 1996). However, most of these management strategies fail to provide satisfactory control or are not economical, environmentally safe or feasible on a large scale (Goldwasser & Kleifeld, 2004; Joel *et al*., 2007). Breeding for resistance is still one of the most effective, feasible and environmentally friendly management strategies against this weed (Rubiales *et al*., 2003). Considerable efforts have been invested in many crops, such as chickpea (*Cicer arietinum* L.) (Rubiales *et al*., 2003), faba bean (Nassib *et al*., 1982; Román *et al*., 2002; Rubiales *et al*., 2006), pea (*Pisum sativum* L.) (Rubiales *et al*., 2009; Valderrama *et al*., 2004) and sunflower (*Helianthus annuus* L.) (Pérez-Vich *et al*., 2004), to develop molecular markers for resistance and to breed crop varieties resistant to various species and races of *Phelipanche* and *Orobanche* spp. However, proved success has only been documented in sunflower. Breeding-resistant varieties remains an on-going struggle because a genetic broomrape-resistance background is scarce or unavailable in most crops and because of the parasite's ability to rapidly overcome newly developed resistant varieties (Fernández-Martínez *et al*., 2000).

Although several potential control measures have been developed in the past decades for some crops, any single approach is often only partially effective and sometimes inconsistent and affected by environmental conditions. Therefore, the most feasible way of coping with the weedy root parasites is via the integration of a variety of measures in an integrated management approach, starting with containment and sanitation, direct and indirect measures to prevent the damage caused by the parasites and finally, eradicating the parasite seedbank in the soil.

This review summarises the four most feasible major control measures for the weedy root parasite *Phelipanche* and *Orobanche* spp. in tomato, namely chemical and biological control, resistant varieties and sanitation. The review presents an up-to-date summary of the available knowledge on their use for management in processing tomatoes.

Chemical control

Chemical control of Phelipanche aegyptiaca in processing tomato

Chemical control has been extensively explored since the 1970s. Control is complicated because: (i) chemical control can only be used as a prophylactic treatment, because in most cases the level of infestation is unknown, (ii) the parasite is directly connected to the host and, therefore, only highly selective herbicides can be used, (iii) if the herbicide reaches the parasite through the conductive tissues of its host, the host must be tolerant to the herbicide by mechanisms which are not based on the latter's metabolic degradation or inactivation, (iv) the parasite germinates continuously throughout the season and throughout the entire cultivated soil-depth profile.

Herbicides that currently are in use for *Phelipanche* and *Orobanche* spp. control in various crops are glyphosate, imidazolinones and sulfonylureas. The sulfonylureas and imidazolinones are selective systemic herbicides that inhibit the enzyme acetolactate synthase (ALS), also called acetohydroxyacid synthase (AHAS), a key enzyme in the biosynthesis of the branched-chain amino acids leucine, isoleucine and valine (Schloss, 1995). Sulfonylurea herbicides are absorbed through the plant foliage and roots with rapid acropetal and basipetal translocation. Imidazolinone herbicides are absorbed and translocated through the host to the meristematic tissues where the ALS enzyme is highly active. Some data support the assumption that imidazolinone herbicides leak from the plant roots to the rhizosphere (Shaner & O'Connor, 1991).

Herbicides should be applied during the parasite's initial developmental stages, i.e. to germinated seeds or young, small tubercles attached to the host roots. Knowledge of the biology and phenology of *Phelipanche* and *Orobanche* spp. and its hosts are essential for effective chemical control. Application should be repeated at 2 to 4 week intervals, because seeds can germinate throughout the season and therefore parasitise host roots that penetrate into deeper soil layers.

Three main chemical approaches have been used for *P. aegyptiaca* control in tomato: (i) soil fumigation, (ii) application of herbicides to the soil, (iii) foliar application of systemic herbicides.

Soil fumigation

Successful soil fumigation has been achieved with methyl bromide; nonetheless, this chemical has been banned from use because of its harmful environmental effects. Other potential fumigants include metham sodium, dazomet and 1,3-dichloropropene (Foy *et al.*, 1989; Goldwasser *et al.*, 1995). However, their partial efficacy, high cost and complex application methods prevent their widespread use by farmers. Therefore, these means are not discussed any further in this review.

Application of herbicides to the soil

Herbigation (delivery of herbicides through irrigation water) and pre-soil herbigation (herbigation before the crop is sown or planted) with sulfonylurea herbicides effectively control *P. aegyptiaca* in tomato through the soil (Table 1). Saturating the soil with herbicide solution controls preconditioned and germinating seeds and young attachments. The control of germinating *P. aegyptiaca* and *P. ramosa* seeds and young attachments is based on direct exposure of the parasite to the herbicides via the soil solution, or via the host that absorbs the herbicide from the soil solution and translocates it to the attached parasites, the latter serving as strong sinks (Qasem, 1998; Plakhine *et al.*, 2001). The herbicide can be incorporated into the soil by several means (Fig. 1).

Table 1 Rate of sulfonylurea and imidazolinone herbicides used for *Phelipanche aegyptiaca* control in processing tomato

Herbicide	Rate (g a.i. ha^{-1})	Application no.	Application method (from Fig. 1)	Reference
Sulfosulfuron	37.5–75.0	1–3	2, 6; 2, 6	Eizenberg *et al.* (2003b, 2004, 2005, 2007, 2008), Plakhine *et al.* (2001)
Chlorsulfuron	3.75–11.25	3* + 2†	3, 4, 5	Hershenhorn *et al.* (1998a,b,c) Eizenberg *et al.* (2003b)
Triasulfuron	7.5–22.5	3* + 2†	3, 4, 5	Hershenhorn *et al.* (1998a,b,c)
Rimsulfuron	37.5–50.0	3 + 2	2, 6	Eizenberg *et al.* (2003c, d, 2008)
Imazapic	10.0–20.0	2	1	Plakhine *et al.* (2001)
Imazamox	0.8	2	1	Achdari *et al.* (2009)

*Drip herbigation.
†Sprinkler herbigation.
[a]Applications followed by 300 m^3 sprinkler irrigation.
[d]Foliar application.

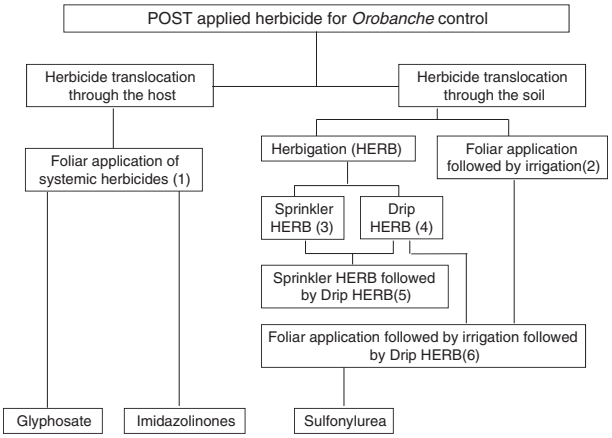

Fig. 1 Methods for post-emergence/post-planting herbicide application in processing tomatoes for *Phelipanche* and *Orobanche* spp. control.

Herbicide application preplanting, or post-emergence or post-planting (via the irrigation system) is effective for *P. aegyptiaca* control in tomato. However, the herbigation method suffers from some weaknesses. Large-scale chlorsulfuron sprinkler herbigation applied under windy conditions can sometimes damage the crop on one hand and not sufficiently control the parasite on the other hand, because the herbicide is delivered unevenly to the tomato and parasite plants. Additionally, most fields in Israel are equipped with drip rather than sprinkler irrigation systems. Hershenhorn *et al.* (1998a,b) have suggested three repeated chlorsulfuron sprinkler herbigation treatments given at 2-week intervals starting 2 weeks after planting to control *P. aegyptiaca* in tomato. However, late in the season, *P. aegyptiaca* inflorescences were seen to emerge at the centre of the tomato beds in the treated plots, near and around the drippers, presumably because the water delivered by the drippers washed the herbicide away from this area. An additional chlorsulfuron herbigation treatment, this time via the drip-irrigation system, prevented this late emergence of *P. aegyptiaca* shoots (Hershenhorn *et al.*, 1998a,b). Eizenberg *et al.* (2003b) suggested sprinkler herbigation in accordance with the level of infestation in the field (as determined by data taken from the field's history); for example, under moderate field infestations, one sprinkler herbigation and four sequential drip herbigations, given at 1-week intervals, were sufficient for almost complete *Phelipanche* and *Orobanche* spp. control (Eizenberg *et al.*, 2003b). Chlorsulfuron was highly effective but the complexity of its mode of application and its long residual effect required the development of a new or improved approach for its uniform delivery to the soil. For this reason, a foliar application of the herbicide was used on tomato followed by sprinkler irrigation in order to incorporate the herbicide evenly in the soil (Fig. 1). One to three foliar applications, in accordance with the field infestation level, of rimsulfuron or sulfosulfuron were needed to control *P. aegyptiaca* in tomato. Again, *P. aegyptiaca* shoot emergence in the centre of the bed late in the season required additional herbigation treatments through the drip system. Under high infestation levels, sulfosulfuron was more effective at controlling *P. aegyptiaca* than rimsulfuron, presumably because of the former's long residual effect in the soil. In addition to *P. aegyptiaca* control, sulfosulfuron was highly effective at controlling some troublesome weeds such as black nightshade, pigweed and nutsedge (Eizenberg *et al.*, 2003a). The herbigation cost for the Israeli farmer is negligible as all the fields are irrigated with drip, sprinkler linear and centre pivot watering machines systems.

The mechanism governing herbicide delivery in *P. aegyptiaca* control was elucidated in surface-activated charcoal-topped pots (Eizenberg *et al.*, 2004). No *P. aegyptiaca* parasitism was observed on the roots of plants treated with herbicide post-emergence, as opposed to high infestation level of plants grown in the charcoal-topped pots. The main activity of the sulfonylurea herbicides was manifested in the soil. The same results were obtained in *in vitro* studies in polyethylene bags (PEB), where the herbicide was highly effective when applied directly to the root zone, but failed to control the parasite when applied only to tomato foliage (Plakhine *et al.*, 2001).

To extend the arsenal of effective sulfonylurea herbicides and rank their potential in controlling *P. aegyptiaca*, several herbicides were applied directly to preconditioned or germinating seeds in Petri dishes. The tested herbicides included bensulfuron, chlorsulfuron, nicosulfuron, primisulfuron, rimsulfuron, thifensulfuron, triasulfuron (Hershenhorn *et al.*, 1998a), sulfosulfuron, flazasulfuron, ethoxysulfuron and rimsulfuron (Plakhine *et al.*, 2001). All of these herbicides significantly reduced *P. aegyptiaca* germination (except nicosulfuron) and radical elongation (except bensulfuron) (Hershenhorn *et al.*, 1998a; Plakhine *et al.*, 2001).

The relationship between *P. aegyptiaca* seed depth and parasitism and the effect of herbicide application on control at depths of 0–30 cm were studied under controlled conditions. *Phelipanche aegyptiaca* emergence was highly correlated with seed depth; the deeper the seeds were buried, the longer the delay in shoot emergence. A four-parameter sigmoid equation described this correlation. No *P. aegyptiaca* shoots were observed above ground when sulfosulfuron was applied to the tomato foliage. However, live and dead attachments were observed at different levels in treatments at all seed depths. No significant difference

in the efficacies of sulphosulfuron at rates of 37.5 and 75 g active ingredient (a.i.) ha^{-1} were observed at depths of 6, 12 or 18 cm. The highest level of control was obtained at a depth of 6 cm at both sulfosulfuron rates. At depths of 24 and 30 cm, no control was achieved with 37.5 g a.i. ha^{-1} sulfosulfuron, and only low control efficacy was obtained at 75 g a.i. ha^{-1} (Eizenberg *et al.*, 2007).

Foliar application of systemic herbicides

The post-emergence herbicide application concept for *P. aegyptiaca* control includes the various application methods described in Fig. 1. Two main issues need to be considered when using systemic herbicides that are translocated through the host phloem to the attached parasite, which serves as a strong sink. One is that the herbicide will not lose its activity while in the plant tissues, therefore, *P. aegyptiaca* underground development should be monitored because the very low rates used will not be effective if the attachments are too large. The other is that if the herbicide is applied too early, new attachments forming long after herbicide application will not be controlled. With these issues in mind, a predictive model for tomato control was recently developed (Eizenberg *et al.*, 2008). It was determined that foliar herbicide application for *P. aegyptiaca* control requires lower herbicide rates than those used for direct weed control. Low rates of imazapic and imazamox applied to tomato foliage were effective for *P. aegyptiaca* control (Table 1). However, these two herbicides injure the reproductive meristems of tomato plants, specifically, the flowers and fruit buds. Thus, to reduce the risk of damage, the herbicide should be applied after termination of fruit set. If imazapic or imazamox are applied on tomato foliage 45 days before harvest or later, the damage is prevented (Eizenberg *et al.*, 2008).

Developing a decision support system for Phelipanche aegyptiaca management in processing tomato

The integration of approaches 'Application of herbicides to the soil' and 'Foliar application of systemic herbicides' has been tested over the last 10 years in many field experiments, producing excellent results for the control of *P. aegyptiaca* in processing tomatoes applying sulfosulfuron through the soil solution and imazapic on tomato foliage. These experiments demonstrated that the efficacy of *P. aegyptiaca* control is highly correlated with the rate and timing of herbicide application. Based on these data, a decision support system (DSS) termed PICKIT for *P. aegyptiaca* control in processing tomato was developed. PICKIT is based on risk assessment and the following submodels: (i) growing degree days and (ii) herbicide rate optimisation. Both models were validated under field conditions using a minirhizotron camera (Fig. 2) (Eizenberg *et al.*, 2005). The alpha version of PICKIT will be evaluated under commercial processing tomato field conditions in the summer of 2009 (Eizenberg *et al.*, 2008).

Conclusions

Twelve years of research have led to the development of a successful chemical approach to *P. aegyptiaca* control in processing tomato. Farmers have adopted the DSS methodology of PICKIT and implemented it in *P. aegyptiaca*-infested tomato fields.

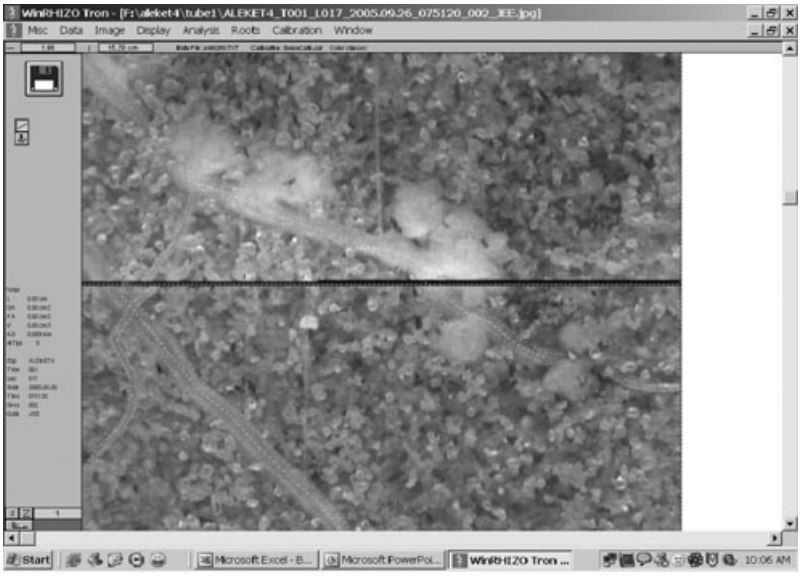

Fig. 2 Minirhizotron observation of *Phelipanche aegyptiaca* attachments on tomato roots.

The DSS PICKIT recommends the use of sulfonylurea herbicides, which are highly vulnerable to the development of sulfonylurea-resistant races. Unfortunately, this group of herbicides is the only group available for *P. aegyptiaca* control in tomato because of its high selectivity for tomato, as well as it high efficacy in controlling the parasite.

The next generation of *P. aegyptiaca* management approaches in tomato should take the environment into consideration by encouraging reductions in herbicide use. One of the most promising directions is the precision-agriculture approach of site-specific weed management. In this approach, herbicide is applied only in the infested area according to the spatial variation of *P. aegyptiaca* infestation in the field.

Biological control

Biological control is particularly attractive for the suppression of root parasitic weeds in annual crops because of the high specificity of the fungal pathogens used as biocontrol agents (Gressel, 2003).

Most fungi that demonstrate high potential as mycoherbicides for *Phelipanche* and *Orobanche* spp. control are *Fusarium* spp. (Amsellem *et al*., 2001b; Shabana *et al*., 2003; Nemat Alla *et al*., 2007). *Fusarium arthrosporioides* and *F. oxysporum* have been found to be pathogenic to *P. aegyptiaca, O. cernua* and *P. ramosa* (Amsellem *et al*., 2001b; Cohen *et al*., 2002). *Fusarium solani*, *Alternaria alternata*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Bacillus* spp. were isolated from *P. aegyptiaca*. A strain of *F. moniliforme* was isolated from *O. cumana* parasitising tomato plants in a PEB system (*O. cumana* is able to parasitise tomato roots under specific conditions in the PEB system) in Israel (Hershenhorn *et al*., 2004; Dor & Hershenhorn, 2009). However, only *F. solani* was recommended for further evaluation because of its high level of pathogenicity and specificity for *Phelipanche* spp. (Dor & Hershenhorn, 2009). More than 50 isolates belonging to 16 different species were isolated from *P. ramosa* in Southern Italy, 12 of them belonging to the genus *Fusarium*, with 17 *F. oxysporum* and 18 *F. solani* strains. Among them, one isolate of *F. oxysporum* and one of *F. solani* showed promising results for *Phelipanche* biocontrol (Campagna & Rapparini, 2002; Boari & Vurro, 2004). Two isolates of *F. oxysporum* were isolated from *P. ramosa* and *O. crenata* in Egypt and were found to be pathogenic (Nemat Alla *et al*., 2007). The only non-*Fusarium* species that demonstrated an acceptable level of control as a mycoherbicide against *Orobanche* spp. was a strain of *Ulocladium botrytis*. This fungus was isolated from *O. crenata* in Egypt and significantly reduced *O. crenata* seed germination and caused disease in *O. cumana* parasitising sunflower in a glasshouse experiment. The isolate had no effect on the development of *O. crenata* or *P. aegyptiaca* attached to the roots of tomato plants, and was therefore only proposed as a biocontrol agent for *O. cumana* (Müller-Stöver & Kroschel, 2005).

Overall, *Fusarium* spp. are the most prominent species associated with diseased broomrapes and of these, *F. oxysporum* is the predominant species. *Fusarium solani* was isolated and tested only by Boari & Vurro (2004) in Italy and by Dor and Hershenhorn (2009) in Israel as potential candidates for *P. aegyptiaca* and *P. ramosa* control in tomato. As soil-borne fungi, *Fusarium* spp. possess several advantages that make them suitable for the bioherbicide approach (Sauerborn *et al*., 2007). In the soil, they are relatively protected from the environmental stresses of drought and heat that commonly prevail in areas of *Phelipanche* and *Orobanche* distribution. The saprophytic nature of *Fusarium* spp. allows them to be cultured in liquid as well as solid media. In addition, soil-borne fungi, like *Fusarium* spp., are preferable for the biocontrol of root parasites, such as *Phelipanche* and *Orobanche* spp., because broomrapes exert their greatest damage prior to emergence of their flowering shoot. Therefore, high priority should be given to targeting the parasite in its initial underground developmental stages.

Despite the effort invested in determining and enhancing the efficacy of broomrape biocontrol agents, at present, there is no commercial product used to control this parasite. The main obstacle to the use and development of biocontrol agents is the poor field efficacy of the known pathogens. Soil-active biocontrol agents for *Phelipanche* and *Orobanche* must be able to contend with soil microorganisms without negatively affecting the host crop (Gressel, 2003). Several approaches to increasing the efficacy of broomrape biocontrol agents have been suggested.

Effective application methods

One of the main problems in using biocontrol agents is finding application methods that provide suitable environmental and nutritional conditions for the microbial agent, allow its uniform distribution and can be applied multiple times to improve control efficacy. Fungi can be applied in the field together with solid growth media (such as wheat, corn or rice grains), or in granules containing the biocontrol agent and nutrients. However, solid formulations have the serious drawback of limiting the number of applications to one and then only at planting. On the contrary, application of soil-borne fungi, such as *Fusarium* spp., in the field does not necessarily require nutrients in the formulation. In most of the experiments using *Fusarium* isolates to control

Phelipanche spp. on tomato, the biocontrol agents were applied in aqueous solutions (Amsellem *et al*., 2001a; Dor & Hershenhorn, 2003; Boari & Vurro, 2004). Therefore, the application of a *Fusarium* spp. suspension in water through the drip-irrigation system, which was proposed by Boari *et al*. (2008), may serve as a good alternative, allowing repeated fungal applications at an appropriate concentration. Conidial suspensions of *F. oxysporum* and *F. solani* pathogenic to *P. ramosa* and *O. crenata* were shown to pass freely through irrigation filters and the narrow passages of commercial drip irrigation systems in tomato fields (Boari *et al*., 2008). Microbigation may serve as an excellent alternative for repeat applications of biocontrol agents, providing them with the moisture that in most cases is the main limiting factor in biological control success.

Multiple pathogen applications using a mixture of two or more fungi

This approach (Charudattan, 2001) has been applied successfully for the control of *O. cumana* in sunflower (Dor & Hershenhorn, 2003). However, application of a mixture of two *F. solani* isolates, one from Israel (Fs) and the other from Italy (ET4), did not increase the effectiveness of the mixture against *P. aegyptiaca* on tomato, relative to each fungus alone. Addition of *F. oxysporum*, isolated from *O. crenata* in Italy (FT2), to *F. solani* completely prevented the damage caused by *F. solani,* indicating a strong antagonistic effect between these two fungi (Dor & Hershenhorn, 2003). It may be that successful pairs of fungi with a synergistic effect against *Phelipanche* and *Orobanche* spp. are the exception rather than the rule.

Mycotoxins

Toxins produced by phytopathogens can play an important role in pathogenicity and virulence processes (Goyer *et al*., 1998; Arnold *et al*., 2005; Kers *et al*., 2005). Therefore, knowledge of the chemical nature of the toxins and the dynamics of their production could contribute to our understanding of their pathogenicity and virulence, and of their relevance to the relationship between the fungus and the host plant. *Fusarium* spp., the most prominent fungi associated with diseased broomrapes, produce a range of toxic compounds – fusaric acid (FA), fumonisins, beauvericin, enniatin, moniliformin and trichothecenes (Abbas *et al*., 1991; Bacon & Hinton, 1996; Capasso *et al*., 1996; Amalfitano *et al*., 2002; Idris *et al*., 2003; Hershenhorn *et al*., 2004). Many of these toxins show a variety of metabolic effects, including phytotoxic activities that cause necrosis, chlorosis, growth inhibition, wilting and inhibition of seed germination, and have been proposed as mycoherbicides. In addition, an evaluation of the *in vitro* production of toxic metabolites could provide a basis for selecting the most aggressive strains for use in biocontrol (Amalfitano *et al*., 2002). More than 50 fungal strains, most of them *Fusarium* spp., were isolated from *P. ramosa* seeds. The correlation between their pathogenicity and virulence towards this parasite on tomato plants and their ability to produce toxins was tested to use the toxic metabolites as biomarkers for selection of the most active strains. FA was proposed as such a biomarker (Amalfitano *et al*., 2002). On the contrary, some metabolites, such as moniliformin and beauvericin, produced by fungi, especially *Fusarium* spp., have been found to be toxic to humans and animals (Wakulinski, 1989; Van Asch *et al*., 1992; Desjardins *et al*., 1998). Fungal production of compounds that are toxic to humans may jeopardise or completely prevent the use of such agents for biological control.

Genetic engineered biocontrol agents

The use of biocontrol agents engineered with hypervirulence genes, such as genes encoding enzymes that degrade metabolites involved in plant defence mechanisms or coding for the production of fungal toxins, has been suggested (Gressel, 2002). *Fusarium arthrosporioides*, a specific pathogen of *P. aegyptiaca*, transformed with the *NEP*1 gene greatly improved virulence towards its host, with little or no damage caused by the phytotoxic product to tomato plants (Amsellem *et al*., 2002; Gressel, 2003). A higher level of virulence can be achieved by transferring factors to the microorganisms that will tip the evolutionary balance in their favour, with the knowledge that if they are truly successful, seasonal applications will be required. *F. oxysporum* and *F. arthrosporioides* were transformed with two genes of the indole-3-acetamide pathway leading to indole-3-acetic acid (IAA), in an attempt to enhance virulence (Cohen *et al*., 2002). The transformants produced more IAA and were more effective at suppressing the number and size of broomrape shoots on tomato plants grown in soil mixed with *P. aegyptiaca* seeds than the wild type. Because of the fear of spread of transgenic organisms and the additional fear that genes will introgress from an *Phelipanche* or *Orobanche*-specific *Fusarium* to crop pathogens, measures to prevent the distribution of transgenic microorganisms should be applied. Hypervirulent host-specific organisms that disappear after killing their host have the advantage here. They do not leave residues in the soil and the likelihood of changes evolving in the host is reduced (Gressel, 2000). A double failsafe mechanism was recently proposed: (i) to generate asporogenic mutants that can be applied only as

formulated mycelia and (ii) to flank the hypervirulence transgene with transgenes that are neutral vis-a-vis *Orobanche*, but are deleterious to crop pathogens (anti-melanin genes, anti-appressorial genes, etc.) (Gressel, 2000, 2002, 2003).

Conclusions

The final goal in improving biocontrol efficacy is the production of an effective, easy-to-use commercial product. To reach this goal, efforts should focus on methods that enable multiple (repeated) applications, formulations that provide long shelf life and high control efficacy, fungi that produce high levels of phytotoxins for high virulence and strong competitive ability in the soil, and the transformation of biocontrol agents with hypervirulence genes. A biocontrol product that combines all of these components could likely be marketed as an efficient and easy-to-use product.

Tomato resistance to *Phelipanche* and *Orobanche*

Efforts to understand different crops' resistance to *Phelipanche* and *Orobanche* spp. have revealed the independent or coordinated induction of several different defence mechanisms. These include low stimulation of broomrape seed germination, Pre-haustorial resistance, phytoalexin induction, high levels of peroxidase activities, lignification of host endodermis and xylem vessels, cell wall deposition, development of an encapsulation layer in the cortical parenchyma, induction of pathogenesis-related proteins, and sealing of host xylem vessels by deposition of mucilage. Callose deposition and reactive oxygen species production and accumulation are also typical responses to biotic and abiotic stresses and are involved in the interaction with parasitic plants. (Dörr *et al*., 1994; Echevarria-Zomeño *et al*., 2006; Joel & Portnoy, 1998; Goldwasser *et al*., 1999; Labrousse *et al*., 2001; Serghini *et al*., 2001; Pérez-de-Luque *et al*., 2005a,b, 2006a,b;). Recently, Lejeune *et al*. (2006) demonstrated the involvement of a *Lycopersicon esculentum* wall-associated kinase in the interaction of *P. ramosa* and tomato roots that triggers the activation of defence reactions.

Much of the effort to find resistant tomato genotypes has not had any appreciable outcome (Dalela & Mathur, 1971; Abu-Gharbieh *et al*., 1978; Foy *et al*., 1988; Qasem & Kasrawi, 1995; Avdeyev *et al*., 2003; El-Halmouch *et al*., 2006). The most promising tomato line reported to date as resistant to *P. ramosa* and *P. aegyptiaca* was obtained in Russia (Avdeyev & Scherbinin, 1977). However, this line failed to show resistance in other locations (Foy *et al*., 1987; Y. Goldwasser, pers. comm.; J. Hershenhorn, unpubl. obs.). Nevertheless, Avdeyev *et al*. (2003) reconfirmed this observation and the resistance shown by this tomato line (PZU-11) has since been used in breeding programmes to introduce broomrape resistance in tomato varieties destined for growth in the southern regions of the Russian federation.

As the genetic variability available to support extensive resistance breeding programmes in existing tomato cultivars is small, several approaches have been taken to improve the situation: (i) search for resistance in wild tomato relatives and (ii) application of different mutagenesis methods to create a mutated population that extends the natural genetic variation. In the first approach, although no sources for high resistance to broomrape have so far been declared, attenuation in susceptibility has been observed. Significant variation in *L. esculentum* genotypes for root exudates that affect broomrape seed germination has been reported (El-Halmouch *et al*., 2006). In this study, it was demonstrated that this trait is influenced by the age of the host plant (e.g. germination-stimulating capacities peaked at 4 weeks for *L. hirsutum* and 6 weeks for 'Tresor' and 'Momor'), indicating variations in stimulant production. Wild tomato relatives (*L. pimpinellifolium hirsute*, *L. pennellii* LA 716, *L. chilense* LA 1969 and *L. hirsutum* PI 247087) showed differences in susceptibility because of attenuation of broomrape seed germination. Interestingly, when *L. pennellii* roots grown hydroponically were exposed to germinating broomrape seeds, the percentage of tubercle necrosis on *L. pennellii* roots reached 91.7%. It was concluded that *L. pennellii* could resist boomrape by two different ways: low stimulant exudates and inhibition of tubercle development, most probably because of necrotic induction on these tubercles (El-Halmouch *et al*., 2006)

Conventional mutation techniques are often used to improve crop yield, quality and resistance to diseases and pests, or to increase the attractiveness of flowers and ornamental plants. In an effort to expand the natural variation, large numbers of tomato M_2 (second generation of the mutagenised seeds) plants created by ethyl methane sulphunate (EMS) mutagenesis were screened for resistance to *P. ramosa* L. (Kostov *et al*., 2007). Six lines with significantly increased levels of resistance were identified in both greenhouse and PEB screening systems. Recently, a successful screening programme to locate a resistant tomato line from a fast neutron-mutagenised M_2 tomato population was reported in Israel (Hershenhorn *et al*., 2005). The screening procedure included screening the M_2 generation in a heavily broomrape-infested field. The resistant M_3 and M_4 generations were screened in pots in the glasshouse and M_5 was screened again in a heavily *P. aegyptiaca*-

infested field in the Golan Heights. The *Lycopersicon esculentum Phelipanche Resistant Trait 1* (*Sl-ORT1*) mutant was self-propagated for another two generations for homogeneity. *Sl-ORT1* showed the highest resistance to *P. aegyptiaca* under both field and greenhouse conditions when compared with its parental line M82. The number of emerging *P. aegyptiaca* inflorescences on *Sl-ORT1* plants was significantly lower than on the wild type (Hershenhorn, 2006). Based on this successful example, it is clear that resistant tomato varieties may be developed by screening mutagenised populations.

Conclusions

Mutants have been proved important either for establishing biochemical pathways or to identify the physiological role of mutated genes and processes. Cloning of the corresponding genes has often provided important, and sometimes unique, information on the mode of action of these genes and offered the possibility of reintroducing these genes into plants, thereby overexpressing or underexpressing the genes. Not much work has been published so far on broomrape-resistant tomato varieties. However, it should be kept in mind that resistance in such varieties may be subjected to rapid breakdown because of broomrape variation and flexibility (Pharan *et al.*, 1997; Verkleij *et al.*, 1991; Verkleij & Pieterse, 1994). More resistant mutants should be isolated that control different steps of the *P. aegyptiaca*–host interactions. Nevertheless, other strategies to delay, or even prevent, this breakdown should be incorporated in the management strategy in *P. aegyptiaca*-resistant tomato fields (e.g. other control methods described in this chapter). At the same time, continuous effort should be invested in developing the next resistant tomato generations.

Disinfection of agricultural equipment

The tiny seeds of *Phelipanche* and *Orobanche* spp. are produced in vast numbers and are easily dispersed by wind, water, animals and humans. Human practices are a major factor in distributing broomrape seeds, including the transport and use of contaminated agricultural materials, vehicles, farm implements and produce containers (Parker & Riches, 1993). Once seeds reach a new site, they remain viable for many years and can ignite a new infestation outbreak once they meet a potential host.

A common means of seed spread and a significant source of new infestations is the seeds' adherence to agricultural equipment that is transported from infested to non-infested fields. Although short- and long-range spread of seeds by agricultural equipment is a significant source of new parasitic infestations, publications regarding seed eradication on farm equipment are scarce. First reports of the efficacy of quaternary ammonium products in killing parasitic weed seeds were reported following the Federal Striga Eradication Program conducted for infestations in North and South Carolina, USA (Eplee, 1992). A National Branched Broomrape Eradication Program was established in 2000 to eradicate *P. ramosa* (branched broomrape) from southern Australia. In this programme, the commercial product 'NiproQuat', containing 120 g L^{-1} didecyl dimethyl ammonium chloride at 1% (w/v) effectively controlled *P. ramosa* seeds on farm vehicles and equipment (J. Virtue, pers. comm.; Panetta & Lawes, 2005).

Preliminary laboratory studies

Ammonium-containing solutions were screened for their ability to kill *P. aegyptiaca* seeds in a Petri dish in the laboratory. Soaking *P. aegyptiaca* seeds for 5 min in 1% (w/v) ammonium chloride or 1% (w/v) ammonium bromide completely inhibited their germination (Kleifeld *et al.*, 1994).

Alkyl dimethyl benzyl ammonium chloride

The commercial product 'Zoharquat 50', containing 50% (w/v) of an alkyl dimethyl benzyl ammonium chloride, is a bactericidal and fungicidal agent. It can also be used efficiently for the control of algae in swimming pools and cooling towers.

Zoharquat 50 was used to disinfect *P. aegyptiaca* seeds on a tomato harvester. Seeds were placed in nylon mesh bags on different parts of the harvester and then the harvester was sprayed with a hand-held sprayer until drainage. Zoharquat 50 at 1% (w/v) caused an average 20% reduction in seed germination tested by the artificial stimulant GR24 in the laboratory. Increasing the application rate to 10% resulted in complete inhibition of seed germination in most parts of the harvester (Hershenhorn *et al.*, 2007).

Didecyl dimethyl ammonium bromide

Didecyl dimethyl ammonium bromide (DDAB) is a twin long-chain quaternary ammonium disinfectant with a broad activity spectrum which proved to be effective against bacterial, viral and fungal pathogens of both veterinary and human importance. The commercial product 'Bromosept 50', containing 50% (w/v) DDAB, was tested for *P. aegyptiaca* control in the laboratory, a spray chamber and a commercial disinfecting facility.

Eppendorf-tube studies

Phelipanche aegyptiaca seeds were soaked for 5 min in 0.01–1.00% (w/v) DDAB in 1.5 mL Eppendorf tubes.

Seeds were then washed, plated on 6 mm Whatman GF/A Glass Microfibre Filter paper discs, placed on 5 cm diameter filter paper in a Petri dish, moistened and kept for 7 days of preconditioning period at 25°C in a dark room. All treatments were replicated five times. After 7 days, 5 mg L^{-1} GR24 was applied and 5 days later, germination was determined. Results of the laboratory studies showed that soaking of *P. aegyptiaca* seeds in DDAB at 0.1% and higher concentrations for 5 min produces complete *P. aegyptiaca* seed kill.

Spray-chamber studies

Phelipanche aegyptiaca seeds in sealed mesh bags were placed on metal trays and sprayed with 0.02–1.00% DDAB in a laboratory spray chamber. Nine hours later, bags were washed and treated and put under the same conditions as in the Eppendorf tube experiment. *Phelipanche aegyptiaca* seeds were completely controlled by 1% DDAB, via complete inhibition of germ-tube germination, as observed under a stereoscopic microscope. DDAB applied at 0.2% resulted in 24% of *P. aegyptiaca* seed germination and when DDAB was applied at 0.02%, 43% of *P. aegyptiaca* seed germinated. In the untreated control, 81% of *P. aegyptiaca* seeds germinated (Fig. 3).

Experiments in a commercial disinfecting facility

Phelipanche aegyptiaca seeds in sealed mesh bags were placed in different parts of 6.9 m long, 1 m high tomato harvest container. Three bags were placed on each wall/floor of each container, and treated with 20 L volume per container of 0% (water only), 0.5% and 1.0% DDAB in a commercial disinfecting facility prototype in Gan Shmuel (Fig. 4). After 9 h, bags were washed, treated and placed under the same conditions as in the Eppendorf-tube and spray-chamber experiments. DDAB at 1.0% and 0.5% yielded complete control of *P. aegyptiaca* seeds placed on the walls and floor of tomato containers (Table 2).

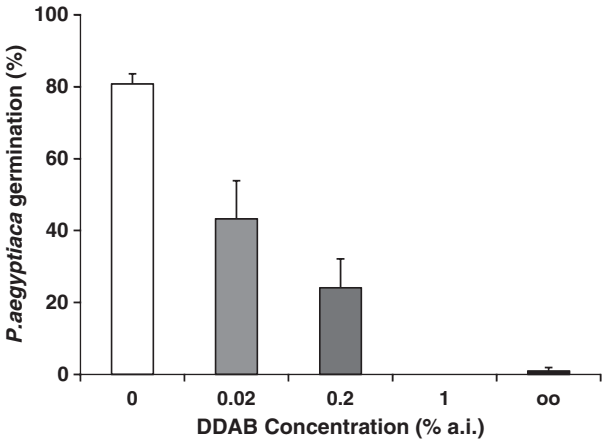

Fig. 3 *Phelipanche aegyptiaca* germination following DDAB application in the spray-chamber study. oo, water control. Error bars represent standard error of the means.

Fig. 4 Commercial disinfecting facility at work at Gan Shmuel. The arrow points on the sealed mesh bags containing *Phelipanche aegyptiaca* seeds placed on the container wall.

Conclusions

The characteristics of *Phelipanche* and *Orobanche* spp. seeds account for much of the difficulty in controlling these parasitic weeds. Extermination of seeds before their spread to new fields and regions is a crucial component in broomrape prevention programme (Panetta & Lawes, 2005). Quaternary ammonium compounds have been found effective in *Phelipanche* and *Orobanche* spp. seed eradication. The broad-spectrum quaternary ammonium disinfectant DDAB proved to be efficient at controlling *P. aegyptiaca* seeds on surfaces under controlled and commercial conditions. Complete seed kill was achieved under commercial conditions using 0.5% a.i. DDAB. Further studies are needed to repeat the results of the commercial experiment and improve coverage of all parts of the tomato container.

Table 2 *Phelipanche aegyptiaca* germination on different parts of a tomato container following treatment with DDAB in the commercial disinfecting facility at Gan Shmuel

	Control	DDAB (0.5%)	DDAB (1.0%)
Floor	56.4	0	0
Outer back wall	58.8	0	0
Inner side wall	52.6	0	0
Outer side wall	50.3	0	0
Control – no GR24	0	0	0

Conclusions and future directions

In this review, we discuss the most advanced methods for *Phelipanche* and *Orobanche* spp. control in tomato. Some of these methods are widely used commercially by farmers in Israel (chemical control), some are in the final stages of development towards commercialisation (resistant varieties and sanitation), and some need further development and improvement before commercial implementation (biological control). *Phelipanche* and *Orobanche* spp. demonstrate a high level of genetic flexibility and it is hypothesised that each of these control methods will be overcome in a relatively short time, if used alone. However, combining these methods in an integrated management approach will contribute to control efficacy as well as protect against the potential breakdown of each control method used separately. Further research is needed to optimise the timing and synchronisation involved in combining the use of these methods, taking into consideration efficacy, cost, ease of use and environmental aspects.

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