An overview of a new technique in *Orobanche* control

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Abstract

***Orobanche* species (broomrapes) are root parasites which cause serious losses in several economic broadleaved crops. Various methods have been developed for broomrape management; these include mechanical, cultural, biological, genetic and chemical control. A new approach to *Orobanche* control is the use of synthetic germination stimulants. Several strigol analogues (GR compounds) have been found to stimulate the germination of *Orobanche* seeds. The highest activity occurred at concentrations of 0.1 to 1 ppm. In vitro soil studies showed a decrease in the activity of GR24 with an increase in soil pH. Greenhouse studies using GR7 applied at 1 and 3 ppm, six weeks before transplanting tomatoes into broomrape inoculated pots, resulted in significant reductions in the dry weight and number of shoots of *Orobanche* and increased fruit yield of the crop. Field applications of GR7 at 0.3 kg ha^{-1} gave good control of *O. crenata* in faba beans grown in acid soils; whereas the application of 1.5 kg ha^{-1} of the stimulant controlled *O. ramosa* in alkaline soils. It may be concluded that these stimulants have good potential to become an important tool for the control of *Orobanche*.**

Introduction

Broomrapes (*Orobanche* spp.) are noxious root-parasitic weeds which cause considerable losses in many broadleaved crops. The seriousness of these parasites emanates from the difficulty of controlling them. Various measures have been employed for *Orobanche* control; these are mainly managerial, biological, genetic and chemical.

Managerial methods tested include deep sowing of crop seeds below the infested soil horizon (Kott 1969), delayed crop sowing (van Hezewijk *et al.* 1987) ploughing at a depth of 40–50 cm to bury the parasite seeds (Kasasian 1973), the use of the "spear", an implement which is manually operated to cut young broomrape unflowered shoots up to 5 cm deep or more in the soil (Krishnamurthy and Nagarajan 1991), and the use of various fertilizers particularly nitrogen (Abu-Irmaileh 1979, ter Borg 1986, Jain and Foy 1987). Another measure is the use of catch crops (natural parasite hosts) and trap crops (non-hosts). These stimulate parasite seed germination, but do not allow the resultant plant to complete its life cycle (Krishnamurthy *et al.* 1977, Musselman 1980, Al-Menoufi 1991). Solar heating of the soil (solarization) through covering it with polyethylene mulches, has also provided good control of *Orobanche* spp. (Jacobsohn 1980, Sauerborn and Saxena 1987, Abu-Irmaileh 1991a, b, Linke *et al.* 1991).

Biological control measures were also tested using fungi such as *Fusarium orobanche*, (Kott 1969), and insects such as *Phytomyza orobanchiae* which proved to be very effective against the parasite (Kapralov 1974, Lekic 1974, Mihajlovic 1986, Horvath 1987).

Success in the control of *O. cumana* in sunflower was achieved by the use of resistant cultivars (Omrior and Sharova 1969, Iliescu 1973, Cherzhantseva 1978, D'yakov and Antonova 1978, Buchvarova and Velkov 1979). Selection for resistance to broomrape has only been done in a small number of host species (Cubero 1991). However, it was recently reported that some of these cultivars had lost their resistance to the parasite (Buchvarova and Velkov 1979). Screening of 108 tomato cultivars at the University of Jordan, Amman, revealed that eight of them were slightly tolerant to *O. ramosa* parasitism (Abu-Gharbieh *et al.* 1978). Foy *et al.* (1987) screened the world collection of tomato germplasm and found that all lines were susceptible to broomrape to varying degrees. In the case of other host plants, several broadbean cultivars were reported to be resistant to *O. crenata* (Cubero *et al.* 1988); however, none of the hundred lines of lentils screened at ICARDA, Aleppo, Syria, was found to be resistant (Basler and Haddad 1979). Significant differences were found among tobacco lines with regard to tolerance to *O. ramosa* in Turkey (Emiroglu *et al.* 1987). Since the cost of developing new lines or cultivars with resistance to certain herbicides is much cheaper than the cost of developing new herbicides, breeding for herbicide resistance becomes economically attractive (McWhorter 1984). Wegmann *et al.* (1991) explained the fundamental biochemical mechanisms of resistance and tolerance to *Orobanche*. They showed that tolerance was due to higher osmotic potential in the tolerant host plant, whereas resistance can be caused by the formation of phytoalexins in response to *Orobanche* infection .

Many chemicals have been tested for possible control of *Orobanche* in several crops (Garcia-Torres *et al.* 1987, Garcia-Torres 1991, Nemli *et al.* 1991). The fumigant methyl bromide was found to give satisfactory results, but was uneconomical to use on a large scale (Wilhelm *et al.* 1959, James and Frater 1977). Of many herbicides tested for *Orobanche* control, only glyphosate at sublethal doses has given consistent control of *Orobanche crenata* in *Vicia faba* (Kasasian 1974, Saghir 1977, Basler 1979, Schmitt and Weltzien 1979, Schluter and Aber 1980, Zahran *et al.* 1981, Abdalla *et al.* 1983, Khalaf 1991, El-Masry *et al.* 1991). In tomato, CGA 14397 (N-propyl-N-tetrahydrofurfuryl-4-trifluoromethyl-2,6-dinitroaniline), was shown to give selective control of *O. ramosa* in greenhouse experiments (Saghir *et al.* 1973), and low doses of glyphosate controlled *Orobanche*, but was phytotoxic to tomato (Janudi and Saghir 1984). In the case of sunflower, *O. cumana* was successfully controlled with glyphosate application (Petzoldt and Sneyd 1986) whereas glyphosate was not safely tolerated by the sunflower crop when used to control *O. cernua* (Castejon *et al.* 1987).

Linke and Saxena (1991) combined several single methods into an integrated control system. The elements of this integrated management program included the use of less infected and early maturing cultivars, slightly delayed sowing, application of herbicide, hand weeding and soil solarization. This system gave efficient control of *Orobanche* spp. in some legume crops in a Mediterranean environment.

Synthetic germination stimulants

A new approach to broomrape control is the use of synthetic germination stimulants known as strigol analogues or GR compounds. These are analogues of the natural germination stimulant of *Striga* seeds named strigol (Cook *et al.* 1972). These compounds induce the germination of *Orobanche* seeds in the absence of a host which normally secretes the stimulant. Being obligate parasites, the germinated parasite seeds will be unable to support their growth and will eventually die, thus reducing the number of their viable seeds in the soil.

Strigol was isolated from cotton root exudates, and was able to induce more than 50% seed germination in *Striga lutea* at a concentration of 10^{-11} M. Johnson *et al.* (1976) at Sussex University in the United Kingdom, first reported on the synthesis of strigol analogues. These chemicals are characterized by having one or more unsaturated lactone groups (Al-Menoufi *et al.* 1987 b). Strigol analogue compound V initiated germination of *S. hermonthica* at concentrations as low as 0.1 ppm. Compound VII induced germination of

S. asiatica and *S. hermonthica* at concentrations of 7×10^{-5} ppm and above. Compound VIII was most active at 0.01 to 0.1 ppm, while compounds IX and X were less active than other strigol analogues. Under field experiments in boxes, the application of compound V at 10 ppm and compound VII at 5 to 10 ppm reduced *S. asiatica* infestation in sorghum. It was observed that the presence of the end ether function in the structure of the strigol analogue increased their activity in inducing the germination of *Striga* and *Orobanche* seeds. Compounds lacking this function were less active against both parasites.

It was observed that *Orobanche* seeds require a preconditioning period before they respond to a germination stimulant. Under laboratory conditions, preconditioning consisted of placing *Orobanche* seeds on 8 mm glass fibre paper discs over moist filter paper in 9 cm sterile petri dishes. Each disc contained 30–50 seeds. After preconditioning for 14 days at 25°C, the discs were transferred into other dishes which had a filter paper saturated with the strigol analogue being tested. The petri dishes were then incubated at 25°C for 12 days, after which the discs were examined under a binocular microscope, and the germination percentage of the seeds was determined.

In vitro studies

In vitro studies were conducted at the American University of Beirut, Lebanon, on the efficacy of various strigol analogues (GR compounds) on *Orobanche* seeds placed in the petri dishes. It was found that GR24 increased the germination of *Orobanche* seeds at all concentrations tested (0.001–10 ppm) as compared to the control (water + alcohol); no significant differences in germination were observed among the treatments at various reading dates (3–12 days) with maximum values recorded 12 days after treatment (Saghir 1986).

Maximum germination percentages were also obtained 12 days after treatment in the case of GR28. A consistent increase in germination was observed as the concentration used was increased between 0.001 to 1 ppm. The germination percentages decreased as the concentration of the stimulant was increased beyond 1 ppm (Saghir 1986).

Previous in vitro studies indicated that GR7 (Al-Menoufi and Zaitoun 1987a), GR28 and GR41 were most active at concentrations ranging from 0.1 to 1 ppm; GR45 showed its highest activity at 1 to 10 ppm, whereas GR53 gave higher germination of *Orobanche* seeds at a range between 1 and 100 ppm. All the aforementioned compounds tended to inhibit germination at concentrations higher than their respective optimal concentrations (ICARDA 1978).

GR24 at 0.01 to 10 ppm caused significant increases in the germination of *O. ramosa*, while a concentration of 100 ppm of the compound inhibited seed germination. Plotting the germination percentages against the concentration of the stimulant revealed a hormonal response curve; germination being reduced at very low as well as very high concentrations (Janudi 1982).

Activity in soil

In vitro soil experiments were carried out at the American University of Beirut using plastic cups filled with soil. Discs carrying the preconditioned *Orobanche* seeds were placed inside muslin cloth bags and then buried in the soil. Different methods of application of strigol analogues to the soil were tested. GR28 was applied in three treatments, either to the top of the soil, embedded as a layer, or incorporated in the soil. Results indicated that the stimulant was effective in stimulating germination of *Orobanche* seeds with all methods of application used, and at both concentrations tested (0.1 and 1 ppm). GR41 embedded in the soil gave maximum activity at 1 to 5 ppm (Saghir 1986).

Studies on the effect of soil pH on the activity of GR24 in different soils showed that a decrease in GR24 activity accompanied the increase in soil pH (Janudi 1982). This decrease in GR24 activity was probably due to cleavage of the enolic linkage under alkaline soil conditions. This conclusion agreed with earlier findings by Johnson *et al.* (1976), who reported a decrease in the activity of the stimulants as the pH was increased. Whitney (1986) explained that inactivation of stimulant in the soil was due at least in part to microorganisms but this effect varied with different soil types.

The activity of GR7 was tested at three moisture levels, namely soil saturation, normal field capacity and permanent wilting point. Maximum activity of the stimulant in the soil was observed under normal field capacity, with no activity being detected at the permanent wilting point (Saghir 1986).

In studies on the effect of soil texture on the activity of GR24, it was observed that the germination percentages of *O. ramosa* seeds were higher in sandy and loamy soils than in a clay soil. However, it was suggested that this was due to conditions which favoured seed germination in sandy soils, such as oxygen availability, rather than a direct effect on GR24 activity related to adsorption to clay particles (Janudi 1982). Seeds of *Striga hermonthica* responded more to ethylene in sandy soils than in a heavy soil (Babiker *et al.* 1987).

Greenhouse studies

In pot experiments on the control of *O. ramosa* in tobacco, compounds II and III were applied as 10 ppm solutions, 32 days before transplanting tobacco. Both germination stimulants failed to reduce *O. ramosa* parasitism (Puzzilli 1976).

Pot experiments conducted in the greenhouse of the University of Jordan, Amman, using tomato as a host plant, and treated with drench applications of GR7 at 1 and 3 ppm, six weeks prior to tomato transplanting, reduced significantly the number and dry weight of *O. ramosa* plants and increased fruit yield of the crop (Saghir *et al.* 1980). This waiting period would allow enough time for *Orobanche* seeds to germinate and die before the host was introduced. It was observed that waiting periods of less than four weeks between treatment and tomato transplanting were not sufficient; in these cases, no reduction in *Orobanche* infestation was obtained. In the case of GR24 applied at 1 and 2 ppm, four and six weeks prior to tomato transplanting, it was found that this synthetic stimulant reduced significantly the number of *Orobanche* haustoria and their dry weight compared to the untreated control (Janudi 1982).

Field studies

Research on GR7 in Egypt, indicated that the stimulant reduced *O. crenata* infestation in broadbeans (ICARDA 1978). A rate of 0.3 kg ha^{-1} was sufficient to reduce parasitism in acid soils (pH 4.5); whereas 1.5 kg of the stimulant was needed per hectare in alkaline soils (pH 8.0). This confirmed earlier reports by Johnson *et al.* (1976) that GR7 was short-lived under alkaline conditions.

Conclusion

It may be concluded that these synthetic compounds have good potential to become important tools for the control of *Orobanche*. The optimal range of active concentrations generally lies between 1 and 10 ppm. The rate and time of application prior to planting is governed by the soil pH. The duration of the waiting period should be long enough to allow *Orobanche* seeds to germinate and die before a host is introduced. Accordingly, a long waiting period should be allowed prior to planting at low soil pH, since the stimulant would not be deactivated rapidly and consequently more *Orobanche* seeds would be stimulated to germinate. However, with high pH, sequential applications of the stimulant over several weeks before planting might compensate for its rapid deactivation.

Additional research on the methods of application of the stimulants, length of the waiting periods, rates to be used under different soil pHs and textures is needed. Field trials that take into consideration the various ecological and biotic variables that usually occur under actual

production conditions should be conducted before definite recommendations can be made.

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