Crenate Broomrape Control in Pea by Foliar Application of Benzothiadiazole (BTH)

A. Pérez-de-Luque,*[,1] J.V. Jorrín[2] and D. Rubiales[1]

Parasitic plants are becoming a severe constraint on major agricultural crops in Mediterranean and tropical countries and the efficacy of available means of control is minimal. The problem is particularly severe in field pea, which is very sensitive to standard glyphosate treatments and in which little resistance has been identified. Systemic Acquired Resistance (SAR) has proven to be effective as a tool for controlling plant pathogens, including fungi, bacteria and viruses, but only recently has this phenomenon started to be evaluated as a control strategy against parasitic weeds. The present studies were conducted to evaluate the potential of SAR activation for broomrape control in pea. The effect of salicylic acid, glutathione and benzothiadiazole (BTH) in three different application methods was studied. Foliar application of 0.6–1.0 mM BTH, in the form of Bion 50 (50% a.i.), reduced broomrape infection under controlled conditions (growth chamber and greenhouse) by limiting the success in attachment and retarding the development of established tubercles.

KEY WORDS: Parasitic plants; broomrape; *Orobanche crenata*; SAR; BTH; induced resistance; pea; *Pisum sativum*.

INTRODUCTION

Broomrapes (members of the genus *Orobanche*) are parasitic plants that threaten agricultural production in many parts of the world. The most damaging species are *O. crenata, O. cumana, O. aegyptiaca, O. ramosa* and *O. minor*. *O. cumana* is an important constraint for sunflower production. *O. aegyptica* is very damaging in legumes, tomato, eggplant and potato among others. *O. ramosa* infects mainly tobacco, rapeseed, hemp and tomato. *O. minor* can be a problem on fodder legumes. *O. crenata* is the most dangerous and the most widespread *Orobanche* species in the Mediterranean region and western Asia. It is a major constraint for faba beans, field peas, lentils, vetches and various forage legumes (18,20). Several control strategies have been employed but none has enjoyed unequivocal success. The methods are not feasible, uneconomic, hard to achieve, or result in incomplete protection. The integration of several control measures is most desirable.

Resistance to *O. crenata* is scarce and complex in nature, making breeding for broomrape resistance a difficult task (4,22). This is particularly true for pea (*Pisum sativum* L.), in which little resistance is available (20). In addition, pea is very sensitive to the standard glyphosate treatment recommended for broomrape control in faba bean (23). Although some control can be achieved by imazethapyr treatment, it is not complete and its

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[1]Instituto de Agricultura Sostenible, CSIC, E-14080 Córdoba, Spain. *Corresponding author [e-mail: bb2pelua@uco.es].

[2]Dpto. Bioquímica y Biología Molecular, Universidad de Córdoba, Campus de Rabanales, E-14071 Córdoba, Spain.

efficacy is influenced by sowing date and environmental conditions (20). Thus, broomrape control ought to be approached by combining several control methods in an integrated management program. Alternative or supplementary methods for enhancing plant health or inducing the plant's defense system might be applicable for control of crenate broomrape.

Chemically induced resistance (IR) is a suitable strategy to utilize the natural defenses of the plant to control pathogens. The phenomenon has been studied at the molecular level and has proven to be mediated by salicylic acid and associated with a number of defense responses and genes (5). IR can be activated by exogenous application of salicylic acid or its synthetic functional analog benzo(1,2,5)thiadiazole-7-carbothioic acid S-methyl ester (BTH). Glutathione has also been reported to delay broomrape development on legumes (2). To date, SAR has been demonstrated to be effective against a broad range of pathogens, including bacteria, fungi and viruses (29). However, evaluation of its capability to control parasitic plants began just recently (25). The aim of the present work was to evaluate the potential of chemically induced resistance in the pathosystem pea / *O. crenata.*

MATERIALS AND METHODS

The pea cultivar 'Messire', highly susceptible to crenate broomrape (20), was used in this study. The peas were inoculated with an *O. crenata* population collected from infected fields at Córdoba in May 1998 and stored in the dark at room temperature. Seed viability was proven by germination in the presence of the synthetic strigol analog GR24 (50% germination).

Pot experiments Pea seeds were germinated for 5 days on wet fiber paper in petri dishes in the dark at 20°C. Seedlings were transplanted to 0.5 *l* pots containing a mixture of sand and vermiculite (3:1 by volume) mixed homogenously with 30 mg of *O. crenata* seeds. There were eight replicates per treatment. This experiment was carried out in a greenhouse at 20±3°C with supplemental light to give 16:8 L:D. Plants were watered when necessary and fertilized six times with modified Hoagland solution during the course of the experiment (12).

BTH [in the form of Bion 50 (50% a.i.) (Syngenta)], salicylic acid and glutathione (Sigma) were applied to the plants in three different treatments: (i) seed soaking, (ii) foliar spray and (iii) irrigation. (i) Pea seeds were germinated in petri dishes containing a 1 mM water solution of the corresponding compounds. Control seeds were germinated in water alone. (ii) 15-day-old seedlings were sprayed with an atomizer, with a 1 mM solution of each compound to which Tween 20 was added as wetting agent (6 drops per liter). Each plant received 1 ml of the solution. Two additional sprays were performed, at 20 and 30 days after transplanting (dat). Control plants received water plus Tween 20. (iii) Plants were watered with a 1mM solution of the corresponding compound in nutrient solution. Each plant received 100 ml of the solution. Two applications were given, at 20 and 40 dat. Control plants were watered with nutrient solution.

Two months after planting, plants were removed from the pots, the roots gently washed under tap water, and the number of broomrape attachments and their development stage recorded. Broomrape development stages were classified according to Ter Borg: S1, tubercles smaller than 2 mm; S2, tubercles bigger than 2 mm but without root formation; S3, tubercles with crown-root; S4, sprout already visible remaining underground; S5, shoot emergence; S6, flowering; S7, setting of seeds (31).

Petri dish experiments *In vitro* studies were performed in a petri dish experiment (21). Petri dishes (15 cm diam) were filled with perlite and covered with fiberglass paper (Whatman GF/A). Broomrape seeds, previously disinfested with ethanol (70%, 30 s) and bleach (20%, 20 min), were sprinkled all over the paper to give a density of ~15 seeds cm^{-2} and then conditioned for 10 days in the dark at 20°C (32). Pea seeds were germinated as described above. When pea roots reached length of 4–5 cm, plants were placed in the petri dishes with the roots in contact with the broomrape seeds. Petri dishes were sealed with parafilm, and wrapped in aluminum foil prior to vertical storage in trays in a growth chamber at 20°C, 14-h photoperiod, for 40 days. Hoagland nutrient solution was added to the trays.

Plants were sprayed with 0.6 mM BTH in either a single treatment at 5 dat or a double one, at 5 and 25 dat. There were ten replicates per treatment. In a preliminary experiment we observed signs of toxicity on the plants treated with BTH at the concentration used in pots (1 mM), so it was reduced to 0.6 mM. Plants sprayed with water were used as control.

At 20 dat, 300 broomrape seeds that were close (<3 mm) to the pea roots were studied per petri dish under a dissection microscope at 20× magnification. Germination percentage was determined by counting the number of seeds with an emerged radicle. Ten days later, the number of broomrape attachments per plant was recorded. Host root length was estimated by the intercept method of Tennant (30) and, in order to exclude effects due to differing amounts of roots produced per genotype, the number of broomrape attachments per plant was calculated as the number of attachments per unit host root length. The attachments were also classified according to their development stage. Finally, 40 dat the number of tubercles that became necrotic and died were recorded and expressed as a percentage of the total.

Field trials were conducted during the 1997–1998 and 1999–2000 seasons. Pea seeds were sown at the beginning of November, in a field with a heavy infestation of *O. crenata* known from previous seasons. The experimental units were 2-m-long rows of 20 plants, with 0.7 m inter-row spacing. Each test row was surrounded on four sides by rows of non-treated plants. Rows were arranged in a fully randomized design with four blocks of ten rows in each block. Plants were sprayed with a 1 mM solution of BTH using an atomizer. The first spray was done in mid-January, the second at the beginning of February and the third in mid-February. Each plant received 5 ml of the solution (100 ml per row). A few drops (5–6 per liter) of Tween 20 were added to the solutions. Non-treated plants were used as control.

At the end of the crop cycle, in May, the number of emerged broomrapes per pea plant was recorded and expressed as the percentage of emerged broomrape shoots in non-treated rows in order to avoid differences due to lack of uniformity of broomrape seeds in the field.

Statistical analysis Statistical analyses (ANOVA) were performed with SPSS 10.0 and Statistic (SX) 1.0 for Windows. Percentages were transformed according to the formula:

$$Y = arcsin(\sqrt{(X\%/100)})$$

RESULTS

The effect of 1 mM of salicylic acid, BTH (Bion 50, 50% a.i.) or glutathione on the biological cycle of the parasite was evaluated in pot experiments and three different

TABLE 1. Effect of BTH (0.6 mM) sprayed on pea leaves on *Orobanche crenata* infection in a petri dish experiment

BTH treatment	Germination (%)	Number of broomrape attachments per plant	Root length (cm)	Number of broomrape attachments per cm host root length	Necrotic attachments (%)
None (Control)	48.5 a[x]	241.3 a	281.4 a	0.88 a	7.5 a
Single[z]	46.9 a	194.5 b	238.7 a	0.83 ab	4.1 a
Double[y]		175.1 b	274.2 a	0.66 b	8.5 a

[z] 15 days after transplanting.

[y] 15 and 25 days after transplanting.

[x] Within columns, data followed by a common letter do not differ significantly (Duncan, $P<0.05$).

application methods: seed soaking, foliar spraying and irrigation. The 1 mM concentration was chosen because higher concentrations were toxic for the plants. Still, signs of toxicity were observed in pea plants irrigated with 1 mM of BTH and glutathione.

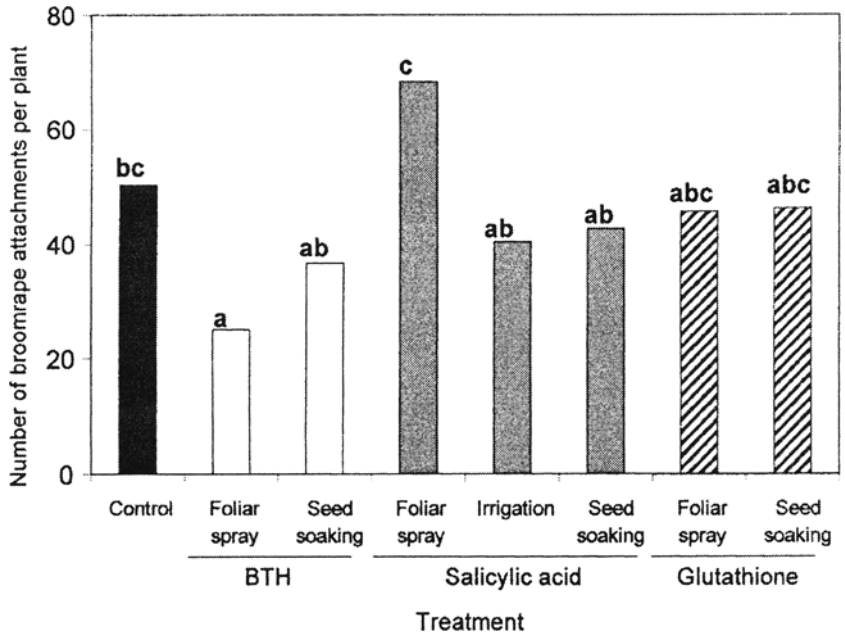

Fig. 1. Effect of 1 mM BTH, salicylic acid and glutathione applied by foliar sprays, seed soaking or irrigation on *Orobanche crenata* infection on pea in pot experiments. Columns with a common letter do not differ significantly (Duncan, $P<0.05$)

The number of attachments formed (Fig. 1), and their development stage (Fig. 2), were measured 2 months after transplanting. Up to 50 broomrape attachments per pea plant were recorded in control, non-treated plants. A significant reduction in the number of attachments was observed in BTH-sprayed plants, with fewer than 25 attachments per plant. No significant decrease in broomrape infection was observed after salicylic acid

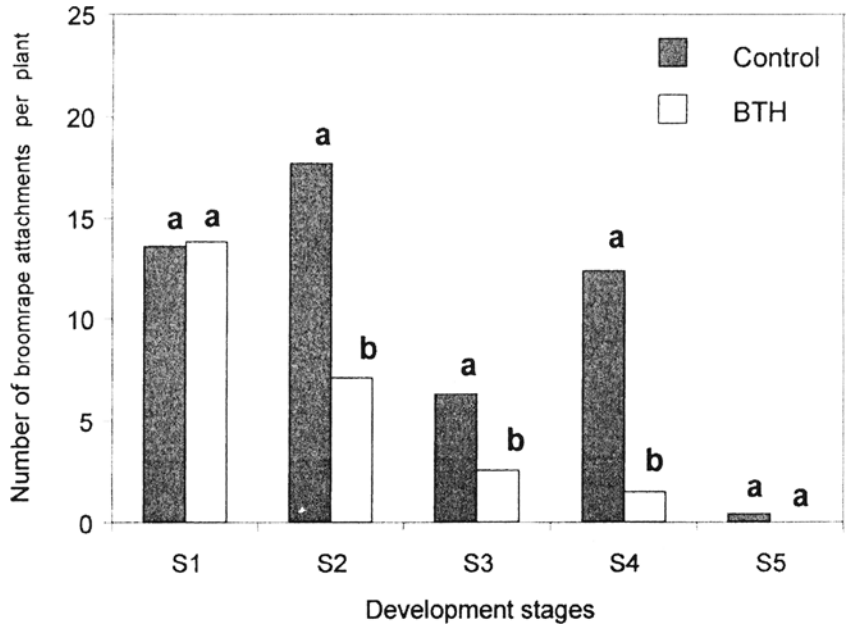

Fig. 2. Total number of broomrape attachments according to their development stage (S1–S5) determined in pot experiments. Plants were treated with 1 mM of BTH by foliar sprays and evaluated 2 months after *Orobanche crenata* infection. Within the same development stage, columns with the same letter do not differ significantly (Duncan, $P<0.05$).

TABLE 2. Effect of BTH, salicylic acid, or glutathione on *Orobanche crenata* after one, two or three foliar sprays with 1 mM compound to pea plants in the field

Season	Treatment	Number of applications	Emerged broomrapes as % of control[z]
1997–1998	Control		100.0
	BTH	3	75.4
1999–2000	Control		100.0
	BTH	1	70.6
		2	85.3
		3	78.9
	Salicylic acid	1	82.8
		2	85.0
		3	65.0
	Glutathione	1	95.1
		2	126.5
		3	98.0

[z]Evaluated at the end of the crop cycle. There was no significant difference between treatments except between BTH and control in the 1997–1998 season ($P<0.05$).

or glutathione sprays. Foliar application of BTH not only reduced the total number of broomrape attachments, but also retarded their development. Most attachments were at the S1 stage, and the number of those reaching stages S2–S5 was significantly reduced (Fig. 2).

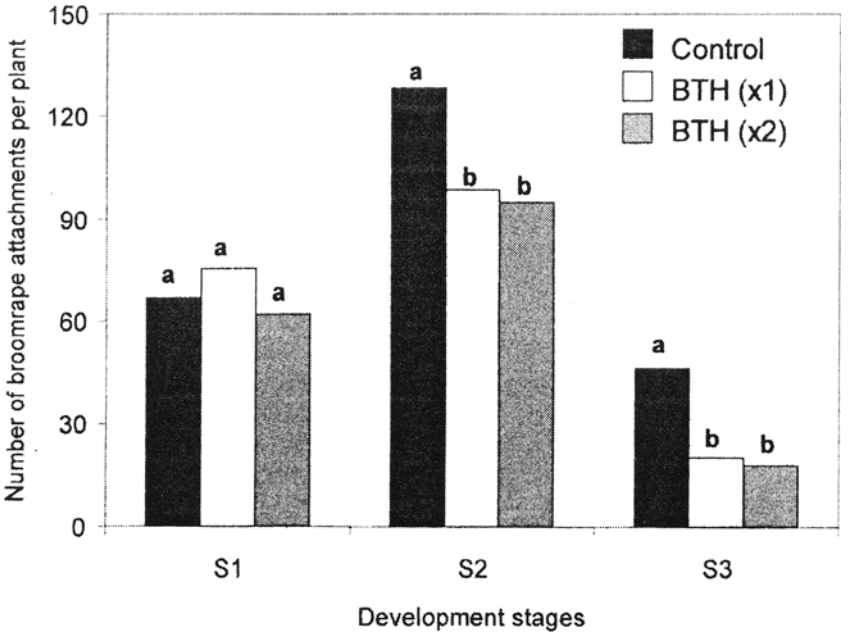

Fig. 3. Total number of broomrape attachments according to development stage (S1–S3), as determined in a petri dish bioassay. Plants were foliar-sprayed once or twice with 0.6 mM BTH, and evaluated 30 days after infection. Within development stages, columns with the same letter do not differ significantly (Duncan, $P<0.05$).

BTH applications did not reduce *O. crenata* seed germination (Table 1), but the number of broomrape attachments per pea plant was significantly reduced. Pea root growth was not disturbed by BTH. The number of broomrape tubercles that became necrotic and died was not increased by BTH treatment. As observed in the pot experiments, fewer attachments developed into stages S2 and S3 after BTH treatment (Fig. 3).

BTH sprays under field conditions significantly reduced infection during the 1997–1998 season, but no effect was observed in the 1999–2000 season (Table 2).

DISCUSSION

Orobanche infestations can be devastating to crops and remove otherwise productive land from effective use for very long periods of time. Crenate broomrape (*O. crenata*) is well known as a major constraint for faba bean, vetches and lentils, being of importance also for other grain and forage legumes. It is at present acknowledged as the major constraint for pea cultivation in the Mediterranean region and Middle East (3,15,20). The problem of broomrape in this area is dramatic due to its broad distribution, the long survival of the seed-bank in the soil, and the extreme susceptibility of the cultivars available to the farmer. Yield loss can be huge, reaching 80% (15) or even 100% (3,22).

Some strategies of broomrape control have been developed, from cultural practices to chemical control (16,20), but none with unequivocal success. The standard glyphosate treatment recommended for broomrape control in faba bean is not applicable in pea,

which is far more sensitive to the herbicide (23). Although some control can be achieved by imazethapyr treatment, it is not complete and efficacy is influenced by sowing date and environmental conditions (20). Breeding for resistance is still the most economical, feasible, and environmentally friendly method of control, but little resistance is available in pea so far (20). Supplementary methods for strengthening plant health or inducing the plant's defense system might be applicable for control of crenate broomrape.

SAR activation has been used successfully in controlling fungal and bacterial plant pathogens (29). There are close similarities between fungi and parasitic plants at the very early stages of the infection process and the same array of defense mechanisms seems to operate in both cases, being those induced in response to SAR activators like salicylic acid, its functional analog BTH, and glutathione. It is therefore possible to hypothesize that SAR activators might play a role in preventing broomrape parasitism as they do against fungal and bacterial plant pathogens. This has recently been shown for the *O. cumana* / sunflower system (25). With these precedents in mind, in the present work we evaluated the effect of host plant defense activators, salicylic acid, BTH and glutathione on broomrape parasitism in pea by using classical pot and petri dish bioassays and field trials.

Plant infection and broomrape development varied greatly depending on the chemical and the means of application. In this study only foliar application of BTH significantly reduced crenate broomrape infection. In contrast to BTH, salicylic acid did not decrease the *O. crenata* infection. This supports the idea that in addition to salicylic acid another systemic signal(s) exists for SAR activation (9,14,24).

More attention should be paid to formulations for seed dressing, because this would be easily applicable by farmers. Sauerborn *et al.* (25) reported an effect of sunflower seed soaking with BTH on *O. cumana* infection, which could not be reproduced by us on pea infected by *O. crenata*.

Broomrape seeds germinate only in response to stimulants released by the host into the medium (7). Excretion of toxic compounds inhibiting germination or necrosing germinated seeds has been reported in sunflower (26). In view of the fact that BTH did not modify the percentage of germinated broomrape seeds, we can conclude that BTH does not reduce production of stimulant substances or increase the release of inhibitory substances by the host.

BTH treatment caused a reduction in the number of broomrape attachments per plant and retarded their development, most of them being arrested at the S1 or S2 stage. This suggests host defense activation by BTH, which would prevent root tissue penetration, connection to the vascular system and/or tubercle development, and could include cell wall reinforcement by lignin and protein (*i.e.,* extensin) deposition (10,11,27,28) and induced synthesis and accumulation of phytoalexins. Lignification has been reported as a defense reaction against *Orobanche* spp. penetration (2,6,8,26), but Sauerborn *et al.* (25) did not find differences in the content of lignins in roots between BTH-treated and non-treated sunflower plants infected with *O. cumana*.

BTH application induced the synthesis of the coumarin phytoalexins scopoletin and ayapin in sunflower and its increased synthesis seems to be part of the defense response against broomrape (17,25,26). Other mechanisms like Pathogenesis-Related Proteins, induced in response to broomrape parasitism and fungal infection, can not be disregarded (9,13,33).

Despite this clear effect of BTH on broomrape infection in pot and petri dish experiments, the effect was weaker under field conditions, being significant in only one season. This can be explained by the strong influence of environment on infection of pea by *O. crenata* (20) and on expression of resistance (19). Similarly, a weak effect of BTH on *O. cumana* infection on sunflower has been observed by us in some years, but not in others (unpublished results). A clearer and reproducible effect has been observed in BTH-treated faba bean plants (unpublished results). Using SAR strategies requires repeated applications of activators and its effect is transient. In this respect it would be necessary to adjust doses, treatments and/or formulations and more research on these key points is necessary. Perhaps combining SAR with other control measures such as resistant cultivars, could provide good control without resorting to agrochemicals which may be toxic, like herbicides. The application of SAR on genotypes with some degree of resistance could boost and increase that resistance.

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