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The effect of plant growth promoting rhizobacteria on *Datura stramonium* L., *Abutilon theophrasti* Med., *Onopordon acanthium* L. and *Verbascum thapsus* L. seed germination

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SUMMARY

The effects of several bacterial media [*Bacillus licheniformis* population 1 (MO_1); *B. licheniformis* population 2 (MO_2); *B. subtilis* (MO_3); *B. megatherium* (MO_4); humates (MO_5)] on seed germination of *Datura stramonium* L., *Abutilon theophrasti* Med., *Onopordon acanthium* L. and *Verbascum thapsus* L. were tested. Seeds were germinated in Petri dishes containing solutions with different bacterial media. The highest germination percentage in all treatments was recorded for *V. thapsus* seeds (100.0%). Different treatments had diverse effects (stimulative or inhibitory) on seed germination of *D. stramonium* [from 5% (MO_1) to 13.3% (MO_3), with 10.0 % in H_20], *A. theophrasti* [from 28.3% (MO_3) to 65.0% (MO_5), with 43.3 % in H_20] and *O. acanthium* [from 10.0% (MO_2) to 13.3% (MO_1 and MO_3), with 6.7% in H_20], depending on the type of media and weed species.

Keywords: Weeds; Rhizobacteria; Germination

INTRODUCTION

Increasingly frequent development of weed resistance to herbicides and an increase in environmental concerns and pressure to reduce pesticide use have encouraged a search for non-chemical alternatives in weed control. Stimulation of seed germination by plant growth promoting rhizobacteria (PGPR) can be a component in weed control programmes. Namely, seedlings of weed species emerge more uniformly when seed germination is stimulated, so that they can be killed in a next step of weed control. That is a way of reducing seedbanks in soil and seedling growth.

Beneficial free-living soil bacteria are usually reffered to as the PGPR (Kloepper et al., 1989). The group includes different bacterial species and strains belonging to the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* (Rodriguez & Fraga, 1999; Sturz & Nowak, 2000; Sudhakar et al., 2000), which may either directly or indirectly facilitate rooting (Mayak et al., 1999) and growth of plants (Karlidag et al., 2007). On the other hand, deleterious rhizosphere-inhibiting bacteria (DRB) have a potential to decrease plant growth (Ying & Williams, 2000; Flores-Vargas & O'Hara, 2006). Many of such DRB have been identified as members of the genera *Achromobacter, Citrobacter, Enterobacter*, *Flavobacterium* and *Pseudomonas* (Schroth & Hancock, 1982; Suslow & Schroth, 1982). However, in the case of *Striga hermontica* (Del.) Benth. seed germination has been inhibited by a product of the plant-promoting bacterium *Azospirillum brasilense* that is beneficial for sorghum growth (Miché et al., 2000). In addition, *Pseudomonas fluorescens* has sometimes been classified as DRB (Zdor et al., 2005) and sometimes as PGPR (Abdul Jaleel et al., 2007).

Many researchers (Harper & Lynch, 1980; Bhat & Alagawadi, 1998; Mayak et al., 1999; Egamberdiyeva, 2007) have essentially been focusing on the effects of microorganisms on germination and seedling growth of crops. At the same time, studies on the effects of microorganisms on seed germination and young seedlings of weed species have been scarce (Ryu et al., 2003; Sarić & Božić, 2009; Vrbničanin et al., 2008, 2011). Mechanisms of plant growth stimulation by associative bacteria include: (1) bacterial synthesis of plant growth-promoting substances, such as indoleacetic acid, cytokinin, gibberellin, B-group vitamins, ammonia, etc. (Revillas et al., 2000; Ping & Boland, 2004); (2) breakdown of plant-produced ethylene by bacterial production of 1-aminocyclopropane-1-carboxylate deaminase (Ryu et al., 2003); (3) improved nutritional assimilation by plants (total N, P, K, microelements) (Wu et al., 2005; Karlidag et al., 2007); (4) antagonism against soil-borne plant pathogens (Bevivino et al., 1998); (5) resistance to different stress conditions, etc. (Carrillo-Castañeda et al., 2002). Today, PGPR are increasingly used as inoculants for biocontrol, biofertilization and phytostimulation (Ping & Boland, 2004).

Abutilon theophrasti Med. (velvetleaf) and *Datura stramonium* L. (jimsonweed), are widespread and very important weeds in many row crops in Serbia and worldwide (especially problematic in corn, sunflower, soybeans, sugar beet or cotton as major crops), causing serious yield and economic losses (Vrbničanin et al., 2008a,b). Both of them are annual weed species that reproduce by seeds. Most seeds fall near the parent plant, but some disperse to greater distances with water, mud, soil movement, and especially through agricultural operations. Seed production can be high with *A. theophrasti* and *D. stramonium* producing up to 50,000 and 45,000 seeds per plant, respectively. Buried seeds are long-lived. A large proportion of buried jimsonweed seeds can survive for 40 years or more, while velvetleaf may survive for 50 years or more. Once established, velvetleaf and jimsonweed are difficult to control because of their long-lived seeds and sporadic germination pattern. In addition to chemical control, manual removal of individual plants before seeds develop can be of great help in controlling small or sparse populations. For more troublesome infestations, integrated weed management may substantially reduce soil seedbanks as soon as in the next couple of years.

Onopordon acanthium L. (Scotch thistle) and *Verbascum thapsus* L. (common mullein) spread along roadsides, annual grassland, pastures, rangeland, and other disturbed habitats. Sometimes both of them penetrate into forest clearings and arable fields, as well as orchards, vineyards, etc. Both of them reproduce by seeds. Most seeds fall near the parent plant. Soil disturbance facilitates germination and seedling growth. Under field conditions, some common mullein seeds are able to survive for up to 35 years or more, while Scotch thistle can remain viable in soil seedbank for at least 7 years and possibly up to 20 years or more. Establishing or encouraging perennial grasses can increase Scotch thistle and common mullein seedlings mortality due to increased competition for moisture. Both of these plants are difficult to control even with systemic herbicides because of their very hairy foliage.

The present study was conducted to determine the ecological interaction between different bacterial media and four weed species: *D. stramonium*, *A. theophrasti*, *O. acanthium* and *V. thapsus*. The main objective of this study was to determine the effects of those media on seed germination and assess whether the bacteria tested can be used as a means of seedbank reduction.

MATERIAL AND METHODS

Seed Source. Seeds of *D. stramonium*, *A. theophrasti*, *O. acanthium* and *V. thapsus* were collected from arable fields in 2010**.** The collected seeds were cleaned and stored at room temperature (approximately 20-25°C) and were put on +4°C for 30 days before studying. Immediatelly before imbibition**,** seeds were sterilized with 1 % (v/v) sodium hypochlorite solution for 10 min, and then rinsed three times with distilled water.

Treatments. All bacterial strains and humates used were isolated from different media. *Bacillus licheniformis* population 1 (MO_1) and population 2 (MO_2) were isolated from manure; *B. subtilis* (MO_3) was isolated from compost; *B. megatherium* (MO_4) was isolated from maize rhizosphere (ZP6); and humates (humic and fulvic acids) (MO_5) were extracted from peat.

Imbibition. A hundred seeds of each species were selected and their dry mass was measured. During imbibition the seeds were put in Petri dishes and treated with solutions of different bacterial strains and humates. Only water was added to control seeds. Water uptake (imbibition) by seeds was measured after 24 h by drying their surface on filter paper and then re-weighing.

Germination. The experiments were carried out in an incubator (Binder CE). Imbibed seeds were germinated in Petri dishes in the dark at 25°C. Twenty seeds were placed in each of three dishes per treatment. In treatments, 5 ml of solution containing different bacterial strains at a concentration of 10^8 cells ml^{-1} were added. In the control, only water was added. Seeds were considered to be germinated after the emergence of radicles. Germinated seeds were counted and the percentage of germination was calculated daily over a period of eight days. Germination rate was calculated using the formula described by Maguire (1962):

$$M= n_1/t_1+ n_2/t_2 +....+ n_x/t_{x,}$$

where n_1, n_2, ...n_x represent the number of germinated seeds at times t_1, t_2, ...t_x in days. Each experiment was conducted three times.

Statistics. The results were processed using the software Statistica 5.0 by descriptive statistics and LSD test.

RESULTS AND DISCUSSION

Continuing our systematic studies of PGPR and seed germination of weed species (Vrbničanin et al., 2008, 2011; Sarić & Božić, 2009) we have reported the effects of different bacterial media on seed germination of several weed species. The reports have shown that different PGPR (*A. chroococcum*, *B. megatherium*, *B. circulans* and *B. pumilus*) and their combinations have a great potential as promoters of seed germination of the weed species *Iva xanthifolia* Nutt., *Amaranthus retroflexus* L., *Sorghum halepense* L.(Pers.) and *Ambrosia artemisiifolia* L. (Vrbničanin et al., 2008, 2011). On the other hand, Sarić and Božić (2009) found *Bacillus* species to have an inhibitory effect on germination of *Cuscuta campestris* Yunck seeds. Many other researchers have also reported stimulative effects of this group of bacteria on seed germination or plant growth of various crops and weed species (Shishido et al., 1996, Gutiérrez-Mañero et al., 2001; Ryu et al., 2003). In this experiment, seeds of four weeds were incubated on media with different *Bacillus* species and humates. The effect of the tested bacterial media on seed mass increase varied depending on the species and media after 24 hours of imbibition (data not shown). Seed germination of *D. stramonium*, *A. theophrasti*, *O. acanthium* and *V. thapsus* differed in responses to different media. The effects of bacterial inoculation on seed germination of weed species are presented in Figure 1. The results show an obvious lack of uniformity in effects of the tested bacterial media on seed germination. For example, *B. subtilis* (MO_3) increased germination of *D. stramonium* and *O. acanthium* and at the same time decreased germination of *A. theophrasti*. Some of these results agree with the findings of Bhat and Alagawadi (1998), Harper and Lynch (1980) and Vrbničanin et al. (2008), who had found PGPR to stimulate seed germination and seedling growth. However, the results indicating contrary effects of bacterial media on seed germination contradict the results of the mentioned studies, but agree with the findings of Miché et al. (2000), who had found the seed germination of *Striga hermontica* to be inhibited by a product of the plant-promoting bacterium *Azopirillum brasilense* known to be beneficial for sorghum growth.

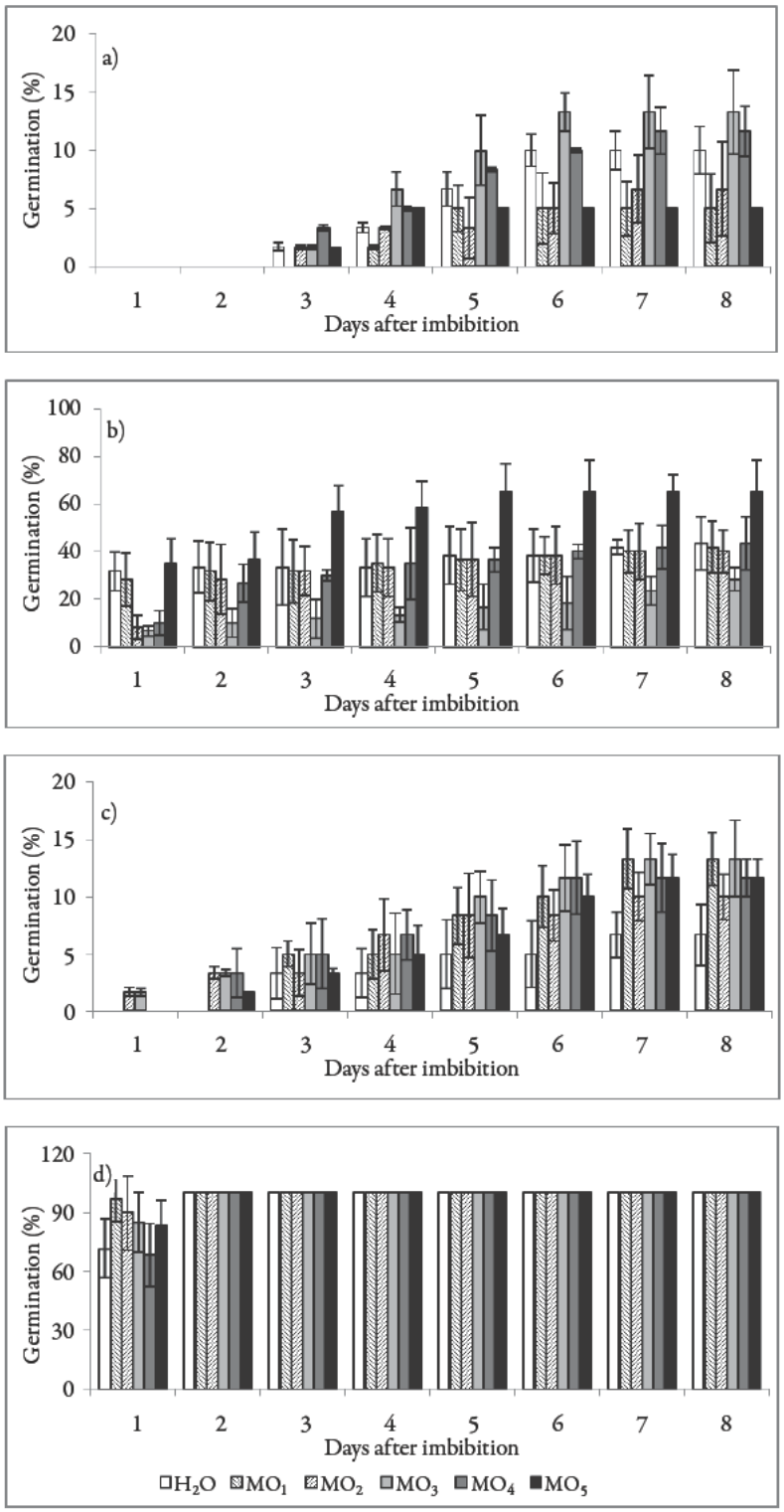

Figure 1. Dynamic of seed germination of *D. stramonium* (a), *A. theophrasti* (b), *O. acanthium* (c) and *V. thapsus* (d) on different bacterial media.

MO_1 - *Bacillus licheniformis* population 1; MO_2 - *B. licheniformis* population 2; MO_3 - *B. subtilis*; MO_4 - *B. megatherium*; MO_5 - humates

Generally, the highest germination (100.0%) in all treatments was recorded for *V. thapsus* seeds. Therefore, it was not possible to analyze the bacterial effect on germination of that species as all its seeds germinated, control included. Comparing all treatments, we found germination to vary among the three other species. For instance, the lowest germination in treatments MO_1 and MO_2 (two populations of *B. licheniformis*) was scored by *D. stramonium* seeds (5.0% and 6.7%, respectively). On the other hand, the highest germination in treatments with the two populations of *B. licheniformis* was recorded for *A. theophrasti* (41.7% in MO_1 and 40.0% in MO_2). In MO_3 and MO_4 treatments, *A. theophrasti* had the highest percentage of germinated seeds (28.3% and 43.3%, respectively). In MO_5 treatement, *A. theophrasti* germinated the best (65.0%), while the lowest germination was recorded for *D. stramonium* (5.0%).

The treatments had diverse effects (stimulative or inhibitory) on seed germination, depending on the type of media and weed species, in that order. The effect was positive in some treatments and species and negative in others (Figure 1). For example, *B. licheniformis* (MO_1 and MO_2) inhibited *A. theophrasti* and *D. stramonium* seed germination, while the results were opposite for *O. acanthium*.

Many researchers have reported stimulative effects of *Bacillus* on seed germination and plant growth as a result of their production of plant growth-promoting substances such as gibberelins, indoleacetic acid, ammonia, hydrogen cyanide, etc. (Gutiérrez-Mañero et al., 2001; Ahmad et al., 2008). In our study, both populations of *B. licheniformis* exerted stimulative effects on *O. acanthium* seed germination. On the other hand, the effect was negative on the germination of *D. stramonium* and *A. theophrasti* seeds, which is in line with the effect of that bacterial species on *A. artemisiifolia* (Vrbničanin et al., 2011). Except for *B. licheniformis*, contradictory results have been reported for *B. subtilis* (as mentioned above) and *B. megatherium*, which had stimulated germination of *D. stramonium* and *O. acanthium*, while seed germination of *A. theophrasti* was not effected.

Both the germination rate (Table 1) and the final percentage of germination varied similarly for all species tested. In contrast to the final percentage of germination, differences in germination rates were found among treatments of *V. thapsus*. Thus, the highest germination rate regarding all studied microorganisms was shown by *V. thapsus* seeds (in H_2O - 48.7, MO_1 - 53.7, MO_2 - 52.4, MO_3 -51.4, MO_4 - 48.0, and MO_5 - 51.0 seeds day^{-1}). The results showed that statistical differences in germination rates between treatements of this species were not as prominent as for the other three species, but they were significant ($p<0.05$) and very significant ($p<0.01$) in some treatments (Table 2). The germination rate of *A. theophrasti* varied considerably in some treatments (in H_2O - 18.6, MO_1 - 17.6, MO_2 - 3.2, MO_3 - 6.4, MO_4 - 13.8, and MO_5 - 25.6 seeds day^{-1}). The highest value was recorded in treatment MO_5, and it very significantly differed ($p<0.01$) from all other treatments. The germination rates of *D. stramonium* and *O. acanthium* seeds (0.7-2.0 seeds day^{-1} and 1.1-2.7 seeds day^{-1}, respectively) were much lower than the rates of the other two species. All treatments had very significant ($p<0.01$) effects on seed germination of both species, compared to the control (Table 2). Treatments MO_1 and MO_2 decreased and all other treatments (MO_3, MO_4 and MO_5) increased the germination rate of *D. stramonium*, while all treatments had stimulative effects on *O. acanthium* germination rate.

Table 1. Effects of plant growth-promoting bacteria on germination rates *of D. stramonium*, *A. theophrasti*, *O. acanthium* and *V. thapsus*

Weed species	Treatments					
	H_2O	MO_1	MO_2	MO_3	MO_4	MO_5
D. stramonium	1.4±0.9	0.7±0.3	0.9±0.2	2.0±0.9	1.8±0.5	1.0±0.2
A. theophrasti	18.6±5.1	17.6±4.2	13.2±4.0	6.4±1.9	13.8±3.0	25.6±7.6
O. acanthium	1.1±0.1	2.0±0.3	2.4±0.2	2.7±1.0	2.3±0.6	1.9±0.4
V. thapsus	48.7±2.1	53.7±1.2	52.4±1.1	51.4±1.2	48.0±2.0	51.0±1.6

MO_1- *Bacillus licheniformis* population 1; MO_2- *B. licheniformis* population 2; MO_3-*B. subtilis*; MO_4- *B. megatherium*; MO_5- humates

Table 2. Germination rates of *Datura stramonium*, *Abutilon theophrasti*, *Onopordon acanthium* and *Verbascum thapsus*

		H_2O	MO_1	MO_2	MO_3	MO_4
MO_1	*D. stramonium*	**				
	A. theophrasti	N.S.				
	O. acanthium	**				
	V. thapsus	**				
MO_2	*D. stramonium*	**	**			
	A. theophrasti	**	**			
	O. acanthium	**	**			
	V. thapsus	*	N.S.			
MO_3	*D. stramonium*	**	**	**		
	A. theophrasti	**	**	**		
	O. acanthium	**	**	**		
	V. thapsus	N.S.	N.S.	N.S.		
MO_4	*D. stramonium*	**	**	**	**	
	A. theophrasti	**	**	N.S.	**	
	O. acanthium	**	**	N.S.	**	
	V. thapsus	N.S.	**	*	*	
MO_5	*D. stramonium*	**	**	N.S.	**	**
	A. theophrasti	**	**	**	**	**
	O. acanthium	**	N.S.	**	**	**
	V. thapsus	N.S.	N.S.	N.S.	N.S.	N.S.

Aqueous suspensions of different bacteria were used in this experiment but the results cannot be extrapolated to soil or rhizosphere conditions because various conditions in soil (pH, microelements and salinity) can influence the excretion of plant growth-promoting substances by PGPR strains and their effect on seed germination (Narula & Gupta, 1986; Egamberdiyeva, 2007).

CONCLUSIONS

In conclusion, the results of this study indicate that: 1) the bacteria tested may have opposite effects (stimulative or inhibitory) on different weed species; 2) the bacteria had both promoting and inhibiting potential for seed germination, depending on plant species. Therefore, screening tests for bacterial effects on many weed species are needed. Based on such results it would be possible to evaluate the use of PGPR bacteria in weed management practices. Interesting new perspectives have opened, helping us to implement the acquired knowledge on PGPR-seed germination interactions as a biological weed control method in sustainable agricultural practices.

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Uticaj zemljišnih bakterija na klijanje semena *Datura stramonium* L., *Abutilon theophrasti* Med., *Onopordon acanthium* L. i *Verbascum thapsus* L.

REZIME

Testiran je efekat bakterijskih kultura [*Bacillus licheniformis* populacija 1 (MO_1); *B. licheniformis* populacija 2 (MO_2); *B. subtilis* (MO_3); *B. megatherium* (MO_4); humati (MO_5)] na klijanje semena *Datura stramonium* L., *Abutilon theophrasti* Med., *Onopordon acanthium* L. i *Verbascum thapsus* L. Semena su naklijavana u Petri posudama u rastvorima različitih bakterijskih kultura. Najveća klijavost zabeležena je kod semena *V. thapsus* (100%). Različiti tretmani pokazali su različiti uticaj (stimulativni ili inhibitorni) na klijanje semena *D. stramonium* [od 5,0% (MO_1) do 13,3% (MO_3), u H_20 10,0%], *A. theophrasti* [od 28,3% (MO_3) do 65,0% (MO_5), u H_20 43,3%] i *O. acanthium* [od 10,0% (MO_2) do 13,3% (MO_1 i MO_3), u H_20 6,7], u zavisnosti od tipa kulture i vrste korova.

Ključne reči: Korovi; rizobakterije; klijanje