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Effect of Plant Growth Promoting Rhizobacteria on *Ambrosia artemisiifolia* L. Seed Germination

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SUMMARY

Soil bacteria are able either to stimulate or inhibit seed germination. If seed germination is stimulated, the seedlings of weed species emerge more uniformly, so that they could be killed in the next step of weed control. This investigation focused on testing the germination of *Ambrosia artemisiifolia* L. on several media: *Pseudomonas fluorescens* (B_1), *Azotobacter chroococcum* (B_2), *Bacillus licheniformis* (B_3), *B. pumilus* (B_4), *B. amyloliquefaciens* (B_5). In control, seeds germinated in water. Seed germination varied depending on bacterial media. Germination was inhibited by bacterial treatments B_1 and B_3, treatments B_2 and B_4 stimulated germination, while germination in treatment B_5 was similar to control.

Keywords: *Ambrosia artemisiifolia* L.; Plant growth promoting rhizobacteria; Seed germination

INTRODUCTION

Common ragweed (*Ambrosia artemisiifolia* L.) is an Asteraceae originating in North America (Basset & Crompton, 1975). During the last two centuries, it is spreading in Europe because of international trade and wars. This annual broadleaf weed species was first observed in France and Germany in the middle of the 19th century (Heckel, 1906). It was also reported in Yugoslavia and Hungary during the 1920s (Be´res & Hunyadi, 1984). *A. artemisiifolia* presents a two-sided problem. First, its pollen causes allergies that often develop into asthma (Plavšić, 2007). Secondly, it is also a weed that can cause substantial yield losses. Its weediness is particularly conspicuous in spring crops such as maize, sunflower, soya bean, sugar beet and, in some respects, pea and cereal crops (Chollet et al., 1999). As a result of its late emergence, it can also grow during the inter-crop period in rapeseed or cereal stubbles, as well as on fallow or set-aside land. However, few studies have been conducted on the control of *A. artemisiifolia* during the inter-crop period. In many crops *A. artemisiifolia* can be controlled by different herbicides (glyphosate, isoxaflutole, acetochlor + isoxaflutole, isoxaflutole + terbuthylazine, dicamba, fluroxypyr, bentazone + dicamba, prosulfuron,

foramsulfuron + iodosulfuron-methyl-Na, topramezone, topramezone + dicamba, tritosulfuron + dicamba, triasulfuron + dicamba, S-metolachlor+ mesotrione + terbuthylazine, lactophen, fomesafen, clopyralid, clopyralid + phenmedipham + desmedipham, triflusulfuron + phenmedipham + desmedipham + clopyralid, etc.) (Janjic et al., 2011). Mechanical control often gives poor results, because *A. artemisiifolia* can produce new stems after being cut (Delabays et al., 2005). In most cases, a second mechanical operation is thus necessary. Also, this species is increasing in importance due to development of herbicide resistance (Zheng et al., 2005).

In Serbia, *A. artemisiifolia* is on the list of invasive weed species. Vrbničanin et al. (2008a) confirmed its widespread all around Serbia, especially in Vojvodina, Mačva and Šumadija (Figure 1) row crops, orchards, vineyards and rarely grain crops, as well as based grasslands, ruderal, settlement and areas along roads are infected.

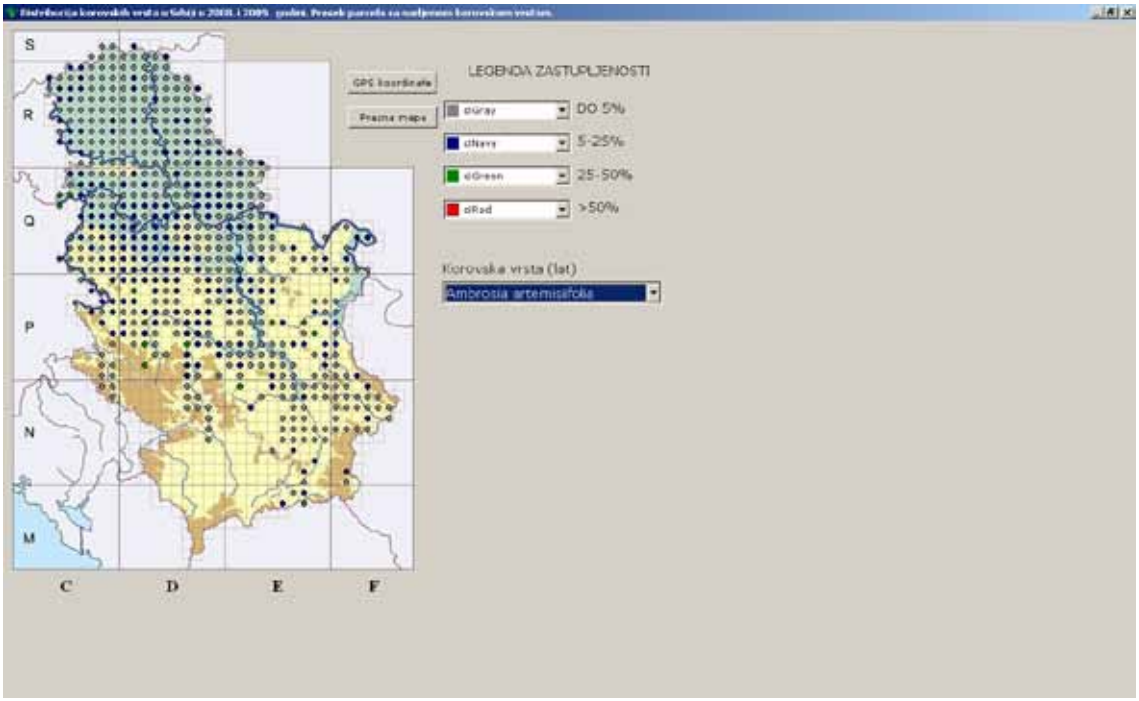

Figure 1. Geographic distribution and widespread of *A. artemisiifolia* in Serbia (Vrbničanin et al., 2008a)

Beneficial free-living soil bacteria known as plant growth-promoting rhizobacteria (PGPR) includes different bacterial species and strains belonging in genera *Acetobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Herbaspirillum*, *Pseudomonas*, *Rhizobium* and *Serratia* (Glick, 1995; Probanza et al., 1996; Rodriguez and Fraga, 1999; Sudhakar et al., 2000). That group of bacteria can either directly or indirectly facilitate seed germination and growth of plants. Essentiall effects of PGPR on germination and seedling growth of crops were studied by many researchers (Rodelas et al., 1999; Egamberdiyeva, 2007), while their effects on weed species seed germination was studied by a very few (Vrbnicanin et al., 2008b,c).

Modern crop production technology include increasing concern about the environmental impact of arable farming, and of the effects of herbicides in particular. This concept lead to the development of more integrated approaches in weed management. Use of PGPR as stimulants for seed germination of weed species can provide more uniform weed emergence. Killing of these plants in the next step of weed control can be a good way for reducing the seed bank in the soil. Before that, it is necessary to investigate basic ecological interactions between different bacterial media and seed germination of economically important, especially invasive weed species. Therefore, the main object of this work was to determine the effects of bacterial media on germination of seeds of *A. artemisiifolia*. The results obtained will show potential for using studied bacterial media in reduction of seed bank of those species in the future.

MATERIAL AND METHODS

Seeds of *A. artemisiifolia* were collected from arable fields in 2009 for testing effects of five different rhizobacteria on germination. Immediately before germination, seeds were sterilized with 1% (v/v) sodium hypochlorite solution for 10 min, and then seeds were rinsed three times with distilled water. The next rhizobacteria were selected: *Pseudomonas fluorescens* (B_1), *Azotobacter chroococcum* (B_2), *Bacillus licheniformis* (B_3), *B. pumilus* (B_4), *B. amyloliquefaciens* (B_5). In control, seeds germinated in water. Bacterial strains used in the study were isolated from rhizosphere of wheat (*P. fluorescens* and *A. chroococcum*) or from manure (*B. licheniformis*, *B. pumilus* and *B. amyloliquefaciens*). Incubation of weed seeds was done with 24h old inocula with cell concentration of 10^8 ml^{-1}.

Twenty seeds of *A. artemisiifolia* were selected and placed in each Petri dish and treated with solutions containing the above mentioned bacterial media. In control, only water was added. In treatments, 5 ml of solution containing different bacterial media was added. Three dishes were used for each treatment and control. Germination took place in an incubator (Binder CE) at 25°C, in the dark. The seeds were considered to be germinating at the moment of radicle emergence. The number of germinated seeds was recorded daily (germination rate), and the final percentage of germination was measured after 8 days. Germination rate (sum of germinations per day) 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 are the numbers of the germinated seeds at times t_1, t_2, ...t_x in days. Each experiment was conducted three times.

All data were processed by analysis of variance (ANOVA) and means were separated by least significant differences (LSD) test using statistical software Statistica 5.0.

RESULTS

In this experiment, seeds of invasive species *A. artemisiifolia* were incubated on media with different bacterial species. Dynamic and final percentage of seed germination (Figure 2) and germination rate (Figure 3) differed depending on the treatment. Generally, seed germination of *A. artemisiifolia* was very low in all treatments, as well as in control. The highest percentage of germinated seeds was recorded for treatment B_2 (10.67%), while the lowest germination was scored for treatment B_3 (1.33%). In the treatment B_1 seeds did not germinate.

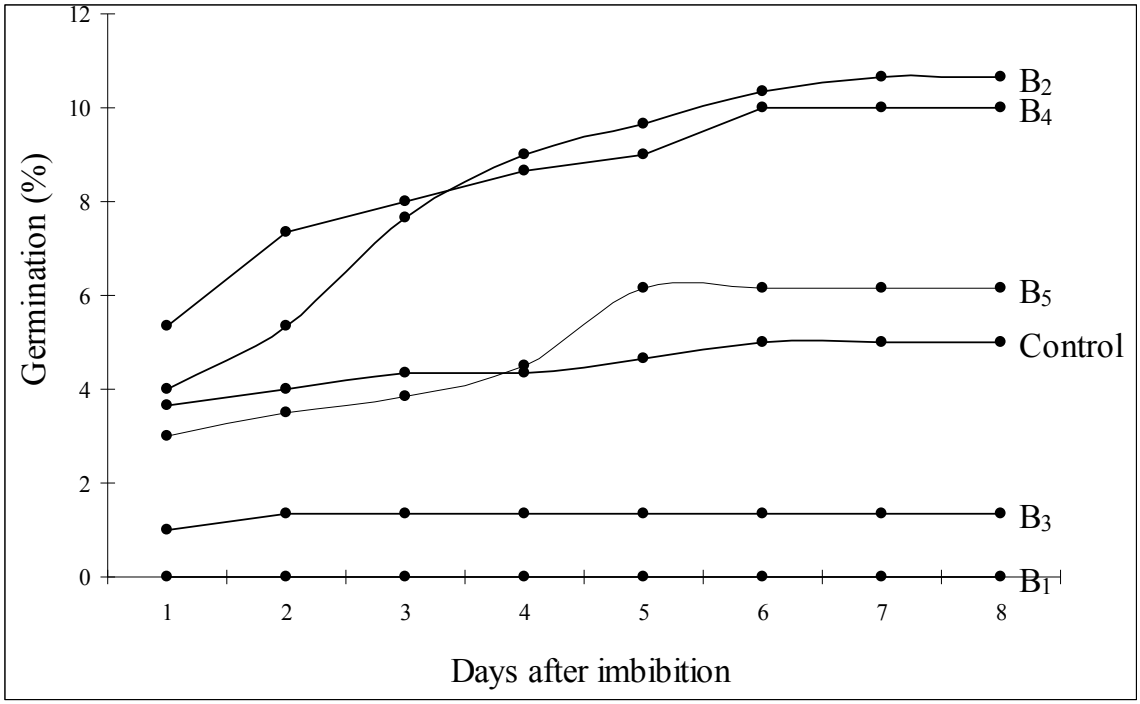

Figure 2. Dynamic of seed germination of *A. artemisiifolia* on different media. B_1 – *P. fluorescens*, B_2 – *A. chroococcum*, B_3 – *B. licheniformis*, B_4 – *B. pumilus*, B_5 – *B. amyloliquefaciens*

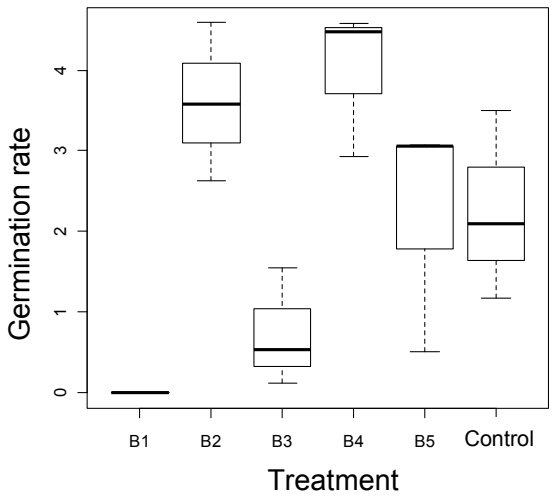

Figure 3. Germination rates of *A. artemisiifolia* on different media. B_1 – *P. fluorescens*, B_2 – *A. chroococcum*, B_3 – *B. licheniformis*, B_4 – *B. pumilus*, B_5 – *B. amyloliquefaciens*

Different bacterial treatments had diverse effects (stimulative or inhibitory) on seed germination of *A. artemisiifolia*. For example, treatments B_2 and B_4 stimulated seed germination, while opposite results were obtained for treatments B_1 and B_3. Seed germination in treatment B_5 was similar to control. Obtained effects were statistically significant ($P<0.05$) only for percentage of germination (Table 1). Germination rate for treatment B_1 statistically significant differed from germination rate in control, while differences in germination rate between other treatments and control were not found (Table 1).

Table 1. Results of one-way ANOVA for percentage of germination and germination rate

	Germination (%)	Germination rate
Control: B_1	0.031405*	0.016239*
Control: B_2	0.017265*	$0.124352^{N.S.}$
Control: B_3	0.044731*	$0.085257^{N.S.}$
Control: B_4	0.031405*	$0.053090^{N.S.}$
Control: B_5	$0.580295^{N.S.}$	$0.954685^{N.S.}$

A one-way ANOVA was performed for seed treatments as the main effect to compare samples of seeds incubated on bacterial media with samples germinating in distilled water. N.S. ($P>0.05$) – no significant differences; *($P<0.05$) – significant differences

DISCUSSION

In continuation of our systematic studies of the PGPR on seed germination of weed species (Vrbničanin et al., 2008b,c; Sarić and Božić, 2009) we reported effects of different bacterial media on seed germination of several weed species. Besides this, many studies showed that microorganisms can promote germination and growth of different plant species (Gutierrez-Manero et al., 2001; Egamberdiyeva, 2007). In order to evaluate effects of this group of bacteria, we have studied effects of five different bacterial media on germination of *A. artemisiifolia*.

P. fluorescens was sometimes classified as deleterious rhizobacteria (DRB) (Zdor et al., 2005) and sometimes as PGPR (Jaleel et al., 2007). This species (B_1) totally inhibited germination of *A. artemisiifolia*, although Ahmad et al. (2008) confirmed plant growth promoting traits of this bacteria. Carrillo-Castaneda et al. (2002) showed that effect of *P. fluorescens* on alfalfa germination depend on conditions in which bacterial cultures develop.

A. chroococcum have different effects on the seedling growth of crops as well as on seed germination of several weed species (*Iva xanthifolia* Nutt., *Sorghum halepense* (L.) Pers., *Amaranthus retroflexus* L., *Datura stramonium* L., *Abuthilon theophrasti* Medik., *Onopordon acanthium* L., *Verbascum thapsus* L., *Cuscuta campestris* Yunck.) (Rodelas et al., 1999; Vrbničanin et al., 2008b,c; Sarić and Božić, 2009). In our study, *A. chroococcum* (B_2) promoted germination of seeds of *A. artemisiifolia*. These results are consistent with findings reported by Vrbničanin et al. (2008b) and Sarić and Božić (2009).

It was found that *Bacillus* species have variable effects on the seed germination. Namely, Egamberdiyeva (2007) reported positive effects of *Bacillus* on seed germination and plant growth as a result of their production of plant growth-promoting substances. On the other hand, Sarić and Božić (2009) found *Bacillus* species to have inhibitory effect on germination of *C. campestris* and alfalfa. In this experiment only effect of *B. pumilus* (B_4) on germination of *A. artemisiifolia* were stimulative. Opposite results were obtained for *B. licheniformis* (B_3), which inhibited seed germination and for *B. amyloliquefaciens* (B_5), which had no effect. Results for *B. pumilus* are consistent, while results for *B. licheniformisa* are oposite with those reported by Gutierrez-Manero et al. (2001) showing that both promoted growth of alder trees.

In conclusion, results from this study showed different PGPR have diverse (stimulative or inhibitory) effects on germination of *A. artemisiifolia*, but it may not be possible to extrapolate the results of these *in vitro* studies to soil or rhizosphere conditions. The reason is influence of different conditions in the soil (pH, microelements, salinity) on excretion of plant growth-promoting

supstances by PGPR (Narula and Gupta, 1986). Some (*A. chroococcum* and *B. pumilus*) bacteria tested have potential as promoters of seed germination, while some of them (*P. fluorescens*, *B. amyloliquefaciens* and *B. licheniformis*) have not. So, it is necessary to do screening of effects of many bacteria on seed germination of *A. artemisiifolia,* and based on that to evaluate possibility of their use in this weed species management.

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Uticaj zemljišnih bakterija na klijanje semena korovske vrste *Ambrosia artemisiifolia* L.

REZIME

Zemljišne bakterije mogu imati stimulativno ili inhibitorno delovanje na klijanje semena mnogih biljaka. Ukoliko je klijanje semena stimulisano, ponici korova se javljaju znatno uniformnije, što pruža realnu mogućnost da se u nekoj od narednih opreacija nege useva korovi eliminišu. U ovim istraživanjima ispitivan je uticaj nekoliko zemljišnih bakterija (*Pseudomonas fluorescens* (B_1), *Azotobacter chroococcum* (B_2), *Bacillus licheniformis* (B_3), *B. pumilus* (B_4), *B. amyloliquefaciens* (B_5) na klijanje semena alohtone invazivne korovske vrste *Ambrosia artemisiifolia* L. U kontrolnu varijantu je dodata česmenska voda. Na osnovu dobijenih rezultata može se konstatovati da je klijanje semena *A. artemisiifolia* variralo u zavisnosti od toga na kojoj bakterijskoj podlozi je vršeno naklijavanje. Naime, utvrđen je manji procenat klijavosti semena na podlozi B_1 i B_3, odnosno veća klijavost je postignuta na podlogama B_2 i B_4 u odnosu na čistu vodu. Osim toga, klijanje semena *A. artermisiifolia* na podlozi B_5 je bilo gotovo istovetno kao i u čistoj vodi (kontroli).

Ključne reči: *Ambrosia artemisiifolia* L.; zemljišne bakterije; klijanje semena