Filtrates of rhizosphere bacteria suppressive to *Centaurea solstitialis* seed germination

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**Summary**

*Centaurea solstitialis* (yellow starthistle) is an invasive weed in the United States that infests millions of hectares of rangelands, roadsides, and recreation areas. It is not completely understood why weeds often become invasive when transported to a new ecosystem. Many factors may be involved including soil microorganisms that influence plant growth and seed germination.

Yellow starthistle (YST) roots removed from four sites in southern France were washed in distilled water. Individual bacterial colonies were isolated from the root wash and plated on nutrient agar. Sixteen isolates were grown on two different media for 1 week at 25°C at either 150 or 250 rpm. The resulting culture was centrifuged and the supernatant adjusted to pH 7.2. The effect of the cell-free filtrates on the germination of YST seeds from France and the United States were tested by placing seeds on filter paper moistened with the bacterial filtrate in Petri plates. Five isolates showed some inhibition dependent upon the shaker speed and media used. Isolates 2(8) and 3(11) shaking at 250 rpm and 1(10) and 4(15) shaking at 150 rpm reduced germination of only French YST seeds. Isolate 2(10), identified as *Streptomyces gri-seus*, grown in a medium recommended for *Streptomyces* spp. at 250 rpm and isolate 4(15), identified as an Actinomycete, grown in a basic nutrient broth medium at 250 rpm inhibited both French and American YST seeds. The other eleven isolates showed no or limited inhibition of germination. These results suggest soil bacteria produce metabolites that inhibit YST seed germination and there is some response difference in the French and American seeds.

**Keywords:** Actinomycete, bacteria, *Centaurea solstitialis*, rhizosphere, seed germination, *Streptomyces*, yellow starthistle

**Zusammenfassung**

Filtrate von Rhizosphärenbakterien mit hemmender Wirkung auf die Keimung der Samen von *Centaurea solstitialis*

*Centaurea solstitialis* (Gelbe Sommerflockenblume) ist ein sich aggressiv verbreitendes Unkraut, das in den USA Millionen Hektar Weide, Straßenränder und Freizeitgebiete einnimmt. Es ist noch nicht geklärt, warum ein Unkraut sich aggressiv verbreitet, wenn es in ein neues Ökosystem versetzt wird. Viele Faktoren können in Betracht gezogen werden, wie zum Beispiel Mikroorganismen in der Erde, die das Pflanzenwachstum und die Keimung der Samen beeinflussen.


Stichwörter: Aktinomyzet, Bakterien, Centaurea solstitialis, Gelbe Sommerflockenblume, Samenkeimung, Rhizosphäre, Streptomyces

Introduction

Centaurea solstitialis L. (yellow starthistle) is native to the Mediterranean region (ROCHE and TALBOTT 1986). In the 1870s, it is thought to have been brought to the United States in contaminated alfalfa seed (SHELEY et al. 1999). Now it is considered a noxious weed and it infests over 10 million hectares of land in California alone. Although invasive weeds have been studied intensively, it is not clearly understood why they are so problematic in their new environment. One hypothesis is that some species increase uncontrollably when introduced into new areas because there is a lack of competition with native species and of attack by predators in the new environment. One study showed that plant species were infected by 77% fewer fungi and virus pathogen species in its naturalized range than in its native range (MITCHELL and POWER 2003).

In terms of biological control, insects and fungi have been used most frequently to manage weeds (KREMER and KENNEDY 1996). Soilborne bacteria that suppress plant growth have often been overlooked because symptoms may not be as obvious as traditional bacterial pathogens. However, bacteria found on root surfaces have been found to decrease seed germination or root growth of barley (Hordeum vulgare L.) (HARPER and LYNCH 1980), sugar beet (Beta vulgaris L.) (SUSLOW and SCHROTH 1982), and wheat (Triticum aestivum L.) (FREDRICKSON and ELLIOTT 1985, CHERINGTON and ELLIOTT 1987). In addition, many deleterious rhizobacteria are plant specific, which makes it a desirable agent for biological control (ELLIO NT and LYNCH 1985, CHERINGTON and ELLIOTT 1987, SCHIPPERS et al. 1987). One mechanism of action towards suppression of plant growth is the production of phytotoxins by bacteria (TRANEL et al. 1993, GEALY et al. 1996). Cell-free supernatant from various bacterial isolates were shown to inhibit radical emergence of several grass species (KENNEDY et al. 1991). Depletion of the seed bank is important in limiting weed infestations and methods to reduce seed production and germination need to further investigated.

In a diverse community, yellow starthistle is not considered a good competitor (DUKES 2002). Therefore, if a biological agent that reduced yellow starthistle plant size or vigour could be found it may allow a more diversity of native plants to establish. Yellow starthistle exists in France but populations are low and not problematic. In some natural sites, it was observed that populations disappeared over time. This observation has led to the conclusion that some factor(s) are either inhibiting seed germination or growth of the plants, thereby reducing its competitiveness and causing a decline in the population. The current study was undertaken to isolate bacteria from the soil that might inhibit the germination of yellow starthistle seeds or reduce plant growth. The results presented here pertain to the initial screening of the culture filtrates from isolated rhizosphere bacteria against germination of yellow starthistle seeds.
Materials and methods

Isolation of bacteria from the rhizosphere of yellow starthistle roots was conducted by collecting plants at the flowering stage from four separate sites near Pic St. Loup, France. The roots from each collected site were placed separately in beakers containing 1 L of sterile distilled water and stirred with a magnetic stirrer for 30 min. The roots were removed by filtration through a layer of gauze. 1 ml of the filtrate was added to 20 sterile Petri plates (90-mm-diameter). Tryptic soy broth (TSB) medium was prepared by adding 3 g of TSB and 15 g of agar in 1 L of water and autoclaving for 30 min at 121°C. After cooling to 50°C, 50 mg of cycloheximide was added and mixed by swirling. The molten agar (approximately 20 ml per plate) was poured into the Petri plates containing 1 ml of the root washing and swirled to mix the agar and root washing. After the agar hardened, the plates were placed in an incubator at 25°C for 1 week. Fifty distinct individual colonies were removed from the plates and streaked on nutrient agar (5 g peptone, 3 g yeast extract, 15 g agar, 1 L water). An individual colony from each plate was removed and streaked on a new nutrient agar plate to assure a pure culture. After 3 days growth, autoclaved 10% skim milk was added to the agar surface of the bacterial cultures. The cultures were scraped and suspended in the skim milk and frozen at -80°C until needed.

Sixteen bacterial cultures from the four sites were selected at random. The cultures were thawed and streaked on nutrient agar plates. Individual colonies were removed and added to 100 ml (in a 250 ml non-baffled Erlenmeyer flask) of either liquid medium A: 5 g peptone and 3 g yeast extract per liter of water; or medium B: 20 g cottonseed flour, 10 g glycerol, 2.5 g cellulose, and 2 ml of Czapek's mineral salts (100 g KCl, 100 g MgSO4, 2 g FeSO4, 1 L water) per liter of water (GERWICK et al. 1997). The cultures were placed on an orbital shaker at 25°C at 150 or 250 rpm for 1 week. The cultures were centrifuged at 4000 rpm for 30 min. The supernatant was saved and the pH was adjusted to 7.2 with 1 N HCl. The filtrate was stored in a closed tube at 4°C until needed.

Filter paper was placed on the bottom of a plastic Petri plate and 2.4 ml of the bacterial filtrates or non-inoculated media A or B (controls) were pipetted onto the filter paper. Twenty pappus bearing seeds, collected from yellow starthistle plants grown in France or the United State (California), were placed on the filter paper. The plates were covered and placed in an incubator at 25°C with 16 h of daylight. Germination of the seeds was recorded after 96 h. The test was repeated one time.

Lettuce (Lactuca sativa L.) seed germination was tested by exposing 20 lettuce seeds to the culture filtrates from isolates 1(10), 2(8), 2(10), 3(11), and 4(15) in the same manner as described above. Germination was recorded after 96 h.

Results

A total of 50 bacterial colonies were isolated from the rhizosphere of yellow starthistle roots at the four different locations. Sixteen of these isolates were chosen at random for further study on inhibition of yellow starthistle seed germination. Preliminary studies showed that 1 week of shaking on a rotary shaker was necessary for metabolite production (data not shown). The results of the filtrate inhibition on yellow starthistle seed germination of the 16 isolates tested are shown in Table 1.

Five isolates [1(10), 2(8), 2(10), 3(11), and 4(15)] showed some inhibition of seed germination dependent upon the shaker speed and media used. These isolates were identified as Streptomyces sp., Streptomyces griseus, Comamonas sp., and an unidentified actinomycete, respectively. Isolate 2(10) was active towards germination inhibition only when grown in a media known to induce phytotoxin production by Streptomyces spp. (GERWICK et al. 1997). When this isolate was cultured in a general nutrient broth medium, no seed germination inhibition occurred. Isolate 4(15) shaking at 250 rpm inhibited the American seeds from germinating but only slightly inhibiting the French seeds. When this isolate was grown at 150 rpm, only the French seeds were reduced in germinating. All five isolates completely inhibited lettuce seed germination (data not shown).
Tab. 1: Percent Centaurea solstitialis seed germination after exposure to filtrates of bacterial isolates collected from the rhizosphere.

Tab. 1: Prozent Keimung der Samen von Centaurea solstitialis nach der Behandlung mit Filtraten von Bakterienisolaten, die von der Rhizosphäre gesammelt wurden.

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Growth characteristics</th>
<th>Percent seed germination&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Media&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Shaker speed (rpm)</td>
</tr>
<tr>
<td>1(2h)</td>
<td>A</td>
<td>250</td>
</tr>
<tr>
<td>1(10)</td>
<td>A</td>
<td>250</td>
</tr>
<tr>
<td>1(10)</td>
<td>A</td>
<td>150</td>
</tr>
<tr>
<td>1(12)</td>
<td>A</td>
<td>250</td>
</tr>
<tr>
<td>2(1)</td>
<td>A</td>
<td>250</td>
</tr>
<tr>
<td>2(2h)</td>
<td>A</td>
<td>250</td>
</tr>
<tr>
<td>2(7)</td>
<td>A</td>
<td>250</td>
</tr>
<tr>
<td>2(8)</td>
<td>A</td>
<td>250</td>
</tr>
<tr>
<td>2(10)</td>
<td>A</td>
<td>250</td>
</tr>
<tr>
<td>2(10)</td>
<td>A</td>
<td>150</td>
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<tr>
<td>2(10)</td>
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<td>3(1)</td>
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<td>4(5h)</td>
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<td>150</td>
</tr>
<tr>
<td>4(16)</td>
<td>A</td>
<td>250</td>
</tr>
<tr>
<td>4(19)</td>
<td>A</td>
<td>150</td>
</tr>
</tbody>
</table>

<sup>1</sup> Centaurea solstitialis seed germination, originating from France or the United States, expressed as a percentage of the control germinated in autoclaved medium.
<sup>3</sup> Media type A: 5 g peptone, 3 g yeast extract, 1 L water; media type B: 20 g cottonseed flour, 10 g glycerol, 2.5 g cellulose, 2 ml Czapeks mineral salts (100 g KCl, 100 g MgSO₄, 2 g FeSO₄, 1 L water), 1 L water (GERWICK et al. 1997).

Discussion

Although the impact of the culture filtrates produced by the isolates collected from the rhizosphere of yellow starthistle roots were variable, some did show activity in reducing seed germination. What is most interesting is that there was a different response to some filtrates between the French and American yellow starthistle seeds. Perhaps this gives some explanation as to why this weed is so invasive in the United States; the seeds may be less prone to microbial or phytotoxin attack. This gives YST a competitive advantage since the seeds start germinating in October after the first autumn rains and the seedlings can be well established before other plant species. One hypothesis for this difference between the French and American seeds is that the seed coat for the American seeds are thicker or contain some other chemicals that help to protect it. Although no known studies involving yellow starthistle seeds have been conducted in terms of this concept, several studies reviewed by KREMER (1993) have concluded that the physical structure of the seed along with phenolic compounds inhibited attack. One specific example showed that shattercane (Sorghum bicolor (L.) Moench) seed survival was positively correlated with glume tightness, caryopsis lignin, and glume tannin (FELLOWS and ROETH 1992). The tannin and lignin appeared to function as barriers to microbial invasion. To the authors knowledge, no studies have compared the seeds from the same weed in this manner grown in two separate ecosystems.
The most active isolate, 2(10), was identified as *Streptomyces griseus*. Herbicidal activity has been reported for a number of metabolites produced by *Streptomyces* strains (SETO et al. 1983, HEISEY and PUTNAM 1990, SCACCHI et al. 1994). One strain of *S. hygroscopicus* produced a culture broth that strongly inhibited germination of garden cress (*Lepidium sativum* L.) by production of two herbicidal compounds (HEISEY and PUTNAM 1986). The identity of the active compound(s) in isolate 2(10) is unknown and may lead to novel chemistry against this weed. Although the discovery of new compounds would be very useful, it is not very sustainable in the long term without the living microorganism producing it under natural conditions. It was the main goal of this research to discover new biological control agents that can be used in a classical biocontrol system without the need for constant input. Further studies will have to be conducted to determine if these bacterial isolates will produce the inhibiting metabolites that reduce seed germination in the soil environment.

The information provided in this study is useful in that it shows that some metabolite(s) produced by certain bacteria species can inhibit yellow starthistle germination. More work will need to be done to determine if the same results can be achieved by supplementing soil with live bacteria. Positive results would lead to an integrated approach with deleterious rhizobacteria (DRB) to manage yellow starthistle. KREMER (2000) found that when DRB were incorporated with a cover crop further weed suppression resulted. Other studies showed that rhizobacteria inhibitory to downy broom (*Bromus tectorum* L.) and jointed goatgrass (*Aegilops cylindrica* Host.) exhibited higher suppressive activity in soil when combined with herbicides at reduced rates of application.

The results from the initial screening of rhizosphere bacteria on yellow starthistle are encouraging in finding a potential agent that might limit the spread of this invasive weed. Additional work needs to be conducted to screen the remaining isolates, optimize production and purification of the active metabolite(s), examine host specificity, and determine if the same affect can be achieved by supplementing soil with live bacteria.

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**References**


