Biocontrol of \textit{Poa annua}

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Crop & Soil Sciences  
Washington State University

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Suncadia, WA  
Oct. 1 – 3, 2017
‘D7’ biocontrol – How it all started

Poor growth of winter wheat in spring

(Lloyd Elliott)
Poor growth of winter wheat in spring

High numbers of *Pseudomonas* on roots

Toxin was selective against cultivars (*Elliott*)

- What about grass weeds?
Weed-suppressive bacteria

- Colonize residue/root
- Survive well at low temperatures
- Not competitive at warm temperatures
- Go dormant during hot, dry summer
- Produce plant-suppressive compounds
- Members of the genus *Pseudomonas*

Research by Ann Kennedy, USDA, WSU
Plant-Suppressive Compounds

- No visible lesions
- No signs of pathogenicity
- Inhibit root cell elongation
- Interrupt tiller initiation
- Root stunting
- Reduce seedling vigor
- Reduce tiller number
- Reduce seed number
*P.f.* D7 colonization of root

*P.f.* D7 on wheat seed

*P.f.* D7 is carried down wheat root, intercepts and grows down downy brome root
Pseudomonas fluorescens strain D7

Active Compound(s):
Chromopeptide, peptides, fatty acid esters & polysaccharides

All must be present for activity

Reduces lipopolysaccharide production - No cell elongation

Genes: Multiple loci (very unlikely to mutate)
SCREENING >10,000+ isolates

Isolate Soil/Root Bacteria after freeze/thaw

100%

Bioassay weed

Does not inhibit weed

Inhibits weed

50%

Bioassay beneficial plants

Inhibits beneficial plants

Does not inhibit beneficial plants

Does not inhibit weed or inhibits beneficial plants

Does not inhibit weed, inhibits beneficial plants, or does not survive

Test in greenhouse soil

Inhibits weed, not beneficial plants

1%

Field tests

Inhibits weed, not beneficial plants

0.5%

Autoclave

Autoclave

BIOCONTROL AGENT
D7 - Good match for biocontrol

Bacteria produces selective toxin at low temperatures that inhibits root growth.

Reduces competitiveness of weed in fall and spring.

Allows other plants to be more competitive.
Species inhibited by *P.f. D7*

**Greenhouse studies**

**Downy brome**  
**California brome**  
**Mountain brome**  
**Jointed goatgrass**  
**Medusahead**

*Bromus tectorum*  
*Bromus carinatus*  
*Bromus marginatus*  
*Aegilops cylindrica*  
*Taeniatherum caput-medusae*

*P.f. D7 DOES NOT INHIBIT*

*Pf. D7 inhibits some grasses, but little else*
Characteristics of downy brome are similar to *Poa annua*:

- Invasive, competitive weed
- Germinate in the fall and spring
- Competitive low temperature root growth
- Produce many tillers and seed
Field Studies
Winter wheat no-till planted
Natural infestation of downy brome; Benge, WA

Inoculated Plot

Control Plot

Downy brome was inhibited by bacteria in the field
WA Winter Wheat Field Trials

*P.f. D7* applied in fall as spray; counts taken following spring.

<table>
<thead>
<tr>
<th>% Reduction of Downy Brome</th>
<th>1% SOM</th>
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<th>3% SOM</th>
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<td>Ritzville</td>
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<td>Pullman</td>
<td>50</td>
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<td>Dayton</td>
<td>10</td>
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<td>5</td>
<td>No D7 Survived</td>
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* = P < 0.05

**Pf. D7 must survive to suppress downy brome**
• Herbicide efficacy - growers wanted weed reductions in first year one
• Years later - growers mention patch-work of downy brome and no downy brome in treated fields
Pasture Field Trials, Pullman, WA

*P.f.* D7 applied as spray in fall

| Control | Applied 2004 | Pictures 2007 | + Bacteria |
|---------|--------------|---------------|------------|------------|

![Pasture Field Trials Image](image-url)
Cheatgrass Reduction with *P. f. D7*

Over time, *P. f. D7* and other plants reduced weed to near zero

5 replicate plots @ each site; *P*=0.05 for all years except *t*=0

Kennedy, Hansen, Stubbs (In preparation, 2011)
Early turfgrass research by Ann Kennedy’s group

Palouse Ridge GC
2011
Downy bromegrass control in rough
SCALE UP - Fermentors
For suppression of Downy Brome (cheatgrass), Medusahead, Japanese Brome, and Jointed Goatgrass on Wheat, Barley, Triticale, Oats, and Rangeland

**ACTIVE INGREDIENT:**
Pseudomonas fluorescens, strain D7*: ................................................................. 95.00%
Other ingredients: ........................................................................................................ 5.00%
.................................................................................................................................... 100.00%

*Contains a minimum of 2x10^{11} cells/g Pseudomonas fluorescens strain D7

**KEEP OUT OF REACH OF CHILDREN**

**CAUTION**

See directions for use in attached booklet

EPA Reg. No. 71975-4
EPA Est. No. 71975-WA-001

Net weight: 0.44 lb. (200 grams)
Batch #

Northwest Agricultural Products
P.O. Box 3453
Pasco, WA 99302
(509)547-8234
2013

2015
Jim Connolly - planet turf
Verdesian Life Sciences
Two Proposals:

1. Biological Control of *Poa annua* in Fairways

2. *Poa annua* Control in Fairways – Chemical Programs

NTA April 7, 2016

“The Research Committee and Board feel that [the] two submitted studies are related closely enough to be considered as one study.”
### 3-year studies

Chemical followed by Biocontrol (2016)

Fall (2015)
Biocontrol vs. Chemical

Biocontrol vs. Chemical

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3-year Study:
Chemical control followed by biological control
3-year Chemical / Biocontrol Study @ Palouse Ridge G C

D-7 1 app fall 2016 and **still to do** fall 2017 @ 3 rates 
(1, 5, and 15 fl oz/A; same as 2, 10, and 30 g/A) 
Tenacity + Xonerase; 3 apps fall 2016 and spring 2017 
@ 4 and 1.4 fl oz/A 
PoaCure; 3 apps spring 2016, fall 2016, spring 2017 
@ 1.26 fl oz/M and **still to do** 3 apps fall 2017
Percent change of *Poa annua* in a Kentucky bluegrass stand

- 6/13/13 (6 WAIT)
- 7/24/13 (12 WAIT)
- 9/6/13 (18 WAIT)

**Bars:**
- Tenacity 4 fl oz/A + Trimmit 16 fl oz/A + NIS
- Tenacity 5 fl oz/A + Turflon 16 fl oz/A + NIS
- Tenacity 4 fl oz/A + Xonerate 1 oz/A + NIS
- Tenacity 5 fl oz/A + Trimmit 16 fl oz/A + NIS
- Tenacity 5 fl oz/A + Xonerate 2 oz/A + NIS
- CHECK
Tenacity 5 fl oz/A + Xonerate 2 oz/A

CHECK

Herbicides applied May 2, May 23 and June 13
Tenacity 4 fl oz/A + Xonerate 1 oz/A

Herbicides applied May 2, May 23 and June 13
Tenacity/Xonerate treatments did not eliminate *Poa* but did result in significant reductions of *Poa* in the Kentucky bluegrass stand. However, the high level of *Poa* injury and depressions caused by the Tenacity 5 fl oz/A + Xonerate 2 oz/A treatment leaving pock marked fairways may result in undesirable playing conditions during the peak of the summer when play is highest. (Eventually KBG did grow into and fill these depressions by the end of the study).

Tenacity 4 fl oz/A + Xonerate 1 oz/A also resulted in high levels of *Poa* reduction. However, this treatment did not cause *Poa* to quickly disappear, thus not creating open areas (depressions) in the fairway. This may be a more desirable *Poa* control option to consider in terms of playability.

Complete *Poa* control was not achieved with any treatment in this study, therefore, a multi-year program may need to be looked at to possibly achieve this goal or maintain *Poa* at lower levels.
PoaCure

Methiozolin

Moghu Research Center

South Korea

Registered for turf in Korea
April 2010
Pine Ridge Country Club, South Korea

>40% *Poa annua*  2013. 05. 16  After 3 apps  2014. 05. 11
Photo taken 5/16/15
Baltusrol GC - site of 2016 PGA Championship

Mark Kuhns, CSGS  Director of Grounds

psu149@aol.com

June 2017
Poa annua control with PoaCure on bentgrass greens

3 applications @1.2 fl oz/1000 ft² (4 L/ha) at 2 week intervals; Fall 2011

Colfax Golf Course
Picture taken 9-28-12
Annual Bluegrass Germination Test with ‘D-7’ and PoaCure
Annual Bluegrass Emergence Test with ‘D-7’ and PoaCure
25 days after planting

CHECK
‘D-7’ 2 g/A
‘D-7’ 20 g/A
PoaCure 4L/ha
Methiozolin Residual Soil Activity Study 2012

4 L/ha = 1.2 fl oz/1000 ft²
Fall Applied PoaCure to Control *Poa annua* in KBG Seed Fields

PI 371775 KBG Seed Increase TARC Plot 12. 5-6-13.
Fall Applied PoaCure to Control Poa in KBG Seed Fields
PI 371775 KBG Seed Increase TARC Plot 12. 5-6-13.

All PoaCure treatment - 2L, 4L, 2L + 2L, or 4L + 4L/ha controlled *Poa annua* Fall 2012
PoaCure Questions?

More WSU turfgrass information: TURF.WSU.EDU

Updates on PoaCure research from different regions:
  • www.moghu.com/en/index
  • Twitter @PoaCure, @MoghuUSA

Other questions regarding PoaCure, contact: PoaCure Development Manager - Kyung Han
  • kyungmhan@moghu.com
  • 717-350-9829
3-year Chemical/Biocontrol @ Palouse Ridge G C

- Poa annua (% stand)
- Ten. 4 fl oz + Xon. 1.4 fl oz (D-7 1 fl oz/A)
- Ten. 4 fl oz + Xon. 1.4 fl oz (D-7 5 fl oz/A)
- Ten. 4 fl oz + Xon. 1.4 fl oz (D-7 15 fl oz/A)
- PoaCure 1.26 fl oz/M (D-7 1 fl oz/A)
- PoaCure 1.26 fl oz/M (D-7 5 fl oz/A)
- PoaCure 1.26 fl oz/M (D-7 15 fl oz/A)
- Check

Sp ’16 PC 3 apps Fall ’16 PC 3 T+X 3 D7 1 app Fall ’17 PC 3 T+X 3 apps D7 1
3-year Chemical/Biocontrol @ Palouse Ridge GC

Turf quality (9 = excellent)

- Ten. 4 fl oz + Xon. 1.4 fl oz (D-7 1 fl oz/A)
- Ten. 4 fl oz + Xon. 1.4 fl oz (D-7 5 fl oz/A)
- Ten. 4 fl oz + Xon. 1.4 fl oz (D-7 15 fl oz/A)
- PoaCure 1.26 fl oz/M (D-7 1 fl oz/A)
- PoaCure 1.26 fl oz/M (D-7 5 fl oz/A)
- PoaCure 1.26 fl oz/M (D-7 15 fl oz/A)
- Check

Bar charts showing turf quality on 7/6/17 and 9/15/17.
D7 or PoaCure (Fall 2015) @ Palouse Ridge G C

To do: PoaCure 3 apps fall 2017 and fall 2018
D7 or PoaCure (Fall 2015 – Spring 2016) @ Palouse Ridge GC

To do: PoaCure 3 apps fall 2017, spring 2018, and fall 2018
3-year Study; chemical/biocontrol
3-year Study; chemical/biocontrol
PoaCure 1.26 fl oz/M
(3 apps Spring, Fall 2016 and Spring 2017)
D-7 15 fl oz/A (1 app Nov. 2016)
3-year Study; chemical/biocontrol
Tenacity 4 fl oz/A + Xonerase 1.4 fl oz/A
(3 apps Fall 2016 and Spring 2017)
D-7 15 fl oz/A (1 app Nov. 2016)
3-year Study; chemical/biocontrol

CHECK
3-year Study; chemical/biocontrol
PoaCure 1.26 fl oz/M
(3 apps Spring and Fall 2016 and Spring 2017)
D-7 15 fl oz/A (1 app Nov. 2016)
3-year Study; chemical/biocontrol
Tenacity 4 fl oz/A + Xonerate 1.4 fl oz/A
(3 apps Fall 2016 and Spring 2017)
D-7 15 fl oz/A (1 app Nov. 2016)
2018

Fading interest in D7 for *Poa annua* control in turf
Cheatgrass Reduction with *P.f. D7*

- **Years after application**
- **Cheatgrass Plants (Percent)**

**Over time, P.f.D7 and other plants reduced weed to near zero**

- *Saddle Mt, WA*
- *Rock Island, WA*
- *Colton, WA*
- *Pullman, WA*

5 replicate plots @ each site; *P*=0.05 for all years except *t*=0

Kennedy, Hansen, Stubbs
(In preparation, 2011)
New biocontrol product for testing:
  Three *Pseudomonas fluorescens* strain mixture

Excellent control on *Poa annua* root growth
(agar, greenhouse, and field trials)

Excellent control on *Poa annua* germination
(antidotal?)

New formulation

WSU will continue current ongoing D7 and chemical studies fall 2017 and through 2018
<table>
<thead>
<tr>
<th>Root growth inhibition</th>
<th>Strain</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td><em>Poa annua</em></td>
<td>100</td>
</tr>
<tr>
<td><em>Poa trivialis</em></td>
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<tr>
<td>Creeping red fescue</td>
<td>10</td>
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<tr>
<td>Tall fescue</td>
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<tr>
<td>Creeping bentgrass</td>
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<td>Kentucky bluegrass</td>
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<tr>
<td>Perennial ryegrass</td>
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<tr>
<td>Downy brome</td>
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No effect on dicots (32 evaluated)
2018 research protocol:

Effect on germination

Effect on seedling growth (growth chamber/green house

Effect of strain ratios other that 1:1:1

Effect on mature *Poa annua* on golf course field sites
WSU turf position – Status update

1. Grass Ecology position
   a. Turf
   b. Seed
   c. Currently on hold; budget crisis at WSU
   d. Teaching – all current students have had all 3 turf courses
   e. Research - Charles Golob and I continue onward
   f. State funding of technicians ended July 1, 2017

2. Turf Plots
   “Moving again”; 3rd time in one career
   Grass Ecology Research & Teaching Laboratory
Thanks for the mentoring, support, and friendship.

Interacting with the NTA has been a great.

All the best!