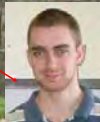
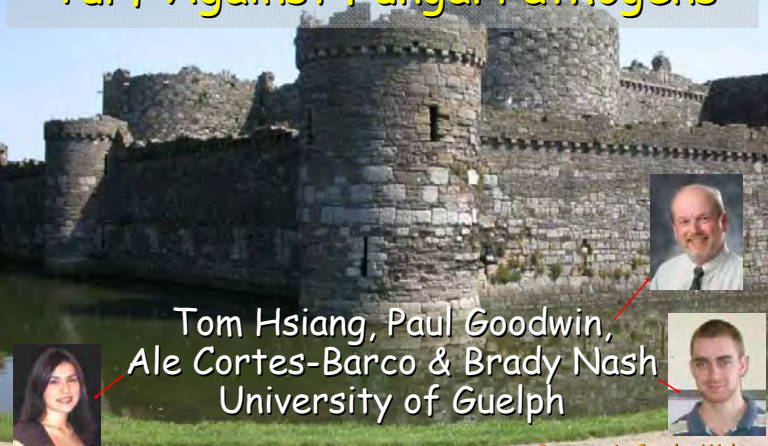


Activating Disease Resistance in Turf Against Fungal Pathogens



Tom Hsiang, Paul Goodwin,
Ale Cortes-Barco & Brady Nash
University of Guelph

Beaumaris Castle, Wales

Activated resistance

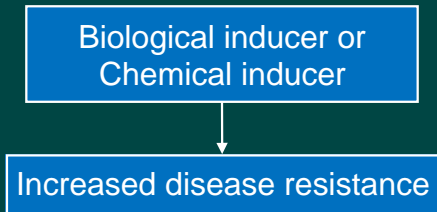
- Utilize a plant's natural defenses
 - against insects
 - against diseases
 - against abiotic stresses

Activated resistance

- Certain chemicals or organisms are known to activate natural resistance mechanisms
 - Phosphites
 - » Aliette, Apear (in U.S.)
 - Benzothiadiazole/Acibenzolar
 - » Actigard (Tomato & Tobacco diseases)
 - » Daconil Action (Dac + Actigard, in U.S.)
 - Chelated metals?
 - » Chelated iron
 - » Chelated copper
 - Civitas + Harmonizer

Stimulating disease resistance response

- Two main forms:
- Systemic acquired resistance (SAR)
- Induced systemic resistance (ISR)



Activated Resistance - induction

- After the signal, the soldiers in the castle are all given extra rations of caffeine to keep them awake and shooting; constant production of new arrows and boiling vats of oil....



Activated Resistance - priming

- After the signal, the cannons are loaded, the guns cocked, bows armed, oil vats set to boil, and sentries on alert so action can begin as soon as enemies get close



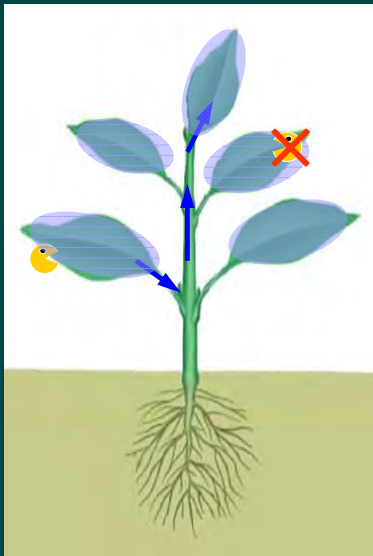
SAR

activator or pathogen attack triggers salicylic acid pathway

↑ systemic signals induce defense response genes which may also respond more strongly upon attack

these produce PR proteins & other defense chemicals

✗ can reduce colonization by the pathogen



ISR

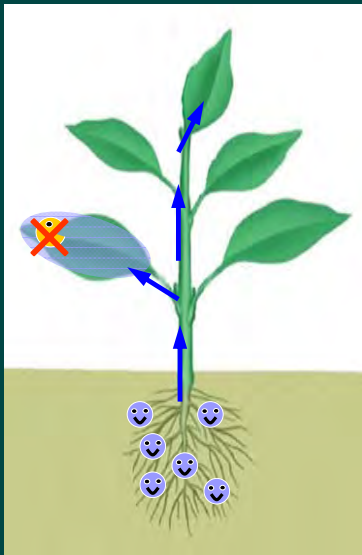
beneficial root microbes (or activators applied to roots/leaves) increase sensitivity

to jasmonate/ethylene systemic signals cause more rapid defense gene expression (**priming**)

after pathogen attack

these produce phenolics, proteinase inhibitors & other defense chemicals

can reduce colonization by the pathogen



Defense activator?

- Civitas (civitasturf.com)



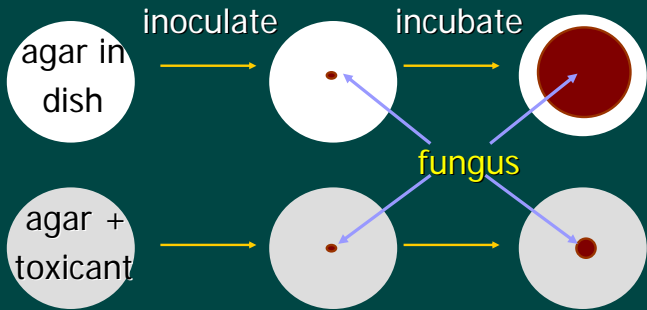
What is Civitas?

- Civitas is a mixture of food-grade synthetic isoparaffins and a food-grade emulsifier produced by Petro Canada and registered in US & Canada
- Clear, colourless liquid at room temperature, with 16 to 36 Carbons
- Label rates of ~5% in 4-20 L water per 100m²
- High Rate = 16 oz / 1000ft²
- Low rate = 8 oz / 1000ft²

How does Civitas work?

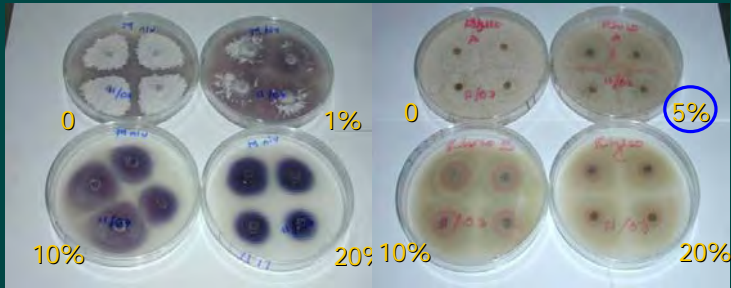
- Direct antifungal activity?
 - kill fungi in petri plates?
- Activate disease resistance in plants?
 - make plants more resistant in lab tests?
 - suppress disease in the field?
 - alter gene expression in treated plants?

Direct antifungal activity



Antifungal activity

- 16 turf pathogens tested on 0 to 20% Civitas added to culture media



Microdochium nivale
(causing pink snow mold)

Rhizoctonia solani
(causing brown patch)

How does Civitas work?

- Direct antifungal activity?
 - kill fungi in petri plates? ✘
- Activate disease resistance in plants?
 - make plants more resistant in lab tests?
 - suppress disease in the field?
 - alter gene expression in treated plants?

Civitas applied to *Agrostis* roots 7 days before dollar spot fungus, and rated 5 days after inoculation

Untreated Control

Inoculated Control

10% Civitas

20% Civitas



Civitas applied to *Agrostis* roots 7 days before pink snow mold fungus, and rated 5 days after inoculation

Untreated Control

Inoculated Control

10% Civitas

20% Civitas



Civitas applied to *Agrostis* roots 7 days before brown patch fungus, and rated 5 days after inoculation

Untreated Control

Inoculated Control

10% Civitas

20% Civitas



How does Civitas work?

- Direct antifungal activity?
 - kill fungi in petri plates? X
- Activate disease resistance in plants?
 - make plants more resistant in lab tests? 😊
 - suppress disease in the field?
 - alter gene expression in treated plants?

Field test: May 2005 snow mold trial

Agrostis stolonifera at greens height (inoculated)

An aerial photograph of a golf green, which is a large, roughly rectangular area of grass. The green is divided into two sections by a diagonal line. The upper-left section is labeled 'Civitas' and the lower-right section is labeled 'PCNB'. Both sections show some light-colored, irregular patches on the grass, likely due to the snow mold inoculation. The background is a dark green color.

Civitas

PCNB

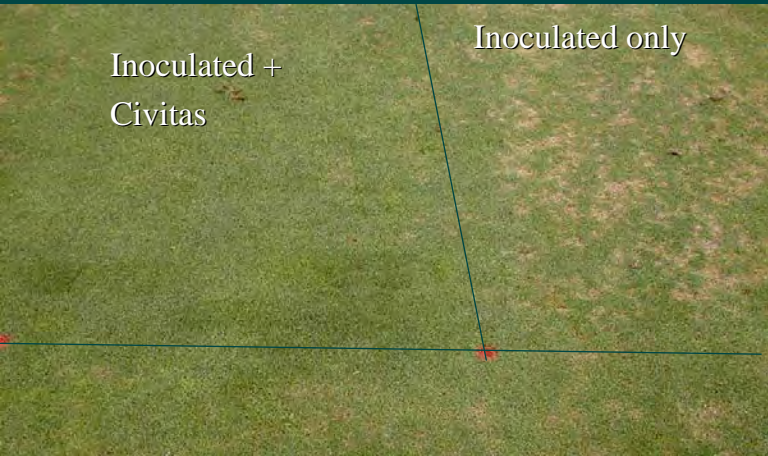
- Civitas worked as well as PCNB in this test

Field test: Aug 2006 Dollar spot trial

Agrostis stolonifera at greens height

Inoculated +
Civitas

Inoculated only



Field test: April 2008 snow mold trials

Agrostis stolonifera at greens height

1/2-rate Banner Civitas+ 1/2 rate Banner

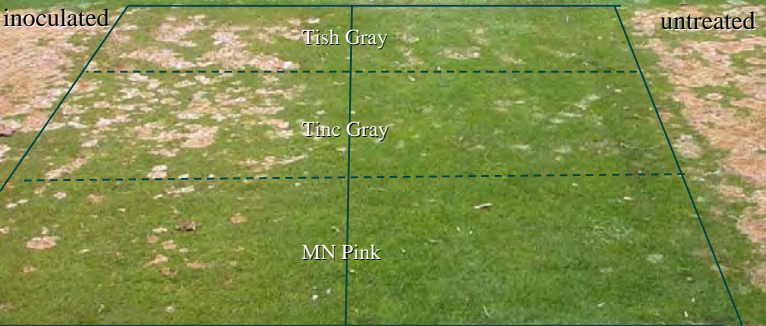
inoculated

untreated

Tish Gray

Tinc Gray

MN Pink



How does Civitas work?

- Direct antifungal activity?
 - kill fungi in petri plates? X
- Activate disease resistance in plants?
 - make plants more resistant in lab tests? 😊
 - suppress disease in the field? 😊
 - alter gene expression in treated plants?

Gene Expression Analysis

■ Treatments

- butanediol (ISR inducer in broadleaf plants)
- Civitas

■ Identify marker genes for ISR by looking for genes known to be activated during ISR in other monocots

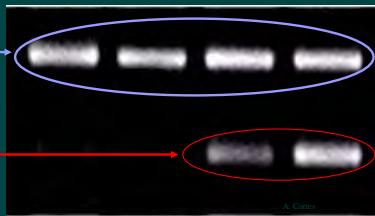
- **Induction** = activation before pathogen
- **Priming** = enhanced activation after pathogen

Methods: relative gene expression

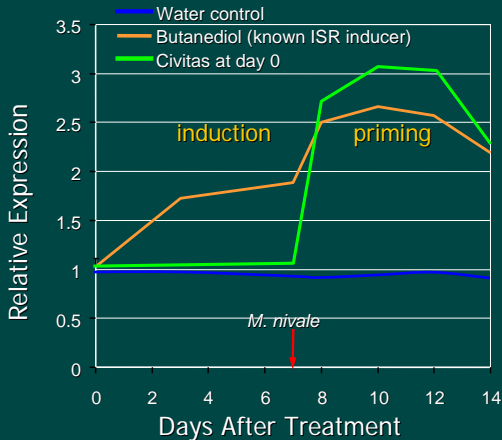
- Gene expression with relative RT-PCR
- Band strength was determined relative to a constitutive creeping bentgrass gene

Control gene

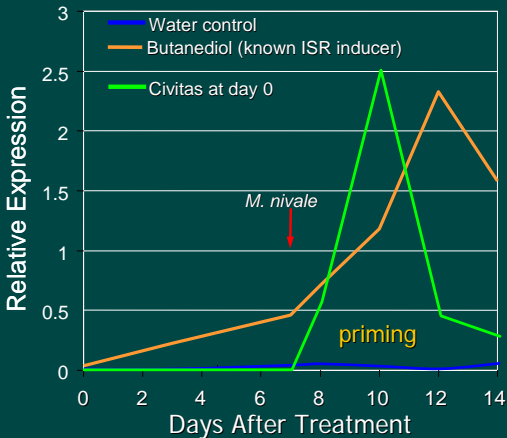
Gene of interest



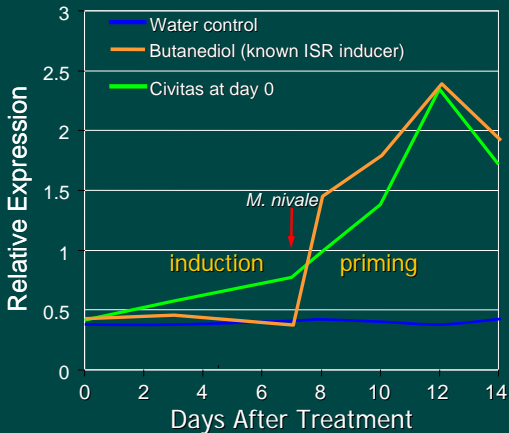
AsOPR4 expression in *A. stolonifera*



AsGNS5 expression in *A. stolonifera*



AsOS1 expression in *A. stolonifera*



How does Civitas work?

- Direct antifungal activity?
 - kill fungi in petri plates? **X**
- Activate disease resistance in plants?
 - make plants more resistant in lab tests? 😊
 - suppress disease in the field? 😊
 - alter gene expression in treated plants? 😊

Conclusions on Civitas

- some disease suppression in lab & field
- Mode of action of Civitas is **ISR** based on expression analysis of several genes and comparison with known ISR activator
- Cortes-Barco AM, Hsiang T, Goodwin PH. 2010. Induced systemic resistance against three foliar diseases of *Agrostis stolonifera* by (2R,3R)-Butanediol or an isoparaffin mixture. *Annals of Applied Biology* 157:179-189.

pigments & colorants used on turf for >50 yrs
do they just add color or do they affect the turf?



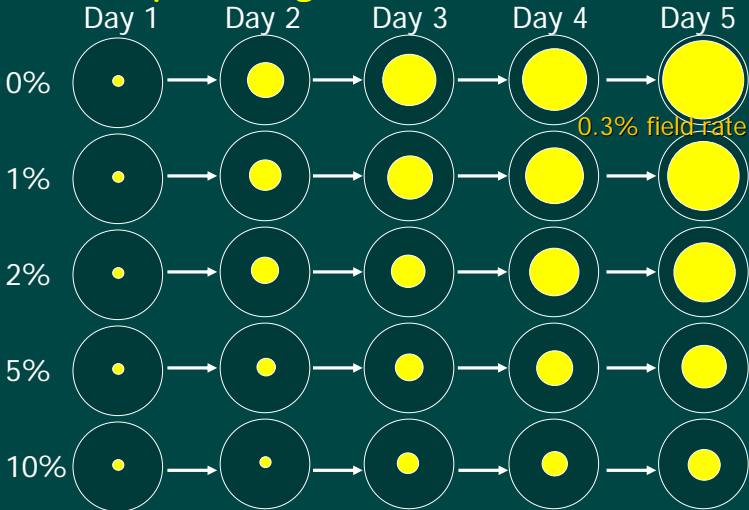
What is Harmonizer?

- Pigment dispersion containing chelated copper
- Produced by Petro Canada and registered in US & Canada
- Dark green solution which strongly stains
- Label rates of ~0.3% in 4-20 L water/100m²
- Mixed with Civitas in a 16:1 ratio (C:H)

How does Harmonizer work?

- Direct antifungal activity?
 - kill or inhibit fungi in petri plates?
- Activate disease resistance in plants?
 - make plants more resistant in lab tests?
 - suppress disease in the field?
 - alter gene expression in treated plants?

Dollarspot fungus on Harmonizer



How does Harmonizer work?

- Direct antifungal activity?
 - kill fungi in petri plates? **X**
- Activate disease resistance in plants?
 - make plants more resistant in lab tests?
 - suppress disease in the field?
 - alter gene expression in treated plants?

Harmonizer sprayed on *Agrostis* leaves,
7 d before dollar spot fungus, and rated
7 d after inoculation

non-inoculated

inoculated
control

5% Harmonizer



60 ± 2.2 %
yellowing

34 ± 2.3 %
yellowing

Harmonizer applied to *Agrostis*
roots 7 d before dollar spot fungus,
and rated 7 d after inoculation

non-inoculated

inoculated
control

5% Harmonizer



53 ± 1.1 %
yellowing

33 ± 1.3 %
yellowing


How does Harmonizer work?

- Direct antifungal activity?
 - kill fungi in petri plates? **X**
- Activate disease resistance in plants?
 - make plants more resistant in lab tests? 😊
 - suppress disease in the field?
 - alter gene expression in treated plants?

October 08 dollar spot trial

Agrostis stolonifera at greens height

inoculated
with dollarspot
fungus



inoculated
+ Civitas
+ Harmonizer

April 2008 snow mold trials (*Agrostis stolonifera* at fairway height)

half rate

Banner

Dac/Rov

untreated PCNB

Inoculated

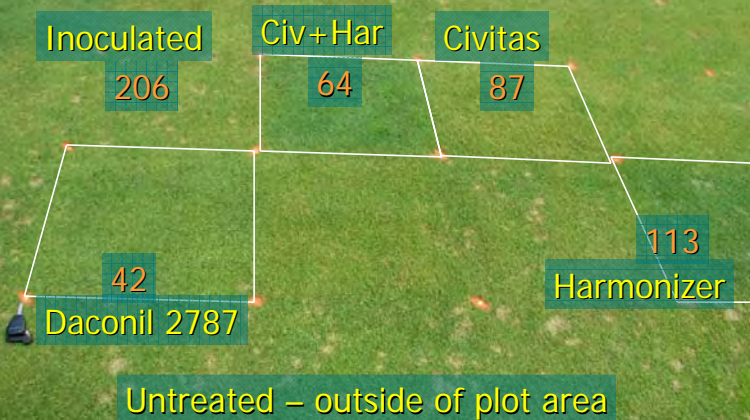
Dac/Rov

Civitas & Harmonzier
at different rates

Untreated - outside of plot area

August 2010 dollar spot trial

Agrostis at greens height, 3 wk after last treatment
numbers reflect dollar spot level over entire trial



How does Harmonizer work?

- Direct antifungal activity?
 - kill fungi in petri plates? X
- Activate disease resistance in plants?
 - make plants more resistant in lab tests? 😊
 - suppress disease in the field? 😊
 - alter gene expression in treated plants?

Effect of Harmonizer on expression of SAR and ISR marker genes (RT-PCR) in *Agrostis*

Gene	Mode of action	Expression
Lipoxygenase	SAR	no change
Aspartic protease	SAR	no change
Hypersensitive-response induced gene	SAR	no change
Glucanase 5	ISR	no change
12-oxo-phytodienoic acid reductase 7	ISR	no change
Allene oxide synthase 1	ISR	no change

SAR = Systemic Acquired Resistance (induced by BTH in *Agrostis*)

ISR = Induced Systemic Resistance (induced by Butanediol in *Agrostis*)

Large scale gene expression (RNAseq)

- 10,000+ genes in *Agrostis* detected
- 1,000+ >2x expression by Harmonizer (7 d pre)
less than two-thirds had annotations
- Manually selected 20 related to resistance

Effect of Harmonizer on gene expression

(Harmonizer 7 d pre vs. water)

Genes used in RT-PCR

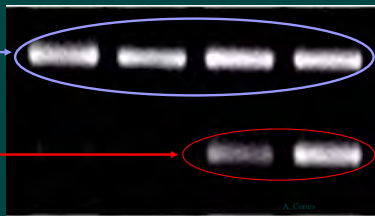
Gene	Expression
Cell wall-associated hydrolase	2.0x
PAL-4	2.0x
PAL-1	2.1x
PR class i	2.1x
WRKY-6	2.1x
Bacterial-induced peroxidase	2.2x
Harpin-induced protein	2.2x
Ethylene responsive protein	2.3x
Hypersensitivity related protein	2.5x
Disease resistance protein	2.8x
PR-5	2.8x
Chitinase	3.0x
Jasmonate-induced protein	3.2x
Lipoxygenase	3.3x
Nematode-resistance protein	3.5x
Endo-beta glucanase	3.6x
Rapidly elicited protein	4.1x
4-coumarate coenzyme A ligase	4.5x
PR-10	4.5x
PR-1 (basic)	20x

Methods: relative gene expression

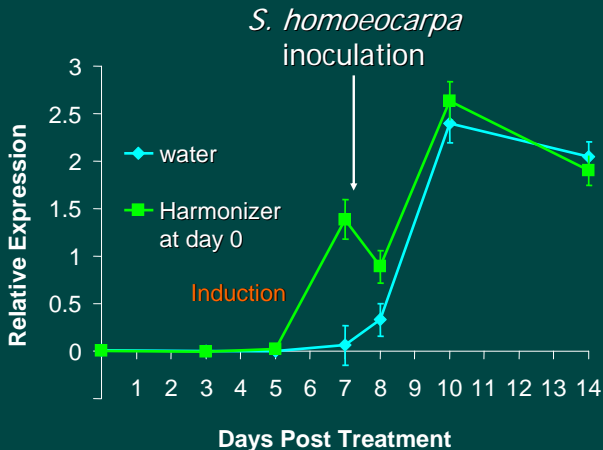
- Gene expression with relative RT-PCR
- Band strength was determined relative to a constitutive creeping bentgrass gene

Control gene

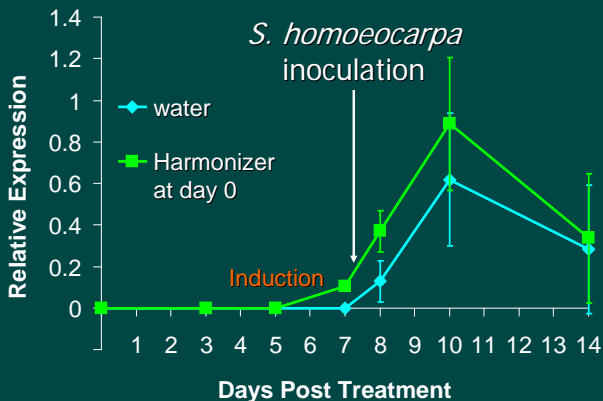
Gene of interest



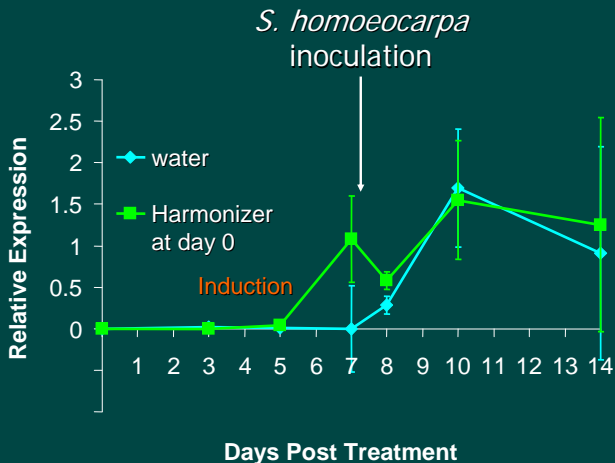
Effect of Harmonizer on *AsPR-1* basic (RT-PCR)



Effect of Harmonizer on *AsPR-5* (RT-PCR)



Effect of Harmonizer on *AsPR-10* (RT-PCR)



How does Harmonizer work?

- Direct antifungal activity?
 - kill fungi in petri plates? **X**
- Activate disease resistance in plants?
 - make plants more resistant in lab tests? 😊
 - suppress disease in the field? 😊
 - alter gene expression in treated plants? 😊

Conclusions on Harmonizer

- some effect against diseases
- affects genes differently than Civitas
 - Civitas primes more (ISR related)
 - Harmonizer induces more (SAR related?) but maybe different mode of action than ISR / SAR
- Harmonizer combined with Civitas has greater effects than either alone against turf diseases
 - High Rate = 16 oz CIVITAS + 1 oz Harmonizer or 17 oz of CIVITAS ONE
 - Low rate = 8 oz CIVITAS + 0.5 oz Harmonizer or 8.5 oz of CIVITAS ONE

Activating defenses - consequences

- can reduce growth rates
- can slow plant development
- can lower crop yield
- stressed plants show even more negative effects
- can cause plant death (if too much)

- Think of activators like steroids