

Goal: Find grass seed varieties that are high in endophytes that are toxic to insects but low in those that are toxic to mammals, such as livestock and other herbivores that might consume them.

Background

- ❖ The sod webworm (*Chrysothoechia topiaria* Zeller) is one of the most destructive insect species found within grass seed operations in Oregon.
- ❖ Pest management can sometimes cost up to \$15/acre (personal communication), for limited success.
- ❖ Eggs (above) are laid by females in the fall which hatch into larvae that burrow into the soil, spinning fragments of grass into a cocoon for the winter, earning the nickname "webworm".¹
- ❖ Larvae (left) emerge from the soil in the spring and begin consuming the grass, with damage apparent by late June.² Damage is characterized by discoloration and thinning (below).³
- ❖ Larvae develop into adult moths which are active in June and July.⁴ Adult moths do not cause damage to grass species which are often being harvested at this time.⁵
- ❖ Larvae will feed until late fall, before burrowing into cocoons.⁴
- ❖ Insecticides are often applied at this point to target the larvae freshly emerged from eggs, but rainfall is essential to ensuring effective translocation to the roots of grasses.⁴



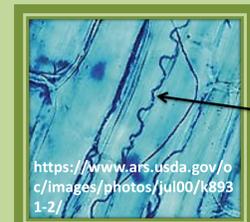
What is an

within

ENDO

PHYTE?

plant



Endophytes are fungi that live within plants in a symbiotic relationship.⁷

Endophytes can protect plants by producing metabolites that deter insects, acting as "natural" insecticides.⁶



It's important to find grass seed varieties that can be used as effective insecticides but pose no risk to mammalian herbivores that might consume the grass.

❖ In the Willamette Valley September averaged 1.58 inches of rain from 2000 to 2020, however, larvae may have already gone underground by the time insecticides are applied, due to the average low temperature for that time frame being 48°F, rendering them almost useless.⁵



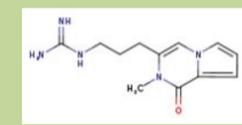
Damage by the sod webworm (left) versus unaffected, healthy plant growth (right)



Types of Endophytes

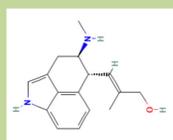
- ❖ Fungal biosynthesis of the ergoline and ergot alkaloids begins with the same four ring carbon-nitrogen structure.
- ❖ The addition of specific side groups defines each unique compound, with ergot alkaloids additionally incorporating an amide bond into their structure.
- ❖ Ergoline alkaloids are effective insecticides, while ergot alkaloids pose a larger toxicological threat to mammalian herbivores

Peramine



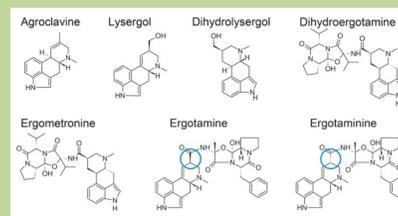
Chem ID plus, Peramine, RN:102482-94-0

Chanoclavine



PubChem Compound Summary for CID 5281381

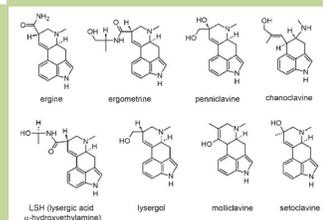
Ergoline Alkaloids



Nowak et. al, "Identification and determination of ergot alkaloids in Morning Glory cultivars", 2016

- ❖ Peramine has been associated with insect resistance and was found in a few perennial ryegrass samples that were found to have high insect mortality.
- ❖ Chanoclavine is another compound that has shown promise as an insecticide that is safe for mammalian herbivores.

Ergot Alkaloids



Zhang et. al, "Analysis of Ergot Alkaloids in Cereal Samples by Liquid Chromatography-Tandem Quadrupole Mass Spectrometry", 2017

- ❖ 32 samples were selected from grass cultivars that were grown under standard greenhouse conditions using a standard 16:8 photoperiod.

- ❖ These samples were comprised of tall (*Festuca arundinacea*) (n=10, TF) and fine fescue (*Festuca spp.*) (n=8, FF), orchard grass (*Dactylis glomerata*) (n=3, OG) and perennial ryegrass (*Lolium perenne*) (n=11, PRG) species.



- ❖ The samples were then hand ground and prepared for extraction via the Sulyok method, and were then quantified via LC-MS/MS.⁸

- ❖ Samples were screened for concentrations of ergot and ergoline alkaloids as well as the compound peramine.



Interested in learning more about Endophytes?



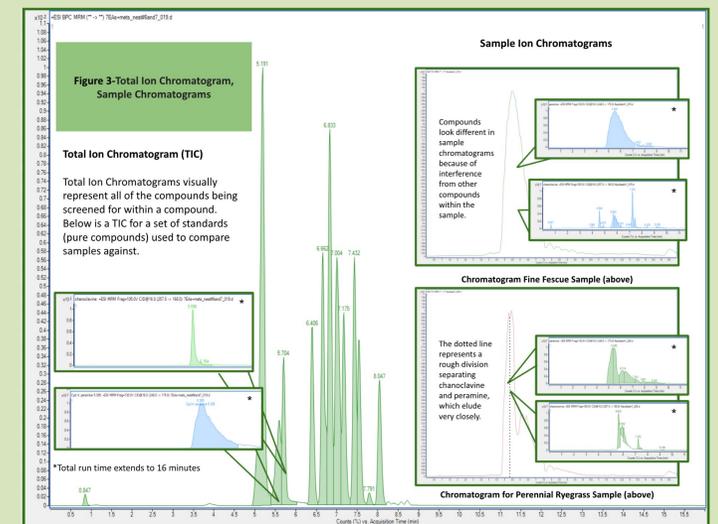
Visit this link to view some of OSU's publications on endophytes and learn more about the Endophyte Service lab.

Results

Table 1-Survey for Ergot and Ergoline Alkaloids in Grass Cultivars and Insect Mortality Rates

Grass Species	Ergot Alkaloids Average Concentration (ng/g)* ± STDEV	Ergoline Alkaloids Average Concentration (ng/g)* ± STDEV	Insect Mortality
Fine Fescue (n=8)	24 ± 28	2,479 ± 7,740	35%
Tall Fescue (n=10)	23 ± 13	173 ± 228	51%
Orchard Grass (n=3)	39 ± 26	1,665 ± 5,170	52%
Perennial Rye Grass (n=11)	91 ± 87	275 ± 479	49%

Discussion



- ❖ When correlating percent mortality to mycotoxins detected, it was found that plants with over a 50% insect mortality had elevated levels of peramine and chanoclavine.
- ❖ Peramine is a compound of interest because it has shown promise as an insecticide in New Zealand in combatting predation by the Argentine stem weevil and has no known toxicity to mammalian herbivores.⁹

Table 2-Ranking of Grass Species[^]

Ergot Alkaloid Concentrations	Ergoline Alkaloid Concentration	Insect Mortality
1. Perennial Rye Grass	1. Fine Fescue	1. Orchard Grass ⁺
2. Orchard Grass	2. Orchard Grass	2. Tall Fescue ⁺
3. Fine Fescue	3. Perennial Rye Grass	3. Perennial Rye Grass
4. Tall Fescue	4. Tall Fescue	4. Fine Fescue

⁺Within 2%
[^] Approximate rankings, individual samples and varieties will vary

- ❖ Peramine has been associated with insect resistance and was found in a few perennial ryegrass samples that demonstrated high insect mortality.
- ❖ Chanoclavine has also shown promise as a potent insecticide with low mammalian toxicity.¹⁰
- ❖ The standard deviation is high in these samples due to the fact there is a considerable range in positive concentrations.

Conclusions and Future Work

- ❖ This project is part of a larger goal to preserve Oregon's grass seed industry.
- ❖ Further work will be conducted to study insect tolerance against plant species cultivars and mycotoxin products.
- ❖ Utilizing endophytes within plants to combat insect pests could be a natural alternative to current synthetic pesticides.
- ❖ Surveying for indigenous entomopathogenic nematodes (EPNs) could serve as a two-pronged approach in development of an integrated pest management program for the sod webworm.

- ❖ The potential use of CRISPR is also being explored as a method to modify genes in the fungi that allow the production of ergoline alkaloids but prevent the production of ergot alkaloids.

Sources

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1-Uga.edu- UGA Extension, 2019, "Sod Webworms: Biology and Management in Turfgrass"
2-Unh.edu- New York State IPM, 2020, "Sod Webworm: A variety of snout moths..."
3-UNL Department of Entomology, "Turf Grass- Sod Webworms"
4-Pacific Northwest Pest Management Handbooks, "Grass seed- Sod webworm (cranberry girdler)"
5- <https://www.ncei.noaa.gov/orders/cdo/2694035.pdf>

6-Vega, 2008, Journal of Invertebrate Pathology, Vol. 98
7-Saikkonen et al., 2006, Trends in Plant Science, Vol. 11
8-Sulyok, 2007, Anal Bioanal Chem 389
9-AgPest, "Choosing a Ryegrass Endophyte"
10- Finch et al., Toxicity Studies of Chanoclavine in Mice. *Toxins*. 2019; 11(5):249. <https://doi.org/10.3390/toxins11050249>