The Grass is Greener in Kentucky

by
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Part I – Super Grass

Summer had arrived, and Nathan was looking forward to the research project he would be working on in Dr. Miller's lab. He had done well for himself, having been awarded one of only a handful of funded summer studentships. Other than mentioning that he would be working with "super grass," Dr. Miller hadn't really told him much about the project. His dad commented, "You'll be working with Gaz and Rob? Wow," he said, and then started to sing, "You are young, you run green, ..., you're alright!" Nathan just shrugged at his dad's attempt at a pop musical reference.

After meeting the lab members over coffee, he was shown around the plant growth rooms. Tufts of grass grew in small pots; elsewhere, individual grass seedlings were lined up in upright petri dishes with clear jelly. That was supposed to be "super grass"?

"You don't seem impressed. Let me tell you a story," Dr. Miller started. "One fine October day almost a hundred years ago, agricultural experts from the University of Kentucky went to a county fair in Frenchburg. After judging a sorghum syrup contest—sorghum is a *big* deal in Kentucky—one of the experts was approached by a farmer who talked excitedly of a 'wonder grass' growing on his neighbour Bill Suiter's farm. Dr. Fergus was persuaded to visit, and the wonder grass did not disappoint; growing on a steep hillside pasture, it had remained green and lush despite the severe drought that summer. Bill claimed it had even stayed green through most of the winter. Dr. Fergus identified it as tall fescue, a grass species that originally came from Europe and had been introduced to the United States sometime in the 1800s.

"Starting with a good handful of seed from Suiter's farm, Fergus and colleagues developed the cultivar 'Kentucky 31' and released it to farmers in 1943. Fescue came to be viewed as a wonder grass that grows on a wide variety of soil types, slows down erosion and resists both summer drought and winter cold. This meant nutritious fresh grass for cattle at times when it used to be in short supply. Kentucky 31 became wildly popular and is today still one of the most widely grown forage and turf grasses in the U.S."

"All right, sounds like a super grass," said Nathan.

"Not quite," replied Dr. Miller. "Soon after the release of Kentucky 31, reports came in of dairy farmers who claimed it was unpalatable for their cattle. Beef producers noticed that animals who fed on fescue were slow to gain weight. Dairy cows produced less milk. Sometimes the animals developed particularly nasty sores and ultimately a kind of gangrenous condition that came to be known as 'fescue foot.' Some experts noticed that the symptoms look a lot like ergot poisoning, also known as St Anthony's fire. Both humans and animals notoriously convulse and develop gangrene after eating grains contaminated with the ergot fungus, *Claviceps purpurea*. However, the ergot fungus is easy to spot even for non-experts, and there was never any outward sign of ergot or other fungi on the fescue grass that caused toxic symptoms."

"So, what makes it toxic?" asked Nathan.

"Why don't you do what scientists do and form a testable hypothesis?" she replied. "This has been a tough nut to crack, in part because the toxic effects weren't always seen. I'll give you some more pointers for your detective work.

- On some farms, two cattle herds were grazing two tall fescue pastures separated only by a fence. Only one of the herds exhibited fescue toxicity symptoms; the other was fine.
- If you harvest fescue seeds from the "toxic pasture" and sow them, those plants are toxic again. Seeds from the non-toxic pasture produce non-toxic plants.
- When seeds from toxic plants are stored for a year or more, especially in very warm conditions like a grain silo, most plants grown from them are less toxic. Hay from toxic meadows gradually becomes less toxic during winter storage."

Questions

1. Try to think of multiple hypotheses that explain why tall fescue is generally, but not always, toxic.
2. Consider which hypotheses you can dismiss because they contradict one or more of the findings.
3. Choose your best hypothesis and outline how you would test it experimentally.

Note: You may be reassured to learn that it took experts several decades to find the answer! Don't be discouraged if you find no satisfying explanation; this is difficult, and the "true" cause is not obvious. Explore as many angles as you can. Make sure your hypotheses are concrete, not vague; follow each idea to its logical conclusion and articulate clearly why it can or cannot explain fescue toxicity.

Part II – Endophytes

"Okay, so what's the answer?"

"It took scientists a long time to find it. Turns out that all of the symptoms of fescue toxicosis were caused by infection with a fungus, *Epichloë coenophiala*, that grows entirely within the plant; a so-called *endophyte* (Figure 1). You can't see it from the outside.

"This fungus is closely related to one that causes choke disease where a furry 'collar' of fungal mass seems to strangle the plant (Figure 2, 3). The choke pathogen at first grows inside the plant without harming it, but then switches to a sexual life cycle and breaks out to form that 'collar.' This attracts small flies that help with fertilisation; sexual spores form and can be spread around.

"However, 'our' endophyte *E. coenophiala* does not do that. As far as we know, it stays within the plant, causes no damage, never breaks through and never produces sexual spores. It can't spread from one plant to another, except to the next generation via seeds.

"But our endophyte does produce toxic compounds that are very similar to those of the ergot fungus *C. purpurea*, to which it is also related. It makes perfect sense that cattle grazing on fescue with the endophyte had symptoms of ergot poisoning."

"So, can you get rid of the fungus?"

"You can, and it's been done; just treat the seeds or seedlings with a fungicide. People call it E– fescue, as opposed to the endophyte-infected E+ version. But E– was not a success; it was not nearly as drought and cold tolerant as E+. Grazing cattle preferred the taste of E– fescue, but insect pests and root

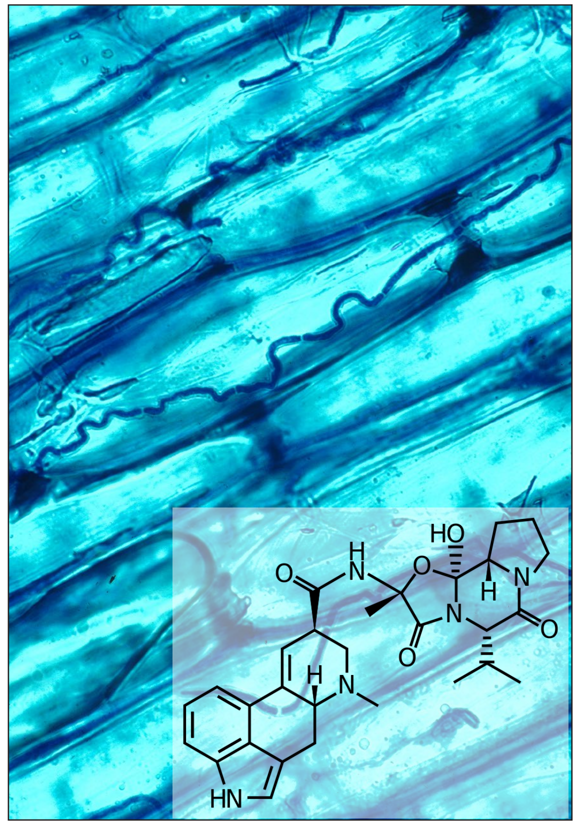

Figure 1. Hyphae of *Epichloë coenophiala* growing within tall fescue *(Festuca arundinacea)* leaf sheath tissue (400× magnification). Inset shows structure of ergovaline, one of the main alkaloids causing toxicosis. *Credit:* Nick Hill, USDA Agricultural Research Service, PD, <https://commons.wikimedia.org/wiki/File:Neotyphodium_coenophialum.jpg>.

Figure 2. Choke disease caused by *Epichloë typhina* growing on bentgrass *(Agrostis sp.)*. Image by M.J. Richardson, CC BY-SA 2.0, <https://www.geograph.org.uk/photo/1410180>.

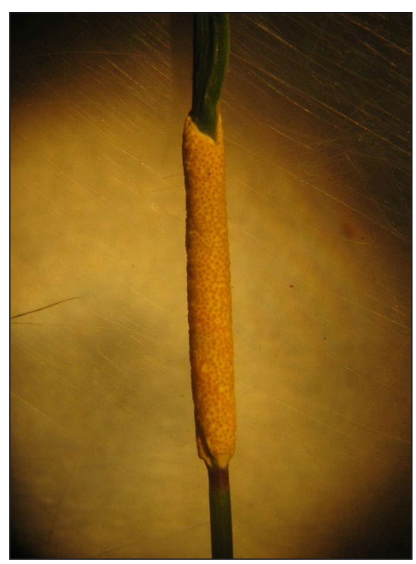

Figure 3. Detail of *Epichloë typhina*. Image by M.J. Richardson, CC BY-SA 2.0, <https://www.geograph.org.uk/photo/1410230>.

parasites attacked E– much more than E+. Pastures seeded with E– could easily be contaminated with E+ if farmers weren't careful. E+ would then overgrow the pasture because it was so much more robust. It seems that most of the 'wonder grass' properties of tall fescue were due to the hidden fungus. E– was not a great success."

"I still can't get my head around a plant having a fungus growing inside it; is this a disease? Okay, grazing cattle get sick, but are the plants sick?"

... to be continued ...

Questions

You will be assigned to one of two teams: "Team Plant" or "Team Fungus." Discuss how (or if) each partner in this symbiosis can work the interaction to its advantage. Based on what you have learned about this symbiosis so far, answer the questions below for your team.

For Team Fungus:

1. Are you a pathogen (do you make the plant sick)?
2. Are you a parasite (do you take from the plant and give nothing in return) or a mutualist, meaning you do give something in return? If so, what is that something?

For Team Plant:

1. Does the fungus make you sick?
2. What has that fungus ever done for you? Would you be better off without it?

Part III – Do I Know You?

How does a plant "know" whether friend or foe knocks on the door? Recognition is key.

After watching excerpts from the short video clips below, try to reconstruct how this partnership between tall fescue and *Epichloë coenophiala* came about.

Videos

- *Central Concepts of Plant Pathology: The Role of Recognition in Host-Parasite Interaction*
  https://youtu.be/2yevwh2paS0
  Produced by the University of California, Davis. Running time: 4:59 min.
  Watch from 0:48 to 2:02, and from 2:47 to 3:41.

- *Mycorrhiza II: What Is It and How Does It Work?*
  https://youtu.be/QYmrOrTM-FA
  Produced by UFZde, 2014. Running time: 10:06 min.
  Watch from 1:57 to 4:26.

Questions

For Team Fungus:

1. How did you get into the plant?
2. How do you spread from one plant to another?

For Team Plant:

1. How did you let the fungus get into you? Did it force its way in, or did you allow it in? If the latter, how did you know this was a "good guy"?
2. How do you stop the fungus from spreading? (Or are you okay with it spreading?)

Part IV – Presentations

In your small group, study the material for one of the symbioses below and prepare a brief in-class presentation. Make sure to introduce both partners in their ecological context. Explain how the host acquires the microbial partner and how the latter affects the host's fitness.

Weevil and Nardonella

- Yong, E. 2018. The grain weevil's bacterial body armor [video].| *I Contain Multitudes.* Running time: 1:00 min. <https://youtu.be/PMXlT-YvFeg>
- Silvis, M. 2017. Beetles exploit bacteria labor to grow their exoskeletons [webpage]. *Massive Science.* <https://massivesci.com/articles/bacteria-symbiosis-beetles-proteins/>
- Anbutsu, H., *et al.* 2017. Small genome symbiont underlies cuticle hardness in beetles. *PNAS* 114 (40) E8382–E8391. <https://doi.org/10.1073/pnas.1712857114>

Termites and Gut Microbes

- Yong, E. 2018. Termites digest wood thanks to microbes. [video].| *I Contain Multitudes.* Running time: 7:11 min. <https://youtu.be/e02keFYEWeU>
- Kraft, L. 2017. Nutritional symbionts: why some insects don't have to eat their vegetables [webpage]. *Entomology Today.* <https://entomologytoday.org/2017/11/15/nutritional-symbionts-why-some-insects-dont-have-to-eat-their-vegetables/>
- Okinawa Institute of Science and Technology (OIST) Graduate University. 2018. Evolutionary origin of termite gut microbiome revealed [webpage]. *ScienceDaily*. <https://www.sciencedaily.com/releases/2018/02/180216110530.htm>
- Bourguignon *et al.* 2018. Rampant host switching shaped the termite gut microbiome *Current Biology* 28(4): 649–54. <https://doi.org/10.1016/j.cub.2018.01.035>

Panic Grass, Curvularia and Virus

- Yong, E. 2018. Can a fungus save plants from global warming [video]? *I Contain Multitudes.* Running time: 8:02 min. <https://youtu.be/2f5aru4103I>
- Jones, S. 2007. Three pieces in the puzzle. *Nature Reviews Microbiology* 5: 168–9. <https://doi.org/10.1038/nrmicro1627>
- Marquez, L.M., *et al.* 2007. A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. *Science* 63: 545–58. <https://doi.org/10.1126/science.1136237>

Bobtail Squid and Vibrio

- Yong, E. 2018. Nature's cutest symbiosis: the bobtail squid [video]. *I Contain Multitudes.* Running time: 8:05 min. <https://youtu.be/3ivMSCi-Y2Q>
- McFall-Ngai. 2008. Quick guide: the Hawaiian bobtail squid. *Current Biology* 18(22): R1043–44. <https://doi.org/10.1016/j.cub.2008.08.059>

Tubeworms and Thiotrophic Bacteria

- Yong, E. 2017. How giant tube worms survive at hydrothermal vents. [video]. *I Contain Multitudes.* Running time: 10:20 min. <https://youtu.be/8W_ywzhkR90>
- Zahn, N. 2017. Anatomy of a giant tube worm [infographic]. *Tangled Bank Studios*. <https://medium.com/hhmi-science-media/anatomy-of-a-giant-tube-worm-fe779af23975>
- Cavanaugh, C. 2016. Chemosynthetic symbioses: living together can be fun [video]. *iBiology*. Running time: 20:49 min. <https://www.ibiology.org/microbiology/chemosynthetic/>
- Bright, M. *et al.* 2013. Giant tubeworms. *Current Biology* 23(6): R224–5. <https://doi.org/10.1016/j.cub.2013.01.039>

Bedbugs and Wolbachia

- El-Showk, S.2014. How *Wolbachia* learned to help bedbugs [blog post]. *Scitable.* <https://www.nature.com/scitable/blog/accumulating-glitches/how_wolbachia_learned_to_help/>
- Nikoh, N., *et al.* 2014. Evolutionary origin of insect–*Wolbachia* nutritional mutualism. *PNAS* 111(28): 10257–62. <https://doi.org/10.1073/pnas.1409284111>

Coffee Berry Borer and Pseudomonas

- Yong, E. 2015. This beetle is ruining your coffee with the help of bacteria [webpage]. *National Geographic.* <https://www.nationalgeographic.com/science/phenomena/2015/07/14/this-beetle-is-ruining-your-coffee-with-the-help-of-bacteria/>
- Ceja-Navarro, J.A., *et al.* 2015. Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. *Nature Communications* 6, 7618. <https://doi.org/10.1038/ncomms8618>

Aphids and Secondary Symbionts

- Muhlrad, P. 2002. Ecologists reveal war triangle among aphids, wasps and bacteria [webpage]. University of Arizona, *UA News* Aug 5th, 2002. <https://uanews.arizona.edu/story/ecologists-reveal-war-triangle-among-aphids-wasps-and-bacteria>
- Walker, M.S. 2015. Bacteria can aid aphids against predation and parasitism [webpage]. *Entomology Today*. <https://entomologytoday.org/2015/04/22/bacteria-can-aid-aphids-against-predation-and-parasitism/>
- Oliver, K.M., *et al.* 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *PNAS* 100(4): 1803–7. <https://doi.org/10.1073/pnas.0335320100>

Internet references accessible as of March 15, 2021.

Part V – Back to the Story

Nathan started to feel that Dr. Miller was going to make him work hard for every piece of the puzzle.

"That's pretty interesting stuff. What's my project going to be then?"

"You know what? We've got so many interesting questions that I'm happy for you to work on any of them, or even better, your own question! Are you more interested in the biology of the symbiosis or do you want to research an applied question? Take your time to think about it. I'll tell you what else you need to know and what kind of tools we have:

- You can stain the fungus to see it under the microscope as blue strands in the leaf. You won't be able to tell different fungi or strains apart, though, and the staining procedure kills both leaf and fungus, but of course you need only one leaf.
- You can analyse gene expression in infected or uninfected plant tissue, but bear in mind that you can't physically separate the fungal tissue from the plant.
- You can surface-sterilise the plant, cut it into sections and place the sections on a petri dish to culture the fungus. We are lucky here; normally you can't culture fungi that naturally grow exclusively in living organisms (obligate biotrophs), but it works for *Epichloë*. If you want to characterise the fungus genetically there's generally no way around culturing it first.
- You can create endophyte-free plants with a fungicide. Seeds from those plants will remain endophyte free in the next generation. Pollen does not transmit the symbiont.
- Conversely, endophytes are passed down from a plant to its seed, so if you have characterised the endophytes in one plant, its seeds will have the same endophytes.
- You can try introducing an endophyte strain of your choice into E− seedlings by making a small incision into a seedling and inoculating it with some cultured mycelium. It is time-consuming, pretty inefficient and doesn't work well with all combinations of plant and fungus, but if you are patient it can be done.
- We have a protocol for making transgenic fungi! So you could introduce a gene into a fungal strain you are interested in.
- We have produced some strains of *E. coenophiala* that express GFP, and we have plants that contain this "glow-in-the-dark" strain as symbiont. You can detect the fungi in the living plant with a fluorescence microscope and follow it over time. You can also transform GFP into any fungal strain you choose.
- We are beginning to learn how to knock out genes, and we also have an *E. coenophiala* mutant collection that was produced with a shotgun approach.
- We have a collection of fungal strains from all over the world, which includes some that don't produce one or more of the toxic alkaloids. We think there are strains with other interesting properties, but haven't had a chance to look at all of them yet in detail."

Question

1. What research question would you suggest to Nathan? Help him write a research proposal.