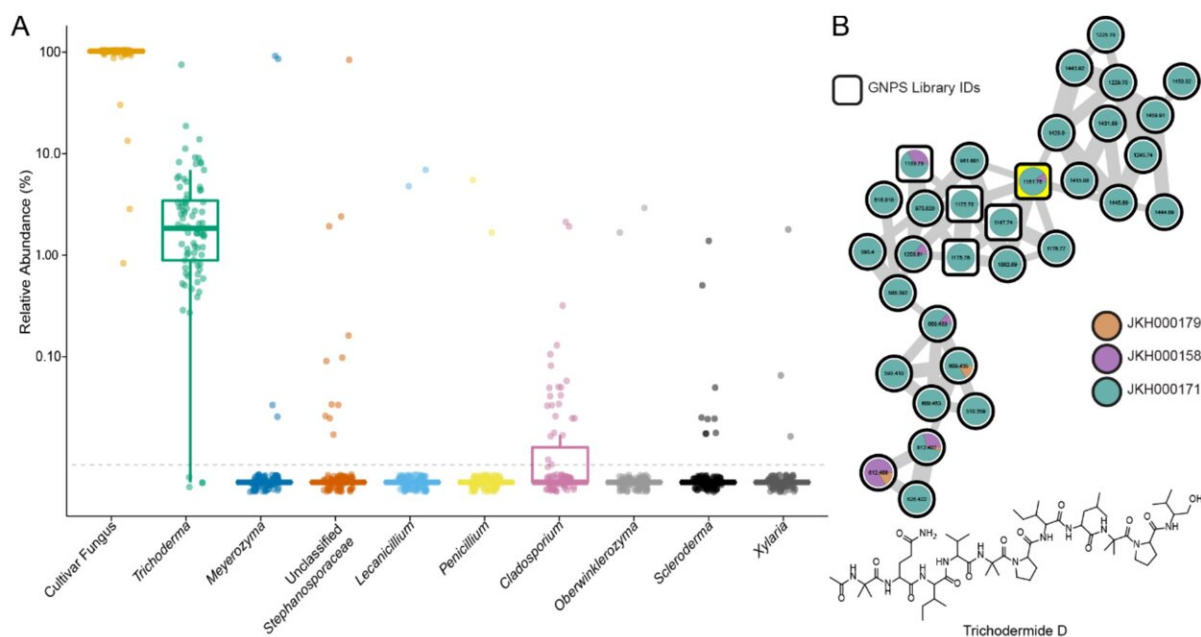


Research hints at how fungus farming ants keep their gardens healthy

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Molecular evidence supporting *Trichoderma* spp. presence in wild *T. septentrionalis* fungus gardens. (A) Relative abundance of ITS2 community amplicon sequencing ASVs shows *Trichoderma* to be the most abundant and most prevalent fungus in wild fungus gardens besides the cultivar fungus. Boxplot center bars show the median relative abundance of ITS2 amplicon reads grouped by genus and the 1st/3rd quartiles are shown by the top/bottom notches respectively. Boxplot whiskers are 1.5 times the interquartile range from the top and bottom notch. Only genera with ASVs that are present at $\geq 1\%$ in at least one sample are shown. The limit of detection (dotted gray line) was determined to be $100 \times (1/15000)$. Taxa on the x-axis are ordered from left to right by decreasing mean abundance. (B) Molecular family of peptaibols detected from environmentally-collected fungus garden extracts (SI Appendix, Fig. S3),

including the node with m/z 1161.76 (highlighted in yellow) whose molecular weight and fragmentation pattern were consistent with trichoderme D (SI Appendix, Fig. S4), a peptaibol previously isolated from *Trichoderma* spp. (37). This molecular family contains related peptaibol metabolites from three fungus garden colonies, all from North Carolina. Square nodes represent spectral matches in the GNPS public libraries. Credit: *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.22193731

"Weed early and often" is the key to a productive garden. Interestingly, certain species of ants are also avid gardeners, a practice they've refined over 50 million years. They too weed their underground fungus gardens, but how they know what to weed out has been a mystery. Now, a multidisciplinary team of scientists report in *PNAS* on June 15 how ants distinguish the good fungus from the bad.

People rely on sight to identify [weeds](#) but ants grow [fungus](#) underground in the dark and must have other ways to sense undesirable garden denizens. A team led by Jonathan Klassen, Ph.D., at the University of Connecticut and Marcy Balunas, Ph.D., at the University of Michigan has found that the ants sniff out diseased fungus by detecting chemicals called peptaibols.

The team focused on the ant species *Trachymyrmex septentrionalis* whose habitat follows the pine barren ecosystem from Long Island all the way south to East Texas. *Trachymyrmex* ants grow their fungus below ground and feed it fresh organic detritus. The fungus acts almost like an external gut for the [ant colony](#); the fungus grows up and around the [fresh food](#) laid on top of it in honeycomb shapes, produces digested food for the ants as it grows, and then secretes waste.

Klassen Lab graduate student Katie Kyle, a co-first author on the paper, experimentally infected [ant nests](#) with *Trichoderma*, a naturally

occurring, disease-causing fungus that infects the ants' gardens and found that the ants began working overtime to remove the infection from the nests, increasing their waste output.

Over the winter, while the ants were dormant, the team analyzed the fungal biomes of several different ant nests collected from different locations and found *Trichoderma* in all of them.

Co-first author Sara Puckett, Ph.D., a recent graduate from the Balunas' UConn lab, prepared extracts of *Trichoderma* containing the organic compounds of the fungus to determine if the weeding was triggered by one or more of these compounds or simply by the presence of the pathogen's cells.

"We were curious to see if the ants were weeding because of compounds produced by the infecting fungus," Balunas said.

The team found the *Trichoderma* extract, when applied to the fungus garden, sent the ants into frenzied weeding activity just as actual *Trichoderma* infections had.

Working with scientists from University of California, San Diego and University of North Carolina, Greensboro, they discovered the nests contained peptaibols, a family of compounds known to be produced by *Trichoderma*. However, finding which specific peptaibols were causing ant weeding proved more challenging since these extracts contained many compounds.

The researchers tested pure peptaibols, including two new compounds called trichokindins VIII and IX.

It turns out that all the peptaibols tested caused some level of ant weeding, a finding that implies it may not be one particular peptaibol but

rather than the whole suite of peptaibols can induce the ants to weed their garden.

"This suite of Trichoderma compounds inducing ant behavior is in contrast to many other [natural products](#) whose activity can often be attributed to one compound," Balunas says.

Although their data support peptaibols as a signal to weed, it's not clear what exactly the ants are perceiving. It may be that the invading Trichoderma fungus produces the peptaibols and the ants detect them and then weed, note the researchers. Or perhaps the [ants](#) are detecting a secondary response from the fungus garden itself.

The next step is to figure out those details of ant-fungus communication, Klassen says.

"Maybe the fungus is signaling 'I'm sick'. Maybe the fungus is detecting the peptaibols. We need to flesh out the chain of signaling," Klassen says.

The findings highlight one of the few known systems where an animal responds to a disease of its beneficial symbiotic partner instead of a disease of its own body, a phenomenon that Balunas and Klassen are calling an extended defense response, and one that they look forward to continuing to tease apart.

More information: Kathleen E. Kyle et al, *Trachymyrmex septentrionalis* ants promote fungus garden hygiene using Trichoderma-derived metabolite cues, *Proceedings of the National Academy of Sciences* (2023). [DOI: 10.1073/pnas.2219373120](https://doi.org/10.1073/pnas.2219373120)

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