Podcast Interview: Maggie Wagner and Manuel Kleiner

PNAS: Welcome to *Science Sessions*, the podcast of the *Proceedings of the National Academy of Sciences*, where we connect you with Academy members, researchers, and policymakers. Join us as we explore the stories behind the science. I'm Taylor Gedeon, and I'm speaking with Maggie Wagner of the University of Kansas and Manuel Kleiner of North Carolina State University. In a recent PNAS article, Wagner, Kleiner, and colleagues report that the interaction between maize and soil microbes influences hybrid vigor, or heterosis. However, mixed results from further experimentation suggest that the composition of the microbial community may be an important factor. PNAS caught up with Wagner and Kleiner on the implications of their findings.

Maggie, what is hybrid vigor, and what role does it play in commercial farming?

Wagner: Hybrid vigor refers to this phenomenon, which is really common among a lot of different plant species, and particularly crop species where, when you crossbreed between two inbred lines of a plant, their offspring are much more vigorous, healthier, more productive than either of the two parents.

Most commercial corn production is using just hybrids. And so when you look out at a cornfield, most of those plants will be hybrids, and probably the exact same hybrid between the two parents. The use of hybrid cultivars for commercial agriculture was one of the most important innovations in agriculture in the early- to mid-20th century. Making this transition to using hybrids has been responsible for a lot of the huge increases in grain production that we've seen over the last century.

PNAS: Why did you focus on the potential role of local microbial communities in the hybrid vigor of maize?

Wagner: For a while my research has been focused on looking at how plants with different genotypes interact differently with microbes, and specifically how plants with different genotypes will form microbiomes that differ from each other. One of the experiments that I ran when I was a postdoc at North Carolina State involved comparing hybrids and inbreds and comparing the composition of their root and leaf microbiomes. And we did find that, in the field, the composition of their rhizosphere microbiomes in particular, which is the soil immediately surrounding their roots, differed quite a lot between inbreds and hybrids. So when we set up that experiment, we were going to just compare how those seven bacterial strains colonized the inbreds and hybrids in possibly different ways. And we were assuming that the hybrid vigor of the phenotype would still be there; we assumed that that was just inherent to the genotypes that we were using. And so it was really surprising when we measured the biomass of these plants just to use as a covariate in our statistical models, and, to our surprise, we did not see the hybrid vigor up for root size in our sterile controls. That was kind of an accident, but it sort of launched this whole project.

PNAS: Manuel, how did you compare the growth of inbred maize lines and their hybrid?

Kleiner: A lot of our experiments really rely on being able to grow plants with and without microbes. That is really critical to understand the interactions between microbes and plants. We need to grow systems where we can actually remove all the microbes, and that's what we call the gnotobiotic growth system. And so what that system really is, it's basically a large plastic bag that is clear. And it's fully sterile; we buy those plastic bags already fully sterilized—I think they are gamma irradiated to kill everything inside. And then we take corn seeds, and we sterilize these corn seeds by applying a pretty harsh treatment of bleach and ethanol and different things to really try to remove all the microbes from the surface. And then we add a sterilized grow substrate; we just use a calcinated clay which is basically just the same powdery substance that people use for baseball diamonds. We autoclave it; we kill everything; we add that in there; the plant has kind of a substrate to grow in; and we add our sterile seed. And then, depending on what treatment we want to apply, we apply the microbes by watering with a solution that has nutrients, like a plant fertilizer solution with the microbes of choice added to it.

PNAS: In your paper you highlighted seven microbial strains used in the bacterial treatments. How did you determine this simplified synthetic microbiome for conducting your analysis?

Kleiner: Ultimately, we actually ended up using different types of things in our growth system. Maggie, for example, came up with the idea to later use soil slurries, where she extracted microbes from a natural soil and added them to the bags. But the original seven came actually from a laboratory in Harvard from Roberto Kolter's lab. There's another PNAS paper on that, actually, from 2017, where they had gone through an extensive process of trying to identify a simplified, fully defined community for corn roots, that is corn-root associated. They tried to find which microbes are present in the corn that they grow, and which are most representative of the community in terms of taxonomic diversity and abundances that we find by growing corn and things like that.

PNAS: Was there a difference in the inbred and hybrid lines when grown in sterile versus bacterial-treated soil?

Wagner: Specifically, we saw that the hybrid lines didn't really care whether they were in sterile conditions or in the presence of bacteria, but we saw that the inbred lines did worse when they had these seven bacterial strains in the soil with them. That difference in the inbred versus hybrid response to these microbial communities is what drove that difference in hybrid vigor between those two treatments. And so that was interesting, too: We didn't see the hybrid getting some sort of boost from the bacteria. Instead, what we saw was a negative effect of the bacteria, but only on the inbreds.

Immediately one thing that we thought of that could explain this, that possibly the hybrids have a much better immune system, and maybe these bacteria are sort of weakly pathogenic to the corn and that could explain the pattern we were seeing. But it's not the only explanation. It's also possible that these bacteria are actually harmless, but maybe the inbreds think that they're dangerous. If the inbreds have sort of a hyperresponsive immune system and start to defend themselves against these harmless bacteria, that comes with a cost. It requires energy from the plant to do that, which could result in less growth in the presence of live microbes. There's even more possible explanations. For example, the relative ability of the inbreds and hybrids to compete for nutrients in the soil with those microbes and things like that.

PNAS: To determine whether natural, complex soil microbial communities also induce heterosis, you conducted a second growth-chamber experiment and two further field experiments in North Carolina and Kansas. What did you find?

Wagner: The gnotobiotic growth system that Manuel was describing earlier is really an extremely simplified system that bears not much resemblance to the types of microbial communities that these plants are experiencing when they're actually on the farm. We did a series of experiments of increasing complexity to try to get closer and closer to those realistic conditions that the plants are dealing with on the farm because we wanted to know if what we were seeing was just a fluke related to these seven bacterial strains and really simplified conditions, or is this something more general that actually is happening on the farm as well. First, we kept it in the lab, but we added a full soil community, which is a whole lot more than seven bacterial strains. And we found the same thing. And then we took it to the field. We actually used a couple different methods to sterilize soil right there on the farm, and then grow the corn in the sterilized soil or in a control treatment, and we saw a similar pattern in North Carolina. We did something similar in the field in Kansas and we found that those treatments did affect heterosis, but the story got a lot more complicated at that point because the direction of the effect was opposite, where we actually saw heterosis, or hybrid vigor, getting stronger after soil sterilization.

Kleiner: You have to sterilize the soil to a certain depth, and we did steaming as one treatment. And then another one was a chemical treatment and [an] ultimate combination of chemical treatment with a steaming treatment. And for the steaming treatment, you have to imagine you have to have this giant steam generator that you have to probe with a tractor or something that generates the steam, and then the steam gets pushed through hoses into little nozzles that you stick into the field. And basically, then you pump steam into the field for a while to really kind of heat the soil to kill everything.

PNAS: What are the implications of these findings for future research and sustainable agriculture?

Wagner: I think we're still pretty far off from any direct application of this. I think the significance of our paper is that it's the first time anyone's reported that just soil microbes are involved in the expression of hybrid vigor. And we have to figure out what's the best way to leverage that. And to do that, we need to understand it a lot better. We have plans to follow up on all of this and figure out how it works from the molecular level all the way up to testing whether this happens in many different hybrids or just some and also to try to understand which microbes are responsible for this and how that works.

We've known about hybrid vigor for a very long time, but we still don't really understand very well how it works at the genetic level. So we're excited about this project, in part because of the potential applications for agriculture, but also, we're hoping that this is going to provide some clues for folks, not just us but in other labs, to follow up with so that we can understand hybrid vigor a little bit better. Ideally, maybe sometime in the future, if we can figure out how to get high-performing plants at the level of current modern hybrids, without having to go through the process of hybridizing every single year to produce the seed that gets planted; that would be hugely beneficial for agriculture in general.

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