

# Understanding how weeds are resistant to herbicides

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In a little over seven hours, University of Illinois weed scientist Patrick Tranel got more genetic information about waterhemp than in two years time in a lab. The genetic information was obtained using pyrosequencing technology in the Keck Center at the U of I. The genetic sequence will allow scientists to study herbicide resistance in waterhemp.

Ten years ago genomics was reserved for what Tranel refers to as "important species" such as humans, cows, fruit flies, and mice. "That's changed now that those species have been sequenced. Now we can start doing genomics on weeds to start understanding weeds better.

"With this type of technology, you can generate all of this [genomic data](#) relatively cheaply and quickly, so it's worthwhile doing in some of these non-model species like weeds. We're able to start generating data now that five years ago would have been cost-prohibitive." Tranel believes waterhemp is the first [weed](#) to be partially sequenced using this technology.

The pyrosequencing machine emits a light signal that's captured every time a [nucleotide](#) is incorporated into a growing DNA strand. "The reason it's so fast is that it's done in parallel," said Tranel. "The plate has thousands of tiny wells, and a sequencing reaction going on in every one of them simultaneously. There's a camera that monitors the light for each of these wells simultaneously and so in one seven and a half hour run you generate a million reads."

Tranel explained that although more traditional herbicide resistance research takes years, it's more gene-specific. "We sampled plants, brought them back to the green house, grew them up, confirmed that they were resistant and then we started crossing a resistant plant with a sensitive plant. We look at its [progeny](#) to see if the resistance is inherited to understand the genetics - if it's a dominant trait or a recessive trait.

"Pyrosequencing is more like just throwing out a fishing net -- we know we're going to get continued resistance to other herbicides which can affect other genes. And we don't want to spend two years culling to find that gene every time. This is a way that we can get all of the genes at once."

All of the data is publically available. "There's a website where you can go and get the 43 million base pairs of sequence. So anyone can get it and use that information."

Tranel said that identifying weed resistance is an immediate outcome of having the genetic data. "Once we obtain the sequence of a resistance gene, we can develop molecular tests that are specific for the resistance mutation. "We can take a sample of waterhemp from a field that was sprayed and the waterhemp hasn't died and we can confirm whether it is resistant or not because we know the gene sequence and we know the mutation and the mechanism."

Having the complete genomic data on waterhemp will help scientists not only to identify but also to understand resistance and how resistance evolves. "If you understand how it evolves, that can help you devise strategies that cannot prevent it from evolving, but at least slow the rate at which it happens," said Tranel.

"If you use the same herbicide year after year, you're exerting selection pressure -- you're selecting for that rare plant or mutation that will

survive. When you do that, and you kill all of the siblings that are weaker, the mutant survives and all of its progenies will survive and that's how resistance evolves. It's evolution in action," said Tranel.

This genomic data will also help in answering broader questions about weeds such as: Why are some plants weeds? What makes a plant a weed? Is it certain genes? Is it the way the genes are expressed? "These are questions that 10 years ago we couldn't address because we didn't know the genes. Now we're at the point where we can start doing that and on a broad scale. We can do this with waterhemp and we can do this with another weed and we can compare the two - are there things that these two weeds have in common that make it different from a corn plant or a soybean plant which explains why it's weedy? This is sort of a first step in that direction - starting to generate the type of information that will allow us to ask these sorts of questions."

Waterhemp is a Midwestern problem, Tranel said, but it's a member of the genus *Amaranthus* which includes weeds that are a problem worldwide such as pigweeds. "Because they all belong to the same genus, their genomes are very conserved. So if we have the sequence for the PPO gene in waterhemp you can use that information to get the PPO gene in redroot pigweed. It would be a similar sequence."

Having this information is like building a tool kit, said Tranel. "We're developing all of these resources and putting these resources in our freezer. When we have an interest in resistance to herbicide A which targets enzyme B, we can go to the freezer, or to the computer and get the sequence of the gene for that enzyme."

Tranel said that because waterhemp is in the group of *amaranthus* weeds, it's a good model for weed genomics. "A weed scientist in Georgia, where there's a lot of Palmer amaranth - another pigweed evolving resistance, can go straight to that data base and get gene sequence data."

Another outcome of having this genomic data is to be able to design markers - so you can fingerprint individual waterhemp plants and use that information to do population genetic studies.

"If you see herbicide resistance in northern Illinois and a year later you see the same resistance in a population in southern Illinois, one of the things you want to know in managing resistance is, did resistance evolve or occur here once and then a farmer moved a combine and that's how resistance got down here? Or did resistance occur here and independently down in southern Illinois?"

Understanding how the resistance occurred has implications for weed management. "If it's evolving multiple times, you need to pay attention to what you're doing in your field whereas if it evolved once and is moving around then you've got to pay attention to what your neighbors are doing. It's important to know how it's evolving, how it's spreading.

"If you have these genetic fingerprinting tools, which we're able to do because of this research, you can go look at these populations and see, are these genetically similar, which would suggest that it was a movement event. If they're completely different, then that would suggest that it's evolving independently."

More information: Sampling the Waterhemp (*Amaranthus tuberculatus*) Genome Using Pyrosequencing Technology will be published in a 2009 issue of *Weed Science*.

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