Land Report

Number 105, Spring 2013 · The Land Institute



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When people, land and community are as one, all three members prosper; when they relate not as members but as competing interests, all three are exploited. By consulting nature as the source and measure of that membership, The Land Institute seeks to develop an agriculture that will save soil from being lost or poisoned, while promoting a community life at once prosperous and enduring.

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LAND REPORT

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- To select a needle in a haystack
 How plant breeding adds up bits of hidden potential to produce a winner
- 8 Leaves and seeds and genes of grass Transforming a wild relative of wheat to become a profitable perennial grain
- 15 Growing Kernza in Minnesota Crop candidate benefits from research, marketing power, and cooler climate
- 19 Kernza in food and drink Brewing, baking, and the search for heavy molecules to make light bread
- 22 Perenniality trumps farm method For capturing nitrogen, more vital than how it's applied is the plant's roots and longevity
- 24 Combing the genome Land Institute collaborators mark DNA to speed and refine breeding
- 26 Thanks to our contributors

Cover

Maximilian sunflowers, bagged in the field, are brought to the lab for analysis. The bags contain the sunflower head and fallen seeds, enabling researchers to accurately determine seed yield. The Land Institute is developing Maximilian as a perennial oilseed crop. In the picture above, seeds are emptied into a pan for weighing and analysis. The bags also collect plant residue, which must be removed before seed analysis. Scott Seirer photos.

To select a needle in a haystack

How plant breeding adds up bits of hidden potential to produce a winner

LEE DEHAAN, LAND INSTITUTE RESEARCHER

he genetics of perennial grains is counterintuitive. But I like it that way, because counterintuitive things fascinate me. Because I get a thrill out of figuring out how something actually works when at first glance it appears to function very differently, the world of science holds endless opportunities for exciting investigation.

A simple example of a counterintuitive situation is sailing vessels that can exceed the speed of the wind that powers them. Many who are unfamiliar with sailing, if asked, would say the top speed of a yacht would be somewhat less than the wind that is powering it. Similarly, we would expect that the top speed of a sailing vessel would be reached by running in precisely the same direction as the wind. I remember trying this as a kid with a small sailboat and being disappointed with the results. I was sure that running straight downwind should be the fastest, but it always seemed surprisingly sluggish relative to running at other angles. Only later did I learn that experienced sailors know top speeds are reached by going several degrees off the direction of the wind. Although the principle is counterintuitive, it is widely understood, or at least used, by anybody who sails.

Now let's consider perennial grains. I've run across people who believe it would be impossible to domesticate a wild perennial grass to make a grain crop, or at least that the process is going to be too slow or risky to be worth trying. I've spent years trying to trace the root cause of concern among those who are leery, and I think I've finally found the sticking point: it's simply counterintuitive.

Those who spend their time improving crop plants usually don't have difficulty imagining a perennial grain crop in the future. After all, they realize that humans have been able to increase the yields of both annuals and perennials through all sorts of clever techniques, so they don't see anything different in the idea of a perennial grain.

But people who mostly experience wild plants see the world differently. They may have spent thousands of hours in prairies, examining millions of perennial plants. And all of those countless interactions with perennial plants have added up to reinforce one idea: perennial plants produce very little seed. It is not surprising that someone who looked at so many plants and never saw a single one with even reasonably abundant or large seeds would conclude it hopeless to breed them for high seed yield.

Now, it can be reasoned that it is survival of the fittest in nature, and the fittest plant would be the one that produces lots of large seed, right? This is an intuitive idea, but the counterintuitive reality is that natural selection can actually end up favoring plants that live a very long time and possibly spread vegetatively, while mostly disregarding seed yield. The counterargument goes: "But aren't they producing as much seed as they can while living as long as they can?" The quick answer to that is "no," but the genetic explanation is complex.

Let me offer some evidence. Our annual grain crops have experienced yield increases of 500 percent or more relative to their wild ancestors. These gains in yield were achieved without having to sacrifice lifespan in the annual grain. Similarly, there is no reason to believe that wild plants have maximized their seed producing capabilities.

The next intuitive observation is, "Occasionally wild annuals have high seed yields. These plants were the forerunners of our crops. But no wild perennials have high seed yield. You can select all you want, but you will never make any progress because the plants clearly don't have the genes available to allow increased yield."

Now this is where my interest in coun-

terintuitive phenomena is aroused, and I realize that the counterintuitive nature of genetics lies at the root of the misunderstanding. To explore this, let's consider a population in which there are five genes that add to seed yield, and each occurs at a frequency of only 1 percent. The species is diploid, like corn, and can have two versions of each gene. So for each high-yield gene, 2 percent of plants will have one copy. The chance of a plant having both copies is only 1 percent of 1 percent – one in 10,000.

So what would be the chance of coming across the outstanding producer with all 10 yield genes? This is the joint probabilities of the individual genes, which turns out to be 1 in 100 quintillion. To evaluate this many plants, you would need to look at one per second, continuously, for about 3 trillion years. Or if you wanted to grow this many plants, you would need to cover the entire land surface of the earth about 40 million times over.



Figure 1. Response of 10 genes to selection in a computer-simulated population of 1,000 individuals. Five of the genes had a starting frequency of 50 percent (dashed lines), but a small beneficial effect. Another five genes (solid lines) had a starting frequency of 1 percent and a large benefit. After nine cycles of simulated selection of the top 10 percent, the genes of large effect were present in all individuals. Heritability was assumed to be 25 percent.

Perhaps the numbers are hard to envision, but we can see that even with five genes which are just slightly rare, we will never just happen upon the outstanding specimen. No wonder we don't see highyielding plants springing up by accident in the prairie.

If it's impossible to stumble upon the high yielder, what makes us think we can obtain yield through selection? To answer this question I considered a simulated population of 1,000 plants having the same theoretical five genes described above. For this hypothetical population, in addition to the major-effect genes there were five other small-effect genes contributing to yield, and these had a starting frequency of 50 percent. Yield had a heritability of 25 percent, meaning that 75 percent of the variation observed was due to non-genetic, environmental factors. By adding in genes of small effect and environmental factors, we make the simulation a much better approximation of

a real group of plants. There were also some genetic assumptions, such as all the genes being on different chromosomes.

Now, instead of just looking for the high-yielding needle in the haystack, we set about creating it. We do this through the process of selection. In the first year we don't expect to find really high-yielding plants, but we take the 100 highest-yielding plants that we do find and mate them together at random. Then we grow out 1,000 of their offspring, measure them, and again select the best 100 to intermate. Through this process we rapidly increase the frequency of the large-impact yield genes. (See Figure 1). After only nine generations, all of the plants contain only high-yield genes! The time to this achievement will depend on the lifecycle of the plant, but it will be closer to 20 years than to 3 trillion.

We've explored the theoretical basis of selecting for a trait not evident in a population. Now let's compare the theory to what's



Figure 2. Genotype distributions of a simulated population responding to selection. This shows how quickly new genotypes can be developed through selection that would be nearly impossible to find in nature.

happening in one of our actual breeding programs at The Land Institute.

In Figure 2 we can see what happens to the distribution of genotypes in response to selection. These are the genotypes because they are the predicted distributions of how the plants would grow without any environmental influences. What we see initially is the population tightly clustered around low values. As selection rapidly increases the proportion of plants having the highvalue genes, the population average begins to increase quickly while also spreading out - some plants are found to be very high while others remain low, and the number of plants near the average is reduced. After about eight cycles of selection the variation has been exhausted, and now the plant types bunch up at the high end of the range, roughly mirroring distribution at the low end before selection began.

Figure 3 illustrates actual response to selection with Kernza (intermediate wheat-

grass). In this case the number of plants used, the selection method, and the number of genes involved differ from the theoretical example. Nonetheless, it is interesting to see how closely the overall trend of response to selection matches the theory. After just three cycles of selection the average seed weight more than doubles. In fact, the average of the last population is greater than the seed size of the highest plant in the starting population.

Although we're well on our way to high-yielding perennial grains, and the genetic explanations are sound, can we expect everyone to be convinced? Certainly not. It's simply in the nature of the human mind to cling to the intuitive. Even when perennial grains follow the sailing ships and "outrun the wind," many will remain skeptical. Indeed, if I'm honest I must count myself as one who doesn't easily let go of the intuitive. After all, just this morning I mentioned the sun "rising."



Figure 3. Response of Kernza seed weight to selection over three generations. Selection procedures varied among the generations, and the selection always was for traits – yield and threshability – in addition to seed weight.



Bob Reeves bags samples during harvest of Kernza, a perennial grass once used only for forage, but now gaining in food seed size and yield through selection and breeding at The Land Institute. Reeves, from Louisville, Kentucky, spent two weeks volunteering at the institute last July. Scott Seirer photo.

Leaves and seeds and genes of grass

Transforming a wild relative of wheat to become a profitable perennial grain

STORIES BY SCOTT BONTZ

ee DeHaan's boyhood farm in Minnesota raised corn. Compared with pre-agricultural ancestors, the ears of grain were giants. The leap between forms came after humans reached what is now Mexico, and began picking favorites from corn's progenitors. This way, as with wheat, rice, and other grains, humans changed a species' face and fate. But the possibility for these dramatic changes lay within each plant's nature. "The grasses are all really very similar genetically," said DeHaan, a Land Institute researcher. "There is an elegant developmental pathway that they follow that works amazingly well in many situations." When people want lots of seed to eat, these organisms have the flexibility to provide. "The overall developmental program is basically the same as a wild grass, with just a few modifications, and we have a grain plant."

The fine pointers of that elegant pathway have been a mystery. Now DeHaan and others are drawing on a map unavailable to their own ancestors. They do so with plants long overlooked, but poised for status in remaking the face and roots of agriculture. DeHaan is developing a relative of wheat, plant of the poet's amber waves of grain. For the land, and potentially for the farmer, this relative may prove more beautiful. Intermediate wheatgrass is a perennial. All grain crops until now have

been annuals. Annuals are carpetbaggers that thrive when soil is disturbed, and when they die every year, soil can blow. Natural landscapes are dominated by perennials. Perennials are solid citizens vastly better at tapping water and soil nutrients, and at holding that soil to keep the community of a landscape intact. "A perennial grain has potential to simultaneously address environmental, social, and economic issues in agriculture," said DeHaan. "Unlike most approaches, we don't have to choose between production and conservation. Perennial grains are the obvious outcome of combining my two primary interests in agriculture - agroecology and genetics. Once you open up the breeding of new plant types, ecological principles will dictate an attempt to find plants that will cover the soil year around while meeting our food needs."

Compared with what millennia of coaxing has brought from humanity's other grains, DeHaan's plants are as yet short on seed. Those "few modifications" that he wants are buried in complex genetic instructions. Even with means now to genetically read a plant's future instead of waiting until it makes grain, developing a crop remains an endeavor of years. Other perennial-crop candidates at The Land Institute are expected to require two to three decades. DeHaan hopes to have Kernza, the institute's trademark name for wheatgrass, ready in one. He has already doubled the seed size."It is much easier than I ever imagined it would be," he said. "Clearly, the genetic variation for the traits we have been selecting on is very large." (For his essay on the counterintuitive power of selection, see page 4.)

ike all of the major cereal grains but corn, wheatgrass is an Old World species. The plant first came to the United States as a forage crop candidate in 1907, but it didn't take off until a variety from the Russian Caucasus arrived in 1932. Now, though not generally considered invasive, and co-existing well with native plants, it grows wild across western America and Canada. It is one of the most productive US forage crops. Compared with another, smooth brome, it is more resistant to drought and makes much larger seed. It quickly forms a protective mat of roots and underground stems called rhizomes that raise soil carbon, even on soils degraded by earth moving and mining. It can be crossed with wheat, to transfer disease resistance, and be the wild parent for hybrid perennial wheat.

Beginning in 1983, the Rodale Institute in Pennsylvania evaluated close to 100 species of perennial grasses for development as grain. The researchers looked for seed size and flavor, synchronized maturity for harvest, heads held above leaves and stems for harvest ease, threshability, resistance to falling in storms, resistance to dropping seed before harvest - shattering - and for perenniality. (The last might seem a given, but for perenniality there are degrees.) Their winner was intermediate wheatgrass. From the USDA and elsewhere Rodale obtained wheatgrass originally collected in the Soviet Union, Iran, Turkey, and other parts of the eastern Mediterranean. In 1989 the 20 best of some 300 plants were intermated, and 380

progeny were evaluated until 1994. The best 11 plants of those plus three from another evaluation were intermated. Seed from that cycle's premiers went to The Land Institute. "They pretty much did the work for us" to decide on a plant and get started, DeHaan said. "I have come to believe that they made a good selection."

DeHaan was a graduate school fellow with funding from The Land Institute, and joined the staff in 2001 after earning his doctorate from the University of Minnesota. His first work at the institute focused on perennial wheat. Depending on the yardstick, wheat is the No. 1 or No. 2 crop in the world, and making it a perennial has great potential payback. DeHaan also took on side projects, including wheatgrass. Most of the side projects dried up. The plants were not well adapted to central Kansas, or were difficult to work with. By comparison, Kernza is easy to grow and manage in research plots. In September 2010 the institute hired Shuwen Wang to take over wheat, and DeHaan turned solely to a side project that had blossomed.

DeHaan's first big effort with wheatgrass was to split each of 1,000 plants into three and grow them at random places in the field. He would average differences that resulted from location, and better see the contenders' genetic values. The genetic differences among plants turned out to be so large that DeHaan decided cloning's precision was not worth the extra year it took to grow plants for splitting. This would also spare him reducing by twothirds the number of plants available for finding those rare individuals that combine valuable traits. Chances are better with numbers. Since that first planting, selecting the best results along the way, he has set out increasing numbers of seedlings. Last year the population reached 14,000.

What a decade of selection has achieved encourages DeHaan. Seed size in widely spaced plants has increased more than 100 percent, in tighter field plantings by about 45 percent. Seed heads are bigger, and seed quantity has increased. Yield, the weight of seed, has doubled both per head and per field area. On average, threshing ease shows a small increase. Lodging, when plants fall over, hasn't been measured, but selection for yield and seed size seems to have reduced it.

DeHaan's progress came from an initial gene pool of 14 Rodale plants. Compared with the wild collections available from the USDA, these made much bigger seeds, and more of them. But while his population grew, his selections shrank the gene pool. "This is the proverbial 'domestication bottleneck," he said. In recent years he worried about lost genes needed for improving certain traits. So he combed the USDA collections. He found varieties that mature early - desirable in a plant that will shut down come summer heat. "I have since crossed these with some of the best plants for seed yield in an attempt to create earlymaturing plants with high yield," DeHaan said. This is one example of how plant breeders, even for an established crop like corn, tap strengths from wild or old stock.

They must take care. Progress in selection does depend on a population's genetic diversity for a particular trait, and the larger the variety, the faster the change. But there is a flip side. "You could cross my very best plants with some terrible ones, which would increase genetic diversity and then increase the rate of progress from that point," DeHaan said. "But you would have gone backwards in order to pick up that increased progress. In the end, you may have been better off sticking with less genetic diversity." For studied reclamation of something lost in the bottleneck, however, he is confident in the USDA collections. "We have come nowhere near exhausting the diversity available," he said.

Though DeHaan is concerned about losses, the diversity remaining in his refined population might be larger than guessed. Wheatgrass the species is outcrossing. An outcrosser can't pollinate itself, but mates with a different plant, a different genotype. In contrast, wheat's flowers do pollinate themselves. This makes for easier breeding of a consistent crop. Outcrossing continually shuffles genes to keep that spice of wild life, variety.

researchers at other institutions are **N**working to read Kernza's genome and help breeding work. (For more on the technicalities of that work, see page 24.) Meanwhile DeHaan expands his field, looks for the best plants, intermates them, and keeps a detailed pedigree. Breeders of perennial grasses usually use either phenotypic selection or progeny testing. Phenotype is how the plant appears, what it actually becomes. This results both from genotype, the specific but somewhat plastic instructions from its parents' combined DNA, and from molding by a particular place and time's weather, water, soil, sun, and other plants and animals. In phenotypic selection, a group of select plants is mated, and their seeds are thrown together and planted at random. Then another round of selection begins. The population improves, but the breeder loses track of lineage and is uncertain whether particular star plants were born or made.

In progeny testing, breeders put seed from, say, 500 mother plants in six plots of differing place and kind. The plants in these 3,000 plots still are open-pollinated – fathers unknown. But by watching the results over varying space and years, the breeder can see which lines most consistently perform best, and credit this to the known mothers. "You go back to the holding pen where you've been keeping those," DeHaan said. You dig up the best ones, and then cross them together for the next round of selection and improvement. "You see that it takes lots of resources and time, but you get a very accurate measure of the true genetic value of those 500 individuals."

When DeHaan traced the lineage of his plots' proven best performers, he found that most came from just a few families. So he went on to crossing plants by hand, and could keep track of not just mothers, but fathers. He learned to read results in a way never covered in his college plant breeding courses, but used by animal breeders, and increasingly by tree breeders. "It is not phenotypic selection, or progeny testing," he said. It's based on predictions using a statistical model that includes pedigree information. "You use the information from relatives in the year that you make the selection, rather than growing progeny out in later years in order to obtain the genetic information," he said. "All that is required is careful crossing, record keeping, and statistical analysis."

This will help take him to a dependably productive plant. But when farmers take to Kernza depends on more than productivity. Above all is whether the crop's value exceeds the production cost. Because they'll take less time, inputs, fuel, and equipment wear, perennials could become profitable before their sale value matches other crops. But DeHaan said, "Not only must prices and costs be compared, but risks." Not only are there the risks of production, but also the risks of price and influence of government programs and crop insurance. Also, a farm is not just numbers, but an ecosystem. Crops and livestock can interact in unpredictable ways for good or ill. This remains to be worked out. "The only thing we can be certain of is that we will not be able to anticipate all the effects," DeHaan said. Because of all the complexities, he's taken a simpler approach to estimating when Kernza's time will arrive. "Just look at the current specialty crops that are grown," he said. "How much do they yield per acre? Consistently, the minimum yield for specialty grain crops is about 1,000 pounds per acre. So I think we can be pretty confident that if Kernza isn't yielding this much we shouldn't recommend its use. If it can consistently attain this level of production, then it will probably be up to marketing strategies to determine at what point the crop catches on." Yields now vary widely, depending on weather and management. Test plots in the upper Midwest have produced more than 1,000 pounds per acre, but in Kansas yields are generally less than 300 pounds.

Charlie Melander raises wheat, milo, soybeans, and cattle several miles south of The Land Institute. For three years he let DeHaan keep 30 acres in Kernza. It largely kept itself, with no cultivation or herbicides. "The Kernza was so competitive, it kept all weeds out," Melander said. Each summer the grain was harvested, and proved no trouble for the combine. In winter Melander put about 30 cattle on the stubble. Melander said the crop made excellent groundsheltering growth for control of erosion by wind and water. It also attracted wildlife. For the first time near his house he saw quail.

Last year drought continued in central Kansas, and the Kernza field finally suffered for it, with a little less cover, and much less grain, even as Melander's wheat produced a decent 40 bushels per acre. Kernza can make more mass of leaves and stems, but it transpires nearly all year. "Grain is very sensitive to resources," DeHaan said. "This is really true of perennials. Annuals under stress will set seed because if they are going to die, only seed will see their genes into the next generation. Perennials under stress will cease growth, hoping to live for many years to come. Seed production in a perennial is generally the first thing to go when stress sets in." For one year of drought a perennial might outperform the annual. "It has a larger bank account to draw from," DeHaan said. Deeper roots reach more soil. This year he saw high plant growth in some Kernza fields - higher than an annual wheat field would have - but almost no grain was produced.

A second part of this story is that Kernza matures later than wheat, which has been bred to quickly use spring moisture to make grain. "Kernza grew luxuriously for months, producing all of that biomass, and then tried to head," DeHaan said. "At that moment, the stored spring moisture was gone." In the short term, Kernza probably will be better adapted to areas where rainfall more regularly exceeds evaporation. As the earlier yield figures show, DeHaan's test plots in Minnesota and Wisconsin have done well. But just as wheat breeders did before him, he is selecting for plants that mature earlier, and he also wants to make them thriftier with water.

Of his experience with Kernza, including the bad year, Melander said, "I think it was really worthwhile, because you can't test for this in a lab or a greenhouse." He said that if Kernza is developed for better grain yield, while still needing no tillage and no herbicides, and allowing cattle to eat stubble, it will become competitive.

Even then, breeding will continue, and farmers of perennial grains will still replant. After a decade the worst weeds in a Kernza field are likely to be volunteer Kernza plants, competing with neighbors instead of making grain. And with evolution of pests and diseases, and of insight, a plant breeder's work is never done. "There is always something new to deal with," DeHaan said. "The new varieties will almost certainly be better than the old in some way. A decadesold variety will likely be worth replacing."

But the replanting should come far less often than with annuals, as should cultivating, spraying, fertilizing, and possibly irrigating. Farming will be made more elegant. From people outside The Land Institute, DeHaan said, "I've heard things like it would be the most important change in agriculture ever."

He guides visitors through his field plots or greenhouse pots, and with a tour given at certain lush time, he can see in their eyes a beholding of beauty. "They are inevitably attracted to particular plant types at just the right growth stage," he said. DeHaan sees beyond this. "I like to say that these plants are like puppies - cute when they're little, but they grow up and become big dogs quickly," he said. Green fades to tan, leaves dry, supple stems stiffen. Atop them heads grow fragile. But here are the amber waves, and under the husks may be riches. Each year DeHaan searches there for big seeds. "The process of threshing, weighing, and analyzing data is analogous to the rush of scratching a lottery ticket," he said. On second thought, he said,"We are confident that we're not going to have a sudden odds-defying breakthrough." But he is certain of making steady progress. "It's really more like getting that envelope in the mail from your 401(k) when you know the stock market has had a good year," he said. "You're certain the results are going to be good, but actually seeing it with your own eyes feels gratifying."



Xiaofei Zhang with Kernza at the University of Minnesota. Genetic analysis can help him predict early in a plant's life what traits it will develop. This should speed breeding progress. Scott Bontz photo.

Growing Kernza in Minnesota

Crop candidate benefits from research, marketing power, and cooler climate

uch of The Land Institute's work to develop perennial grains has been solo. Help toward spreading these plants over world cropland comes through collaboration. The help can be particularly strong when a partner has the resources of a land grant university, plus soil and weather where different lines of a new plant excel and deliver bigger, market-enticing yields sooner. Institute Kernza breeder Lee DeHaan found this combination by going north from Kansas to the University of Minnesota.

At the St. Paul campus, with acres of open land surrounded by big city, Don Wyse leads plant breeders, soil researchers, and food scientists, including 14 graduate students. He also makes deals with businesses. lobbies the legislature, and hoes weeds. This is all to get more plants covering northland soil for more of the year. The effort is called Forever Green Agriculture Initiative. It's not just about perennials, but includes overwintering annuals. It also is not just about grains. Key is making sure that your chosen plant has a profitable end use, and better yet, has more than one. Kernza could make grain, forage, and biofuel. Maybe it could make cosmetics. "I don't really care what the hell it is," Wyse said, as long as the land gets covered, the soil and water protected. If you can get farmers to earn money, he said, they'll plant.

Corn and soybeans dominate upper Midwest agriculture. They are annuals, and Wyse said their roots in Minnesota are active for just two and half months, mostly after the region's heaviest rains. "That's pretty wasteful," he said. "The annual cropping system is leaky." It loses water, it loses soil, and, with less chlorophyll production, it loses solar energy. "Capturing more of that energy is part of what we need for global food energy." He wants "high-efficiency agriculture."

With a land grant school's staff, land, laboratories, and equipment, his team has more than the ability to breed new plants. "We have the capacity to build enterprises," Wyse said. They pursue science, production, end uses, and economic framework. Potential partners include General Mills, which Wyse said is interested in sustainable food cropping. "They would have to build a product off of a sustainably grown grain," he said. The university has deals with the cosmetic companies Estee Lauder and Aveda to use compounds from native plant species. Wyse is confident the Minnesota legislature will soon approve for his broad project \$15 million over 10 years.

His team is working on these perennial plants: Flaxseed is a Minnesota native rich in nutritious omega-3 fatty acids. Perennial sunflower is a North America native also being developed by The Land Institute. Hazelnuts have a native species, a European species, and hybrids. Willows can go to construction and energy. Alders can could be used for energy but grown on land unsuitable for food crops. Kura clover makes living mulch for grain crops. Kernza can

We know that for most of wheat disease, intermediate wheatgrass has resistance.'

Kathryn Turner, University of Minnesota

make food, forage, and fuel. Take the valuable grain for food, Wyse said, and use the residue for fuel. DeHaan said that doing this with annuals will ruin soil. Study of perennials such as hayfields show that almost everything aboveground can be cut and hauled off for decades with no soil degradation and no need for fertilizer. The Forever Green project also develops plants to fit between annual crops, and cover fields through winter and early spring. Field pennycress is seeded after harvest of corn or soybean and resumes growth in spring after winter dormancy. After suppressing weeds and protecting soil, it can be cut and its seed used for oil and protein meal. Malting barley also could be double-cropped with soybeans, if it can be bred to overwinter. Early screening of plant collections have found individuals with winterhardiness.

DeHaan took his Kernza plants to Minnesota to take advantage of the university's resources, but the trip had additional benefits. Growing the crop there showed some plants that had been standouts in Kansas were mediocre up north, and plants unremarkable in southern Plains heat shone in the cooler clime. This was not a surprise. The same happens with other grains, and farmers choose their varieties accordingly. But it showed which Kernza lines to pursue in Minnesota.

It also showed that Minnesota is an easier place to see big yields from Kernza, which should bring quicker economic success. The cool-season grass does better in a cooler, wetter place, exceeding Kansas production by threefold. If the 1,000 pounds of grain per acre obtained on Minnesota plots is born out in field-scale plantings, the plant might already be productive enough for the specialty grains market, though not yet for mainstream commodities.

Several Minnesota researchers are helping develop Kernza. (For the food science story, see page 19.) Most have other projects too. One scientist focuses on Kernza full time. Xiaofei Zhang is a plant breeder who previously worked with wheat. He breeds Kernza by traditional means, selecting plants from how they appear in the field, but this year will also begin exploring genomes. This should help him predict early in a plant's life what traits it will have, and so speed breeding progress – though he'll still need to compare those predictions with actual performance in the field. (For details on the genetics work, see page 24.)

With seeds from DeHaan's third selection cycle to improve Kernza, he and the Minnesota team planted a field in 2011 and raised more than 2,000 plants, in 69 families – each family having the same mother plant. Last year Zhang measured traits like biomass, grain yield, and seed shape, then picked the best 200 plants. The seeds from these plants were sowed last fall and will be evaluated this fall. The breeding cycle continues with more selection and crossing of the best plants, including over winter in the greenhouse. He plans to begin amassing DNA sequences from his plants this spring, and this summer begin combining that information with field observations for a model to guide genetic selection over several generations.

Zhang will use genetic sequencing for more than this pioneering selection. Over evolutionary history chromosome sets from three species combined to make intermediate wheatgrass, the wild plant being cultivated as Kernza. A similar interspecies hybridization made bread wheat. These two modern plants are both from the wheat tribe. The genomes of wheat and other major crops are known in much more detail than the still largely wild upstart Kernza. So if there is a common subgenome, Kernza breeders will have much useful genetic work already done for them. This year Zhang will look for that connection.

Kathryn Turner does not work directly to increase a grain crop's yield. She works to keep that yield in the face of attack by pathogens with names like bacterial leaf streak, stem rust, and Fusarium head blight. The University of Minnesota's work with wheat is primarily about disease resistance. Turner, who is earning her doctorate, said, "I think it is the most important trait we can give growers."

Minnesota's barley crop was devastated by Fusarium head blight 20 years ago, and it is making a comeback only now. Head blight is hard to control with a fungicide. It's also the biggest disease affecting wheat. But Kernza is resistant. It also stands up to eye spot, tan rust, and a new threat from African varieties of stem rust. "We know that for most of wheat disease, intermediate wheatgrass has resistance," Turner said.

Before graduate school Turner spent a summer working at The Land Institute. For her master's degree she studied perennial wheat's disease resistance and over-wintering ability in Minnesota. For her doctorate she's looking at resistance to leaf rust in bread wheat and durum wheat. She also helps Zhang with Kernza, both for making it a perennial crop and for improving wheat.

Growing Kernza as a grain crop might increase its risk for disease, Turner said. Accumulation of soil pathogens is more likely with a perennial than with rotations of an annual. She wants to address this potential problem now. Devastation of a new crop by disease for just one year would not only hurt the food supply, it would discourage farmers from planting a crop with potential to conserve their land and water, and so make more food in the long run.

Turner said Kernza fields in Kansas, Minnesota, and Canada have not shown much disease. The university's field planted in 2010 turned brown the next summer, but the cause remains unclear – it might've been dryness or heat stress. But the occurrence inspired close monitoring of disease. In 2012 Turner walked the field, looked at every plant, and sampled tissue. Most plants showed very low levels of infection. Of plants affected, the primary disease was bacteria leaf streak, a relatively new disease in wheat.

Unlike self-pollinating wheat, Kernza plants must breed with other Kernza plants, which makes every individual different. Even within a family susceptible to leaf streak there was much variation. The individuality makes difficult the replication needed for a scientific solution. But it allows selection of resisters. Turner dug up tillers, side sprouts, from plants that resisted the disease, and from plants that succumbed. She divided and replanted the vegetation for replication. This spring she'll infect them with leaf streak. The goal is to associate genetic markers with resistance. Then breeders can better work toward attaining strength in numbers.



Marty Christians prepares holes for transplanting Kernza at The Land Institute. Scott Seirer photo.

Kernza in food and drink

Brewing, baking, and the search for heavy molecules to make light bread

o succeed in solving the problem of agriculture and save soil, a perennial grain like The Land Institute's Kernza must be able to succeed financially, and to succeed financially it must work not only as a crop in the field, producing lots of manageable seed, but as a food, handling well in the kitchen and appealing to the palate. Land Institute staff members have made and been pleased by Kernza baked goods including pancakes and quick breads. Kernza flour can't yet rise as well as bread wheat flour, however, and a food scientist said that it needs flavor improvement to go mainstream. But Kernza appears to have the potential for bread making, and fermentation during bread dough rise eliminates the perceived "green" flavor. Breeding might bring the mild flavor favored by the big market. Meanwhile, eaters who want success for the plant already are happy with what it makes. It also has augmented a beer, and is soon to be used for whiskey.

Heartland Mill in Marienthal, Kansas, has turned thousands of pounds of Kernza seed into whole- grain flour. General Manager Mark Nightengale said that though the seed is as yet much smaller than other grains, there are no problems in milling. Heartland can use a hammer mill or a stone mill for Kernza. Before milling there is a challenge. Ninety percent of the seed keeps its hull through the farmer's combine, like with oats and spelt. Land Institute researcher Lee DeHaan wants to make Kernza free-threshing like wheat, so it leaves the combine naked. Meanwhile, Heartland's challenge isn't great. Most oat seeds also keep their hulls, and mills have de-hulling machines. Nightengale said the Kernza hulls come off much easier than oat hulls.

DeHaan has used the Kernza flour to make pancakes, biscuits, quick bread, cake, cookies, and scones. For The Land Institute's annual Prairie Festival, Wheatfields Bakery of Lawrence, Kansas, makes yeast bread combining Kernza and wheat flours. For the same event the institute sells sample bags of the flour, and includes recipes. The institute's managing director, Scott Seirer, said buyers report pleasing results. DeHaan says his results have been unpredictable, but that baked goods often have an extra moistness.

To learn what's inside the seeds and what they can do for eating, he returned to his alma mater, the University of Minnesota, and its Department of Food Science and Nutrition. Tonya Schoenfuss, along with three other university scientists, is working through Kernza's food qualities. With thousands of flavor and texture compounds, which may transmogrify depending on preparation method, flour is as complex as any fine wine. Schoenfuss' goal boiled down is this: "Can you make something out of it? If you can get something, how does it taste?" And can you get mainstream interest - can you attract farmers and businesses like General Mills?

• That stuff's great for you. But it's not what people eating at McDonald's every day are going to eat.'

Tonya Schoenfuss, University of Minnesota

She presented on her computer screen photos comparing whole-wheat cookies with Kernza cookies. Slight color difference, same flat discs. Another photo compares bread loaves. A whole-wheat loaf rises higher than Kernza, and a white flour loaf rises well above both whole grains. "It doesn't form that structure," Schoenfuss said of the Kernza. It's like cookie dough, not tenacious like wheat bread dough. It isn't that Kernza lacks gluten - unfortunately for people with gluten intolerance. But it hasn't yet combined the heavy protein polymers that make dough glutinous, able to trap carbon dioxide from fermentation, and to rise. Xiaofei Zhang is the university's Kernza breeder. He studied the plant's seeds, and found that Kernza had a similar number and type of heavy proteins as common wheat. This suggests potential for a wide range of food products such as bread and cakes. But the minimum of three of the proteins, possessed by bread wheat, doesn't seem to have come together in Kernza test flour. The flour so far has been from fields of diverse individual plants. A Kernza plant can only breed with another plant, not with itself like wheat does, so its seeds remain genetically diverse. Even the seed on the head of one plant can somewhat vary, because each might've come from a different father's pollen. But when all of the seed in a baking experiment come from a single plant, Zhang said, the results might be better than anything seen yet in the food science kitchen labs. In coming tests the Minnesota team will enjoy the luxury of grinding nine

distinct lines of DeHaan's Kernza.

This still might not collect enough heavy hitters. Then might come selection and breeding. DeHaan said that complex job, of gathering genes for the rare proteins without losing genes for other trait gains made over years, would take time and a big investment.

Another possible explanation for Kernza dough's weak rise, Zhang said, is a high bran content. This can cut gluten formation. Kernza seeds are bigger because of breeding, but still smaller than other grains, and small seeds have a relatively high proportion of bran, the outer coating, to endosperm, the white, meaty portion with most of the protein and starch. This ratio will improve as DeHaan and Zhang select their way to plants with bigger seed.

Kernza might have less gluten-forming protein than wheat, but its total protein content is higher, Schoenfuss said, 17 percent versus 13 percent. This could be seen as a nutritional advantage. As breeding enlarges Kernza's seeds, the endosperm growth might come more in the form of carbohydrate, and the protein proportion might lessen. DeHaan doesn't know yet, but predicts that selection could produce either higher or lower protein types, depending on what is desired.

Schoenfuss knows that Kernza's small seed has less starch, about 44 percent versus 60-70 percent in wheat. The starch quality is fine, she said. But there's not yet enough of it for a light cake leavened with baking powder, in which baking gelatinizes starch to hold risen structure. She has an undergraduate student working on cakes. "I told her, watch them while they're baking." Eggs add volume to the Kernza cakes, but they still collapse. Cake mixes already add starch, however, and this could benefit Kernza. Schoenfuss said that with larger seeds the starch level probably will go up. "It works fine in cookies," she said. Cookies have lots of fat and sugar, so the starch doesn't gelatinize.

Both fermentation and heat can dissipate compounds that give foods particular flavors, and Schoenfuss said that yeast bread made with Kernza has no taste to give one pause. But in chemically leavened products like cakes and cookies, tasters detect a "green note," somewhat grassy. She said whole wheat has similar issues.

If a staple ingredient like whole wheat or Kernza carries a curious flavor, Schoenfuss said, "You find a way to make it taste better" through process or ingredients. "You can do that now." Pancakes with maple syrup might taste fine. She said that a specialty marketer like Hudson Mill might go for Kernza as is.

But that's not the aim of the Minnesota team. They want to make a product that's easy to use, easily marketable. If you really want to get more perennial grain on the landscape for the sake of its health, Schoenfuss said, you will want to do well in the mainstream commodities market. Bulghur wheat, quinoa, barley: "That stuff's great for you," she said. "But it's not what people eating at McDonald's every day are going to eat."

Chuck Magerl owns Wheatfields, the bakery that puts Kernza flour in bread for The Land Institute's Prairie Festival. He also manages Free State Brewing Co., which has made and sold a couple of small batches of Kernza beer at its brewery and restaurant in Lawrence. The project has not spread yet to retail with other Free State products in stores in Kansas, Missouri, and Nebraska. Grain supply is low, and efforts are still experimental.

Also planning to try Kernza is Boulevard Brewing Company, which makes beer in Kansas City, Missouri, and reaches 25 states. Brewmaster Steven Pauwels said the company wants to operate as sustainably as possible, and in a way most beneficial to farmers. He said it also wants to find new grains and flavors. The first Kernza will go to a test batch of about 1,000 gallons. Like wheat, Kernza will be brewed with the essential malted barley.

Much of what brewers and bakers want from grain is the opposite, Magerl said. Bakers like protein, brewers carbohydrate. Kernza now is relatively high in protein. DeHaan now is working out basic domestication traits. Later he may address the protein : carb ratio. Though there are obstacles with Kernza, as with any novelty, Magerl said it has been used as an adjunct to other malted grains, such as barley and wheat, adding flavor that he called "nutty" and reminiscent of roasted sunflower seed. The beer shows a haze that might result from Kernza's higher protein. This won't appeal to drinkers who favor something like a sparkling light lager. But Magerl said adventurous craft beer drinkers put flavor over visuals and won't care.

Kernza's appeal to Magerl is flavor and, number one, that it is a perennial. "It offers huge possibilities," he said. "I think Kernza has a solid place in our food future, and we just need to see how many niches we can find for that to be valued in."

Another place in that culinary ecosystem is spirits, such as whiskey. A young man who visited The Land Institute a few years ago is on the verge of a commercial, blended Kernza whiskey. Henry Tarmy and two other men, brothers, built a 300-gallon still in the basement of an old industrial building in Ventura, California. Ventura Spirits doesn't want to make a new whiskey or vodka using only, as Tarmy put it, "regular GMO commodity grains." Their plans are for Kernza and Southern California's prickly pear cactus fruits, persimmons, and kelp. The aim isn't just originality. It is to add value to products of the distiller's home place. Tarmy sees the distillery as a vehicle of community. Many spirits originated in inland farming cultures, he said. When farmers had surplus, alcohol was a way to keep their labors' fruit. Now let's say distilling was invented today, Tarmy said: "Well, looking around, what would be interesting in this place?"

Kernza is not obvious in that sense, but it can grow in the West, and Tarmy said that as a perennial grain it meshes well with the goal of Ventura Spirits. Paraphrasing Land Institute President Wes Jackson, he said he and his partners want to see in the cropping of their plants "conservation as a result of production." "We really don't want to be a net negative," he said. "I would really love what we do to add value, and not just to our product, but to the earth."

For liquor Kernza has desirable traits. Before distilling, a mash of grains is first fermented, and this fermentation requires enzymes, either synthetic or from malted grains. Tarmy said malted Kernza is powerfully enzymatic, possibly because of high protein. Also, mashes typically are combinations of plants, which mingle flavors, and from Kernza the distiller will get what Tarmy called "sweetness." They plan to mix it with "spicy" rye.

First they must get permits from three levels of government. Tarmy hopes to begin production in late spring.

Plant trumps farm method

For capturing nitrogen, more vital than how it's applied are the plant's roots and longevity

he first study of its kind showed that within two years after planting, the perennial grain called Kernza beat annual wheat at tapping groundwater, building soil carbon, and, by a huge margin, conserving nitrogen fertilizer. For absorbing rather than leaking the nutrient, Michigan State University researchers found, plant type was even more important than fertilizer amounts and kind.

Research already had shown that pastures and hay fields, landscapes covered by perennials for decades, are better than annual crops at conserving soil nutrients, soil life, and soil itself - so-called ecosystem services. The Michigan State study was made with the help of Land Institute scientist Lee DeHaan and was led by former institute graduate fellow Steve Culman. It compared a perennial treated not as forage but as grain, a crop fertilized and, though perennial, every several years still in need of rotation and starting over. The study showed how quickly a perennial can pay off, which will be important in winning the way to market.

Researchers sowed seeds of the two plants in fall of 2009. By 2011 the wheatgrass had reduced moisture deeper in the soil and reduced nitrate leaching 86 to 99 percent – depending on how much was applied – compared with wheat. This means the perennial roots put more water and fertilizer to use. In the second year one form of gauging soil carbon formation showed Kernza 13 percent higher than wheat. Increasing organic carbon improves soil, as organic matter is about 50 percent carbon. First-year Kernza grain yields were just 4.5 percent of annual wheat's, but second-year yields increased to 33 percent. DeHaan continues each year to get more and larger seed from his selection and cross-breeding of the best plants.

The study treated plots of each crop three ways: organic, which was fertilized with chicken manure, and synthetic nitrogen applied at rates moderate and heavy. Organic and synthetic nitrogen applied moderately leached past wheat roots at about the same rate. The big difference came with perennial vs. annual plants. "Annual organic crops using legume or manure as a nitrogen source can leak, but it's the big perennial root system that really stopped the leaking," DeHaan said.

In 2010, the study's wheat yielded more than 20 times more grain than did Kernza. But in total aboveground biomass, with leaves and stems, there came a near draw. Next year both crops made more grain, but although wheat biomass changed little, Kernza's increased dramatically, to two times that of the annual. A plant breeder can transfer that mass to boost seed production. The Michigan report said that if total aboveground productivity like Kernza's in the second year is kept for several more years, and if breeding brings the ratio of seed to total biomass near 30 percent wheat gives about 50 percent - the new crop's yield would be comparable.

The study appears in Agronomy Journal.

Yield in kilograms per hectare



Comparison of how Kernza and wheat performed side by side in Michigan, the summer after initial planting and the following year, with nitrogen fertilizer applied at a typical rate.

Combing the genome

Land Institute collaborators mark DNA to speed and refine breeding

and Institute plant breeder DeHaan doesn't need to find and fully understand all of the genes needed to turn his Kernza plants into a successful perennial grain. But finding links to genes could bring success sooner. So while he selects star plants and builds a pedigree, scientists elsewhere sift through the Kernza genome.

Steve Larson is a research geneticist for the USDA's Agricultural Research Service office in Logan, Utah. He specializes in forage crops, and wants not only to help DeHaan make wheatgrass a grain crop for humans, but also get from it more and better leaves and stems for grazing and hay. In Kernza Larson found 50,000 sequences of genetic code that were more than 400 molecules long and identical among the plants but for variation at one molecule position. For example, using the letters that represent the four types of these molecules, a sequence could start in one plant with GATC, in another with GATA, in another with GATT. These varying markers can be associated with varying plant growth. The markers needn't define genes, the codes for individual proteins. But good markers are near enough to the gene sequence on the chromosome that they stick with it over generations of mating. If Larson sees that plants with erect leaves always have the same form of a certain sequence, he has a helpful marker.

Seldom will the key be so simple.

Jim Anderson, a wheat breeder overseeing Kernza work at the University of Minnesota, said that dozens or even hundreds of genes might interact for a trait. But with enough markers, no longer will breeders need to wait months to find the best looking plants, then work months more at measurement and analysis to estimate how much of the good result was due to genes and how much to growing conditions. Instead they will pull sequences from a mere seedling and see particular strengths laid out in the cards. Anderson compared it to a fingerprint. Correlation of field traits with genetic data will allow prediction by fingerprint. "It's very powerful," said Xiaofei Zhang, a University of Minnesota scientist breeding Kernza originally provided by DeHaan, with the help of sequencing work complementary to Larson's. (For more about Minnesota's "Forever Green" program, of which this is just a part, see page 13.)

Here is another way to look at the power of markers. In traditional breeding, the "selection unit" is a plant within a population. A breeding population might have thousands of genetically diverse plants, and an unending possibility of diversity over generations. The going with such a complex is slow. In molecular breeding the selection unit is reduced to a gene or marker. For any given gene controlling differences in growth form, such as variable seed size, scientists expect only about 1 to 10 of the gene variants called alleles. Or there might be no variation. Likely a trait will be influenced by



Kernza breeding plot harvesters, led by scientist Lee DeHaan, left, take a break from their work. The harvest is done by hand to segregate wheatgrass heads for seed analysis. Scott Seirer photo.

more than one gene's contiguous segment of molecular code. But by reducing the examination to one select segment at a time, the scientist can see and act with unprecedented clarity and precision. Together enough markers will allow useful reading for desired genes scattered throughout Kernza's 42 chromosomes.

"The objective is to try to simplify things," Larson said. But he referred to consultant Bill Jensen, who said, "The paradox of simplicity is that making things simpler is hard work." Getting the markers takes a great deal of time. His team spent six months collecting and scanning genetic material. He has tried to devote several hours each week for the past year for analyzing the information. And he has yet to start the task of connecting markers with traits. He must wait for and find mature plants showing those traits across various field plots. To this end DeHaan established plots in Kansas last year. Larson will set out clones of the same plants this spring, after northern Utah's colder winter. Then the established plants will need another winter before they set their first seed. Preliminary analysis to connect markers with simple traits might begin next year.

Meanwhile, Larson develops "highthroughput assays" to read the markers, to see which SNP variants appear in each plant's DNA. The technology will enable him to check 96 sequences from 96 plants at once – 9,216 genotypes in that "one relatively simple experiment." The laborious groundwork will have made reading the genotype very efficient. With improved machines, the per marker price of such assays has plummeted from as much as \$5 to about 5 cents.

Minnesota's Zhang said the approach and technology he uses will yield thousands of markers from across one plant's entire genome for less than \$20. This is about 1 percent of what such work cost until a few years ago. Before, cost confined breeders to looking at only a few important genes and markers. Now, Anderson said, "We have the entire genome blanketed with markers." Zhang wants to see the price even lower, since a plant breeder must deal with thousands of plants.

Having a lab find markers will account for only a fraction of plant breeding's cost, and though DeHaan wants to use what Zhang and Larson find, he avoids pinning on it expectation of a revolution. This is not just because he can't afford a quarter of a million dollars to assay the markers of 14,000 plants - a cost that does not include collecting tissue and extracting DNA. Kernza's genome is large and complex. A marker that can be associated with a trait in one breeding cycle might not remain so closely associated in the next. Genes interact. "This is a very new area of science," Anderson said. Much of the work is theoretical. He said the first comparisons between genomic selection and traditional are just now being published. Zhang said, "Genomic selection is certainly not perfect. Maybe there is a little difference between the model and the reality." He'll still need to check his sequencing and statistical analysis against field results. "You always need the field testing for verifying at the end."

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Land Institute scientist Lee DeHaan carries for transplantation a tray of Kernza seedlings. Stories inside tell how he and collaborators in Utah and Minnesota are turning a wild perennial plant into a grain crop. Scott Seirer photo.