

# PNP

PACIFIC NUT PROCESSOR



*Walnut  
Rootstock  
Clones*

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# Walnut Rootstock Clones

## *What's All the Noise About?*

**By Patrick Cavanaugh, PNP Editor**

Could it be true? In the near future, walnut growers may call their nursery and order up rootstocks that are not only identical to each other, but may be superior to any rootstocks previously available. It's the tentative result of a team of researchers (many of them at UC Davis) and many nurserymen that began the work in 1997 on a Paradox Diversity Study (PDS).

The PDS study was conceived by Dr. Gale McGranahan, who heads up the Walnut Improvement Program in the UC Davis Plant Sciences Dept. The study represents a huge commitment by 12 commercial walnut nurseries who submitted about 40 different seed sources, each coveted as excellent and proprietary examples of hybridized walnut rootstock, commonly called "paradox" within the industry.

Three of the nurseries consented to growing the seedlings for four commercial orchard trials and the disease screening tests. This was the equivalent of about 60 acres of trees. The orchard trials were conducted in San Joaquin, Kings, Yolo, and Tehama counties. Chandler and Tulare were the two English walnut cultivars used for the study.

"The goal of the PDS orchard trials was to determine if the family of trees produced from certain seed sources differed significantly in yield performance," said Bob Beede, UC Cooperative Extension Farm Advisor, Kings and Tulare Counties, who participated in the study. Ultimately, while the researchers discovered significant yield differences between the seed sources, there were greater differences among the individuals in a particular seed source,

some of which had superior individual traits including disease resistance or tolerances.

"It's important to note that these are not genetically engineered plants," stressed Beede. "These plants are from the natural gene pool because they are selected paradox seedlings." Cloning is simply any method for reproducing identical plants and has been used for thousands of years. For example, the Cabernet Sauvignon vines growing in California is a clone of a vine selected long ago in France.

"The project's goal was not to develop clonal rootstocks," said Dave Ramos, Research Director for the California Walnut Commission. "We knew that nurseries had special seed sources from black walnut trees around the country, which might produce paradox seedlings with different production characteristics," noted Ramos. "However, when tested, none of the original nursery seed sources had uniform detectable tolerance or resistance characteristics for any pathogen."

In addition to the commercial orchard trials, a team of UC and USDA researchers performed green house and field station screening tests on each paradox seed source regarding their susceptibility to crown gall, *Phytophthora*, root lesion nematode and cherry leafroll virus--the cause of blackline. Ideally, a seed source would be tolerant to all of these ailments, however hitting such a home run will be unlikely, researchers report.

Individual seedlings within a few paradox sources show various degrees  
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## Clones

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of tolerance in the above tests. These have been selected for cloning and development into nursery plantlets.

McGranahan and Chuck Leslie, a UC Davis Staff Research Associate, headed up this initial work. "Clones will eventually become a more dependable source of plant material for the nursery, because they will not depend on the percent paradox derived from natural crossing between selected black walnut and English trees," said McGranahan.

The present cost and complexity of micro-propagation tissue culture limits the number of nurseries capable of multiplying clonal selections in-house. Therefore, Dr. Wes Hackett, Professor Emeritus of the Horticultural Science Dept. at the University of Minnesota joined the team to develop acclimation methods for micro-propagation plants using hardwood cuttings and root grafting techniques. Hackett's specialty by training is plant physiology with a strong knowledge in plant propagation.

Although, Hackett's work at UC Davis and with commercial nurseries is



Wes Hackett in a UC Davis greenhouse with young rooted walnut plants.

on a reliable hardwood cutting system, he noted that the late Bill Stuke (Stuke Nursery), pioneered root grafting about 30 years ago. "These propagation methods may make it easier for the smaller nurseries to get into the action," noted Hackett. The high-tech plantlet cloning requires expensive technology, while root grafting and hardwood cuttings are much easier to do. Hackett is working with David Bonilla Walnut Nursery in

Oakdale, Calif., to improve the hardwood cutting success rate. Leslie Nerli, general manager and research director at Stuke Nursery in Gridley, Calif., adds, "We are currently continuing to root-graft the UC rootstocks in small quantities for grower trials."

Hackett also has been closely involved in the acclimatization for the micro-propagated rootstock plantlets.

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## Research Collaboration Advances Paradox Lines

Nothing with the scope of the Paradox Diversity Study could be done without many researchers representing different disciplines. In order for specific lines to advance in the study, many measurements and observations must be completed.

Working in the UC Davis lab and greenhouse with micro propagation were Dr. Gale McGranahan, Chuck Leslie, and Dr. Wes Hackett.

The PDS project would never have been possible without the tireless effort of Jim McKenna, former staff research assistant to McGranahan. Now participating in the Black Walnut Forestry Breeding program at Purdue University, McKenna worked hand in hand with the nurseries and farm advisors to propagate the paradox seed sources, and established the PDS orchard trials.

Additional work is being accomplished by the following:

- *Phytopthora* screening---Greg Browne, USDA plant pathologist, and his group
- Nematode screening---Mike McKenry, UC Nematologist, and his group
- Crown gall screening---Dan Kluepfel, USDA plant pathologist, and his group
- Nursery propagation---cooperating nurseries
- Nursery cultural practices---cooperating nurseries
- Field trials---Growers and UC farm advisors including Joe Grant, Janet Caprile, Kathy Kelley, Janine Hassey and Bruce Lampinen, a UC specialist.
- PDS Workers ---Jim McKenna, Bob Beede, Joe Grant, Wilbur Riel, Rich Buchner and Bruce Lampinen.
- Funding---Walnut Marketing Board and CDFA/IAB (which collects funds from nurseries for research).

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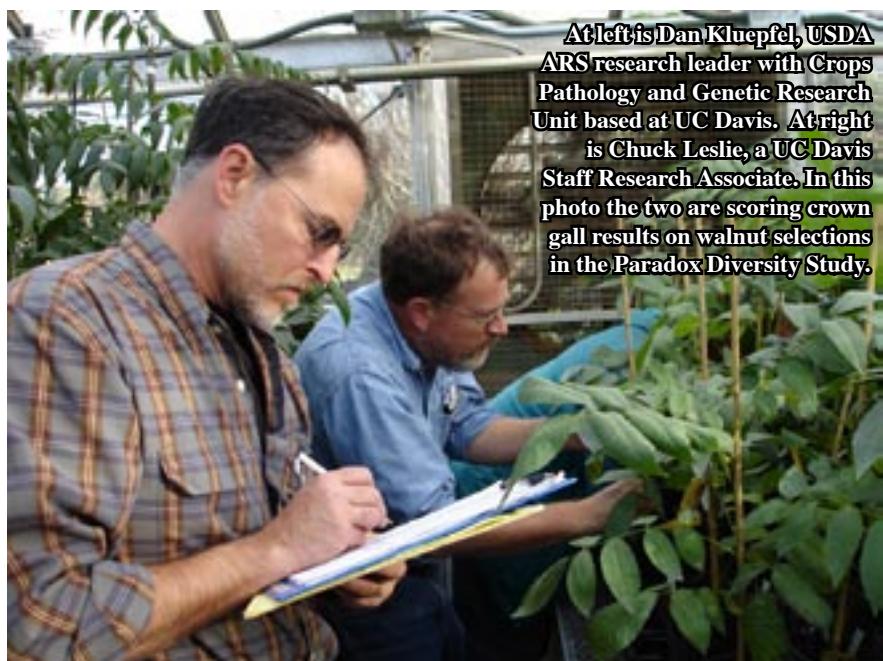
## Clones

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"This was the weak link at the time I became involved in 2000," Hackett said. He noted that the cloned plants coming out of the lab are not able to adapt quickly to a greenhouse environment so they designed a system to interpose the plants in an environment that has a very high humidity produced by a fogging system. The plants are in that environment for about four weeks. After that, they are transferred to a greenhouse bench.

The rootstocks moving forward in the study are all paradox selections and some of them have lower susceptibility to *Phytophthora* root and crown rot, less damage from root lesion nematodes and increased tolerance to blackline disease. It's also been noted that because clonal plants lack genetic variability, their response to these diseases would be more predictable. "And depending upon their sensitivity to any one of the disorders, it could prove to be good or bad," noted Beede.

So far, the following three new clonal selections are being released to walnut nurseries and are described as having the following characteristics:



At left is Dan Kluepfel, USDA ARS research leader with Crops Pathology and Genetic Research Unit based at UC Davis. At right is Chuck Leslie, a UC Davis Staff Research Associate. In this photo the two are scoring crown gall results on walnut selections in the Paradox Diversity Study.

- VX211, a standard paradox rootstock, a cross of Northern California black and English. It is considered root lesion nematode "tolerant" because, unlike "regular" seedling paradox, it survives and grows very vigorously in soils with high lesion nematode populations. This selec-

tion also has moderate resistance to *Phytophthora* and survives well in field tests. A UC Davis patent has been filed on this selection.

- RX1, a Texas Black crossed with English selection. It is moderately resistant to *Phytophthora* with average vigor and is currently being tested for response to nematodes. It has survived well in all field tests. A UC Davis patent has been filed on this selection.
- WIP3, a paradox backcrossed to English, (in essence 3/4 English). It's been selected because it avoids the blackline caused by cherry leafroll virus. However, it is susceptible to *Phytophthora* and may perform more like an English seedling rootstock. It is being tested for response to nematodes.

Hundreds of the selected and cloned plantlets have been given to nurseries to further propagate and grow out and later be grafted to scions. Several nurseries, particularly Duarte Nursery in Hughson are very busy propagating clones.

"We are focusing on Vlach, VX211, and RX1," said John Duarte, company president. "The two standout clones are Vlach and VX211--they both did very well in vigor which relates directly to production and tolerance to wet soil conditions," he noted.

Duarte Nursery owns Dry Creek Laboratories on the nursery site. The founders of the lab have been working on micro-propagation of nut trees for more than 20 years. "Through this technol-

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# Crown Gall Still Elusive

The first round of intense greenhouse screening of the Paradox Diversity Study (PDS) selections have shown that most of them have some degree of susceptibility.

"There is one clone that shows some potential, and it's AZ025," said Dan Kluepfel, USDA ARS, research leader with the Crops Pathology and Genetic Research Unit based in the Plant Pathology Dept. at UC Davis. "However we are at the beginning stages. There are all different levels of susceptibility and that's what we are exploring with AZ025 and other rootstocks."

"Our philosophy has been to subject the selections to very aggressive screening," said Kluepfel. "Anything that makes it through the first test will be interesting to look at."

The screen involves making a "T" cut into the crown of the seedling growing in pot in a greenhouse. The scientist then inject it with millions of *Agrobacterium tumefaciens* cells, the bacteria that causes the tumors at the base of trees. They then wrap this area with film and keep the humidity high--an ideal environment

for crown gall.

ogy base, the company has been able to produce clonal rootstock for many fruit and nut crops," said Duarte. "Having these paradox clones characterized by UC Davis, we can now apply this technology to walnut rootstocks."

Duarte explained that in many crops, clonal rootstocks are delivering growers higher productivity, uniformity and resistance/tolerance over other common seeding rootstocks.

"My job in advising growers is to help them become aware of the state of the knowledge of these clones. The research has been conducted. We let the growers weigh the risks and benefits and decide for themselves," Duarte said. "That's the way growers adopt technology."

Growers may well turn to the first generation UC clones or the Vlach clone because availability for traditional two-year-old (from seed to graft) trees is extremely limited. Nearly all nurseries are sold out until 2010.

## A Call for Caution

But hold the phone. Growers need to realize that despite many apparent  
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for crown gall.

"This type of disease pressure rarely happens in the field," said Kluepfel. "And since we subject the plants to such high pressure, we could end up passing up some clones that may exhibit a low level of tolerance.."

Besides screening through the PDS selections, Kluepfel and his team are screening through a lot of material in the extraordinary Germplasm Repository at Davis.

"We are making multiple observations after infection," noted Kluepfel. "We do this because we know that some selections will develop tumors in three to four weeks while others take months to develop visible tumors. Those selections that develop smaller tumors or are delayed in tumor development are still selections that we have an interest in---down line in the breeding program."

Despite no clear winners yet in the crown gall screening, there are cultural practices that can dramatically minimize infection.

Kluepfel noted that if growers can minimize long term exposure of bareroot trees to warm, moist conditions during planting then they can minimize infection from the *Agrobacterium* pathogen.

Leslie Nerli with Stuke Nursery also noted that the trees must stay cool and dry during the transport and planting. "We'll dig the trees in the winter, but we only send them out in a refrigerated van," she said. "Then, when planting, growers must make sure they avoid damaging the tree. If there is no wound, then the pathogen will not get in."

"Leslie's approach to reduce temperatures and moisture are effective approaches to limit bacterial multiplication and subsequent plant infection," said Kluepfel. "The strategy may be especially important on bare root trees."

Kluepfel also noted that reduced crown gall incidence on walnut trees sold in pots may be the result of limited wounding, stress and less than advantageous environmental conditions for infection by *Agrobacterium*.

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positive aspects of this new frontier in rootstocks, a caution light should be heeded. According to researchers, mature orchard trials are at least five years into the future. At that time there may be the realization that the first generation of clonal rootstocks may have flaws not yet discovered.

Plus, genetic vulnerability may become a problem in that the scion variety is already clonal, and with the rootstock a clone as well, there is a loss of genetic diversity in the orchard.

Researchers at UC Davis, therefore, recommend a conservative approach to clonal rootstocks. "If growers are planting an orchard, be sure to consider it as an experiment," said Hackett. "We recommend that growers mix the new selected rootstocks with the regular seedling rootstocks and observe their growth as well as their disease and pest tolerance over time."

Many nurseries echo the conservative approach to the new clonal rootstocks. Robert Woolley with Dave Wilson Nursery said the new rootstocks released by UC are certainly promising, but are not widely tested, or challenged under various California soil and climate conditions. "Also, the root structure of tissue-cultured plants, or hardwood cutting propagated or root grafted plants, is significantly different than tap-rooted, field grown trees that growers are accustomed to receiving," said Woolley. "This is not to imply that clonally-propagated plants are inferior, but surely it would be prudent to evaluate establishment and anchorage performance prior to widespread use."

The university is developing new and potentially improved rootstock, noted JoAnn Stuke Diethrich, owner of Stuke Nursery. "However we think they are promoting them much too fast, without adequate consecutive years of field testing in known disease and pest pressured areas throughout the state," Diethrich said. "We were the first nursery to put out a trial with the rootstocks RX1 and AZ2 (another selection which later showed problems and did not advance) with a cultivar (Chandler) grafted on top. Prior to this, all trials of these rootstocks for their "tolerances" have only been ungrafted plantlets and most of those trials were plantlets that were

put in as replants in problem orchards with various results."

Diethrich and her research head, Leslie Nerli believe that there is a great need for testing of the new rootstocks with an English cultivar grafted on top to insure that the university's outcome of such "tolerances" are true. "We all know that paradox without a grafted cultivar is a 'growing machine,'" said Nerli. "Ungrafted plantlets grow much faster and more vigorously, and in a trial may give us false indications of the rootstock's true degree of tolerances."

Nerli reported that in a trial two years ago, she put out AZ2, side by side with RX1 in a new walnut planting. "The university thought AZ2 had *Phytophthora* tolerance, but in the end it had a mortality of 50 percent when planted in the grower's field, and the remaining 50 percent had unsatisfactory growth," Nerli said. (According the McGranahan, AZ2 did not die from Phytophthora; it was more of a transplant issue.)

### "We are trying to walk slowly and deliberately"

"However, RX1 satisfied the growers as to growth and survivability," said Nerli. "But I think it needs to be evaluated for at least 10 years and beyond for its yield because the rootstock has the most to do with how the cultivar performs in terms of yield."

Diethrich noted that a prominent grower commented to her that with a traditional nursery seed planting, you at least have some variability, some diversity. "If you have a high stress or disease pressure situation, you might lose 10 to 20 percent of the orchard with the remaining 80 to 90 percent of the orchard producing a good yield," the grower said. "However, in a 100 percent clonally propagated rootstock orchard, if there is a problem affecting the trees, you have a 100 percent problem." However, genetic vulnerability has not been a problem with clonal rootstocks in other fruit crops, noted Hackett.

While the newer paradox selections are gaining attention, there has been at least one other paradox selection that has been propagated and for sale for many years. That selection is Vlach, which grows a big and beautiful tree, and it has been available since the late 1980s.

Tom Burchell, with The Burchell Nursery is an early pioneer in propagating the Vlach selection. In fact, Vlach is one of the first clonal rootstocks commercially available. Working with him was Peter Viss, a Bakersfield-based sales rep for Wilson Nursery and John Driver who at one time worked with Burchell. Driver did the early propagation of Vlach. He also developed a tissue culture medium that is often used today in walnut propagation. Also Vlach is a public variety, requiring no licensing agreements.

Tom Burchell said he is working with Walter Viss (Peter's brother) with V-Tree Nursery, in Waterford on propagating additional clonal rootstocks.

Burchell said "growers will be guinea pigs in the early enthusiasm for clonal varieties. They could certainly choose a variety that is thought to be resistant to many of the *Phytophthora* species and in the long run it may not be," he noted.

"Vlach has been tested in greenhouse studies and so far it is not as tolerant as VX211 to root lesion nematode and *Phytophthora* tests," noted McGranahan, adding, "However, in the long run, Vlach may be better than all the other varieties; we just do not know at this point."

Joseph Grant, UC Farm Advisor, San Joaquin County over the last few years has had three small trials in his county that included the UC Davis clones as well as Vlach. "These sites had documented severe *Phytophthora* problems and it was do-or-die for the selections planted," noted Grant.

"In the worst sites we saw that Vlach and VX211 had success, while others failed," said Grant. "The worst sites were in replanted orchards where there were documented problems with *Phytophthora*. "The next phase of the test will be larger scale trials that will be planted on new orchard land, where there has been no mortality."

In these new trials there will be fewer individual clones tested and they will be compared to the performance of traditional seedlings. The conditions will be more robust and ideal for walnut production, noted Grant.

"We are trying to walk slowly and deliberately," said Grant. "The success and failure of these things will be in the commercial orchards and that means we need 15 or 20 years to know for sure." PNP