

**IN THIS ISSUE:**

The Clegg Collection: A Valuable Resource

Impacts of the recent drought conditions on Central Coast avocado production, and potential impacts of continued drought conditions

Zinc and Phosphorus

Recent findings on polyphagous shot hole borer/*Fusarium* dieback on avocado

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**EDITOR'S NOTE**

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## **The Clegg Collection: A Valuable Resource**

**Vanessa Ashworth, Mary Lu Arpaia, Philippe Rolshausen. Department of Botany and Plant Sciences, University of California, Riverside**

An unusual population of avocado trees may soon suffer the same fate as many commercial orchards elsewhere in California: its water supply will be cut off and the trees fed to a wood chipper. And yet these trees (Fig. 1) potentially hold a key to the avocado's future: they are the cornerstone of scientific research at the University of California, Riverside, aimed at unravelling the genetic underpinnings of agricultural traits and at placing avocado breeding on a molecular footing.

It is well known to plant breeders that the traits observed in a promising selection are rarely transmitted to its offspring. This is because the so called phenotype (what you see or measure) is a poor predictor of the genotype (the underlying genetic machinery). Unfortunately, for breeding to make any progress, phenotypic traits need to have a genetic basis. Traditional breeding, which cannot distinguish between phenotype and genotype—only works because it starts out with a large pool of trees and, by chance, ends up with a few selections that show promise as future cultivars. In avocado, the selection efficiency of traditional breeding is in the order of 0.1-0.2%; in other words, only 1–2 promising selections are recovered for every 1000 trees that have been laboriously (and expensively) screened over the course of a minimum of 5–10 years. Clearly, a better understanding of the relationship between phenotype and genotype would make the breeding process more efficient.

Recognizing this need, Professor Michael Clegg of (then) UC Riverside established a carefully designed experimental population of avocado trees, later known as the Clegg Collection. It consisted of over 200 progeny from a single cultivar Gwen mother tree, and each progeny tree was clonally propagated four-fold, taking the total number of trees to ca. 800. The seedlings were grafted to a uniform Duke 7 rootstock to further reduce the impact of non-genetic variability. Between the fall of 2001 and spring of 2003 half of the trees (two clonal replicates of each unique genotype) were planted out at UC Riverside and the other half at South Coast Research and Extension Center, Irvine.

In this experimental design, every tree genotype is represented twice at each of two locations. Any variation between the clonal replicates at the same location sheds light on how much of a trait is environmental and how much of it is genetic. Only the genetic component is useful for breeding purposes. The environmental component is the “noise” that misleads breeders and, regrettably, often has a large influence on agriculturally relevant traits.

Since 2003 the Clegg trees have been put to good use. First, a quantitative genetic study was initiated to address the mismatch between genotype and phenotype and, specifically, to determine whether certain vegetative growth characteristics are amenable to breeding. This work (Chen et al. 2007) revealed that about 30% of the total phenotypic variation in growth rate and flowering was genetic in origin and thus amenable to breeding.

While the initial value of the Clegg experimental population arose from its utility in teasing apart genetic and environmental effects

on a phenotype, the trees soon acquired additional roles. Genetic markers offer the opportunity of placing phenotypic measurements in a molecular framework. Microsatellite markers were used to determine the pollen parent of each 'Gwen' progeny tree, revealing that approximately three quarters of the genotypes had been pollinated in roughly equal proportions by 'Bacon', 'Fuerte', and 'Zutano', with the remaining quarter sired by miscellaneous cultivars or rogue pollen sources. What better opportunity than to examine genetic variation of traits in the context of the pollen parent. An interesting finding from this line of study was that 'Gwen' × 'Fuerte' progeny had significantly wider canopies and shorter stature than their half-sibs sired by 'Bacon' or 'Zutano' (Fig. 2). The fact that tree width and height is amenable to breeding is an encouraging result in the context of high-density planting such as that commonly practiced in apple.

At a time when avocado was gaining cudos as a healthy fruit with excellent nutritional qualities and beneficial effects in the treatment of high cholesterol and cancer, the Clegg Collection was next harnessed in a study on fruit nutritional composition. Data were gathered on fruit nutrient content in each genotype. Again, taking advantage of the experimental setup, the environmental "noise" associated with each measurement was stripped away to extract the genetic portion that proved to be appreciable (Calderón-Vázquez et al. 2013).

The next step was to connect these data with a new type of molecular marker. These markers—so called SNP markers (Single Nucleotide Polymorphisms)—were developed using gene sequences from a subset of the Clegg trees. They were designed to reside in genes known to control the accumulation of particular fruit nutrients. Statistical analyses revealed that beta-sitosterol contents were being tracked by one of the SNP markers: in other words, the presence of this marker in an

individual was indicative of high beta-sitosterol levels in its fruit.

Markers that are highly predictive of desirable traits and are relatively easy to measure in young seedlings are the nuts-and-bolts of marker-assisted selection, a breeding method that draws on molecular tools. Consequently, a third project was initiated that harnessed the SNP marker that predicted high fruit beta-sitosterol contents. Progeny from trees of the Clegg population were screened using the marker. Out of an initial pool of over 600 seedlings 73 seedlings (12%) were identified that had the desirable form (allele) of the marker, and 12 seedlings (2%) were eventually planted out. The selection intensity of marker-assisted selection therefore is at least 10-fold higher than under traditional breeding.

A loss of the Clegg Collection would surely represent an opportunity lost. Many more projects could be envisaged that address the genetic determination of a trait, its association with SNP markers, the influence of the pollen donor, or the utility of a marker for marker-assisted selection. The Collection has also been the nucleus of a genetic mapping project. Significantly, the SNP markers developed for these trees are also relevant for studies beyond fruit nutrient content because their biosynthetic pathways intersect with those underlying plant stress and disease responses. This property makes the candidate genes equally relevant for studies on pathogen or salinity tolerance and a key resource that could help secure the future of avocado production in California during turbulent times.

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Figure 1: The Clegg Collection: the trees shown here are growing at UC Riverside.



Figure 2: Variation for tree shape observed in genotypes from the Clegg Collection growing at UC Riverside.

## Impacts of the recent drought conditions on Central Coast avocado production, and potential impacts of continued drought conditions

**Ben Faber, UCCE Farm Advisor, Ventura, Santa Barbara**

Avocados are the most salt and drought sensitive of our fruit tree crops. They are shallow rooted and are not able to exploit large volumes of soil and therefore are not capable of fully using stored rainfall. On the other hand, the avocado is highly dependent on rainfall for leaching accumulated salts resulting from irrigation water. In years with low rainfall, even well irrigated orchards will show salt damage. During flowering there can be extensive leaf drop due to the competition between flowers and leaves when there is salt/drought stress. In order to reduce leaf damage and retain leaves, an excess amount of water is required to leach salts out of the roots zone. The more salts in the water and less rainfall, the greater leaching fraction required. Drought stress often leads to diseases, such as black streak, bacterial canker, and blight (stem, leaf, and fruit). Defoliation leads to sunburned trees and fruit which can be severe economic losses.

### Strategies to address drought conditions

- Ensure that the irrigation system is at its greatest potential and is maintained. Avocados are grown on hillsides and pressure regulation is extremely important and is frequently neglected.
- Prune trees to reduce leaf area which requires significant pruning. Avocado can be a very large tree, and if half the canopy is removed, there can be as much as 1/3

reduction in water use. When trees are about 15 feet tall, removing half the canopy can reduce water use by one half.

- In extreme drought conditions, the canopy can be reduced to just the skeleton branches which are white washed to prevent sunburn. Water use drops to zero, and then gradually as the tree leafs out, water can be slowly reapplied, but at significantly less amounts than with the full canopy. Stumping typically results in three years' worth of crop.
- In orchards that have low producing areas, because of recurrent frost, high winds, shallow soils, disease, etc. the grower could decide to completely remove those trees, thereby saving water.
- White kaolin applied to leaves has been shown to reduce leaf temperatures and water loss. This can be used, but under the direction of the packing house, since if it is applied to fruit, it is very difficult to remove.

### **Impacts of the recent drought conditions on Central Coast citrus production, and potential impacts of continued drought conditions**

Citrus is much less sensitive to salts and drought than avocado, partially because of its greater rooting depth. However, it is much more sensitive than deciduous fruit trees, resulting in smaller fruit and lower prices when drought cannot be addressed with adequate irrigation water. Drought also makes the trees more susceptible to leaf drop, and sunburned fruit.

#### Strategies to address drought conditions

- The strategies for citrus are very similar to those for avocado. It is much more sensitive to pruning to reduce water use than avocado. Typically removing half the canopy results in half the water use. Because of this greater control, citrus is rarely stumped.

- By reducing canopy size, production can be maintained, often without loss of fruit size.
- Kaolin clay can effectively reduce water use and can be applied soon after harvest without the problem of coating the fruit making its removal difficult at the packing house. All year round lemon production can have problems with kaolin fruit coating.

## **Zinc and Phosphorus**

**Ben Faber, UCCE Farm Advisor, Ventura, Santa Barbara**

I was recently in an avocado orchard and saw the rounded fruit and small leaves typical of zinc deficient trees. I asked the grower if there were recent leaf analysis of the orchard, and so we looked at them. The leaves were running at 20 ppm which is low. A sufficient level is 50 ppm. The recommendation was to apply zinc sulfate to the soil. The recommendation included, though 200 pounds of phosphorus per acre. Phosphorus and zinc are antagonistic, meaning applying one can limit uptake of the other. In applying phosphorus at such a high rate was probably preventing uptake of zinc. It is also antagonistic to copper, iron and manganese, so all of these micronutrients can be limited by phosphorus applications.

There have only been two documented cases of phosphorus deficiency in fruit trees, walnuts on a volcanic soil in Lake County and oranges on decomposed granite in San Diego. It is an essential element, yes, but applying it when there is a sufficient level in the leaves can lead to other problems which can be hard to correct. Generally speaking, phosphorus does not need to be applied to fruit trees in California. In other states that have peat soils, high carbonates or highly weathered soils,

phosphorus application is a normal practice, but here make sure you need it before applying it.

## **Recent finding on Polyphagous Shot Hole Borer, *Fusarium* Dieback – A pest-disease complex on Avocado**

**Akif Eskalen, Extension Subtropical Plant Pathology Specialist, UC Riverside, Monica Dimson, UCCE Orange County, John Kabashima, UCCE Orange County**

The Polyphagous Shot Hole Borer (PSHB), *Euwallacea* sp. #1, is an invasive beetle that carries three fungi: *Fusarium euwallaceae*, *Graphium* sp. , and *Acremonium* sp. The adult female tunnels galleries into a wide variety of host trees, where it lays its eggs and grows the their symbiotic fungi. The fungi cause the *Fusarium* Dieback (FD) disease, which interrupts the transport of water and nutrients in over 35 tree species that are suitable for beetle reproduction.

Once the beetle/fungal complex has killed the host tree, pregnant females fly in search of a new host.

A separate invasion was recently detected in commercial avocado groves and landscape trees in San Diego county. It has been determined that the damage has been caused by another closely related species of PSHB (*Euwallacea* sp. #2), carrying a new species of *Fusarium* and *Graphium*. The beetle in LA, Orange, Riverside, and San Bernardino Counties are morphologically indistinguishable, but genetically distinct from the beetle found in San Diego County.

### **Signs and Symptoms**

Attack symptoms, a host tree's visible response to stress, vary among host species. Staining, sugary exudate (B), gumming, and/or frass may be noticeable before the tiny beetles (females are typically 1.8-2.5 mm long). Beneath or near these symptoms, you may also see the beetle's

entry/exit holes, which are ~0.85 mm in diameter. The abdomen of the female beetle can sometimes be seen sticking out of the hole. Sugary exudate on trunks or branches may indicate a PSHB attack (photos A-E). Note that exudate may be washed off after rain events and therefore may not always be present on a heavily infested branch.



**Sugary exudate on trunks or branches may indicate a PSHB attack**

### **Hosts**

PSHB attacks hundreds of tree species, but it can only successfully lay its eggs and/or grow their symbiotic fungi in certain hosts. These include: Avocado, Box elder, California sycamore, Coast live oak, White alder, Japanese maple, and Red willow. Visit [eskalenlab.ucr.edu](http://eskalenlab.ucr.edu) for the full list.

*Fusarium* dieback pathogens cause brown to black discoloration in infected wood. Scraping away bark over the entry/exit hole reveals dark staining around the gallery, and cross sections of cut branches show the extent of infection. Advanced infections eventually lead to branch dieback and death of the tree



**Sugary exudate on trunks or branches may indicate a PSHB attack**



**Scraping away stained bark from a PSHB-infested tree reveals discolored tissue**

**How to report a suspect tree**

Please report suspected tree infestations to UC Riverside ([eskalenlab@gmail.com](mailto:eskalenlab@gmail.com)).

Submit the following information:

- Contact information (name, city, phone number, email)
- Suspect tree species
- Description of suspect tree’s location (and/or GPS coordinates)
- Description of suspect tree’s symptoms
- Photos of suspect tree and close-up photos of symptoms (see examples)

Take photos of suspect trees from several distances. Include photos of:

1. the trunk or symptomatic branches;
2. the symptoms (close-up); and
3. the entry/exit hole, if visible, with a ballpoint pen for scale (remove exudate if necessary). If dieback is observed, take a picture of the entire tree.



**PSHB entry/exit-holes on an avocado branch. The small holes are about the size of the tip of a ballpoint pen.**

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**Sonia I. Rios**  
**UC Cooperative Extension**  
**Subtropical Horticulture Farm**  
**Advisor in Riverside & San Diego**  
**Counties**

***Education:***

Rios completed an M.S. in Plant Science with an emphasis in Weed Science from California State University, Fresno, a B.S. in Plant Science from California State Polytechnic University, Pomona

***Experience:***

Prior to accepting her advisor position, Rios served as a staff research associate in UCCE Tulare County where she assisted advisors in all phases of applied agricultural research (field and greenhouse research on cereal crops, cotton and weed management). She was involved in approximately 30 – 40 research projects that included research testing in herbicide resistance, variety evaluations, and pest management including evaluating new herbicides and insecticides. Rios has conducted and reported agronomy research experiments through data collection that is statistically analyzed, translated and disseminated to clientele; maintained research plots; prepared educational materials for research reports and University publications that would benefit California growers, industry clientele; assisted regulatory agencies with science-based information; conducted radio interviews; and she was a regular speaker at the Tulare County Pesticide Safety meetings. In addition to working with UC, she was also her main professors' student research assistant that would help with trials on campus, assist undergraduates with their research projects and spent time in the classroom teaching.

Rios had also worked with the United States Department of Food and Agriculture (CDFA) inspecting airplanes for Japanese beetle as an Agriculture Aide I. She also worked with the United States Department of Food and Agriculture (USDA) Forest Service as a Forestry Technician.

Rios will be working in education and applied research program in tree crops production and marketing in Riverside and San Diego Counties. Primary target crops are citrus, avocados and dates, but also include other subtropical and deciduous fruit and nut crops, such as pomegranates, figs, mangos and walnuts.

She will facilitate interactions and information exchange among campus based academic, Cooperative Extension advisors and community stakeholders. Focus is expected on increasing productivity and efficiency of commercial tree crops operations, thus maximizing the return on invested capital, and at the same time, providing consumers with a high quality, safe and reasonably-priced product. The advisor will address emerging production issues in subtropical fruit crops including: horticulture, entomology, plant pathology, integrated pest management, plant nutrition and variety testing. She will be working closely with the subtropical horticulture industries, local growers and members of the subtropical horticulture and nuts and fruits workgroup to identify research areas of highest priority. Her contact information is as follows:

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