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# Leveraging the Genomic Landscape of Avocado for Breeding Purposes

By Drs. Vanessa Ashworth and Philippe Rolshausen,  
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Advances in molecular tools already have led to the development of large numbers of genetic markers distributed across the avocado genome. These markers are being put to good use, many forming the basis for projects to inventory avocado germplasm repositories and, in our own case, to create a pathway toward marker-assisted selection. Most recently, the first avocado genome was sequenced, marking the beginning of a new era. Genome annotation entails adding functional information about genes and other compartments of the genome, aided by comparisons with model organisms. The challenge is that the avocado occupies a highly divergent position on the evolutionary tree of life, close to such plants as laurel and magnolia, near the base of the flowering plants lineage and far removed from convenient model organisms (such as *Arabidopsis*) for which annotations are most advanced and comprehensive. Therefore, genome annotation in avocado is likely to be slower. While there is great potential for exploring the genome for interesting gene regions, the procedures will not be insignificant and will require bioinformatics and experimental verification before breeding targets can be identified. Additionally, quantitative traits such as yield-related traits are not controlled by a single gene but, instead, by many genes of small effect distributed across the entire genome. Even when the genome becomes thoroughly annotated, it is still only one genome and will not include all the variation present in the large pool of avocado germplasm. In comparison, efforts are underway to sequence 1 million human genomes. Clearly, additional avocado genomes will allow broader comparisons and will lead to an acceleration and broadened scope of breeding efforts.



**Figure 1:** Diversity of shape, size, skin color and surface texture of fruit picked from trees sharing the same maternal parent. Some of these fruit were assayed in the QTL analysis for fruit nutrient content.

A major goal of molecular breeding is to pinpoint which genes are responsible for a trait of interest and to make this relationship accessible for experimental manipulation. In avocado, many genetic markers<sup>1</sup> have been developed in recent years and used widely to characterize scion and rootstock germplasm collections. However, few studies have attempted to examine how genetic markers are related to specific traits and how they can be used to improve breeding material using marker-assisted selection. In a recent scientific article, we reported our findings from a genetic study to identify genes and markers controlling various yield-related and nutritional traits in avocado. It centered on a procedure called Quantitative Trait Locus (QTL) analysis<sup>2</sup> and presents a high-density linkage map<sup>3</sup> for avocado useful for molecular breeding. Our study included quantitative traits (tree height, canopy diameter, and trunk diameter, and contents of vitamin E, beta-sitosterol and carotenoids in the fruit flesh) and a qualitative trait (flowering type) (Figure 1 and 2). Here are some of the major findings of our research.

**Flowering type:** Avocado flowers exhibit either A- or B-type flowering, a mechanism designed to prevent self-pollination and that increases fruit set. We found that a tight cluster of markers on chromosome 10 showed a very strong signal for flowering type. It appeared that a single gene on that chromosome is likely responsible for controlling which flowering type a tree will have when it reaches maturity. In orchards devoted to ‘Hass’, with A-type flowering, inter-planting with B-type pollinizer cultivars is the norm to boost pollination and fruit set. Currently, most pollinizers are green-skin cultivars (especially ‘Bacon’, ‘Fuerte’, and ‘Zutano’) whose fruit do not fetch a good price compared to ‘Hass’, reducing overall market value of the orchard yield. Therefore, when breeding new cultivars for ‘Hass’-like taste and appearance, it would be advantageous to be able to include an early screen for B-type flowering: this would ensure that any promising breeding material with ‘Hass’-like attributes would also be usable as B-type pollinizers that produce marketable fruit. The screen would involve a routine lab procedure performed on DNA extracted from young leaves of the seedlings in a breeding program: the strategy would be to only keep those seedlings that have the particular marker that coincides with B-type flowering and to discard the rest. All seedlings progressing through subsequent tiers of the breeding program would have B-type flowering and there would be no wastage of time and resources by having to cull mature trees once they have been revealed as A-type flowerers.

**Nutrient content of the fruit flesh:** We also found that the content of alpha-tocopherol, a form of vitamin E, is strongly associated with a group of markers on chromosome 3. This finding opens up the possibility of breeding avocado for enhanced vitamin E concentrations and to further elevate its status as a nutritious fruit. Selection for a suitable marker in young seedlings would avoid the long wait until the seedlings have produced fruit, which can take many years. Though weaker, a signal for marker association with beta-sitosterol was also detected on a short section on chromosome 1. This plant sterol has been shown to have anti-oxidative properties and to reduce blood cholesterol levels in humans. The fact that vitamin E and beta-sitosterol are controlled by genes on different chromosomes is a practical advantage because it means that breeding for one nutrient can be performed independently of the other nutrient. It is noteworthy that both vitamin E and beta-sitosterol have been cited as targets for biofortification in other crops.

Avocado production in California, the main avocado-producing state in the US, cannot keep pace with consumption, and the market is supplemented by imports from Mexico and many other countries. For now, almost all fruits imported are of ‘Hass’ or “Hass-like” cultivars, yet taste panels at UC Riverside suggest that consumers are open to new tastes and visuals. Supermarket offerings in the form of a startling abundance of different apple and pear cultivars are in stark contrast to those for avocado, which is essentially synonymous with ‘Hass’ alone. The time for customized breeding may be ripe. As a nutritious and tasty fruit crop, avocado has acquired a strong culinary following and is prized for its healthy attributes. Having shown that vitamin E content is amenable to marker-assisted selection, future breeding could focus on generating nutrient-enriched cultivars, and future elucidation of similar trait-marker associations could create opportunities to generate high-value avocados that would coexist alongside the mainstream crop. Currently, health-conscious consumers and avocado fans are being short-changed, and molecular breeding can play a role in developing new and interesting material.



**Figure 2:** Tree height—a quantitative trait—at different locations in identical genotypes (clonally replicated). The white stick in each photo measures 1 m in length.

### Definitions

<sup>1</sup> Genetic markers: specific locations on the DNA sequence that can be readily identified by molecular methods. They are also known as molecular markers.

<sup>2</sup> QTL analysis: infers which markers on a linkage map influence a trait of interest. Results are shown in a chart with all markers on the map plotted against their (statistical) contribution to the trait.

<sup>3</sup> Linkage map: a map showing the order of markers and genes along each of an organism’s chromosomes (i.e., how they are linked). It is also called a genetic map.



# Fresh Market Raspberry in California

By Natalie Solares, M.S. and Dr. Alexander Putman,  
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Raspberry (*Rubus* spp.) is an important crop for California, where it is among the top 20 commodities with an average annual value of \$448 million from 2015 to 2017 (CDFA: California Agricultural Statistics Review 2017-2018). This represented 82% to 88% of the domestic raspberry production. The four California counties where raspberry is produced are Ventura, Santa Cruz, Santa Barbara, and Monterey. Specifically in Ventura and Santa Cruz counties, raspberries are among the top commodities (California Agricultural Statistics Review 2017-2018). On the West Coast of the United States, raspberry is typically produced in two stages from a single planting that is grown for a maximum of two years. In the primocane stage or first year cycle, harvest generally begins five months after planting of bare root transplants and continues for approximately three months. After harvest, the primocane growth is pruned near the last fruiting lateral or is mown at the soil line. The growth that follows this pruning begins the florican stage or second cycle, which has a harvest period that generally begins three months after pruning and can last approximately two months (personal communication Jose Gomez, Driscolls). Fresh market raspberry production in Ventura County is commonly grown on 3 rows of densely-planted raspberries under one plastic hoop tunnel (Figure 1). Raspberries are grown under protected structures, plastic covered high-hoop tunnels to extend the production season and to protect the delicate fruit from direct sun or rain/fog damage. In Ventura County, a crop can be planted during four periods throughout the year: early spring, late spring, mid-summer, or late summer. Main production challenges include limited availability of farm workers, Phytophthora root rot, Yellow rust, Botrytis fruit rot, Spotted wing drosophila, and Two-spotted spider mites (UC IPM online). In 2018, the raspberry industry in Ventura County produced 64,736 tons on a little over 4,000 acres (Ventura County's 2018 Crop and Livestock Report).



**Figure.** Standard raspberry planting under one plastic hoop tunnel.

**Useful Link:** UC IPM Online: <http://ipm.ucanr.edu/PMG/selectnewpest.caneberry.html> - UC IPM Pest Management Guidelines: Caneberries. Production Cost Study online: [https://coststudyfiles.ucdavis.edu/uploads/cs\\_public/20/e3/20e339eb-2ea7-41f0-8823-26939ff07c06/bpraspberry-cc-finaldraft.pdf](https://coststudyfiles.ucdavis.edu/uploads/cs_public/20/e3/20e339eb-2ea7-41f0-8823-26939ff07c06/bpraspberry-cc-finaldraft.pdf)

# Weed Management in Citrus Orchards

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Proper weed management is important for several reasons, but in general younger orchards are much more susceptible to the negative impacts of weed overgrowth. The full canopies of mature orchards limit the amount of sunlight reaching the orchard floor, which suppresses the growth of many weed species. Younger trees also have less extensive rooting systems, putting them into direct competition with weeds for water and nutrients. The presence of weeds provides habitat for insects, pathogens, and rodents, which can affect trees of all ages through direct damage to vascular tissue and foliage, and as disease vectors. Rank growth of weeds also visually obscures irrigation infrastructure and signage, and in some cases even field roads. Weeds can also limit mobility of laborers during harvest, and snag on ladders, which is a safety hazard for workers. Frost damage is more likely in orchards with weed overgrowth because there is less transfer of heat from the sun to the soil surface and lowers the amount of heat that will radiate back into the air during the night. Further, weeds are a primary source of fuel for fires. Weed management can be broken down into four phases that correspond to different life stages of the citrus orchard: 1) best practices before the trees go in the ground, 2) best practices for young orchards, 3) best practices for established orchards, and 4) monitoring or scouting which is ongoing throughout the life of the orchard. Each of these phases or scenarios has a different set of challenges and somewhat distinct set of management opportunities and constraints.

Monitoring is critical in identifying how effective management practices are, is helpful in identifying what practices may be having unintended consequences (increasing a particularly problematic weed species for example), and provides clues for what can be done differently to achieve more satisfactory results. The first step in effective management is to know what weed species are present, their relative abundance, and where they're located (more on weed id later). This is important because different weed species have different tolerances and susceptibilities to herbicides and other control methods, and knowing where they appear in an orchard in a given year is a good predictor of where they may occur in the future. Keeping accurate records of weed species and locations and reviewing these records periodically will facilitate an adaptive approach when combined with knowledge of past management practices. Under irrigated conditions, weeds can and do germinate, grow, flower, and set seed all year long, but generally the biggest flushes of weed population growth are in the cooler months and warmer months. This is when they will be easiest to detect. However, it is worth noting that this is not necessarily the best time for management – all too often by the time they are noticed, they've reproduced, and in the case of annuals it's too late for control (annuals will die no matter if you kill them or not – what's important is to stop them from setting seed). The University of California Statewide Integrated Pest Management Program (UC IPM) offers some useful monitoring forms available online. These forms contain a section to sketch out the locations of weed species, infrastructure, and other landmarks within an orchard, and are already partially filled in with common weed species by season (summer vs. winter). Pay particular attention for the weeds that are most difficult to control: namely perennials (Johnsongrass, bermudagrass, nutsedge, bindweed, etc.) and species with known herbicide resistant populations in California (horseweed, fleabane, Palmer amaranth, junglerice, etc.). Also, limit monitoring to the orchard itself- weed propagule pressure can and will come from off-site, especially from roads and adjacent areas that aren't actively managed.

Weed management before planting is the best opportunity to reduce the overall weed seedbank, but more critically it is when the most effective management options are available to remove perennial weed species from the site. In the case of perennials this is also the most economically efficient opportunity for management as any perennial weeds left in place may incur management costs for the life of the orchard for ongoing suppression instead of a one-time cost for removal. This is one of the few times that cultivation won't have negative impacts on crop health and productivity. A useful strategy for perennials with underground storage structures is to repeatedly disc the field every few weeks to bring the roots to the surface and allow them to desiccate in the hot sun (this should take place during the summer months). For an integrated approach, supplement this strategy with a systemic postemergence herbicide, applied in the fall when the plants are preparing for dormancy. This is when weeds will have maximum translocation rates of photosynthate (carbohydrates) from the leaves to the roots. Because systemic postemergence herbicides follow this same path within the plants, this is when the weeds are most susceptible to complete kill from these herbicides. This can be repeated in the spring for regrowth and followed by disking 2-3 weeks later to again expose and dehydrate rhizomes. Once irrigation infrastructure is in place, another strategy is to irrigate to "flush" the weed seedbank. For annual species a burndown herbicide application can be used to kill emerging weeds – important to time this properly to catch the two main cohorts of annuals (cool- and warm-season germinators), so plan to repeat at least twice in one year. For perennials a systemic postemergence herbicide may work better, especially if they are not sprayed very soon after emergence when they are susceptible to contact herbicides. Repeat multiple times to greatly reduce weed seed load on site and minimize future management requirements. Additionally, properly timed preemergence applications can further deplete the weed seedbank by killing plants as they germinate in the spring or late summer/early fall. Any weeds that survive should be spot-treated with herbicide or hand rogued.

Once young trees are in the ground, it's important to minimize root disturbance, especially close to the trunks of the trees. This means more reliance on chemical control, but also keep in mind that the trees are also more susceptible to injury from herbicides at this stage from roots and foliage. The bark is also less lignified and herbicide is more easily taken up through this route than on older trees. Protect with wrappers and/or use a spray shield when applying herbicide to minimize potential damage from drift. Because roots are more sensitive to herbicide uptake it is important to select preemergence herbicide products that are labeled as safe for young trees. Postemergence herbicide options are 1) contact herbicides (includes all of the organic products), 2) systemic herbicides that are selective for grass species, and 3) glyphosate. Contact herbicides are only effective when applied to young plants below a certain height (this varies by product so refer to the label, but always better to catch them sooner than later), and are less effective on perennials than annuals. Grass herbicides, although systemic, are most effective on annual grasses, but will kill certain perennial grass seedlings below 4-6 inches in height.

Cultivation should be avoided whenever possible. Several studies demonstrate the negative impact of cultivation in citrus. These include but are not limited to damage to feeder roots, which impacts the trees ability to collect soil resources (nutrients, water, oxygen); increasing exposure to soil pathogens which can enter the vascular system of the tree through damaged tissue; and increased risk of soil erosion, especially in sloped orchards. Further, the additional passes with a tractor can contribute to compaction, especially in finer textured soils, which limits tree root growth and ability to uptake soil resources. Dust generated from cultivation practices can reduce the effectiveness of biocontrol of insects and mites, and also

buries organic matter in the soil, providing a food source for certain insect pests. Cultivation also risks spreading root fragments of perennial weed species throughout the field and making weed problems worse.

A robust preemergence herbicide program can be helpful in managing orchard weeds, but preemergence herbicide efficacy and safety can be greatly modulated by several factors. Finer soil texture can bind up certain herbicides, making them less effective. Herbicides can quickly move beyond zone of seed germination in coarse soils, also making them less effective. Soil pH, especially in CA, can be high (alkaline) when calcium carbonate (aka caliche) is present in the soil, causing increased carryover or herbicide persistence and potential for injury. Knowledge of orchard soil characteristics and a careful review of the herbicide product label are critical in avoiding unintended crop injury. Many product labels will contain special precautions or rate modifications for specific soil characteristics or weather conditions. Herbicides are also broken down by sunlight on the soil surface if not immediately incorporated via rainfall or irrigation. Under prolonged moist soil conditions, certain herbicides are more quickly broken down by soil microbes, and are also moved out of the zone of seed germination. If permitted by the product label (limited by recommended application rate, max rate per year, and pre harvest interval), sequential applications can be made to extend control.

Contact herbicides are not translocated within the weed's vascular system, they require more complete coverage as they only kill the parts they come in direct contact with. This is less of an issue for annuals (always attempt to target annuals before they set seed) but a drawback of contact herbicides is that they are much less effective at controlling perennial weeds unless applied to newly emerged seedlings. Suppression of perennials (not mortality) will likely require multiple applications of a contact herbicide. A positive aspect of contact herbicides is that their efficacy may be less affected by drought conditions than systemic herbicides.

There are few systemic postemergence herbicide products labeled for citrus. Systemic (translocating) postemergence herbicides don't require as complete coverage as contact herbicides, but they do require active growth and are highly impacted by drought. As always, young, succulent plants are more easily controlled than mature plants with waxy leaf cuticles that impede absorption. For many reasons, glyphosate usage may be restricted in some areas or situations. This is problematic because glyphosate is the only postemergence herbicide option for labeled for control of most perennial weed species in California citrus, especially broadleaves weeds (but many grass species as well). When glyphosate is not available, thorough weed management prior to planting and robust preemergence herbicide programs are even more critical to successful and effective weed management. Other postemergence weed management options include mowing (although this tends to select for more perennial weeds) and hand roguing which can be expensive when weed problems are extensive. Cover crops are another potential option, but more research is needed to help identify appropriate cover crop species, recommended cultural practices for their management in citrus orchards, and their potential positive and negative impacts on orchard productivity.

The first category of problematic weeds in citrus are those species that have known herbicide-resistant populations. This topic is covered more thoroughly in other UC publications, but for the purposes of citrus weed management the three main species of concern are horseweed (*Erigeron canadensis*), fleabane (*Erigeron bonariensis*), and Palmer amaranth (*Amaranthus palmeri*). These species are known to have populations in California with glyphosate resistance. To manage these species 1) use herbicides with multiple modes of action, 2) use full label rate of glyphosate (no "burndown" applications), 3) treat when small, and 4) avoid incomplete control of the targeted population and adjacent populations (ditchbanks, roadsides, etc.).



The second category of problematic weed species are those with tubers, rhizomes, and/or a long-lived seedbank such as nutsedge and Johnsongrass (*Sorghum halepense*). Yellow nutsedge (*Cyperus esculentus*) is found throughout California to 3300 ft in elevation. It produces round, smooth tubers about ½ inch in diameter that are usually found in the upper 6 inches of soil, but sometimes much deeper (particularly if buried during cultivation). Yellow nutsedge tubers are only found at the ends of the rhizomes. Purple nutsedge (*Cyperus rotundus*) is found mostly in the southern parts of California (Central Valley, South Coast, and Desert to 820 ft in elevation). Tubers are larger than those of yellow nutsedge (about 1 inch), oblong, rough, and scaly, and are chained together at the ends of the rhizomes. Nutsedge is best controlled by eliminating it from the site before planting the orchard (as described earlier), and past this time can be extremely difficult to manage. Young plants can be controlled with glyphosate, but once plants develop more than 5-6 leaves (often called the “FLS” or “five leaf stage”) translocation of carbohydrate from the leaves to the tubers is limited, meaning that only top-kill occurs. Further, yellow and purple nutsedge also do not typically reproduce from seed, meaning that preemergence herbicides are not very effective. Some sulfonyl urea preemergence herbicide products labeled for citrus are effective at controlling nutsedge when applied foliarly (postemergence), though repeated applications are likely necessary for more complete control. Nutsedge tends to dislike shade and is less of a problem once orchards are established and canopies are mature. Johnsongrass is a rhizomatous perennial grass species with very long-lived seeds that can remain viable in the soil for more than 5 years. Like nutsedge, Johnsongrass is best to control before orchard is planted so that repeated tillage can be utilized that bring rhizomes to the soil surface where they will desiccate and die. This should be done in the summer when the soil is dry. Keep in mind that Johnsongrass can form plants from rhizomes as small as 1 inch long, so cultivation under moist conditions can exacerbate the problem by spreading rhizomes throughout the orchard. Otherwise glyphosate, specifically timed for immediately after plants finish flowering in August and September may be the best control option. This is when the plant will be moving photosynthate into the rhizomes and the herbicide will be more efficiently transported to this structure where it can kill the plant.

The UC Statewide IPM Program has several online resources available for integrated management of weeds in citrus. These include a photo gallery of common citrus weeds to aid in identification, a tutorial on how to identify weed species from common morphological traits (complete with illustrations), a chart of herbicide susceptibility by weed species, and a searchable gallery of herbicide injury photos.

Monitoring forms: <http://ipm.ucanr.edu/PMG/C107/citrus-summerweeds.pdf>  
<http://ipm.ucanr.edu/PMG/C107/citrus-winterweeds.pdf>

Weed ID and photo gallery: [http://ipm.ucanr.edu/PMG/weeds\\_intro.html](http://ipm.ucanr.edu/PMG/weeds_intro.html)

Weed species herbicide susceptibility: <http://ipm.ucanr.edu/PMG/r107700311.html>

Herbicide symptoms: <http://herbicidesymptoms.ipm.ucanr.edu/>

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## Topics in Subtropics



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