Clonal Propagation: The Clean Plant Choice for Orchard Crops©

Michael Vietti
Duarte Nursery Inc., 1555 Baldwin Rd., Hughson, California 95326
Email: michael@duartenursery.com

A BRIEF HISTORY

Duarte Nursery Inc., was founded in 1989 in California's Central Valley near the city of Modesto, as a grapevine nursery supplying potted bench-grafted grapevines to the wine industry. During the nursery's establishment, demand for vines was outstripping availabilities so the nursery grew rapidly in the early years and by the late 1990s was producing 18,000,000 potted vines a year. Even though the company was growing rapidly, there was concern over having only one product line creating sales for the company, so the Duarte family entered into a partnership with John Driver who was operating Dry Creek Laboratory, a biotechnology lab in Modesto, California. Out of the many projects Mr. Driver was working on, his work on clonal propagation of rootstock taxa being used in agriculture, had real potential as a viable commercial enterprise that fit well into the nursery's established practices. With the ability to produce clean clonal rootstocks, the nursery began producing potted, grafted selections of almond (*Prunus dulcis*), apricot (*P. armeniaca*), cherry (*P. avium*), peach (*P. persica*), and nectarines (*P. persica* var. *nectarina*). Eventually, Dry Creek Lab (DCL) became a fully owned subsidiary of Duarte Nursery Inc. (DNI) when John Driver retired and sold his interest in DCL to the Duarte family. The nursery has now grown to encompass 130 acres, with 30 of those acres dedicated to greenhouse production. One hundred percent of our products are container-grown on benches in a soilless medium, which is key to producing plants free from crown gall (*Agrobacterium tumefaciens),* that resides in native soils and can easily infect field-grown trees.

PURPOSE OF A ROOTSTOCK

Almost without exception, most fruit and nut tree taxa being planted as agricultural commodities today, have been grafted to a rootstock, which serves as the trees foundation and supports the scion, that is, the fruiting portion of the tree. The choice of which rootstock to use is generally based on its particular characteristics and its compatibility with the scion. The ability to tolerate a particular soil type, soil-borne diseases, insect pressures, influence over the size and vigor of the scion, or induce precocity are examples of rootstock characteristics.

Many of the rootstocks used in today's commercial orchards are the result of crosses between two species or genotypes. These crosses give rise to a hybrid plant that contains some characteristics from both parent plants, resulting in a new and hopefully better plant. In some cases the resulting plant, while exhibiting desirable characteristics, may also have some inherent problems such as low seed viability or may contain an undetected virus that was passed on from one of the parent plants. Either one of the above examples could keep a potentially valuable new selection from reaching the commercial market place.

The acquisition of DCL provided DNI a world-class micropropagation facility that could address, in house, the issues of commercially available rootstock material that either had become infected with viruses, was difficult to propagate vegetatively under traditional methods, or was being produced from seed and consequently would exhibit some variability after being planted in the field. Therefore, a "Clean Plant System" was developed to address these very situations.

CLEAN PLANT SYSTEM

What do we mean by a "clean plant"? At DNI a clean plant is one that is free of viruses and *Agrobacterium* (crown gall), not infected with any fungal or bacterial disease and is pest and nematode free. The plant should arise from clean stock that is from the mother tree whenever possible and to have been found free of any viruses. Plants will be container-grown in soilless media and will be well-developed before delivery. Finally, the plants are vigorously established in the customer's field under the supervision of our field staff. In order to accomplish this, we follow the following four steps:

Clean Plant System — Step 1: Source or Create Elite High Health Plant Material. Whenever possible we source material from the following:

- Foundation Plant Services (University California, Davis): *Vitis*
- National Research Support Project 5 (NRSP5 program) (Washington State University): *Prunus*
- Lindcove Field Station (University California, Riverside): *Citrus*
- Germplasm Repositories/Collections from U.S.A. and worldwide: olive (*Olea europaea*, fig (*Ficus carica*), walnut (*Juglans regia*), and pomegranate (*Punica granatum*)
- IRTA: olive

Most of the organizations listed above have plant material that meet our criteria for virus-free plants, but if none is available of a particular taxon, we create clean material in our lab by either thermotherapy or meristem/shoot tip culture.

Thermotherapy. Thermotherapy is a method of growing plant material under sterile conditions and high temperatures. A small plant or plantlets that were found to be infected with a virus are grown on an agar medium in enclosed containers, put into a growth chamber, and grown under temperatures of 30–35 °C to encourage rapid growth of the apical shoot. In many instances, the rapidly growing shoot grows faster than the virus' ability to infect the tissue. This new shoot material is then excised from the plantlet, transferred to sterile growing media and allowed to develop into a new plantlet. After a period of growth, a tissue sample is taken and sent to a lab for virus testing and if found free of any viruses, these new "clean" plants become the source material for establishing a new line of the cultivar.

A recent example of this was done at DCL with Bright's Hybrid 5, an almond/peach hybrid rootstock. The material we had sourced was found to be infected with Prunus Necrotic Ringspot Virus, a potentially devastating virus in *Prunus* sp., especially cherries and peaches. Plants underwent thermotherapy treatment and the resulting plants were free of the virus and have been used to reestablish the cultivar in the nursery.

Meristem/Shoot Tip Culture. Meristem/shoot tip culture is another procedure used at DNI to remove viruses from infected plant material. It requires the use of a high-power microscope and a more experienced technician. We are currently using meristem/shoot tip culture on a number of crops at Dry Creek Lab including Grape Heritage cultivars, various selections of figs, the latest selection of olive cultivars from Spain used in high-density plantings, and experimenting with *Citrus* cultivars.

1) The first few cell layers of a shoot tip are virus free
2) Virus-free shoot tip section is cut and placed on sterile media
3) New plant is then propagated further and then retested for viruses

Clean Plant System — Step 2: Propagate Material in a Sanitary Environment. Micropropagation is the technology that allows us to propagate clean, disease-free plants in a sterile environment. It allows us to propagate certain plant cultivars that otherwise might be difficult to produce under conventional methods. It also gives us a tool to rapidly produce a large number of plants in a relatively small area and has the added advantage of allowing us to react quickly to changes in grower demands. Currently, we are producing the following plants using micropropagation techniques: *Prunus* sp., walnuts, pistachio, kiwi, figs, citrus, olive, blueberries, and grape.

Clean Plant System — Step 3: Container-Grown into Healthy, Well-Developed Plants. Micropropagated plants are transplanted into plug trays filled with a sterile medium and placed into an acclimation greenhouse. Once these "plugs" become established they are ready to be sent to the greenhouses. Prior to transplanting, containers are placed on raised benches in temperature-controlled greenhouses, each of which hold ~600,000 trees. The young plants are potted directly into the finish container filled with a soilless mix containing Canadian sphagnum peat moss and horticultural-grade perlite. A custom nutrient charge is also added during the soil-mixing process. Once the rootstock has grown and reached a minimum stem caliper of $^{3}/_{8}$ in., they are ready to be budded with the scion cultivar. Once the grafted tree has grown to its finished height, it is moved to an outside bench, placed on drip irrigation, and allowed to harden-off before being shipped to the field.

Clean Plant System — Step 4: Rapid Establishment in the Field by the Grower. Prior to receiving any of our products, one of our experienced and knowledgeable field reps meets with the grower to make sure proper steps have been taken in prepping the field for planting. Proper irrigation immediately after planting is key to successfully establishing a container-grown tree. Container-grown trees also give the grower the added advantage of planting year-round instead of being limited to the traditional bareroot timeframe of January–April.

CONCLUSION

Thermotherapy and meristem/shoot tip culture, coupled with micropropagation techniques, are invaluable tools that can be used in agricultural nurseries to establish clean, virus-free lines of rootstocks used in grafted fruit and nut tree production. Micropropagation also has the advantage of producing large numbers of plants in a much smaller and sterile environment over conventional propagation methods. Investing assets into a production facility for growing plants in containers, allows us to grow healthy, well-developed trees that establish rapidly once planted in the field. Containerized trees also have the advantage of being available year round for growers.