INTRODUCTION

In 2011, pistachio growers began reporting poor rootstock performance of a UCB1 clone which originated from Duarte Nursery. This problem continued until 2014. Symptoms included stunting, shortened internodes, a bushy appearance, a wavy leaf margin, poor rooting vigor, galling, cracking at the bud union, and failure to take a bud. These collection of symptoms were given the name Pistachio Bushy Top Syndrome (PBTS). The problem re-emerged in 2016, again in UCB1 clones, but originating from Pioneer Nursery. Abnormal bark patterns and stunting were also noted in PG1 rootstock that had been in close proximity to the affected UCB1 clones.

The cause(s) of the poor performance have not been determined to the satisfaction of all involved. Rhodococcus fascians and R. corynebacteriodes, both solely and synergistically, have been described as pathogens that have met Koch's postulates on UCB1 clonal rootstock. However, detection in symptomatic rootstock collected in the field has been erratic – it is more likely to detect Rhodococcus in symptomatic plants than in non-symptomatic but it is not detected in all symptomatic and non-symptomatic/ Rf positive rootstock do not later develop symptoms. Pathogenicity has not been tested on other rootstock but PG1 in the greenhouse appeared symptomatic through accidental transmission.

Mutation during propagation has also been proposed. Several clonal lines have been selected from the original UCB1 clone that develop symptoms similar to those described above. Rhodococcus has not been detected in these mutational selections and graft transmission tests have not revealed a transmissible pathogen.

Meetings with clonal propagators, nurseries that distribute clonal rootstock, and UC faculty have been held several times over the years and most recently May 23, 2017. Below are meeting "minutes" from May 23.

EPIPHYTES

Dr. Johan Leveau gave a short presentation on epiphytes and endophytes, bacteria growing upon plant surfaces and those growing within plants respectively. Virtually all pathogens are endophytic at some point but there is a wide range of host/pathogen endophytic interactions. There needs to be some recognition between the host and the potential pathogen, suggesting a need for a pathogenicity gene. It is not uncommon for a potential pathogen to exist as an epiphyte and become a endophytic pathogen at some stage, presumably due to a change in the environment or the host. In the case of Rhodococcus, the endophytic phase is likely necessary for pathogenicity.

RHODOCOCCUS POPULATIONS

Dr. Florent Trouillas presented research data on the genetic relationships of pistachio isolates of RF to other Rhodococcus isolates for which sequences have been published. The isolates he has collected are

closely related to isolates from ornamental and wild plants and closely related to the PBTS1 isolate collected by Dr. Jennifer Randall and used in the Koch's postulates studies. However, none of the isolates have the linear virulence plasmid described by Randall. Thus, it is uncertain whether the virulence plasmid is required for disease development.

An analysis of the genetics of the isolates from the Duarte symptomatic plants and those from Pioneer indicate that they are unrelated and independent

DETECTION OF RHODOCOCCUS

Dr. Elizabeth Fichtner described how Rhodococcus is detected. Rhodococcus is a relatively slow growing Gram positive bacterium. Care must be taken so the RF is not overgrown by other bacteria fungi and consequently, D2 media is widely used. Depending on the material, it may or may not be surface sterilized- leaf prints are not surface sterilized and consequently represent epiphytic populations of RF. Following isolation, the colonies are tested with PCR using several different primers – 16S, gyrase, vicA, and FasD have been used. The 16S is a very broad test and does not separate among the isolate. Gyrase (?) and vicA are chromosomal genes that are specific for Rhodococcus while the Fas primers (D for most researchers and labs, A for some) are specific for the linear virulence plasmid. Commercially available testing labs have their own methods that differ among the labs.

Detection in plantae is problematic. There are questions about the efficiency of Rhodococcus cell disruption (necessary for nucleic acid release) due to a thick cell wall and the possible erratic distribution of RF in infected plants. Attempted isolations from symptomatic tissue are frequently negative. Because RF pathogenicity may be due to plant hormone modulation, the bacterium doesn't necessarily need to be present in the affected tissue to have been the cause of hormones that created the symptoms. Thus, Dr. Trouillas has been unable to isolate RF from galls but the bacterium could be releasing hormones from elsewhere in the plant that caused the galls.

Direct testing of symptomatic plants with PCR have the same difficulties described above. A serological detection method also have the same issues because these are related to pathogen presence and the pathways through which symptoms are induced.

PHYSIOLOGICAL/MUTATIONAL BASIS FOR PBTS

Dr. John Preece described a number of mutations that have occurred in other plants that give rise to symptoms similar to PBTS. These include witches broom, corking in the branch collars, deformations, etc. He also described the appearance of some UCB1 seedlings in an experimental planting in Davis and how the appearance of these plants are similar to PBTS clonal propagants. Many of the PBTS symptoms could result from more extreme manifestations of known phenotypes – the genes to produce the symptoms are already in the plant and would only require a mutation in a regulatory gene to overexpress the gene that causes the symptom. He also described how corkscrew roots can be hormonally induced during rooting through excess ethylene production.

In the only publication that has some bearing, clonal P. vera cultures showed significant variability after ten generations. However, the origin of the tissue from which the clones were derived was not clear so there were questions about the applicability of the research to UCB1 clonal propagation.

OBSERVED MUTATIONS IN UCB1 CLONES

John Duarte presented information on the various mutations they have detected in the UCB1 clonal line used by Duarte Nursery prior to 2014. Depending on the observer, 3-5 mutations have been detected, based on phenotypes and not sequencing.

PBTS SYMPTOMATOLOGY

Since the initial appearance of PBTS in 2011, virtually any rootstock abnormality has been attributed to PBTS. Consequently, a wide range of symptoms are informally included in PBTS and a more formal symptomatology has not been developed. Dr. Trouillas felt that the symptoms he saw could be divided into two broad classifications, bushy and galling. The bushy was primarily due to loss of apical dominance and the proliferation of branching. The second type was swelling of the buds on the rootstock trunk, giving the appearance of galls. Cracking and overgrowth of the bud union was also characteristic of this symptom type. Both types have poor rooting vigor with perhaps one or two significant roots, commonly angled in line with the tree row. The roots may also be J-rooted but this might be due to having outgrown the pot. Corkscrew roots are also commonly seen but Dr. Preece pointed out that this is a common manifestation of ethylene, a result of abscisic acid application during culture to induce rooting. Thus, the corkscrew roots may not be related to PBTS.

PBTS has only been observed in UCB1 clones but poor rooting has also been observed in other rootstocks, including putative UCB1 seed rootstocks. This may indicate a more extensive problem with root vigor due to potting conditions.

There may be more types as well. Some recent plantings have grown normally until topped and budded which apparently induced the onset of symptoms.

Dr. Neil McRoberts suggested that a comprehensive survey needs to be done to provide a statistically robust overview of the locations and types of symptoms at each location. This would include planting date, soil type, and grower practices. This could help collect all the information in a single form, rather than relying on sharing of anecdotal recollections. However, many of the affected plantings have been removed and it may be too late to collect the information.

PBTS EPIDEMIOLOGY

This was on the agenda but was not discussed as its own topic. PBTS has not been seen to spread in the field nor has it been shown to be graft transmissible. Healthy replanted rootstock planted in the same spot from which a symptomatic rootstock had been removed immediately before replanting has not been known to develop symptoms. No spread = no epidemiology .

The possible spread of Rhodococcus from UCB1 clones to PG1 seedlings in the Pioneer nursery seems to be the the sole exception to this.

FUTURE RESEARCH

The cases for Rhodococcus pathogenicity and/or genetic mutations in culture as the causes of PBTS are not completely convincing and consequently, both possibilities must be considered. The research needs to be done with the goal of developing management options.

PBTS Symptomatology

- Statistically catalog and map the affected plantings by rootstock source, symptom type, etc with enough corresponding nonaffected plantings for comparative purposes
- Determine which symptoms are related to guide research into each symptom group

<u>Rhodococcus pathogenicity</u>

- Perform Koch's postulates with the original isolates, both with and without the linear plasmid
- Test a wider range of rootstock to determine if pathogenicity is limited to UCB1 clones
- Maintain inoculated rootstock for at least 2 years
- Test environmental triggers (heat, hard pruning, etc) for pathogenicity induction
- Better diagnostic techniques, standardize detection (fasD primer?, ELISA?)

Mutations

- Determine if symptoms can be developed through application of plant hormones, particularly cytokinins
- Measure propensity for genetic drift in culture to determine practices that might induce mutation as well as measure longevity of a UCB1 clonal line
- Using primers based on plant growth regulation from other species, determine if the affected rootstocks have altered genes
- There were comments about epigenetics and transposable elements but no suggestions about specific ways to approach this
- Environmental triggers for symptom induction