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Propagation of Citrus Rootstock Cuttings Success Depends on Season

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Abstract

Propagation of citrus rootstock cuttings in a phyto-santitary greenhouse during summer months was highly successful with the right combination of shoot maturity, diluent, and auxin concentration; however, propagation of citrus cuttings over the course of a year has not been reported. Results of a yearlong trial indicate that common citrus rootstocks can be easily and quickly rooted when started from mid-March until early November at latitudes around N 28.5°. However, in December through late February, rooting success of citrus cuttings varied with auxin concentration, but was generally commercially unacceptable (24%) in greenhouses with minimum air temperatures above 19°C. If bottom heat of 27°C was applied during this period, average success was around 60%, achieving above 80% in late in the winter period. Rootstocks propagated during this period exhibit no abnormalities and could be budded when rootstock size and cambium activity are sufficient.

Keywords: Rootstock; Citrus; Citrus cuttings; Propagation

Abbreviations:

HLB: Huanglongbing Disease; NAA: Naphthalene Acetic Acid; IAA: Indole-Acetic Acid.

Introduction

Citrus rootstocks have historically been germinated from the seeds of select trees, harvested in late fall and stored until new rootstock was needed. Over the past decade, citrus canker and Huanglongbing (HLB) disease, commonly known as citrus greening, caused by the bacteria *Candidatus* Liberibacter asiaticus has substantially reduced seed trees and consequentially rootstock seed quantities. Year-round production of seed for rootstocks depends on harvest size in late fall, which is interrupted by canker and HLB depressing seed yield that collapses the pipeline for rootstocks several years ahead by not having enough trees for replanting. Production of rootstocks by cutting propagation can not only reduce the demand for genetically variable seed but also can supply more uniform rootstocks which can be selected for desirable traits and uniformity. Normally production of rooted cuttings is faster and quicker to bud than seedlings [1] or tissue culture plantlets.

Most woody plants require auxin applied to a cutting's proximal end to develop adventitious roots [2]. Even those that develop roots without exogenous auxins usually benefit from auxin treatment [2]. Before 1900 and the discovery of auxins, it was known that some species of the lemon family would root readily in glass covered propagation frames. Minimum temperatures for rooting were suggested to be 24-26°C, but temperatures of up to 43°C were acceptable [3]. Lemon cutting propagation studies showed that more leaves on a cutting produced more roots [4]. The discovery of Indole-acetic acid (IAA) in the early 1930s led to its use as a rooting agent. In one of the earliest uses of auxin compounds to enhance rooting of citrus, researchers compared root development of mature 15 cm lemon stem cuttings treated with three levels of IAA [5]. The 2000 ppm solution was best when at least two mature leaves were retained. Stems treated with 1000 ppm produced limited root mass, similar to the water control. Later Naphthalene acetic acid (NAA) was discovered to also generate roots but at half the concentration of Indole-butyric acid IBA. NAA is degraded much more slowly than either IAA or IBA, so its effect on root generation is more prolonged [1].

Intermittent mist works well for rooting citrus cuttings, as opposed to glass propagation frames [6]. Utilizing the intermittent misting method, researchers found high success of Carrizo and sour orange cuttings taken in September [7]. In this study, 20 cm cuttings were taken from 1.5 year old trees, wounded, treated with 2500 ppm of IBA in water and then placed in vermiculite under mist for two months. Root and shoot mass, along with root length, were greater for cuttings than seed-grown plants. This was attributed to more leaves on cuttings, thus greater leaf area compared to seedlings of the same age. Auxin concentrations of 1000 and 3000 ppm IBA and NAA were evaluated on 12 selections of Citrus genotypes [1]. Cuttings were misted for duration of 10 sec every 5 min during daylight. Carrizo rooted at 83% with 3000 ppm NAA and similar concentrations of IBA produced 67% success. Starting in late September, total root lengths after six weeks were 36 cm with NAA and 24 cm with IBA. The highest reported rooting success was with 2000 ppm IBA when *Citrus limon* cuttings were treated with a 10 sec soak [8]. Both IBA and NAA at 3000 ppm were reported to have stimulated the greatest root production in both juvenile and mature cuttings of *Swingle citrumelo* [9]. They preferred 15 cm stems that retained three or four leaves at the top. Greater root generation occurred with IBA for juvenile cuttings, while mature cuttings produced more roots with NAA. Three node stem cuttings treated with 2500 ppm IAA in water produced more roots than lower concentrations (500 to 2000 ppm), and had higher success rates and root growth using bottom heat [10]. Perhaps part of the variability of rooting citrus cutting has been due to maturity of trees from which cuttings were harvested, which has been reported as important in the successful rooting of citrus [11]. As trifoliate orange trees aged, rooting success declined rapidly. Seedlings up to two years of age had 100% success for rooting, whereas success declined with age for three to five year old trees. After 15 years, trees lost ability for cuttings to root [11].

Another unknowns related to the production of rootstocks, are the questions of which rootstocks to use and the quantities of cuttings needed to match propagation goals. For other hardwood trees, propagation is usually most successful during the spring to mid-fall period, then declining during the late fall to early spring [11]. However, while citrus is a hardwood tree, it is adapted to a subtropical climate and is grown under relatively short day lengths compared to temperate hardwood trees. Propagation of woody plants during spring to early fall usually occurs easily and quickly for most species [12]. For these same species however, especially in higher latitudes, rooting of cuttings is difficult and often impossible without exogenous auxins and often bottom heat during winter months. With winter dormancy carbohydrates of woody plants are stored as starch, and generally unavailable for growth [13]. During spring and warmer temperatures, starch is hydrolyzed to sugars that fuel plant growth. Interestingly, *Ficus infectoria*, a semi-tropical species, exhibited high rooting with renewal of growth and cambial activity from spring to mid-fall but then had poor rooting potential during winter dormancy, despite mild temperatures [14]. Since budding of rootstocks is nearly year round in Florida, the production of rootstocks from cuttings would need to be initiated to match likely anticipated need; and then also to account for common lower rooting success during winter months. This research tracked success of rootstock cuttings for 54 weeks in generally three-week intervals. Use of bottom heat for rooting cuttings during winter months was found to be obligatory for successful propagation.

Most, if not all, of rootstocks used before HLB became prominent in Florida are susceptible to HLB. In recent years there have been many new rootstocks proposed to be resistant to HLB. Most of these were found in dying or abandoned groves as chimeras growing from the rootstock. To evaluate the strength of these chimeras for resistance to HLB and perhaps other diseases, they first have to be propagated, and then propagated in quantities to allow sufficient evaluations. In a previous study in late spring 2005, the effects of stem maturity, diluent and auxin concentrations on rooting success were evaluated [15]. To produce the most rooted cuttings quickly, researchers used single node, single bud cuttings. While small in both shoot and root size, single node cuttings allowed for the greatest number of cuttings to be produced and later reduced the extra labor of removing elongating shoots originated from extra buds of multi-bud cuttings [15]. While some citrus rootstocks have been produced by tissue culture in abundant quantities, it takes time to develop protocols and sufficient size to warrant inclusion in variety trial testing. Producing rootstocks by cuttings is much more rapid and can deliver liners ready to bud much sooner, which would accelerate the selection of viable combinations of rootstock and scion [15].

Materials and Methods

This research focused on understanding how success in rooting citrus cuttings is affected by the interaction of time of year, auxin concentration and bottom heat. On 6th May 2015, 60 single node/single leaf and bud cuttings were made from mature stems of the widely used and seed produced Kuharske rootstock [15]. Half of the cuttings were treated with 4000 ppm Dip&Gro (Dip&Gro, Clackamas, OR), the rest treated with 7500 ppm Dip&Gro. Both concentrations were diluted using a solvent of 60 ml of Cell-u-wett (Hort Specialties Inc., Pinckney MI) dissolved in 270 mL of distilled water. Cuttings were quick dipped (0.5 s) in an auxin solution and immediately inserted into moistened cell trays (IP110, 45 cell trays, Stueve & Sons, Tangent, OR) previously filled with a commercial substrate of 60% Canadian peat moss: 40 perlite (Sun Gro 2P mix, Sun Gro, Agawam, MA). Trays were placed under overhead mist tables in a phyto-sanitary greenhouse. Each mist area consisted of two mist nozzles (Dramm mist 360NW Green; Dramm Corp., Netherlands) spaced to uniformly cover 3.5 m^2 of rooting trays. Identical additional mist systems were utilized as need. Mist timing and duration was controlled by a Sterling Controller 30 (Superior Controls, Torrance, CA). Mist was pulsed 15 seconds every 10 minutes from 6:00 am to 10:00 pm EST from mid-April to mid-October, with mist beds covered with two layers of black shade cloth, first 30%, and then overlain with 50 percent. In winter months, mist occurred from 7:00 am to 7:00 pm, running 10 sec per pulse without shade. During transitions of seasons of mid-October to mid-November and from mid-March to mid-April, mist beds were covered only with the 30% shade. Every three weeks a new set of cuttings using the same procedure was repeated for a total of 17 times.

During the winter months from 4th January to 18th March 2016, a second set of Kuharske cuttings were prepared and placed in benches outfitted with bottom heat supplied by commercial heating cables (Wrap On, model 6306) and controllers (Redi-heat, Phytotronics, Inc. Earthcity, MO). Minimum bottom heat was maintained at 26°C at night. Greenhouse air temperatures were set to a minimum of 19°C and maintained using two natural gas heaters. Two exhaust fans (American Cool Air, Jacksonville, FL) were set at 28°C and 31°C for cooling as needed throughout the year.

Results

From early May 2015 until mid-October, cuttings treated with 4000 or 7500 ppm Dip&Gro rooted at nearly 100%, independent of auxin concentration (Table 1). This success rate was repeated again with cuttings initiated on 23rd February 2016. Between these two dates, rooting success was quite variable during early November to December. Thereafter rooting percentage declined drastically for cuttings in unheated benches. From 4th January until late February, rooting success ranged from 6% to 40%, but mostly below 23% for cuttings treated with 4000 ppm auxin. Rooting success was always marginally higher with cuttings treated with the 7500 ppm auxin compared to the 4000 ppm auxin.

Cuttings initiated after 1st December 2015 until 23rd February 2016 on unheated benches were not commercially viable (Table 1). Most replications during this period produced 20% or less rooting success, with almost no root development for cuttings initiated on 4th January 2016. Higher percentages mostly occurred with the 7500 ppm auxin solution. Beginning with cuttings initiated on 23rd February 2016 and thereafter, propagation success returned to commercially viable levels, increasing from 20% or less rooting to at least 80% in less than three weeks. Rooting success returned to >95% for cuttings stuck 5th April and beyond.

Rooting success was unacceptably low in unheated substrates during the 1st December to 5th February period. Whereas rooting percentages of heated substrates generally ranged from 40% to 63%, during this time. Higher percentages generally occurred when the 7500 ppm auxin solution was used. By late February, rooting percentages were similar between unheated and heated substrates. No cuttings were stuck in heated substrates after 18th March.

While rooting success is important, root quantity accelerates plant growth. Root mass was generally the highest for cuttings stuck from early April through mid-October (Table 1). Root mass was similar between the two auxin concentrations 65% of the time. Mean root dry mass ranged from 0.9 mg for cuttings initiated using 4000 ppm auxin on 5th February, to 182 mg for those initiated on 15th May (Table 1). Throughout the experiment duration, cuttings treated with 7500 ppm never ($P<0.05$) produced less root mass than cuttings treated with 4000 ppm (Table 1). During the late summer to fall months (6th August to 15th September) trees treated with 7500 ppm produced on average 36% more root mass than cuttings treated with 4000 ppm. When root mass differences were significant between concentrations, with one exception, it was due of higher mass generated by cuttings treated with the 7500 ppm auxin.

New cuttings only produced new shoots within the six week window of evaluation when they were stuck from 5th April until 2nd September (Table 1). Within this period most shoot growth occurred from late April until early August. The rest of the year, little shoot growth was observed during the six week propagation period. When shoot grow did occur, it was short, with maximum average length less than 2 cm. In other experiments with longer rooting durations, shoot growth was equal or greater than seedlings of the same age.

Effects of bottom heat during the winter months, January to April, showed clear positive effects of bottom heat and treatment with 7500 ppm for cutting stuck on 12th January and 5th February (Figure 1). However by late February the benefits of bottom heat were no longer effective, nor were there additional benefits of using the 7500 ppm auxin.

	4000 ppm auxin			**7500 ppm auxin**		
Date	**Percent rooted**	**Root Dry mass (mg)**	**Shoot elongation (mm)**	**Percentage rooted**	**Root Dry mass (mg)**	**Shoot elongation (mm)**
6 May	94	110.7 a	11.4 a	100	113.9 a	15.5 a
15 May	92	181.6 a	12.6 a	94.7	139.8 b	30.3 a
6 August	100	129.1 b	19.1 a	100	173.1 a	11.1 a
2 September	100	73.7 b	0.5 a	100	102.1 a	3.5 a
15 September	100	45.6 b	0 a	100	63.0 a	0 a
14 October	100	102.0 a	0.8 a	100	29.9	0 a
3 November	100	43.9 a	0 a	56.7	29.1 a	0 a
1 December	63.3	40.3 a	0 a	86.7	67.2 a	0 a
4 January	10	1.0 b	0 a	40	10.0 a	0 a
12 January	30	4.8 a	0 a	33.3	5.3 a	0 a
5 February	6.7	0.9 a	0 a	23.3	3.0 a	0 a
23 February	83.3	27.8 a	0.6 a	80	25.2 a	0 a
18 March	76.7	54.7 a	0 a	93	83.8 a	0 a
5 April	100	120.2 a	3.1 a	100	117.5 a	4.5 a
28 April	97	79.8 b	9.8 b	100	100.2 a	33.7 a
20 May	97	80.7 a	17.2 a	100	76.9 a	12.5 a
10 June	97	69.4 b	8.4 a	100	93.4 a	3.5 a

Table 1: Rooting percentage, root and shoot dry masses of single node cuttings treated with 4000 or 7500 ppm auxin (Dip&Gro) concentrations diluted with, Cell-u-wett, a spray thickener. Each treatment consisted of 30 cuttings. Dates were when cuttings were inserted for rooting into seedling trays, beginning in 2015. Means with the same letter are statistically similar at $P<0.05$.

Discussion

When citrus rootstock(s) have been identified that are tolerant or resistant to HLB or citrus canker or both, the demand will be overwhelming. While propagation by tissue culture has the ability to ramp-up the number of tissue culture plants rapidly, propagation of these identified plants to verify their attributes will most likely be accomplished by cutting propagation due to its speed and resulting quicker larger plants for budding.

Root generation in citrus appears to be photoperiodic, based on its inability to generate roots or shoots at climate-controlled air temperatures similar to those experienced during growth in mid-fall to late spring. This follows identical trends of no active shoot growth in commercial citrus nursery greenhouses in Central Florida during the winter mid-November to mid-March period. Propagation of mandarins' cuttings was 100% in the late spring in Brazil, but only 23% for cuttings treated with 3000 ppm IBA in the fall in Brazil [16]. Research with night interruption found that 1 hour of 3:1 red:far-red lighting in the middle of the night induced bud break of rootstocks during the deep winter period [17]. However, the response of roots was not noted [17].

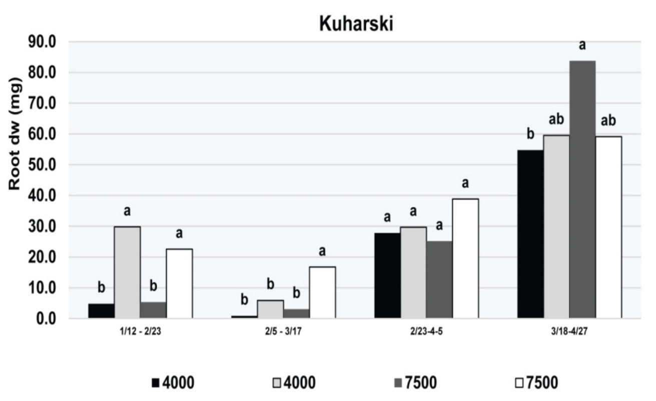

Figure 1: Effect of bottom heat (26°C) on rooting percentage and root dry masses of single node cuttings treated with 4000 or 7500 ppm auxin concentrations diluted with Natrosol. Each treatment consisted of 30 cuttings. Dates were when cuttings were stuck into seedling trays, beginning in 2015. Means with the same letter are statistically similar at P<0.05.

Maintaining higher soil temperatures than which are normally used for woody ornamental propagation (21°C to 27°C) induced higher root generation rates for both auxin concentrations using bottom heat [18]. Root generation was further increased by nearly doubling the level of auxin for the high concentration Researchers have reported optimum soil temperatures for germinating many types of citrus rootstocks was 31°C to 35°C [19] and that rooting of citrus in glass covered propagation frames remained successful up to 43°C [3]. This suggests citrus cuttings, and perhaps other tropical fruit trees, would have higher rooting success during winter months with elevated soil temperatures beyond those used for hardy woody plant production. The introduction of bottom heat in this research occurred late in the winter period, yet it still produced increases in rooting percentage and root mass compared to cutting without bottom heat. Based on the rapid decline of rooting percentage and root mass after 1st December for both auxin concentrations, bottom heat should be considered beginning in mid-November to maintain acceptable levels of root generation.

The auxin used here was a combination of IBA and NAA (2:1). NAA is a man-made compound; it degrades more slowly that either IAA or IBA, thus stimulating cell division longer [1]. The carrier of the auxin, Cell-u-wett, appears to enhance root initiation. The viscus solution retards the evaporation of the alcohol solvent in Dip&Gro. This both greatly slow the evaporation of the alcohol which slows the precipitation of auxin out of solution and aids in the absorption of auxin into the stem.

Conclusion

Single node, single bud cuttings of Kuharske rootstock rooted mostly at 100% during the early spring to late fall period in Central Florida. Root growth was abundant during this period, with some shoot growth also occurring. Shoot growth was small, owing to rooting periods of only 41 days long. When shoot growth occurred, it was almost greater for cuttings treated with 7500 ppm auxin compared to cuttings treated with 4000 ppm auxin. During the winter months of December to late February, rooting was not commercially acceptable, but success was higher using 7500 ppm auxin compared with 4000 ppm.

While citrus is a sub-tropical hardwood tree, in Florida it responds to shorten day lengths with a period of 2 to 3 months of no shoot, and little to no root growth in both propagation beds and for container grown budded trees in greenhouses [20]. The addition of bottom heat during winter propagation greatly improved the number of rooted cutting and generated root mass, but percentages were commercially unacceptable and root mass was marginal compared to spring to fall periods. Most growth, both shoots and roots, occurred between mid-March to late August. Shoot growth was limited by the short time span (41 days) between initiation and harvest.

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