Citrus Section

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Update on 'US Early Pride' in Florida

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'US Early Pride' is a very low-seeded mandarin hybrid that that was released, under patent, by the USDA in 2009. 'US Early Pride' was produced by irradiation of 'Fallglo' budwood in 1989. Other than seed count, 'US Early Pride' has proven to be essentially identical to 'Fallglo'. Seed counts in 'US Early Pride' are typically less than two per fruit, and frequently the fruit are completely seedless, compared to 'Fallglo' which can contain as many as forty seeds per fruit. Industry interest in 'US Early Pride' is evidenced in the fact that it has been the most propagated specialty fruit in Florida in recent years. Early on there was a question regarding the need for a pollinizer for 'US Early Pride', and if so, what would be suitable pollinizers? We have conducted three experiments to address pollination requirements of 'US Early Pride'. To date, results suggest that 'US Early Pride' does not require an alternate source of pollen, but may benefit from it. Several growers have reported that 'US Early Pride' can suffer from a dieback problem. The dieback is not unique to 'US Early Pride', it also seen in 'Fallglo'.

'US Early Pride' is an irradiation-induced, very low-seeded mutant of the 'Fallglo' mandarin hybrid ['Bower' (*Citrus reticulata* Blanco x (*C. paradisi* Macf. x *C. reticulata*) x Temple'] and was released, by the USDA, under patent, in 2009 (McCollum and Hearn, 2011). Although 'US Early Pride' fruit typically contain about two seeds, limited evaluations suggest that its performance is essentially identical to 'Fallglo'. Due to consumer interest in seedless citrus fruit (Vardi et al., 2008), 'US Early Pride' should provide a competitive advantage over 'Fallglo' in the market place. Industry interest in 'US Early Pride' is evidenced in the fact that it has been the most propagated specialty fruit in Florida in recent years (Anon. 2013, 2014).

With all newly released citrus cultivars there are always questions regarding horticultural performance, need for pollinizers, production, pest resistance, and rootstock incompatibility that cannot be addressed thoroughly until commercial plantings have been established and trees come into bearing and production followed over several years (Soost and Roose, 1996). 'Fallglo' is self-compatible and therefore does not require a pollinizer; 'Fallglo' can also serve as a pollinizer for some self-incompatible varieties (Tucker et al., 1987). However, 'US Early Pride' produces pollen that is less abundant and of lower viability than does 'Fallglo'. Although 'US Early Pride' has been fruitful when evaluated in mixed blocks, the question of self-compatibility and its effectiveness as a pollinizer for self-incompatible varieties was not known. To address the pollinizer question, three separate trials were conducted: 1) controlled pollinations of 'US Early Pride' flowers with pollen from several commercially important citrus varieties; 2) a pollinator exclusion trial, plus and minus bees, to address self-compatibility; and 3) a commercial-scale trial to determine effects of proximity to an alternate pollen source on fruit set in 'US Early Pride'.

In addition to questions regarding 'US Early Pride' pollination, a twig dieback problem has been reported in several commercial plantings of 'US Early Pride'. 'Fallglo' is known to suffer a similar dieback (Hearn, 1987), and it is likely that this is the same problem with 'US Early Pride'. An extensive review of the dieback problem with 'US Early Pride' is available on the internet (Rogers, 2014). Because the dieback problem was first observed on 'Fallglo', and, 'US Early Pride' originated from 'Fallglo' and the disorder appears to be similar for both varieties, we have chosen to refer to this disorder as " 'Fallglo' dieback".

In this report we describe results of experiments to address 'US Early Pride' pollination issues, and also report on findings regarding twig dieback and suggested management strategies.

Materials and Methods

Pollination Experiments

Controlled pollinations. In March 2012 controlled pollinations of 'US Early Pride' were conducted at the A.H. Whitmore Citrus Research Foundation Farm, Lake County, FL, and at the USDA–USHRL Research Farm, St. Lucie County, FL. Eleven pollen sources were included in the experiment ('Temple', 'Minneola', 'Early Gold', 'MidSweet', 'Ortanique', 'Fallglo', 'Flame', and 'Murcott' in St. Lucie County and 'Fallglo', 'Hamlin', 'Sunburst', 'Orlando', 'Minneola', and 'Murcott' in Lake County). Pollen parents were selected based on pollen availability at the time of 'US Early Pride' flowering. Anthers of pollen parents were collected prior to anthesis and allowed to dehisce overnight under an incandescent light bulb. Petals were removed from 'US Early Pride' flowers prior to opening and pollen was applied by

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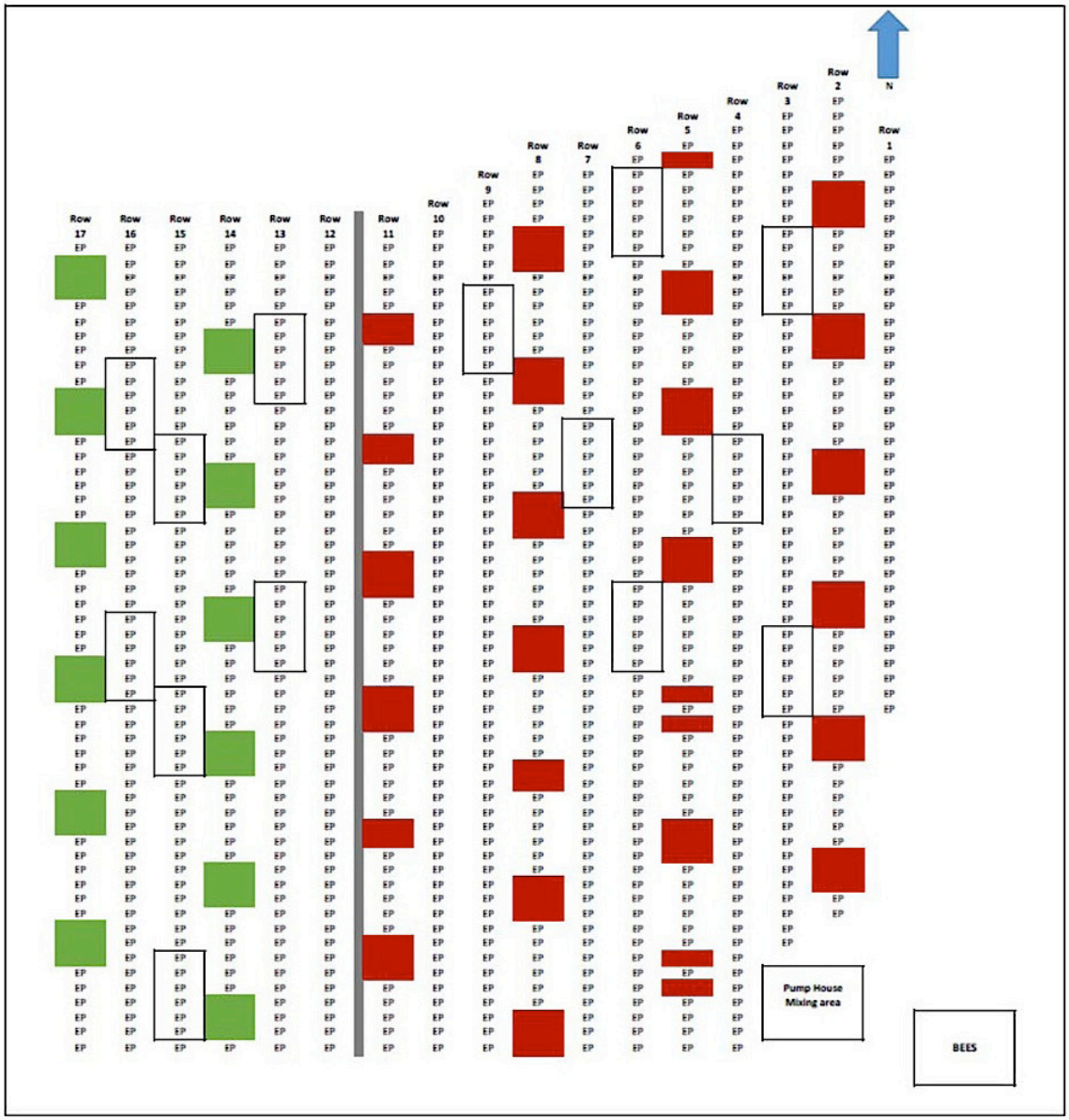

Fig. 1. Layout of field plot for testing the effects of proximity to pollinizer on *Citrus reticulata* 'US Early Pride'. All 'Orlando' trees on the east side of the block were severely pruned to prevent flowering. Red boxes represent 'US Early Pride trees distant from pollinizer. Green boxes represent 'US Early Pride' trees adjacent to pollinizer. Open boxes represent trees used to collect fruit count data.

rubbing stigmas with anthers of the pollen parent. At the time of pollination, each hand-pollinated flower was tagged with a unique identification number. Numbers of fruit resulting from hand pollinations were recorded one and two months following pollination at both locations. In Nov. 2012, numbers of mature fruit and seeds per fruit were recorded.

Self-compatibility. Cage experiments were conducted in the spring of 2012. Prior to flowering (Jan. 2012) four mature 'US Early Pride' trees were enclosed in screened cages to exclude pollinators and pollen from alternate sources. Bee hives were placed inside two of the four cages to ensure pollination ('US Early Pride' on 'US Early Pride'), bees were not placed in the remaining two cages. Cages remained in place until flowering was completed and were; then removed. All mature fruit were harvested from the trees that had been caged, total number and weight of fruit were recorded.

Effects of pollinizer proximity. This experiment was conducted in Spring 2014 in a commercial planting of 'US Early Pride' on 'Swingle' rootstock and inter-planted with 'Orlando' to serve as a pollinizer. The experimental block was located in central Polk County, and was established in 2011. 'Orlando' trees were interplanted among the 'US Early Pride' trees as follows: In every third row, 'Orlando' trees (typically in groups of three) were planted between groups of six 'US Early Pride' trees (Fig. 1). Proximity to pollinizer was the single treatment evaluated in the experiment. In the eleven rows on the eastern side

Table 1. Effects of pollen source on *Citrus reticulata* 'US Early Pride' fruit yields and seed counts.

Picos						
Pollen parent	Total Crosses	Total Fruit	Crosses (%)	Weight (lbs)	Total Seeds	Seeds/fruit (Avg)
Early Gold	12	4	33	7	3	0.8
Fallglo	10	7	70	7	6	0.9
Flame	10	1	10	7	2	2.0
MidSweet	56	20	36	7	33	1.7
Minneola	81	31	38	12	57	1.8
Murcott	6	3	50	7	0	0.0
Ortunique	21	1	5	7	3	3.0
Temple	15	5	33	7	2	0.4

Whitmore						
Pollen parent	Total Crosses	Total Fruit	Crosses (%)	Weight (lbs)	Total Seeds	Seeds/fruit (Avg.)
Fallglo	9	0	0	0	0	–
Hamlin	21	8	38	3.0	23	2.9
Minneola	44	25	57	8.5	68	2.7
Murcott	28	9	32	2.5	35	3.9
Orlando	15	7	47	2.0	21	3.0
Sunburst	68	30	44	10.5	95	3.2

Table 2. Effects of screening and bees on yields of *Citrus reticulata* 'US Early Pride.

	Average per tree	
Treatment	Boxes	Weight (lbs)
Screen		
– Bees	2.0	185
+ Bees	3.0	301
No screen		
'US Early Pride'	1.5	177
1-62-122[z]	2.8	281

[z]1-62-122 was the original tree from which all 'US Early Pride' trees were propagated.

of the block all 'Orlando' trees were severely pruned to prevent flowering. 'US Early Pride' trees in this group were designated distant from pollinizer, with none closer than four rows to the non-pruned 'Orlando' trees. Fruit were counted September 26, 2014 using frames that measured 50 cm x 50 cm 50 cm (0.125 m^3). Frame counts were conducted on 42 trees in each treatment group (Fig. 1). For each tree counts were made on the east side of the tree at chest height, and are expressed as the average number of fruit per 0.125 m^3.

Representative fruit samples were collected from the experimental trees (25 fruit for each treatment group) in October 2014. Numbers of seeds per fruit were recorded.

Observations on Twig Dieback

Reports of a dieback problem with 'US Early Pride' in several commercial plantings prompted field surveys to evaluate symptomology and commonalities among affected trees. The incidence of dieback in a replicated field trial containing "US Early Pride' and 'Fallglo' trees was also conducted. Isolations for potential fungal pathogens were made from three plants of 'US Early Pride' displaying symptoms of tip dieback and stem cankers. Tissue was excised from the necrotic margin of stem nodes and tips, surface sterilized for 60 s in a 10% commercial bleach solution. Sterilized tissue was rinsed twice in sterile deionized water, patted dry on sterile filter paper, and plated onto potato dextrose agar (PDA). Plates were incubated at 24 °C with 12-h diurnal light cycle.

Results

Pollination Experiments

Controlled pollinations. 'US Early Pride' fruit and seed counts resulting from controlled pollinations with eight pollen sources in St. Lucie and Lake Counties, FL, are presented in Table 1. A total 211 pollinations were made in St. Lucie County. 'Midsweet' and 'Minneola' were the two pollen parents for which the greatest number of pollinations were conducted, and also resulted in the greatest numbers of mature fruit, 20 and 31 for 'MidSweet' and 'Minneola', respectively. The fewest pollinations were observed with 'Murcott' and no fruit resulted.

A total of 185 pollinations with six pollen parents were conducted in Lake County. The greatest number of crosses in Lake County were with 'Sunburst' and 'Minneola' (68 and 44, respectively). However, the percent of pollinations that produced mature fruit was 44% for 'Sunburst' vs. 58% for 'Minneola'.

Mature fruit resulted from 34% of all pollinations in St. Lucie County and 43% in Lake County. The average number of seeds per fruit was 3.1 in Lake County. and 1.3 in St. Lucie County.

Mature fruit resulted from 56% of 'Minneola' pollinations in Lake County and 38% in St. Lucie County. Surprisingly, results when 'Fallglo' was used as the pollen parent were contradictory for the two locations. In St. Lucie County from 10 Fallglo crosses, 7 produced fruit; in Lake County, from 9 'Fallglo' crosses, no mature fruit resulted. Because only 9 or 10 pollinations were made with 'Fallglo', making conclusions would be extremely speculative. Why so few 'US Early Pride' pollinations were conducted with 'Fallglo' pollen is not known.

Self-compatibility. To address the question of 'US Early Pride' self-compatibility, four-mature 'US Early Pride' trees were enclosed in screened structures to exclude pollinating insects and the potential for pollination by alternate sources. Bees were added to two of the enclosures to determine effects of pollinator. The effects of screening and pollinators on 'US Early Pride' fruit production are presented in Table 2. A comparison of fruit yields produced by screened trees suggests that the presence of bees resulted in an increase in fruit production. Fruit production on single non-screened 'US Early Pride' and US 1-62-122 (the original version of 'US Early Pride') was similar to yields of screened trees. Concluding that 'US Early Pride' will not benefit from an alternate source of pollen based on this small experiment is not possible, however, the results of the pollen exclusion experiment do suggest that 'US Early Pride' has some parthenocarpic tendency. Although trees were screened, there is always the possibility that pollen could make its way by wind to the 'US Early Pride' flowers.

Effects of pollinizer proximity. To further explore 'US Early Pride' response to pollinizer a study was conducted in a commercial planting of 'US Early Pride' that was interplanted with 'Orlando'. Our hypothesis was that if 'US Early Pride' productivity and seed count are impacted by pollinizer, as the proximity to pollinizer trees ('Orlando') increases, the effect will

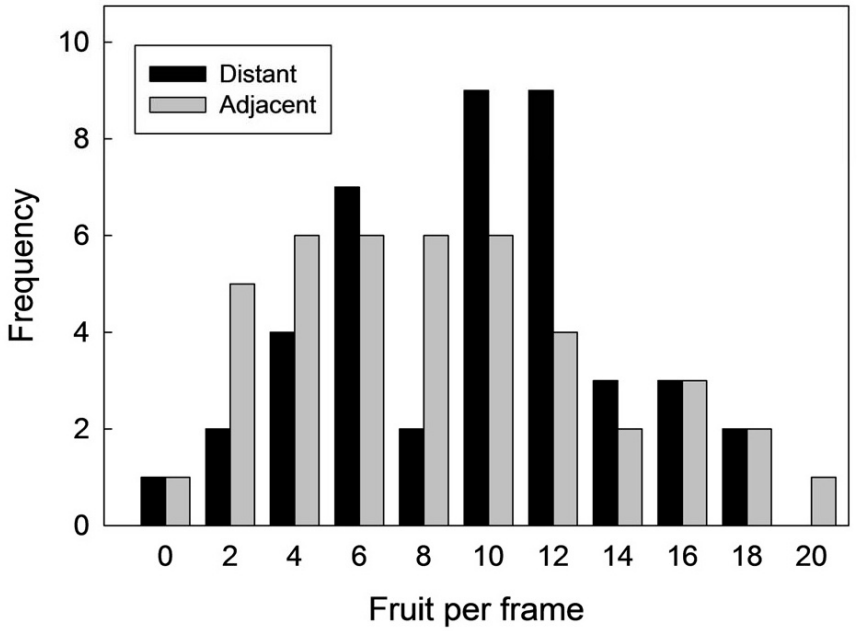

Fig. 2. Effects of proximity to pollinizer on *Citrus reticulata* 'US Early Pride' fruit counts.

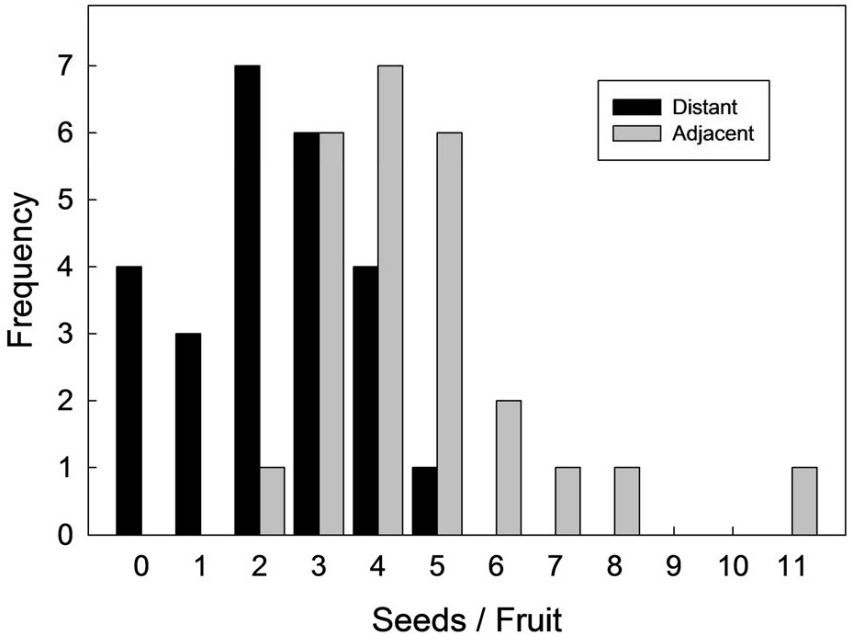

Fig. 3. Effects of proximity to pollinizer on *Citrus reticulata* 'US Early Pride' seed counts.

be greatest in trees adjacent to the pollinizer and least in trees distant from the pollinizer. By removing all of the 'Orlando' flowers in one section of the block we created a group of 'US Early Pride' trees considered 'distant' from the pollinizer. We compared fruit and seed counts in trees distant from the pollinizer with those in another part of the block where the 'Orlando' trees had not been pruned to remove flowers (adjacent to the pollinizer). The effect of proximity to pollinizer on 'US Early Pride' fruit counts is presented in Fig. 2. There was no significant difference in numbers of fruit per frame between trees adjacent to or distant from the pollinizer. This result supports that 'US Early Pride' does not require a pollinizer to be fruitful. The effect of proximity to pollinizer on 'US Early Pride' seed counts is presented in Fig. 3. Although there was a trend toward greater numbers of seed per fruit in the group adjacent to the pollinizer, the difference was not statistically significant. One problem with interpretation of the results for this experiment is that there was poor overlap of bloom time for 'US Early Pride' and 'Orlando'. Perhaps if an alternate source of pollen with better overlap of bloom with 'US Early Pride' had been used treatment differences may occur.

Fig. 4. Dieback symptoms observed on *Citrus reticulata* 'US Early Pride'.

Observations on twig dieback

Reports of a dieback problem with 'US Early Pride' in several commercial plantings prompted field surveys to evaluate symptomology and commonalities' among affected trees. The dieback is typically first observed at nodes where tissue develops a darkened corky appearance (Fig 4). As the symptoms progress gummy sap is exuded from the lesion. Spread of bark discoloration is followed by death of entire stems. Seventeen individual isolates were axenically-cultured and saved for identification. One isolate was putatively identified as a *Colletotrichum* sp., one as *Fusarium roseum*, a third as a pycnidial fungus, and numerous *Pestalotia* sp. The remaining isolates did not produce reproductive structures in culture. One isolate, obtained from tip tissue grew slowly on PDA and was more successfully cultured on lima bean agar. Three isolates from the tip die-back and two isolates obtained from the nodes were selected. These five isolates were transferred to potato dextrose broth by removing three plugs of mycelium from the actively-growing margin of the clean colony from PDA. These were allowed to grow for one week under the same conditions as stated above. The MoBio UltraClean®Microbial DNA Isolation Kit (MO BIO laboratories, Inc. Carlsbad, CA, USA) was used to extract genomic DNA from the fungal mycelium from liquid cultures. Estimation of DNA concentration and purity from the extracted samples were estimated using the NanoDrop ND-1000 spectrophotometer according to the manufacture's methods (Thermo Fisher Scientific, Wilmington, DE, USA). Extracted DNA was then subjected to PCR using internal transcribed spacer region primers ITS 4 and 5 using the standard thermocycler protocol (White and Bruns). The PCR products were sequenced and the resulting sequences were used to search the NCBI database. The Blastn search resulted in the identification of the three tip isolates as *Fusarium* sp., *Guignardia mangiferae*, and *Colletotrichum gloeosporiodes*. The node fungi had ITS sequence similarity to two uncultured, unidentified fungal species. These five isolates were used to inoculate four healthy 'US Early Pride' each, in an attempt to reproduce the observed symptoms. Inoculations were performed using by making a small wound in the terminal shoot of the tree, into which a 1cm square mycelial plug was inserted, wrapped with parafilm and allowed to remain for two weeks. Five control plants were wound-inoculated with clean PDA plugs lacking fungal mycelium and wrapped in parafilm. The inoculated branch was then bagged for 24 h. The inoculation was conducted twice. None of the isolated fungi resulted in the reproduction of the symptoms previously observed on 'US Early Pride'. Although *G. mangiferae* is not a pathogen of citrus, it is commonly found as a fungal endophyte of woody plants. Since *C. gloeosporiodes* can quiescently infect citrus tissue, and result in anthracnose when plants are stressed, it is possible that this was the cause of the symptoms observed in the field, but the stress component was not present in the greenhouse during inoculation.

Controlled inoculations of healthy 'US Early Pride' and 'Fallglo' trees were conducted, but dieback was not induced in either variety by either pathogen. It is likely that all of the fungal isolates were secondary opportunists. In a replicated field trial containing 'US Early Pride' and 'Fallglo' dieback was rated on 25 trees of each cultivar, but in St. Lucie County, FL, the dieback seems to be more prevalent in nursery trees that are smaller rather than larger. In addition, any girdling of the stem seems to exacerbate the problem, making it imperative to remove any type of material that may lead to girdling from around the stem. Although 'US Early Pride' trees affected by dieback typically outgrow the problem, tree death is a possibility if the disorder is left unchecked. Frequent scouting for the appearance of dieback followed by prompt pruning to remove affected tissue appears to be the best approach to manage the problem. Strict attention to sanitation of pruning tools is imperative for preventing spread of microorganisms that may be involved in the dieback problem (Rogers, 2014).

Although 'US Early Pride' is a relatively new cultivar, it has been widely planted in Florida. The low seed character of the variety makes it an attractive alternative to 'Fallglo', which may contain as many as 40 seed per fruit. Although dieback has been observed in some newly planted blocks, the problem appears to be manageable. Based on grower reports, 'US Early Pride' produces acceptable yield. However based on the pollination studies reported here, we cannot say conclusively that 'US Early Pride' does or does not benefit from inter-planting with a pollinizer. One interesting observation regarding 'US Early Pride' is that it tends to be less severely impacted by Huanglongbing (HLB) than are most other varieties. If this proves consistent it will be a tremendous advantage in Florida and other areas affected by HLB.

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