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# Evaluation of Cross Pollination of Zephyranthes and Habranthus Species and Hybrids

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# Evaluation of Cross Pollination of *Zephyranthes* and *Habranthus* Species and Hybrids

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***Abstract.***—The genus *Zephyranthes* and the related genus *Habranthus* in the family Amaryllidaceae are commonly grown ornamental bulbs having new world homologies. Inter-specific and inter-generic hybridizations are complicated by the fact that some of the species are apomictic, cross incompatible, or have widely variable 2n chromosome numbers. A simple, inexpensive method of pollen storage was tested to evaluate the production of hybrid seed. Intact anthers harvested after pollen release and stored at 4°C were used for pollination. Emasculated flowers were pollinated at noon on the first or second day of anthesis. Hybrid crosses were labeled and seed collected when formed. All available parents were used in hybridizations except when species were known to have apomictic or pseudogamous seed development. Hybridization data were recorded for seedpods having successful seed set as well as those that aborted. Development of 'normal' seedpods filled with seed occurred even though viable embryos were not always formed. $F_1$ *Z. grandiflora* was successful 55% of the time, and Pink Trihybrid, a rather infertile seed parent, was successful at least 19% of the time. These data suggest that repeating many crosses ultimately produced a few hybrids in problematic crosses. In addition to producing seed of potentially interesting new hybrids, this study helped to identify successful seed and pollen parents for future breeding efforts. Hybrid seed was sown to test viability of the progeny.

*Key words.— Zephyranthes, Habranthus, Amaryllidaceae,* ornamental bulbs, hybridizations, pollination.

## Introduction

The genera *Zephyranthes* and *Habranthus* include many distinct species commonly called rainlilies or surprise lilies due to their habit of episodic flowering after seasonal rainfall. Species belonging to these 2 genera are native to diverse areas of the new world including Argentina, the Caribbean, Mexico and North America. The North American species range from Florida and the Carolinas to the mountains of western New Mexico and comprise perhaps the most cold hardy and drought tolerant members of these genera. The assessment of the family using cladistical analysis indicates that these two genera only have new world homologies (Meerow et al. 1999).

These plants are perennial geophytes that reproduce either asexually (i.e. via offsets or twin scaling) or by seed. Efficient asexual reproduction of most rainlilies makes preservation of selected clones easy. Production of unique phenotypes via seed, however is complicated by the fact that some species are apomictic or pseudogamous (Gupta et al. 1998). Such species reproduce the maternal phenotype faithfully without variation. Chromosome number within *Zephyranthes* range from 2n = 18-96, obviously a concern for crosses among species (Raina and Khoshoo 1971).

Flowers of *Zephyranthes* are usually solitary, with 6

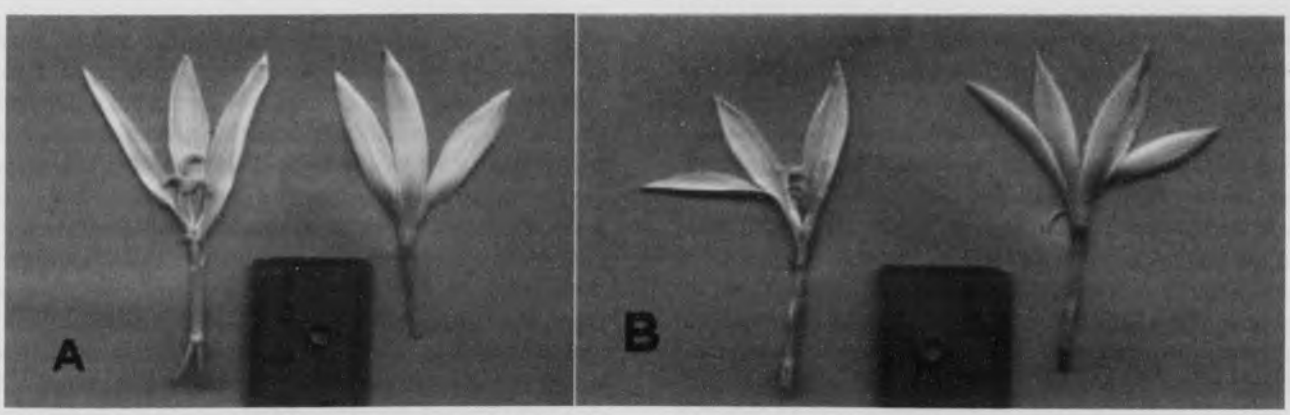



Fig. 1. Dissection of 2 flowers showing arrangement of stigmal surfaces and anthers. A: *Habranthus* flower showing crescent moon shaped anthers and uneven length of filaments typical of the genus. B: *Zephyranthes* flower showing even length of the filaments and more linear anthers. Photos by Joschles H (www.pacificbulbsociety.org/plosesiki/index.php/zephyranthes


Fig. 2. Comparison of seed phenotypes show extreme differences in morphology. A: Seed from *Z. species* with the '*allium*' phenotype. Seed coat is waxy, seed is plump containing lots of endosperm. Typical example would be *Z. citrina.* B: Seed from *Z. species* with the '*lillium*' phenotype. Seed is papery, flat, shriveled and contains only minimal endosperm. Typical example would be *Z. labuffarosea.* C: Hybrid seed often shows an intermediate phenotype. (Big Shot x *Z. citrina*) D: Hybrid seed formation with a robust intermediate phenotype (*Z.grandiflora* x Z. *primulina*) E: Seed from fertile hybrid cross.

petals having colors ranging from white to sulfur yellow to pink. The stamens are often spatially separated with either exserted pistils (i.e. approach herkogamy) or hidden pistils (i.e. reverse herkogamy). This arrangement significantly influences pollination in the wild. Flowers with hidden stigmas are self pollinated unless insect visited. Several species of *Zephyranthes*, such as *Z. jonesii* which have hidden pistils and are mildly fragrant, bloom at night and release pollen at dawn. This arrangement of stamens insures self pollination unless the corolla with attached stamens is excised before pollen release when hand pollinating. *Habranthus* species have similar flowers in colors from white to bronze yellow to pink (some have purple stripes), but the flowers generally remain funnel or trumpet-like not opening fully. *Habranthus* flowers typically have spatially separated stamens and pistils and distinct crescent shaped anthers held on filaments of uneven length (Fig. 1). Seedpods are trilobed capsules and contain D-shaped seed that usually develop rapidly. Species in both genera produce 2 distinct types of seed. Some species produce seed that is flat and papery with a phenotype similar to *Lilium* while other species produce seed that is plump and somewhat waxy similar to *Allium* seed (Fig. 2). Intermediate seed phenotypes are observed in hybrids and such observations are useful in evaluating the result of cross pollinations.

Generally the leaves, bulbs and roots of these plants are toxic. Although the presence of alkaloids has been documented in several species (Kojima et al. 1997), and other species have been evaluated for medicinal value, the primary interest in this breeding program is for the ornamental value of the flowers. Wild collected naturally occurring hybrids such as *Z. sp.* Tenexico have apricot colored flowers and commercially available cultivars now extend the color range from orange to salmon pink and red (Fellers 1996). Although many of the species have small ephemeral flowers some hybrids produce larger flowers that will remain open for up to 3 days. The potential for new cultivars via cross pollination is limited because of some reproductive barriers in these plants. A summary of the barriers to cross pollination among *Zephyranthes* species and hybrids and with the species of *Habranthus* is as follows:

1. Plant structural morphologies: These include length of the floral tube, spatial arrangement of the stamens and length of the pistils.
2. Chromosome number or ploidy level: A wide variety of 2n chromosome numbers is a deterrent to crosses.
3. Pollen production: Certain species and hybrids produce limited amounts of pollen.
4. Self or Cross incompatibility: Apparent sterility might actually be incompatibility.
5. Apomixis and/or Pseudogamy: These species often produce prolific seed which reproduces the maternal phenotype.
6. Flowering season: Some species are once-flowering; others repeat flowering throughout the growing season.
7. Receptivity of the stigma: Some species remain open for more than one day and may actually be receptive on day 2 of anthesis.

While all barriers are noted, the ultimate limiting factors governing pollinations in this study were availability of flowers and pollen during the study period. All crosses were attempted with the exception of apomictic species. Apomictic species were never used as maternal parents; as paternal parents these are good pollen donors which were used freely in crosses. Certain reciprocal crosses are thus impossible. The objectives of this study are as follows:

1. To evaluate a simple pollen storage method that will potentially allow greater flexibility of crossing non-synchronous blooming species.
2. To make as many pollinations as possible and to evaluate the compatibility of inter-specific crosses as well as a few inter-generic crosses.

Table 1. List of species and hybrids used for hybridization.

| Species | Color | Hybrids | Color |
|---|---|---|---|
| *Zephyranthes candida* | white | Apricot Queen | apricot |
| *Z.chlorosolen* | white | Benidama | pink |
| *Z. citrina* | dark yellow | Best Pink Trihybrid | pink blend |
| *Z.clintae* | pink | Big Shot | cream |
| *Z.drummondii* | white | Yellow Big Shot | yellow |
| *Z.grandiflora* | pink | Pink Big Shot | pink |
| *Z.insularum* | white | Orange Big Shot | orange |
| *Z.flavidissumus* | yellow | Salmon Big Shot | salmon |
| *Z.jonesii* | cream | C C Moon | white |
| *Z.labuffarosea* | blush pink | Dark Pink Spider | pink |
| *Z.lindleyana* | variable pink | El Cielo | blush pink |
| *Z. longifolia* | neon yellow | Fireball | orange/yellow |
| *Z.macrosiphon* | pink | Cherry Fireball | cherry red striped |
| *Z.morrisclintae* | blush pink | Goliath | pink |
| *Z.primulina* | lemon yellow | Ivory Star | white |
| *Z.pulchella* | dark yellow | Lemon Pinwheel | light yellow |
| *Z. reginae* | light yellow | Norma Pearl | white |
| *Z. simpsonii* | white | Orange Citrina | orange |
| *Z.smallii* | yellow | Hybrid El Cielo | pink and white |
| *Z.traubii* | cream | Prairie Sunset | salmon |
| *Habranthus texensis* | bronze yellow | Quad Pink | pink |
| *H. texensis var. roseus* | pink w/purple | Quad Orange | light orange |
| | | Sunset Strain | yellow w/red stripes |
| | | Tall Pink | pink |
| | | Tenexico | salmon |
| | | Xzb-H2 | white |

## Material and Methods

A collection of *Zephyranthes* and *Habranthus* species and hybrids is maintained at the Arkansas Biosciences Institute, Arkansas State University, State University, AR 72467. These plants and their seedlings (Table 1) were made available for this project from September to December 2005. It should be noted that maximum flowering of these species normally occurs from April until October, so this project was conducted during the time when flowering normally decreases. The number of crosses attempted strictly depended on the natural blooming capacity of the species and hybrids and was not under the control of the researcher. Successful storage of pollen was critical because flowering flushes normally decrease at the end of the season. The diversity and availability of pollen was expected to be a limiting factor.

Flowers were emasculated using fine tipped forceps at the time of pollination. Entire anthers were collected intact into 25 ml brown plastic sample vials labeled and stored in sealed plastic containers (Rubbermaid ™) in a cold room at 4-6 °C and kept for the duration of the experiment. Details of this storage technique were provided by Mr. John D. Fellers, Rainlily Breeder/ Authority, Auburn, Alabama (personal communication 2005). Flowers were pollinated close to noon which is the average time for anthesis and pollen shed. There are extreme exceptions to this; *Z.traubii,* San Carlos and *Z.labuffarosea* which are nocturnal flowering species. Such species are presumed to be insect pollinated and are fragrant. It was not possible within the scope of this project to pollinate at night or dawn. When these plants were used as female parents, emasculation was done the previous day when possible. After pollination the flower stalks were labeled, dated and left for seed formation. Unsuccessful crosses were often noticed within 3-5 days, but some crosses took as long as 1 week or more before aborting. Successful seed formation took approximately 3 weeks depending on the female parent (Fig. 3). El Cielo pods typically took 4-5 weeks to mature properly. Seedpod formation is not always indicative of normal, viable seed. Seed was sown to check for viability. Rainlily seed (of both *Zephyranthes* and *Habranthus*) is generally sown soon after harvest. Seed of most of these species remained viable for only a short time.

## Results and Discussion

The results of crosses made in Fall 2005 are given in Table 2 and Fig. 4. In total, 215 crosses were attempted; of

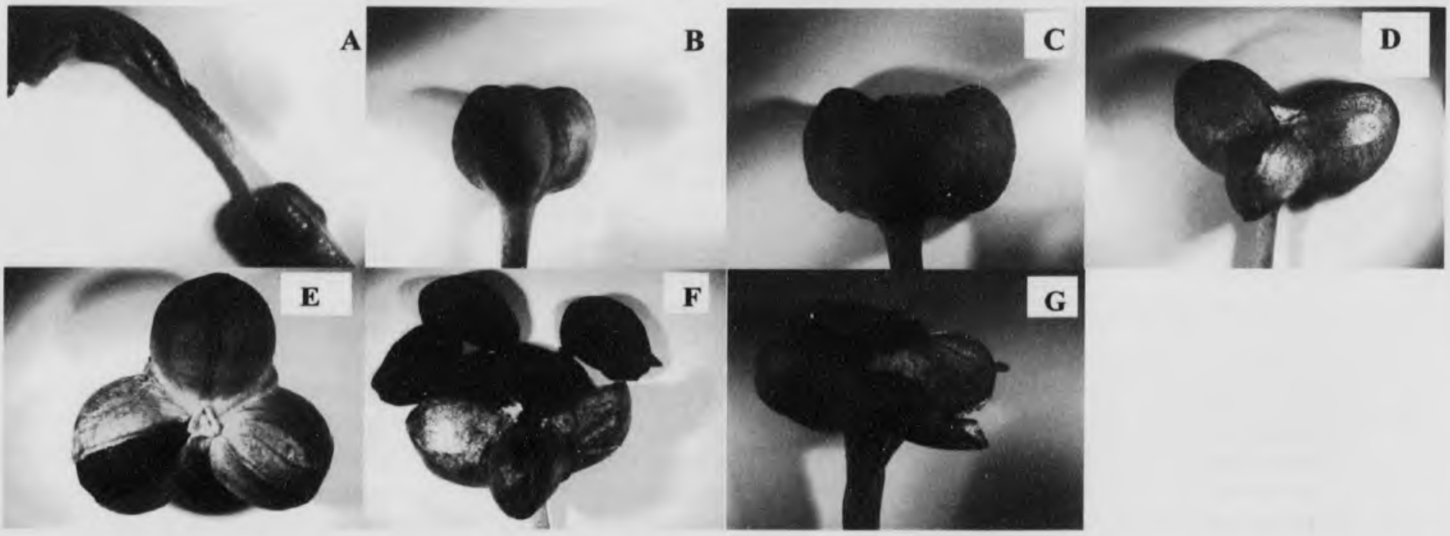


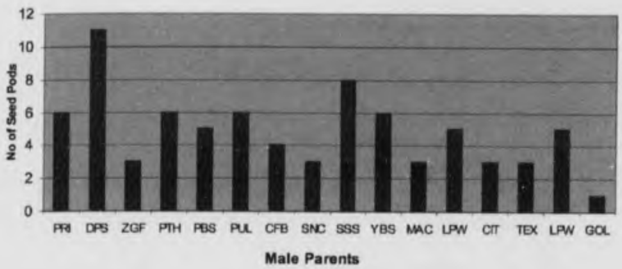

Fig. 3. Development of typical *Zephyranthes* seed from flower to shattering. A: ovary 3 days post pollination; B: developing pod; C: maturing pod two weeks after pollination; D: fully mature pod; E: dehiscence of pod; F: collectable seed; G: empty capsule [all images *Z. citrina*].

these, only 87 or about 41% set seed capsules. These were then harvested. The remainder, about 59% of the crosses aborted. Stored pollen was successful 41% of the time. This provided the breeder with pollen representing a wider variety of genotypes than fresh pollen available on a given day and a greater choice of male parents. Species that are known to be apomictic by experience or via citations in the literature were excluded from use as female parents. These species and/or hybrids include, *Z. primulina, katherinae, macrosiphon, citrina,* Sunset Strain, *jonesii, longifolia,* Ivory Star and *pulchella* (Howard 1996). Such species were all used as pollen parents. Using these parents as females is always successful but never produces hybrid seed.

The choice of female parents other than the apomictic species mentioned above depended strictly on weather and flowering conditions. During this project, episodic flowering events occurred only twice. Some days there were no flowers available, while at least two prolific episodes of synchronous flowering occurred. During these episodes as many as 40-50 flowers were available in a 7 day period. For the remaining bloom season, crosses were accomplished with whatever intermittent flowering occurred. Lack of predictable flowering made experimental design difficult. Considering the relatively small sample sizes, looking at the frequency of successful seed production offers some insight. Successful pollen parents include both species and hybrids; notably, $F_1$ *Z. grandiflora* and a *Z. traubii* hybrid called Yellow Big Shot (Fig. 4). Pollen sources are always more diverse since all the yellow apomictic parents can be used and are responsible for production of orange and red hued hybrids. When seed parents were evaluated, it is notable that although $F_1$ *Z. grandiflora* is considered to have an unusual 2n chromosome number, it was among the best of the female parents tested (Fig. 4). Trihybrid female parents [(*Z. candida* x *Z. citrina)* x *Z. macrosiphon*] were used for pollinations. These plants are sister seedlings and have similar phenotypes but are mostly self and cross sterile. The interesting genetics that they represent (cold tolerance of *Z. candida* x deep yellow color of *Z. citrina* x large pink flowers of *Z. macrosiphon*) makes them desirable as parents. Best Pink Trihybrid and Pink Trihybrid (sister seedlings) made fertile seedpods 46% and 19% of the time respectively with multiple pollen parents. This indicated remarkable fertility never seen before in these desirable parents.

For female parents that were attempted 10 or more times, the rate of fertile crosses ranged from 0-60% (Table 2). All of the hybridizations attempted are either inter-specific crosses or crosses with cultivated hybrids which are usually inter-specific crosses themselves when their ancestry is known. These data indicated that unrelated crosses have potential for success.

Among the crosses recorded in Table 2 which were attempted in extremely limited numbers, there are some exceptional responses. $F_1$ *Z. grandiflora* (which is a seedling code for a fertile *Z. grandiflora* hybrid seedling produced in this breeding program) was crossed with *Habranthus tubispathus* var. *texensis*, which represents an unusual inter-generic cross. The quadhybrids, crossed with Yellow Big Shot, represent at least 6 ancestral species and indicate the potential for making complex hybrids within the genus *Zephyranthes*. At least one female parent, the fertile $F_1$ *Z. grandiflora* hybrid was successful with 9 different pollen donors. This $F_1$ parent is derived from a species that is usually self and cross sterile (Kapoor and Tandon 1963). The fact that this plant will set seed with multiple parents is significant because this is the source of the largest flowers



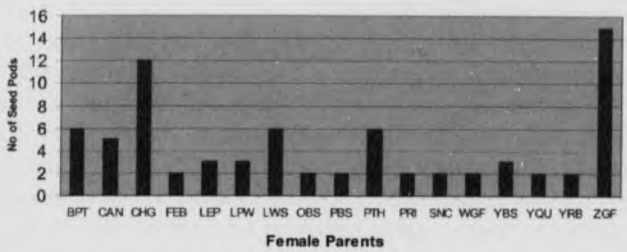




Fig. 4. Relative Fertility of Male and Female Parents. BPT: Best Pink Trihybrid; CAN: *Z. candida*; CFB: Cherry Fireball; FEB: Fireball; LPW: Lemon Pinwheel; LNQ: Lemon Quad; LYP; LWS: Lindlelyana White Star; MAC: *Z. macrosiphon*; OBS: Orange Big Shot; PBS: Pink Big Shot; PTH: Pink Try Hybrid; PRI: *Z. primulina*; DPS: Dark Pink Spider; PUL: Pulchella; SNC: San Carlos; SSS: Sun Set Strain; CIT: *Z. citrina*; LPW: Lemon Pin Wheel; WGF: White Grandiflora; YQU: Yellow Quad; ZGF: *Z. gradiflora*; TEX: *Habranthus tubispsthus* var *texanisis*; GOL: Goliath

within the *Zephyranthes*.

In this study, 44 different female parents were tested. However, only 34 or about 77% of the female parents made at least one fertile cross (Table 2). Testing large numbers of parents gave some insight into the potential for a breeding breakthrough by discovering potential maternal parents that may not be limited by apomixis. In other plant genera there is evidence that apomixis is heritable. In *Zephyranthes* some cultivars, such as Sunset Strain carry this trait. The identification of breeding lines that reliably produce hybrid progeny is necessary to make progress in backcrosses or $F_2$ crosses. These data identify distinct genetic lines that might be pursued which include 1) Hybrid $F_1$ *Z. grandiflora* which are fertile, 2) Trihybrid (partially) fertile lines, 3) *Z.traubii* hybrids (these include all of the yellow and pink Big Shot hybrids, 4) Fireball progeny, 5) *Z. labuffarosea* hybrids and finally 6) *Z. lindleyana* lines. Phenotypic traits of interest arising from these crosses include: ruffled petals, bronze foliage, fragrance, and colors of varying intensity (ranging from apricot, salmon, orange, cherry red, orange red and purple) stripes, picotees, bicolors as well as flowers that remain open for as long as 3 days.

## Conclusions

The use of stored pollen resulted in seed formation on some recalcitrant crosses (e.g. the trihybrids) and suggests that a high number of pollinations and the use of many pollen parents may help to overcome some barriers to hybridization.

These data identify suitable parents for future crosses. Female parents that produce hybrid progeny predictably (and that are ultimately not apomictic) are needed to advance the generations and, in particular, to permit the evaluation of predicted recombinants in the $F_2$ and beyond.

Breeding within the *Zephyranthes* and between Z. species and *Habranthus* species may remain problematic due to obvious difficulties expected in making multiple backcrosses and reciprocal crosses. Identifying reliable fertile hybrids may eventually permit breeding for cold hardiness and adaptability to soil types. Disease resistance, fragrance and persistent foliage are also desirable traits for long term breeding efforts.

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Table 2. Summary of *Zephyranthes* hybridizations –Fall 2005.

| Female Parent | # attempts | # successful | % fertile crosses | # male parents | ID of pollen parents |
|---|---|---|---|---|---|
| Apricot Queen | 1 | 1 | 100 | 1 | SBS |
| Best Pink Trihybrid | 13 | 6 | 46 | 5 | DPS,PRI,LIN,GRA |
| Big Shot | 2 | 0 | 0 | 0 | none |
| Candida | 10 | 5 | 50 | 5 | PTH,PBS,PQU,DPS,DPS |
| Cherry Fireball | 1 | 1 | 100 | 1 | DPS |
| Colorbreak Grand | 1 | 1 | 100 | 1 | PBS |
| Dark Fireball | 1 | 1 | 100 | 1 | SJP |
| Dark Pink Spider | 10 | 0 | 0 | 0 | none |
| El Cielo Pink Hybrid | 4 | 0 | 0 | 0 | none |
| Fireball | 2 | 2 | 100 | 1 | PUL |
| F1 Grandiflora | 36 | 20 | 46 | 10 | CIT, DPS,TEX,PBS,SSS,PRI,SNC, PSS, YBS |
| Goliath | 8 | 3 | 37 | 3 | PRI, CIT, TEX |
| Ivory Star | 2 | 2 | 100 | 1 | SNC |
| Jonz Pink | 2 | 1 | 50 | 1 | DPS |
| Jonz Striped Pink GRF | 1 | 1 | 100 | 1 | PUL |
| Labuffarosea | 1 | 1 | 100 | 1 | SSS |
| Lemon Pinwheel | 11 | 3 | 27 | 2 | GOL, PBS |
| Lemon Quad | 3 | 3 | 100 | 1 | PTH |
| Lilypies | 1 | 1 | 100 | 1 | YRB |
| Lindleyana | 10 | 6 | 60 | 6 | PRI,CFB,DPS,YBS,SSS,PTH |
| Lindleyana 'White Star' | 1 | 1 | 100 | 1 | PTH |
| Lt Pink Grandflora | 1 | 1 | 100 | 1 | PUL |
| Macrosiphon | 2 | 2 | 100 | 2 | CIT,LPW |
| Orange Big Shot | 2 | 2 | 100 | 1 | MAC |
| Pink Big Shot | 6 | 3 | 50 | 3 | SSS, PBS,CFB |
| Pink Yellow Big Shot | 1 | 0 | 0 | 0 | none |
| Pink Quad | 1 | 0 | 0 | 0 | none |
| Pink Spider B S | 1 | 1 | 100 | 1 | DPS |
| Pink Trihybrid | 36 | 7 | 19 | 5 | LPW, DPS,YBS, PRI, WSP |
| Primulina | 3 | 2 | 66 | 2 | ELS,ZGF |
| Pulchella | 6 | 0 | 0 | 0 | none |
| San Carlos | 8 | 2 | 25 | 2 | IVS, GOL |
| Spider Lemon Pinwheel | 1 | 0 | 0 | 0 | none |
| Sunset Strain | 1 | 1 | 100 | 1 | MAC |
| Veined Pink B S | 1 | 1 | 100 | 1 | YQU |
| White Grandiflora | 2 | 2 | 100 | 1 | YBS |
| White Star PBS | 3 | 2 | 66 | 2 | CFB |
| White Pinwheel | 1 | 0 | 0 | 0 | none |
| White Trihybrid | 2 | 1 | 50 | 1 | PUL |
| Yellow Big Shot | 5 | 3 | 60 | 3 | DPS,PQU,YTP |
| Yellow Pinwheel | 1 | 0 | 0 | 0 | none |
| Yellow Quad hyb. | 4 | 2 | 50 | 2 | DPS,YTP |
| Yellow Spider BS | 1 | 0 | 0 | 0 | none |
| Yellow/Red Striped BS | 2 | 2 | 100 | 2 | ZGF,LYP |
| TOTAL | 210 | 93<br>44% | | | |

DSF: Dark Seedling Fireball; APQ: Apricot Queen; BPT: Best Pink Trihybrid; CAN: *Z. candida*; CFB: Cherry Fireball; FEB: Fireball; IVS: Ivory Star; JLP: Jonz Labuffarosea Pink; JZP: Jonz Pink; JSPG: Jonz Striped Pink Grandiflora; LFR: Labuffarosea: LPW: Lemon Pinwheel; LNQ: Lemon Quad; LYP: Lilypies; LDY: Lindlelyana; LWS: Lindlelyana White Star; MAC: *Z.macrosiphon*; OBS: Orange Big Shot; PBS: Pink Big Shot; PKG: Pink Grandiflora; PSBS: Pink Spider Big Shot; PTH: Pink Try Hybrid; SBS: Salmon Big Shot; PRI: *Z.primulina*; DPS: Dark Pink Spider; PUL: Pulchella; SNC: San Carlos; SSS: Sun Set Strain; CIT: *Z.citrina*; LPW: Lemon Pin Wheel; VBS: Veined Big Shot; WGF: White Grandiflora; WTH: White Trihybrid; ZGF: *Z. grandiflora*; PSS: Prairie Sun Set; TEX: *Habranthus Tubispsthus* var *texansis*; ELS: El Cielo Star; YQU: Yellow Quad; YRB: Yellow/Red Striped Big Shot: WSP: White Star Pink Big Shot; YTP: Yellow Throat Pink Big Shot.



 