ed with ORSV. This difference is particularly obvious with *Cattleya* and *Cattleya* intergeneric hybrids in which 42% (83) and 17% (33) of the 197 plants tested were infected with CymMV and ORSV, respectively. Interestingly, the reverse is true with regard to *Cymbidium* species and hybrids in which 21.5% (31) and 2% (3) of the 144 plants tested were infected with CymMV and ORSV, respectively.

The improvement in virus control methods and horticultural practices over the last 20 years would most likely reduce the probability of detecting CymRSV in new collections or commercial orchid businesses. Likewise, the probability of detection should increase with respect to the age of the plant. Therefore, specific emphasis was placed on sampling species and hybrids that have been in cultivation for 30 to 100 years. While this virus was first detected in a few *Cymbidium* plants during a routine survey of a commercial orchid nursery in Great Britain, the species was not reported. Therefore, *Cymbidium* plants which may have been in cultivation since the time of the first report were requested from collections which may have existed at that time. Given these data as well as those of Freitas et al. (1999) and Martelli (pers. comm.), it is our opinion that *cymbidium ringspot* tombusvirus does not infect orchids.

**Literature Cited**


**A SIMPLE LEAF-ASSAY METHOD FOR EVALUATING AGLAONEMA SENSITIVITY TO CHILLING TEMPERATURES**

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Abstract. *Aglaonema* is one of the most popular tropical ornamental foliage plants used indoors. Sensitivity to chilling temperatures is, however, a limitation to its production and successful utilization, especially with some cultivars. With increased releases of new cultivars, it would be beneficial if chilling tolerance of a new cultivar were known prior to its commercial production. In this study, a detached single-leaf assay technique was developed to compare with whole-plant evaluation of chilling injury. Results from 10 cultivars chilled at three temperatures (1.7, 7.2, and 12.8°C) for 24 hours showed that there was a high correlation between the single-leaf assay and the whole-plant evaluation. Among the 10 cultivars tested, ‘Silver Queen’ was the most sensitive, whereas ‘Stars’ and ‘Black Lance’ were the most tolerant. There was also a chilling response difference based on leaf maturity in the following order: mature leaves > old leaves > young leaves. A single detached mature leaf chilled for 24 hours will quickly indicate breeders, growers, and all others concerned of sensitivity of a cultivar to chilling temperatures.

*Aglaonema*, commonly called Chinese Evergreens, belongs to the family Araceae and is comprised of 21 species which are native to southeast Asia where they grow in the humid, heavily shaded tropical forests (Huxley, 1994). *Aglaonema* species have...
become one of the most popular tropical foliage plants in the ornamental plant market due to attractive variegated foliage, tolerance to low light and low humidity, and few disease and pest problems (Jervis, 1980). Sixty-two Florida wholesale nurseries in the FNGA Locator 1998-1999 have listed Aglaonema.

One limitation in the production of Aglaonema is chilling injury when plants are exposed to temperatures between 0°C and 12.8°C (Foosehee and McConnell, 1980; Henley et al., 1998; Hummel and Henny, 1986; Lyons, 1973). Chilling injury, characterized by dark and greasy-appearing patches on injured leaves, can result in unsaleable plants. Chilling injury may also occur during Aglaonema shipment, retail display, and interior decoration.

Recently, new cultivar release and cultivar discontinuations have been in a fast pace. For example, Aglaonema cultivars listed in the FNGA Locator 1997-1998 and 1998-1999 totaled 35 and 36, respectively, eight previously listed cultivars were dropped and nine new cultivars were introduced for 1998-1999. With the increased breeding activities and new cultivar releases, it would be beneficial if the chilling response of a new cultivar could be known prior to its commercial production. The objectives of this study were to characterize morphological changes of Aglaonema cultivars in response to chilling temperatures and to develop a reliable single-leaf assay method for simple and quick determination of cultivars' sensitivity to chilling temperatures.

Materials and Methods

Two experiments were conducted, one in summer and another in early spring, for developing the single-leaf assay method. In the summer experiment, secondary shoots of three Aglaonema cultivars 'Patricia', 'Silver Queen', and 'Stars', each with 10-15 leaves, were separated from stock plants and established singly in 20.3-cm (8-inch) pots containing Vergo Container Mix A (Verlite Co. Tampa, Fla.). The plants were grown in a shaded glasshouse at 1,000 foot candles and a relative humidity ranging from 70% to 95%. Temperatures in the shaded glasshouse ranged from 26.7 to 32.2°C. Forty-five days after rooting, 16 uniform plants of each cultivar were selected and total number of leaves per plant counted.

Temperatures in the walk-in coolers (3 m x 3 m) were set at 1.7, 7.2, and 12.8°C. Twelve volumetric flasks and deionized water for holding detached leaves were placed in each cooler overnight. The next day, the leaves of each plant were divided into three age groups: young (count from the recently expanded leaves up), mature (leaves immediately below the newly expanded leaves) and old (about three to four basal leaves). Using a clean razor blade, one whole leaf with petiole from each age group of a plant was excised from the main plant stem and placed into the flask filled with pre-cooled water. Each flask of leaves was placed into the cooler with its plant-of-origin for 24 hours. The control plants and corresponding excised leaves were placed in the shaded glasshouse. The experiment was a randomized complete design with four replications. The excised leaves and original plants were moved out of the cooler at the same time, and placed in the shaded glasshouse until all chilling sensitive evaluations were completed.

The primary indicator of chilling injury in this study was a visual foliage color blemish that developed after leaves were exposed to chilling temperatures. Chilling damaged leaves were counted every day for up to 10 days after chilling treatments, and damaged leaf area as well as total leaf area were measured using an LI-3100 area meter (LI-COR, Inc., Lincoln, Neb.). Chilling sensitivity in the whole plant assay was measured on the percentage of damaged leaves relative to total number of leaves per plant, and in the detached single-leaf assay was based on the percentage of leaf surface blemished. Differential sensitivity of leaf maturity was also measured.

Based on the results of the above experiment, a single-leaf assay method was developed and tested with 10 cultivars in an early spring experiment. A mature leaf was excised at the base of the petiole from each plant of the cultivars and placed in a flask containing pre-cooled water. The flasks and plants were placed in coolers with a temperature of 1.7°C. Twenty-four hours after chilling, plants and leaves were moved out of coolers and evaluated for sensitivity as described above. The experiment was a randomized complete design with three replications.

Results and Discussion

Visual symptoms of damaged leaves were most pronounced on plants from 1.7°C treatment and least severe in 12.8°C exposure. The percentage of total leaves per plant damaged from the chilling exposures for three cultivars are presented in Fig. 1. Values presented are the means of four plants of each cultivar used in the three chilling temperatures and the control 3 days after chilling. Results from the excised single-leaf assay were matched well with those from the whole-plant assay (Fig. 2).

There was significant genotypic variation in chilling sensitivity among the three cultivars 'Silver Queen', the most popular cultivar in the trade, was most vulnerable to chilling temperatures, with notable damage occurring at 12.8°C. 'Stars', however, developed few visual symptoms from chilling, and the sensitivity of cultivar 'Patricia' fell in between that of 'Silver Queen' and 'Stars' with only slightly damaged at 12.8°C. These results were in agreement with those of Hummel and Henny (1986) that marked cultivar differences exist in the genus Aglaonema in response to chilling temperatures. The chilling tolerant cultivars should have breeding potential for decreasing Aglaonema sensitivity to chilling temperatures.

In addition to cultivar differences, leaves of different maturity responded to the chilling temperatures differently (Figs. 1 and 2). Regardless how sensitive a cultivar is, mature and old leaves were more vulnerable to chilling than young leaves. The physiological basis for differential responses of leaves of different age to chilling temperatures is yet to be determined. However, this finding does provide useful information for developing a single-leaf assay for chilling temperature sensitivity in Aglaonema cultivars.

The correlation between whole-plant and single-leaf assays to chilling temperatures is presented in Fig. 3. The data for the whole-plant responses were the percentages of damaged leaves relative to the total numbers of leaves, and for the single-leaf assay were the percentages of damaged leaf area relative to the whole leaf area. The correlation coefficient was 0.95, suggesting that the single-leaf assay could be used as a quick and simple evaluative method to measure cultivar's tolerance to chilling temperatures.

A total of 10 Aglaonema cultivars were then evaluated using a single mature leaf against its whole plant-of-origin (Fig. 4). The single leaf-assay provided results similar to the whole plant evaluation. Given the results from the two experiments in connection with those of recent refrigerator tests, the pro-

Figure 1. Different maturity of leaves of three Aglaonema cultivars (a. ‘Patricia’; b. ‘Silver Queen’; c. ‘Stars’) responding to three chilling temperatures (1.7, 7.2, and 12.8°C). Plants were chilled in respective temperatures for 24 hours, then moved to shaded glasshouse for symptom development. Data were the percent of damaged leaves relative to total leaves 3 days after chilling.

Procedure of developed single-leaf assay method can then be summarized as follows:

1. Set the temperature of a refrigerator to a specific chilling temperature level within a range of 2 to 13°C and cool 2 liters of water and three cups (or bottles) in a refrigerator overnight. Be sure the refrigerator works properly and the temperature swing above and below the set point is not more than 2°C as the refrigerator cycle on and off. Place a thermometer inside the refrigerator to monitor the temperature.

2. Plants to be evaluated should be grown in a standard environment under the same light intensity, temperature, irrigation system and nutritional program for 4 weeks or more prior to testing. Cut a single mature leaf from a cultivar to be tested at the base of petiole using a clean razor blade, and place into a cup containing the pre-cooled water. At the same time, cut a mature leaf with intact petiole from both cultivars of ‘Silver Queen’ and ‘Stars’ and place the two leaves into the cup with the above leaf that is being tested. The test should have at least three replications, so three cups are needed and each should hold three mature leaves. Place cups into the refrigerator immediately, and avoid leaf contact with the refrigerator wall. Since ‘Silver Queen’ is the most sensitive and ‘Stars’ is the most tolerant cultivar, ‘Silver Queen’ will act as a negative control, while ‘Stars’ will be a positive control to ensure the reliability of the test.

3. Leave the cups with leaves in the refrigerator for 24 hours, and then move them from the refrigerator and place them in a greenhouse or in a room with relative humidity above 70% for about 2 days. If the test is conducted in the summer, chill damage symptoms should develop in 2 to 3 days after the treatment, whereas tests conducted in the winter or early spring may require more time or lower chilling temperature exposure for symptoms to occur.

Figure 2. Different maturity of single leaf of three Aglaonema cultivars (a. ‘Patricia’; b. ‘Silver Queen’; c. ‘Stars’) responding to three chilling temperatures (1.7, 7.2, and 12.8°C). Petioles of leaves were placed in flasks filled with pre-cooled water and chilled in respective temperatures for 24 hours, flasks were then moved to shaded glasshouse for symptom development. Data were the percent of damaged leaf area relative to total leaf area 3 days after chilling.
Figure 3. Correlation between whole-plant assay and single-leaf assay of three Aglaonema cultivars ('Patricia', 'Silver Queen', and 'Stars') in response to three chilling temperatures (1.7, 7.2, and 12.8°C). Data for whole-plant test were the percent of damaged leaves relative to total leaves and for single-leaf assay were the percent of damaged leaf area relative to total leaf area 3 days after chilling.

4. If chilling symptoms appear on the leaves of 'Silver Queen' and none are observed on the leaves of the tested cultivar(s) and 'Stars', results would indicate that the cultivar is more chilling resistant than 'Silver Queen' and tolerant to the temperature level tested. If the tested cultivar and 'Silver Queen' are both damaged, but 'Stars' has no damage or slight injury, it suggests that the tested cultivar is sensitive to the chilling temperature exposure. If none of the plants in the test are injured, the test was not successful; check the temperature of the refrigerator and run the assay again using new leaves from different plants. If all cultivars including Star are severely injured, the temperature was lower than suggested in the above instructions.

Conclusions

Results in this study showed significant cultivar differences within the genus Aglaonema in their sensitivity to chilling temperatures. Differences also occur in leaves of different maturity. Due to the slow rate of transpiration from Aglaonema leaves and tolerance to low humidity, the developed non-destructive single-leaf assay can now be used commercially as an evaluation tool on new Aglaonema cultivars to establish chilling sensitivity before large-scale commercial production is initiated. This method is simple and reliable, and can be used by breeders and growers with minimal investment of time, equipment and space.

Literature Cited


