Postharvest Management of Botrytis During Shipping and Storage

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BACKGROUND
American Floral Endowment considers Botrytis one of the cut flower industry’s most significant postharvest challenges. Temperatures and relative humidity (RH) during storage and shipping of cut flowers are often conducive to Botrytis infection and development, and closed shipping boxes allow ethylene (ET) build-up. This research with cut roses was conducted to better understand the relationship 1) between Botrytis susceptibility and ET sensitivity and 2) between temperature and RH within shipping boxes and Botrytis incidence.

Six experiments provide insight into the relationship between Botrytis and ET in cut roses. Two experiments screened cut rose cultivars for Botrytis susceptibility and ET sensitivity. Two investigated the relationship between Botrytis susceptibility and ET sensitivity and the influence of ET on Botrytis infection and development. Two investigated the influence of within-box temperature and RH during international transport on Botrytis incidence.

MATERIALS & METHODS

Exp. 1. Botrytis susceptibility was assessed for 27 cut rose cultivars. Entire stems were inoculated by spraying to run-off with solution containing 10^5 · ml⁻¹ Botrytis spores and incubated in plastic bags for 24 h. Control flowers were handled the same way except the inoculation solution did not contain spores. Flowers were placed into jars in tap water and observed daily through termination, which was attributed to Botrytis (Figure 1) or other reasons.

Exp. 2. Sensitivity to ET was assessed for the same rose cultivars as in Exp 1 by exposing them for 20 h to 0, 0.1, or 1.0 ppm ET prior to vase performance trials.

Exp. 3. The relationship between Botrytis susceptibility and ET sensitivity in cut roses was investigated using four cultivars based on being relatively Botrytis susceptible (B+) or not (B-) and/or relatively ET sensitive (E+) or not (E-): Cuenca (B+E+), Vendela (B+E-), Daphnee (B-E+)

Figure 1. Reasons for terminating cut roses due to Botrytis (cultivar): A) petal tips (Impression), B) petal bases (Jade), C) entire outer petals (Bonanza), D) entire middle petals (Domenica), E) entire inner petals (Sandra – outer petals pulled back), F) receptacle (Moon Walk).
Stems were exposed to 1.0 ppm ET or to an anti-ET agent: 700 nl · L⁻¹ 1-methylcyclopropene (1-MCP), activated charcoal to absorb ET, or 28.3 ppm silver thiosulfate (STS). After 24 h, half of the stems of each cultivar within each of the five treatments were inoculated with Botrytis and incubated as in Exp 1.

Exp. 4. Entire stems of the cultivars used in Exp 3 were inoculated with Botrytis and incubated as in Exp 1 before treatment with ET and anti-ET agents to gain insight into ET influence on Botrytis incidence and severity by altering post-infection disease development only.

Exp. 5. Prior to the experiment temperatures were recorded every 10 m within standard 200-rose, cardboard shipping boxes on eight occasions (twice each in Aug, Sep, Oct, Apr) for the duration of transport from a production farm near Bogotá, Colombia to the laboratory in Raleigh, NC. Temperature data (e.g. Figure 2a) were analyzed to provide ranges and fluctuations for shipping simulations (Figure 2b).

Exp. 6. Rose cultivars Sweet Unique and Hot Party were used to test the effects of flower packing density and pre-cooling of packed boxes prior to placement in coolers on temperature and RH within shipping boxes and subsequent influence on vase life and Botrytis incidence. Packing density was either 200 stems per box (completely full = densely packed) or 100 stems per box (= loosely packed). Boxes were moved from room temperature (= ambient) to a 3.5 °C cooler, and pre-cooling was accomplished on half the boxes by pulling 3.5 °C air through their vent holes with a high-speed exhaust fan.

RESULTS

Exp. 1. Three cultivars were highly Botrytis susceptible based on 100% of flowers terminated due to Botrytis when inoculated and very high Botrytis frequency when not inoculated: Jade, Ocean Song, Satina. Eleven cultivars were susceptible based on ≥54% termination due to Botrytis and vase life reduction of ≥40% when comparing inoculated to non-inoculated flowers: Bonanza, Cool Water, Cuenca, Dark Lulu, Domenica, Idole, Marisa, Orange Crush, Punch, Sprit, Vendela. Three cultivars were non-susceptible based on inoculated flowers having ≤27% termination due to Botrytis and vase life not significantly reduced for inoculated flowers compared to untreated flowers: Daphnee, Freedom, Hot Princess.

Exp. 2. Eight cultivars appeared to be ET sensitive based on having a significantly shorter vase life when treated with 1.0 ppm ET compared to no-ET controls: Caramba, Cool Water, Cuenca, Daphnee, Dark Lulu, Domenica, Ocean Song, Satina.

Exp. 3 and 4. As in Exp 1, cultivars Cuenca and Vendela were relatively Botrytis susceptible while Daphnee and Freedom were not. All cultivars had greatly increased Botrytis incidence when inoculated with B. cinerea compared to non-inoculated controls, but across all treatments
termination due to Botrytis was lower for the two non-susceptible than for the two susceptible cultivars.

As in Exp 2, cultivars Cuenca and Daphnee were relatively ET sensitive while Vendela and Freedom were not. Compared to 1-MCP and charcoal treatments, ET sensitive cultivars treated with ET had higher leaf drop frequency and increased flower openness, but these responses did not differ between STS treated and water control flowers. Compared to controls, exposure to ET resulted in shorter vase life for ET sensitive but not ET insensitive cultivars when ET was applied before (Cuenca and Daphnee) or after (Cuenca) inoculation.

While Botrytis inoculation did not influence the ET sensitivity variables of leaf drop and flower openness in any cultivar, ET and anti-ET treatments influenced all the Botrytis susceptibility variables of flower Botrytis damage, leaf Botrytis, and flower termination due to Botrytis in at least some cultivars. The ET insensitive and Botrytis non-susceptible cultivar 'Freedom' had more flower Botrytis damage, higher incidence of leaf Botrytis, and higher frequency of termination due to Botrytis when flowers were exposed to ET compared to 1-MCP treated and charcoal control flowers. Treatment of Freedom flowers with STS before Botrytis inoculation reduced flower Botrytis damage and frequency of termination due to Botrytis, but STS had no effect when applied after inoculation.

Data analyses at the finest level of treatment combination, show that ET exposure before or after B. cinerea inoculation and incubation can have a pronounced negative impact on Botrytis response variables. When ET was a pre-inoculation treatment there were 10 instances in which Botrytis responses were influenced by ET exposure compared to charcoal control flowers - all in inoculated flowers, and responses were negatively impacted by ET exposure in each case. When ET was a post-inoculation treatment there were 15 instances in which Botrytis responses were influenced by ET exposure compared to charcoal control flowers. Instances were in inoculated (7) and non-inoculated (8) flowers, and responses were negatively impacted by ET exposure in each case.

**Exp. 5.** No matter the time of year of the shipment, temperatures within shipping boxes during international cut rose transport followed a similar pattern that matched the stages of the shipping process. This pattern was similar to, but took total less time than, the single temperature recording published by Macnish et al. (2010). Our simulated shipping conditions based on the eight recorded temperature logs from actual shipments is shown in Fig. 2b. Compared to flowers stored at constant 3.5 °C for 5 days, flowers subjected to the fluctuating temperatures of simulated shipping exhibited a shorter subsequent vase life in two of the four cultivars, Freedom and Idole, which also had significantly shorter vase lives than

![Figure 3 a-b. Vase life (a) and frequency of stem termination due to Botrytis (b) in four cut rose cultivars after 5 d simulated transport with stable 3 °C or with temperatures fluctuating 4-12 °C as shown in Fig 2b.](image-url)
Cuenca and Vendela (Figure 3a). The latter two cultivars had greater frequency of stems terminated due to Botrytis when exposed to 5 d of fluctuating temperatures compared to stable 3.5 °C (Figure 3b), which was interesting because we had previously found Cuenca and Vendela to be relatively highly Botrytis susceptible.

Exp. 6. Compared to roses packed at typical, commercial density and placed into a 3.5 °C cooler, flowers that were loosely packed cooled more rapidly, particularly at the center of flower bunches (Figure 4 a and c). As expected, pre-cooling allowed flowers at both edges and centers of bunches to attain 7/8th the target temperature (6.3 °C and 3.5 °C, respectively) more rapidly than the flowers that were not pre-cooled (Figure 4 a and c). Bunch edges cooled more quickly but had more temperature fluctuation both while cooling down and once at the lowest storage temperature as compared to bunch centers (Figure 4c). Densely packed flowers remained warmer than loosely packed flowers throughout the cool-down process at both the center of edge of bunches (Figure 4 a and c). The RH within the center of bunches was ≥90% nearly the entire duration of the trial regardless of packing density or pre-cooling (Figure 4b). The RH at bunch edges was higher throughout the cooling period and duration of storage when boxes were densely packed (Figure 4d).

Following 5 d in a 3.5 °C cooler, the benefit of pre-cooling was seen in flowers once placed in vases. Compared to flowers from boxes that were at ambient temperature when placed into the

Figure 4 a-d. Temperature (a and c) and relative humidity (RH, b and d) at the center (a and b) or edge (c and d) of a bunch of roses within a cardboard shipping box that starts at ambient temperature (legend A) or with pre-cooling with forced 3.5 °C air for 30 m (legend P) and has been packed loosely (legend L) or densely (legend D) before being kept in a 3.5 °C cooler for 24 h. Temperature and RH was recorded every 10 m during the 24 h, and the trial was repeated four times.
cooler, flowers from boxes that were pre-cooled had much longer vase life (9.5 vs 6.3 d), slightly less open flowers at termination (1.9 vs 2.2), lower ratings for flower Botrytis damage at termination (2.3 vs 4.2), and much lower frequency of Botrytis on leaves (15% vs 70%). Influence of packing density was not as pronounced with densely packed and loosely packed flowers.

CONCLUSIONS
Screening of 27 cut rose cultivars currently in production in the industry.

- Current cultivars of cut roses vary greatly in Botrytis susceptibility and ET sensitivity.
- Botrytis susceptibility was not predictive of ET sensitivity and sensitivity to ET was not predictive of Botrytis susceptibility.
- Observed levels of Botrytis susceptibility and ET sensitivity for any given cultivar in controlled laboratory testing were not always the same as those observed during commercial production and postharvest handling.

Relationship between Botrytis susceptibility and ET sensitivity in four cut rose cultivars.

- Sensitivity to ET was not influenced by B. cinerea inoculation in any cultivar.
- Exposing cut roses to ET in many cases resulted in significantly more Botrytis damage.
- This response occurred across all of the treatments.

Temperatures and RH during shipping and Botrytis incidence in cut roses.

- The temperature pattern observed within shipping boxes from time of packing in Colombia through unpacking in Raleigh was similar at different times of the year and included two periods of high and fluctuating temperatures: 40 h (range 5 to 12 °C) in transit from the packing facility in Colombia through cargo handling at Miami airport and 28 h (range 10 to 24 °C) in transit from Miami to Raleigh.
- The within-box temperature fluctuations observed during international shipping can significantly reduce vase life and exacerbate Botrytis damage.
- Temperature and RH were in a range conducive to Botrytis germination and development most of the time during transit.
- The benefit of pre-cooling packed boxes before placement into coolers was seen in longer vase life, less rapid bud opening, less flower Botrytis damage, and lower frequency of Botrytis on leaves.

INDUSTRY IMPACT
Our most significant finding is that cut roses exposed to ethylene often exhibited reduced vase life and significantly more pronounced negative Botrytis responses compared to non-ethylene exposed flowers. This was observed in ethylene sensitive and insensitive cultivars, in Botrytis susceptible and non-susceptible cultivars, in Botrytis inoculated and non-inoculated flowers, and in flowers that were ethylene exposed before Botrytis infection and in flowers that were ethylene exposed after Botrytis infection. This means that, even if ethylene does not appear to have a major influence on a cut rose cultivar, ethylene exposure at the Botrytis establishment and/or development stage could have a significant negative impact on that cultivar by exacerbating Botrytis damage. It is also clear that shipping temperatures and methods can have a strong influence on Botrytis damage, with precooling being beneficial.