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Ozone as a Post-harvest Insecticidal Fumigant for Peony Cut Flower Market in Alaska
Preliminary Trial

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Introduction

At least 12 species of thrips have been found in commercial peony fields in Alaska (https://arctos.database.museum; University of Alaska Museum of the North Insect Collection, personal communication, March 2023). Many are incidentals blown in from field and forest edges near the peony fields. At least two species are known pests of many cultivated crops, including floral crops: western flower thrips (*Frankliniella occidentalis* Pergande) and onion thrips (*Thrips tabaci* Lindeman). Thrips insert eggs into immature peony buds with their sharp ovipositor. Additionally, due to their piercing/sucking mouth parts, immature and adult thrips can cause serious scarring to petals and sepals, bud distortion, cell destruction and flower bud abortion while feeding (Figure 1). Some thrips can complete their entire 7- to 10-day life cycle in the flower bud. Because of this short life cycle, thrips eggs may be able to hatch and spread to other buds during shipment (Alaska Department of Natural Resources Division of Agriculture, 2019; Gerdeman & Holloway, 2016).

Figure 1. Thrips on mature 'Sarah Bernhardt' flower.

Some species of thrips, including western flower and onion thrips, may be listed as quarantine species on agricultural products exported to certain foreign countries. During required state phytosanitary inspections, one tiny, living thrips found in a box of peonies can lead to rejection of the entire shipment, or the boxes may be returned to the grower for reconditioning which includes additional pest control treatments. If growers intend to export to foreign countries, they need a reliable method of killing thrips either in the field or after harvest (Alaska Department of Natural Resources Division of Agriculture, 2022).

Alaska growers employ several tools to manage thrips. Weed control in and around the field can minimize movement of thrips into the peony field. Growers may use field-applied insecticides (e.g., flonicamid), some of which are not registered for use on peonies. Other growers have experimented with killing thrips in cold storage using no-pest strips (active ingredient: dichlorvos, an organophosphate) available at home garden centers. Peony storage is not listed on the label of this commercial product and neither are thrips, except under the catch-all phrase, "kills flies, mealy bugs and other insects" (e.g., United States Department of Agriculture Environmental Protection Agency, 2003). It is a fumigant with significant public health concerns and has been banned from sale in the European Union (Fishel, 2019; Okoroiwu & Iwara, 2018; Smilanick, 2003). Scientists at USDA Agricultural Research Service (Davis, California) have studied other kinds of fumigants and aerosols (e.g., ethyl formate, sulphur dioxide, pyrethrum) that may hold promise for killing thrips in storage (Alaska Department of Natural Resources Division of Agriculture, 2022).

Ozone, which has the chemical formula O_3, has many commercial uses in agriculture due to its strong oxidizing power: water purification in hydroponic and irrigation systems, ethylene and pesticide degradation, and improvement of cold storage longevity of fruits and vegetables, mostly through insect and disease control (Abd El-Aziz, 2011). Ozone is also implicated as a powerful atmospheric pollutant in some regions, causing significant crop damage in fields and greenhouses (World Health Organization, 2000). There are few reports of ozone fumigation with cut flowers and ornamental plants in storage, and phytotoxicity (adverse effects of a treatment and/or chemical on plants and/or plant growth) is a major concern (Bumroongsook & Kilaso, 2018; Graham et.al., 2007; Hollingsworth & Armstrong, 2005). It is used mainly as an anti-microbial agent in ozonated water (Almasi et al., 2015; Mansoor et al., 2022; Robinson, 2006; Suslow, 2004) and as an air filter/ethylene scrubber in storage (Mansoor et al., 2022; Scariot et al., 2014). Additionally, ozone can degrade storage facilities that are not made of ozone-resistant materials (Apple Rubber, 2014).

Beginning in 2007, several Alaska growers began testing ozone generators in cold storage to control *Botrytis* gray mold on peonies. The ozone generators used by Alaska growers show concentrations only as high, medium or low, and the optimum duration of exposure is unknown. There are no previously published reports regarding peony cut flowers for ozone concentration and duration of exposure. Two Alaska growers lost their entire crop of stored peonies due to phytotoxic effects of the ozone treatment of unknown concentration and duration. Samples (up to 1.5 hour duration) of ozone concentrations at three farm coolers in 2023 ranged from a maximum of 7-20 ppm ozone (Gary Chastagner, Washington State University, Puyallup, Washington, and Ron Illingworth, North Pole Peonies, North Pole, Alaska, personal communication April 20, 2025).

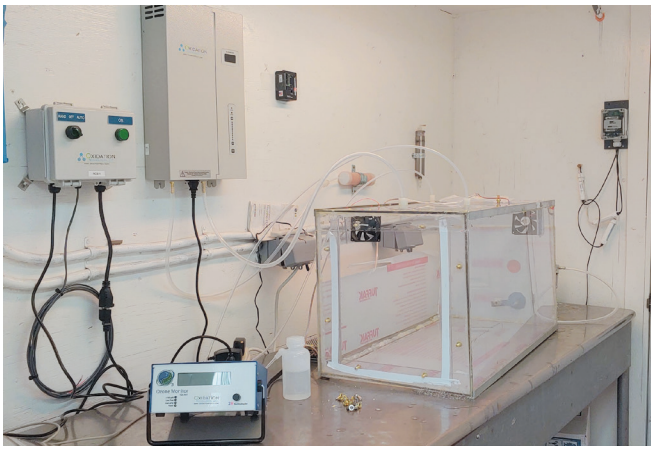

Figure 2. Laboratory setup for phytotoxicity and vase life studies.

The purpose of this project was to design an ozone chamber for treating peony cut stems, identify levels and duration of ozone necessary to kill thrips, and to document phytotoxic effects of ozone on peony cut flowers. This report is the result of a preliminary first-year trial.

Methods

An ozone generator was made with a plexiglass shell and controllers that fit into a cold storage unit's precooler (Figure 2). It was evaluated by multiple cut flower trials

for durability and ozone resistance and to establish treatment conditions for peony cut flower stems.

The chamber was made of nonreactive materials. Sheets of polycarbonate (0.25 in; 8.3 mm thick) were glued together to form the chamber. All seams were sealed using silicone caulking. Dry air was pulled through the system with a Rocker 300 air pump (New Star Environmental). Flow rates were 5 lpm (0.18 cfm). Silicone tubing was used to connect the ozone generator to the monitor, chamber and air pump. Two 3-inch-diameter fans were mounted inside the chamber to mix the air within the chamber. Ozone was produced by a VMUS-4 ozone generator (Azco Industries, LTD). Levels of ozone in the air flow were monitored by a UV-106-M ozone analyzer and controller (2B Technologies, Inc.) that measures ozone concentrations within ± 0.01 ppm in the range of 0 to 1000 ppm. Ozone concentrations and temperature were logged at 1-min intervals. When ozone concentrations in the air stream reached the setpoint, the controller signaled a relay to turn off the ozone generator. After ozone concentrations dropped 10 ppm below the setpoint, the controller signaled the relay to turn on the ozone generator. Ozone concentrations were maintained within ± 10 ppm of the setpoint. After completion of each trial, the air in the system was passed through a catalytic ozone destructor cylinder to eliminate ozone. Ambient air within the room was monitored in the range of 0 to 0.14 ppm with a wall-mounted Eco Sensors C-30ZX monitor (Eco Sensors, Inc.).

Figure 3. Laboratory setup for phytotoxicity and vase life studies.

'Sarah Bernhardt' peonies were harvested from the University of Alaska Fairbanks Agricultural and Forestry Experiment Station from a 10-year-old peony research plot. Stems were cut at maturity scale, 2.0 (Eason et al., 2002) when true petal color first appeared on firm, tight buds. This stage is an optimal cutting stage for export shipments. Stems were immediately moved to a precooler in a cold storage facility (65° ± 4°F; 18 ± 2°C). Each stem was cut to a length of 6-8 in (15-20 cm), and leaves were removed except for one to three single leaflets beneath the bud. Stems were placed upright in a beaker (no water) and inserted into the test chamber. Buds were treated with one of six levels of ozone: 0 ppm (control), 50-70 ppm, 90-110 ppm, 150-200 ppm, 250-275 ppm or 300-350 ppm for one of four durations: 0, 1, 2 or 3 hours. Because ozone dissipates rapidly, final readings were a range of ozone levels rather than a specific level. Treatments consisted of three replicates of 6-10 buds per treatment. Control buds were placed in the chamber for the three treatment times but with no ozone.

After treatment, buds were moved to an insulated portable picnic cooler in 24-hour darkness, 80% relative humidity. The picnic cooler was placed into a cold storage room at 36° ± 2°F (2.2° ± 1°C). Buds were held dry, for 48 hours, to simulate a short-term cold storage treatment. Following storage, stems were moved to the UAF Horticulture Laboratory. The bottoms of the stems were trimmed 1 in (2.5 cm) and placed into Mason jars that had been cleaned, sterilized, (10% Clorox, 30 minutes), rinsed and air-dried. Jars were filled (and re-filled as needed) with tap water (pH 8.2). Each jar contained a maximum of four stems randomly placed in the treatment area with replicates arranged in a randomized design. For each stem, we recorded cutting date, treatment, number of days to full bloom and petal fall/wilt. They were held at room temperature (68°-73°F; 20°-23°C), 80% relative humidity and 24-hour fluorescent lights. Protocols followed previous research (Holloway et al., 2010).

Data included days to full bloom and petal fall/wilt. Full bloom is the stage at which the base of the large guard petals is perpendicular to the stem. Some may never open fully, a condition that may be due to cultivar, cutting age, environmental conditions or phytotoxicity. The second phase was petal fall/wilt. Some flowers may wilt and not lose a single petal, or they show petal abscission of at least the guard petals. The days to each

Figure 4. Glass vials containing 10 thrips each for mortality trials.

event were recorded on each stem tag and any variations from expected were noted.

Each bud (including flowering stem, bracts, sepals, outer guard petals and foliage) was evaluated immediately upon being placed in the water for visible injury due to phytotoxicity. A visual damage score was assigned to each bud:

0 - no damage to flowering stem, bracts, sepals, petals and leaves; commercially acceptable

1 - slight damage consisting of minor surface blemishes; commercial use possible

2 - moderate damage consisting of discoloration, sunken tissues and pock marking on less than 50% of the tissues; commercial use unacceptable

3 - severe damage on >50% of the exposed tissues consisting of severe discoloration, sunken tissues, warped leaves and bracts, pock-markings on sepals, petals; commercial use unacceptable

Days to full bloom, days to petal fall/wilt, total vase life and failure to open were analyzed by analysis of variance for completely randomized design with three replicates and five stems per treatment, and/or regression analysis for ozone visual damage score.

Mortality of thrips was determined simultaneously with ozone exposure tests on peony buds. Because low numbers of thrips were found on flower buds at the harvest stage, adult thrips were collected in the field from open blossoms. Two glass 20 ml (0.7 fl oz) vials containing 10 thrips each were placed into the ozone chamber with the peony buds at 0 ppm, 50-70 ppm, and 90-110 ppm ozone for 1, 2 and 3 hours (Figure 4). After the exposure to ozone, the vials with thrips were removed from the chamber, emptied into a petri dish, covered and allowed to rest for at least 60 minutes before examining for thrips mortality.

Twelve peony stems were harvested from the field and brought directly into the lab. Petals, sepals, bracts (lower, abaxial surface), stems and mature leaves (lower, abaxial surface) were painted with a 1.6 cm^2 (0.5-in^2) square of clear nail polish (ethyl acetate/nitrocellulose). Once dried, the polish was removed with forceps and placed on a microscope slide. The hardened polish formed an imprint of the tissue surface to determine the presence of stomates.

Results and Discussion

Control

Control flower buds showed no phytoxicity, and all buds received a visual damage score of 0 (Figure 5). All buds reached full bloom stage and vase life averaged 7.5 days for control buds (Figures 6, 7).

50-70 ppm

All buds showed phytotoxicity at 50-70 ppm at all treatment times. Every bud received a visual damage score of 3. They were unacceptable as cut flowers. Damage appeared first in the bracts just beneath the buds. They were curled, discolored and papery. Damage also occurred on the flowering stem and sepals: brownish scars, pock-marked sunken areas and bruised appearance (Figure 8). One third of the buds exhibited slight discoloration on the petals at the exposed apex (Figure 8c).

Previous ozone research indicated that damage to tissues might be hastened by the presence of stomates that could act as a conduit for ozone to reach inner leaf tissues (World Health Organization, 2000). In our study, ozone damage appeared first in the bracts/leaflets at 50-70 ppm. However, the sepals also have stomates, and at 50-70 ppm, they showed damage only to the base, possibly due to the waxy cuticle that would protect the surface directly exposed to ozone. Peony tissues with stomates included leaflets/bracts, sepals and leaves (Figures 9a-9c). Tissues without stomates included petals and flowering stems. (Figures 9d, 9e). The presence of stomates was not a predictor of ozone damage at the lowest levels of ozone.

90-110 ppm

At 90-110 ppm ozone levels, major damage occurred on leaves, bracts and sepals, including discoloration, pock marking and sunken areas. All buds were given a visual

Figure 5. The control group of peony buds displaying no visual damage on outer petal (a), bract and sepal (b) or at the full bloom (c). Ozone concentration was 0 ppm for a duration of 2 hours.

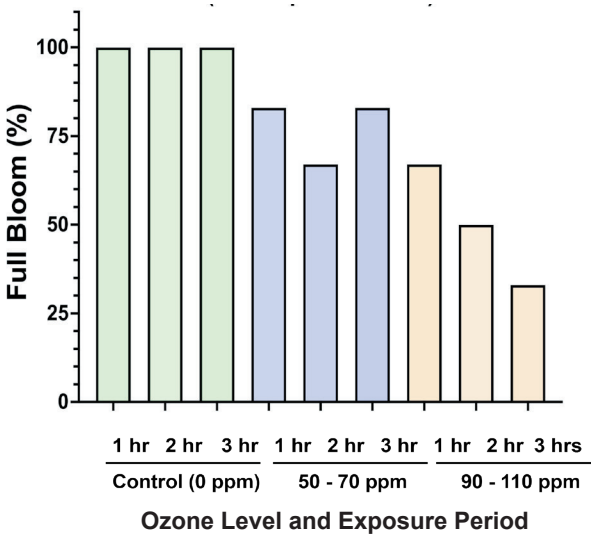

Figure 6. Percentage of buds reaching full bloom stage after treatment with ozone. Six buds per treatment.

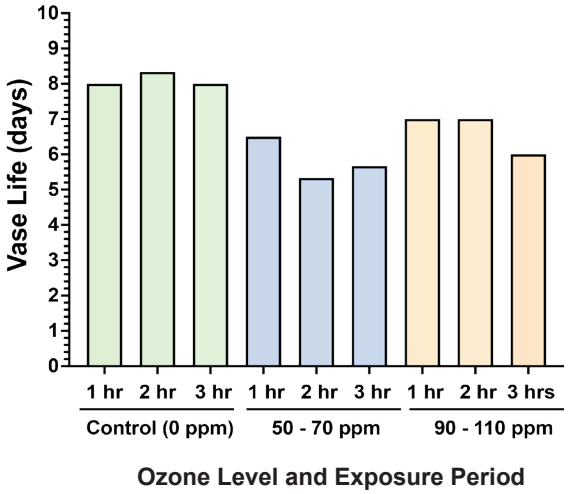

Figure 7. Vase life of buds that opened following treatment with ozone. Data do not include buds that did not open.

Figure 8. A visual damage score of 3 was observed on peony buds exposted to an ozone concentration of 50-70 ppm for a duration of 2 hours. Damage included bracts, flowering stem and base of sepals (a & b) and brownish speckling on outer guard petals (c).

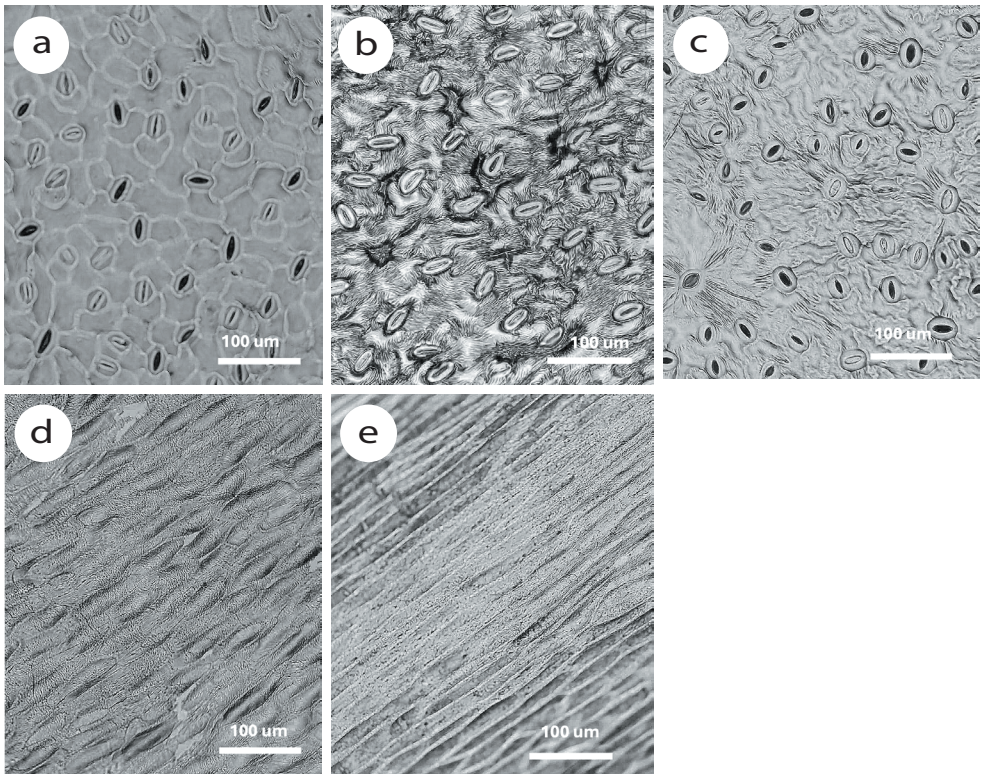

Figure 9. Imprints of peony surfaces denote presence or abscence of stomate:
(a) Stomate on the lower (abaxial) surface of a bract.
(b) Stomate on outer (abaxial) surface of a sepal.
(c) Stomate on lower (abaxial) leaf surface
(d) Cell imprint of outer (abaxial) surface of guard petals, no stomates
(e) Peony flowering stem, no stomates

damage score of 3 (Figure 10). All were commercially unacceptable. Despite this surface damage, most buds continued to open, appearing normal from above at full bloom (Figure 11a). Damage was extensive when buds were examined from below (Figure 11b).

≥150 ppm

At higher concentrations of ozone, phytotoxicity worsened, and all buds were given a visual damage score of 3. Damage occurred on the flowering stems, leaves, bracts, sepals and petals. Ten percent of all buds showed severe stem cracking beneath the buds. Most damage was concentrated on the outer (abaxial) exposed layers. As the flowers opened, inner petals appeared normal (Figures 12, 13, 14). All ozone treatments except the control showed reductions in bud opening to full bloom. However, all treatments had some flowers that reached the full bloom stage (Figure 6). Vase life also decreased with all ozone treatments, but results varied widely. All treatments were unacceptable commercially (Figure 7). Since all buds were given a visual damage score 3, these data are not significant except to guide the design of future replicated experiments.

Thrips mortality

The ozone treatments on vials containing thrips showed that all thrips survived in the control treatments, and mortality was 100% at the 90-110 ppm for all three exposure times. At 50-60 ppm, some thrips survived even after a 3-hour-long treatment (Table 1). Since phytotoxicity occurred at the 50-60 ppm ozone level, all ozone treatments failed to control thrips in this experiment. This preliminary experiment showed the visible effects of ozone damage on peonies at levels greater than 50 ppm for as little as 1 hour. Damage was severe enough to make all treatments except the control unmarketable. Not all thrips were killed at 50-70 ppm treatment for as little as one hour. The levels of ozone in cold storage reported by Alaska growers (0-20 ppm) will not be sufficient to kill thrips unless the duration of treatment is much longer than 3 hours. Future experiments should be designed to test 90-110 ppm ozone for durations of less than 1 hour. Alternatively, low levels of ozone (<50 ppm) for a long duration (i.e., 24 hours) should be tested.

	Thrips Mortality (%)		
Ozone Concentration (ppm)	Ozone Exposure 1 hr	Ozone Exposure 2 hr	Ozone Exposure 3 hr
0 (control)	0	0	0
50 – 70	55	65	75
90 - 110*	100	100	100

Table 1. Thrips mortality (%) after exposure to ozone, n=20 for each treatment.

Figure 10. Damage to flowering stems, leaves, bracts and sepals of peony buds exposted to an ozone concentration of 90-110 ppm for a duration of 2 hours. Damage includes cracked stem just beneath the bud (a) and darkening and speckling of outer petals (b).

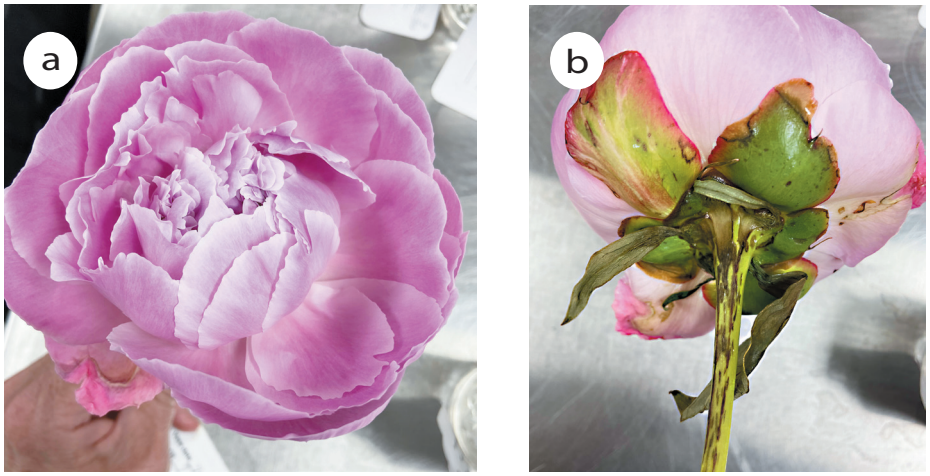

Figure 11. Bud exposed to an ozone concentration of 90-110 ppm for 3 hours appear to open normally when viewed from above (a). Ozone damage to the stem and sepals is visible when observed from below (b).

Figure 12. Peony bud exposed to an ozone concentration of 250-275 ppm for 1 hour showed significant darkening and browning of outer guard petals.

Figure 13. Peony bud exposed to an ozone concentration of 250-275 ppm for 2 hours showed severe darkening of outer guard petals, along with destruction of sepals and bracts. No visible damage to inner petals

Figure 14. Peony buds exposed to ozone concentration of 300-350 ppm for 1 hour resulted in the stem cracking beneath the bud.

Ozone hazards

Growers should be cautious when using ozone in cold storage because of its toxicity to humans, especially children. Ozone generators should not be used when people are loading and unloading flowers or working in the coolers. Additionally, since ozone impacts everything organic, machinery and cold storage facilities should be examined closely for premature cracks and degradation that could happen more frequently unless facilities are constructed using ozone-resistant materials.

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