Preharvest Absciscic Acid Application to Alleviate Chilling Injury of Sweet Basil (*Ocimum basilicum* L.) during Cold Storage

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Abstract. Fresh-cut sweet basil (*Ocimum basilicum* L.) is highly sensitive to low temperatures during postharvest storage. This study investigates whether preharvest foliar application of different concentrations of abscisic acid (ABA) can increase tolerance of the commercial basil varieties ‘Di Genova’ and ‘Nufar’ to chilling injury (CI) during postharvest storage at 3.5 °C and at 7 °C. Experiments were conducted under greenhouse and commercial open-field conditions in southwest Florida during the 2017/2018 growing season. Our results showed that greenhouse-grown plants were less affected by CI during 9 days of storage at 3.5 °C when treated with 1000 mg/L or 1500 mg/L ABA and at 7 °C storage compared with the water control, but effects varied by experiment. Preharvest applications of 1000 mg/L ABA were sufficient in reducing CI during cold storage at 3.5 °C in basil grown under open-field conditions; however, at 7 °C postharvest storage, chilling-induced damage did not differ between ABA and untreated plants. Electrolyte leakage analysis of leaves confirmed the beneficial effects of ABA on alleviating chilling-induced injury. Under greenhouse conditions, preharvest applications of 1000 mg/L ABA were more effective when plants were harvested at 1300 or 1530 hr than at 1100 hr. Our results suggest that 1000 mg/L foliar preharvest applications of ABA in combination with afternoon harvest are an effective strategy to alleviate CI damage during postharvest storage at temperatures less than 4 °C and to extend the shelf life of greenhouse or field-grown, fresh-cut basil.

In commercial herb production, handling, storage, and transportation can greatly affect shelf life and flavor and therefore marketable quality of the product. This study examines strategies to alleviate damage to sweet basil that occurs during low-temperature conditions following harvest. Sweet basil (*Ocimum basilicum* L.), a plant of tropical origin and member of the mint family (Lamiaceae), is grown in many regions around the world. Basil is grown under different climatic and ecological conditions but prefers warm sunny conditions and is susceptible to chilling during transport and storage at temperatures less than 5 to 10 °C (Caliskan et al., 2009; Lange and Cameron, 1997; Vitor et al., 2018). After harvest, commercially grown basil usually is transported from the packing facility to the local grocery store in refrigerated vehicles containing mixed loads of fresh produce.

Transport in these vehicles often occurs under nonoptimal environmental conditions, which can lead to considerable damage and loss of produce (Vigneault et al., 2009). Of particular concern for produce quality is temperature; temperatures that are too high result in spoilage, and temperatures that are too low result in freeze damage or CI. This is especially important for chilling-sensitive crops such as basil, which experience leaf injury and sometimes irreversible wilting or death when transported or stored at suboptimal temperatures. Different strategies have been investigated to reduce CI during cold storage. These include postharvest applications of biochemicals such as 1-methylcyclopropane (1-MCP) and salicylic acid (Berry et al., 2010; Supapvanich et al., 2015), light treatment (Coata et al., 2013), modification of air and temperature conditions (Lange and Cameron, 1997), and use of chilling-tolerant cultivars (López-Blancas et al., 2014).

Chilling stress induces various cellular, physiological, and physical changes in plants that include changes in antioxidant levels, hormonal concentrations, photosynthesis and respiration, lipid peroxidation, and loss of membrane integrity (Berry et al., 2010; Kratsch and Wise, 2001; Meir et al., 1997; Wongseree et al., 2009). In fresh green herbs, postharvest stress is manifested by reduced nutritional value, reduced aroma, off-odor, off-flavor, chlorophyll loss, and decay (Aharoni et al., 1993). In basil, chilling

stress manifests as leaf necrosis, spotting or browning, loss of leaf turgidity, and decay (Aharoni et al., 2010). At temperatures greater than 12 °C, CI symptoms are usually less severe, whereas temperatures less than 12 °C can cause severe damage during prolonged storage (Aharoni et al., 2010). In the basil cultivars Italico a foglia larga, Cammeo, and Italiano classico, in addition to more chilling damage, a reduced release of volatiles was observed during storage at 4 °C compared with storage at 12 °C (Cuzzolino et al., 2016). Depletion of polyphenol content and antioxidant capacity was observed during postharvest storage of basil at 4 °C but not at 12 °C (Fratiani et al., 2017).

Cold stress responses are regulated by various changes in plant metabolism including production of phytohormones (Eremina et al., 2016). ABA is a phytohormone that plays an important role in drought, cold, and salinity stress regulation (Eremina et al., 2016; Fernando and Schroeder, 2016; Verslues et al., 2006). Stressed plants often exhibit decreased water potential along with physiological changes, which include increased production of solutes to avoid dehydration and cell damage (Verslues et al., 2006). The most common response of plants to prevent stress-induced dehydration is stomata closure, a process that is triggered by ABA (Albert et al., 2017). Exogenous ABA applications are therefore often used as a strategy to maintain plant tissue turgidity and reduce cold stress damage in postharvest storage of vegetables and fruits (Parkin et al., 1989). In cucumber (*Cucumis sativus* L. Bet Alfa), ABA application 15 h before chilling showed a significant reduction in chilling-induced ion leakage (Rikin and Richmond, 1979). In mung beans (*Vigna radiata* L.), ABA was shown to reduce CI by lowering lipid peroxidation, membrane leakage, and hydrogen peroxide levels (Saleh, 2007). Exogenous ABA application also induced chilling tolerance in other crops such as rice, grapefruit, and squash (Shinkawa et al., 2013; Wang, 2010).

In addition to ABA application, other methods have been investigated to reduce cold-induced damage in basil but with varying success. Postharvest immersion of cut basil shoots in 1-MCP, an inhibitor of the cold stress-related hormone ethylene, did not significantly reduce CI symptoms in basil during postharvest storage at 5 °C (Berry et al., 2010). However, 1-MCP in combination with increased carbon dioxide levels significantly reduced postharvest decay at 12 °C in summer-grown basil (Kenigsbuch et al., 2009).

The time of day at which harvesting is conducted was found to play an important role in cold-induced damage during postharvest storage in vegetables and herbs (King et al., 1982; Lange and Cameron, 1997). The effect of harvesting time and postharvest storage temperature on the shelf life of sweet basil was demonstrated by Lange and Cameron (1994). Harvesting basil during the evening hours (1800 and 2200 hr) and storing at temperatures of 10, 15, and

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20 °C increased shelf life by nearly 100% compared with harvesting earlier in the day (0200 and 0600 HR). However, later harvesting times did not reduce CI severity during postharvest storage at lower temperatures (0 and 5 °C). Aharoni et al. (2010) demonstrated a similar interaction of harvesting time and temperature during postharvest storage of basil and suggested afternoon or evening harvest in combination with hot air treatment as a strategy to reduce chilling susceptibility. The suggested reason for harvest time-induced reduction of CI damage is the diurnal dynamics of plant photosynthesis and starch turnover leading to higher sugar concentrations, and therefore cell protection, at the end of the day compared with the beginning of the day (King et al., 1988; Stitt and Zeeman, 2012). Studies on *Arabidopsis* demonstrated the critical role of endogenous ABA concentrations in the regulation of carbohydrate metabolism under stressful conditions at the transcriptomic and metabolic level (Kempa et al., 2008; Thalmann et al., 2016).

Chilling-induced damage during postharvest transport and storage is a great concern for herb growers in Florida and other parts of the United States. Most strategies to prevent postharvest damage of vegetables and herbs have focused on the time period and temperature of postharvest storage, and less attention has been directed at developing strategies that may be applied during the preharvest stage. The objective of this study was to evaluate preharvest applications of ABA as a strategy to alleviate chilling-induced damage of commercial open field-grown basil in Florida and provide growers with a simple tool to increase marketable yield. Field studies were complemented by greenhouse studies to optimize applications and validate findings under open-field conditions.

Materials and Methods

Plant material and experiment design

Greenhouse trials. Seeds of the variety ‘Di Genova’ were sown into 4-L pots (five seeds/pot) containing Fafard No. 2 (Sun Gro Horticulture, Agawam, MA) potting medium. Plants were reduced to three per pot after emergence and maintained in a polycarbonate greenhouse at the University of Florida Southwest Florida Research and Education Center (SWFREC) in Immokalee, FL, under natural light conditions at average daytime and nighttime temperatures of 30 °C and 22 °C, respectively. Once seedlings were at the first true leaf stage, they were irrigated as needed with a nutrient solution containing N, P, K (20N–10P₂O₅–20K₂O) at a rate of 150 mg/L N (Peters Professional, Allentown, PA). Three weeks after emergence, plants were fertigated daily by an automated dripper system using the same fertilizer and rate of N. Three greenhouse trials were conducted from Nov. 2017 to May 2018. Treatments were applied five weeks after germination. Details of treatments and date of trials are summarized in Table 1. The experimental design

Table 1. Summary of greenhouse and field trials conducted from Nov. 2017 to May 2018.

Trial	Basil variety	ABA concn	Postharvest temp	Month/yr
<i>Greenhouse</i>				
1	Di Genova	1000 and 1500 mg/L	3.5 °C	Nov. 2017
2	Di Genova	1000 and 1500 mg/L	3.5 and 7.0 °C	Feb. 2018
3	Di Genova	1000 mg/L	3.5 and 7.0 °C	May 2018
<i>Field</i>				
1	Di Genova	1000 and 1500 mg/L	3.5 °C	Dec. 2017
2	Nufar	1000 and 1500 mg/L	3.5 °C	Jan. 2018
3	Nufar	1000 and 1500 mg/L	3.5 and 7.0 °C	Feb. 2018

was complete randomized with six replications per treatment, each replicate consisting of one pot with three plants.

Preharvest treatments of water or ABA (s-ABA, ProTone; Valent, Libertyville, IL) consisted of either 1000 mg/L ABA, 1500 mg/L ABA, or water (untreated control) in combination with 0.05% (v/v) Tween 20 (Sigma-Aldrich Inc., St. Louis, MO) as an adjuvant. An exploratory trial was conducted previously using 1000 mg/L and 2000 mg/L ABA to assess the suitable range of preharvest ABA concentrations. Foliar applications were conducted until runoff using a 1-L handheld sprayer. Time of application was 1000 to 1100 HR, and plants were harvested 24 h thereafter. Four mature fully expanded leaves were separated from each plant with petioles attached and packed into 17 × 10 × 2.5-cm commercial plastic clamshells (Easy-Pak LLC, Leominster, MA). Clamshells were stored for 9 d in the dark at either 3.5 °C or 7 °C. CI symptoms were rated at day 3, 6, and 9 of storage; leaf electrolyte leakage (LEL), total weight loss (TWL), and phenolic content were assessed on day 9 of postharvest storage (see respective sections below). A separate set of leaves was processed in the same manner to determine weight loss of leaves during postharvest storage.

To assess the effect of harvest time on chilling-induced injury during postharvest storage, plants from greenhouse trial 3 were harvested at 1300 and 1530 HR in addition to 1100 HR and processed as described previously.

Field trials. Field trials were conducted at C&B Farms near Clewiston, FL, between Nov. 2017 and Feb. 2018. Basil was sown in five rows on raised 1.3-m × 30-m beds. Three trials were conducted using varieties ‘Di Genova’ (trial 1) or ‘Nufar’ (trials 2–3); the switch in varieties was due to the grower’s response to consumer preference. Details of treatments and date of trials are summarized in Table 1. The experimental design was complete randomized with six replications per treatment with each replicate consisting of a 1.3- × 1.3-m plot. The number of basil plants in each plot was ≈200. Irrigation was by seepage, and fertilization, insecticide, and pesticide application were according to the grower’s standards.

Preharvest foliar applications were conducted when plants were 5 weeks of age. Treatments consisted of water (untreated control), 1000 mg/L ABA, and 1500 mg/L ABA in combination with 0.05% (v/v) Tween 20 as an adjuvant and were applied

using a 15-L backpack sprayer. About 200 mL of each treatment solution were applied to each plot (replicate) of basil (1–1.5 mg a.i. ABA/plant). Applications were conducted between 1000 and 1100 HR. Twenty-four plants were harvested randomly from each plot 24 h after applications. Stems were cut at a length of 20 to 25 cm from the top to include multiple nodes with mature and fully expanded leaves. Plants were immediately placed into macroperforated clear polyethylene bags, placed in commercial harvest crates, and transported to the SWFREC laboratory, where they were cut to size and packed into clamshells. Four mature fully expanded leaves from each plant with petioles attached were placed into each clamshell. Clamshells were stored at 3.5 °C and 7 °C as described previously. CI symptoms were rated at day 3, 6, and 9 of storage; LEL, TWL, and phenolic content were assessed on day 9 of postharvest storage (see respective sections below). A separate set of leaves was processed in the same manner to determine weight loss of leaves during postharvest storage.

CI assessment

CI ratings were conducted on a scale of 0 to 5. Ratings were based on size and extend of necrosis on the leaf surface with 0 = no visible damage, 1 = few small necrotic lesions, 2 = many small necrotic lesions, 3 = isolated large but narrow necrotic areas (1.5–2.0 cm in length) covering less than 50% of the leaf surface, 4 = large (>2 cm in length) necrotic areas covering 50% to 70% of the leaf surface, and 5 = severely damaged leaves with necrosis covering most of the leaf surface (Fig. 1). CI ratings were conducted on day 3, 6, and 9 during postharvest storage.

Leaf electrolyte leakage

LEL was measured on day 9 to assess changes in cell membrane integrity during cold storage. LEL was determined according to the protocol described by McKay (1992). In brief, electric conductivity (EC) of deionized water was measured before adding leaf samples (EC0). A total of 12 leaf discs (0.5 cm in diameter) per treatment combination were excised from leaf interveinal areas with a cork borer and suspended in 20 mL of deionized water. Leaf discs were incubated at room temperature on a shaker (60 rpm) for 20 h before measuring conductivity (EC1). Samples were then autoclaved at 110 °C for 10 min, cooled to room temperature, and conductivity was measured again (EC2). Loss of electrolytes was calculated as (EC1 – EC0)/(EC2 – EC0)

Fig. 1. Chilling injury (CI) score rating. CI scores were assigned on a scale of 0 to 5, with 0 = no visible damage (A); 1 = few isolated necrotic lesions (B); 2 = many small necrotic lesions (C); 3 = isolated large but narrow necrotic areas covering less than 50% of the leaf surface (D); 4 = large necrotic areas covering 50% to 75% of the leaf surface (E); and 5 = severely damaged leaves with necrosis covering most of the leaf surface (F).

and expressed as a percentage. Conductivity was measured with a conductivity meter (Orion Star; Thermo Scientific, Chelmsford, MA) in units of $\mu\text{S}/\text{cm}$.

Total weight loss

A separate set of leaves was used to analyze TWL during 9 d of cold storage. TWL was calculated as percent loss of weight measured before and 9 d after cold storage.

Total phenolics

Total phenolic content was determined on day 9 of cold storage using a modified method by Gajula et al. (2009). Basil leaves were ground in liquid nitrogen using mortar and pestle. Total phenolics were extracted twice from 150 to 200 mg fresh weight of ground leaf tissue for 30 min at room temperature in the dark with 1 mL of absolute ethanol. After centrifugation at 20,000 g for 5 min, combined supernatants were filtered through 0.22- μm spin filters (Corning, Tewksbury, MA) and dried in a Vacufuge plus (Eppendorf, Hamburg, Germany) for 1.5 h at room temperature. The dried extracts were stored at -80°C until further use. Samples were resuspended with 1 mL of water, vortexed, and centrifuged. Supernatants were used to quantify total phenolics spectrophotometrically (Spectramax 190; Molecular Devices, San Jose, CA) at 750 nm using Folin Ciocalteu reagent and gallic acid (Sigma-Aldrich) was used as a standard. Assays were conducted in duplicate in a 200- μL reaction volume. Phenolic concentrations were expressed in microgram gallic acid equivalents (GAE)/mg of tissue.

Statistical analysis

Data from all technical replicates were averaged for each biological replicate. Anal-

ysis of variance (ANOVA) was conducted using JMP Pro v13 software (SAS Inc., Cary, NC) for each trial, day, and postharvest storage temperature. Main effect means were separated using Tukey's honestly significant difference post hoc test. Kruskal-Wallis ANOVA followed by multiple comparison of mean ranks was used for analysis of CI scores. Differences were considered significant when $P < 0.05$.

Results

Influence of preharvest ABA applications on greenhouse-grown basil

Five-week-old 'Di Genova' plants treated with 1000 mg/L ABA before harvest in the exploratory greenhouse trial exhibited significantly less chilling-induced damage (1.88–2.52) than untreated plants (2.75–3.15) after 6 and 9 d of postharvest storage at 3.5°C (data not shown). CI scores of ABA applied at a concentration of 2000 mg/L did not significantly reduce CI compared with the control; for that reason, the following trials were conducted using 1000 mg/L and 1500 mg/L ABA.

Chilling injury. No differences of CI scores were detected in untreated and ABA-treated 'Di Genova' plants in any of the greenhouse trials on day 3 of postharvest storage at 3.5°C ; CI scores ranged from 0.11 to 0.29 in trial 3 to 1.54 to 1.88 in trial 1 (data not shown). Similar to the exploratory greenhouse trial, on day 6 significantly lower CI scores were observed for 1500 mg/L ABA-treated plants in trial 1 and for 1000 mg/L ABA-treated plants in trial 3 (Table 2). The same was found for day 9 of postharvest storage at 3.5°C when 1500 ABA and 1000 ABA reduced CI scores from 2.93 to 2.60 in trial 1 and from 1.96 to 0.72 in trial 3,

respectively. No significant differences between ABA-treated and untreated plants were observed at any time point in trial 2 after postharvest storage at 3.5°C .

Postharvest storage of 'Di Genova' leaves at 7°C did not result in development of severe CI symptoms during the first 6 d of storage and no significant differences of CI scores were found between untreated and ABA-treated plants. Compared with CI scores of untreated plants during storage at 3.5°C which ranged from 0.11 to 1.88 on day 3 (data not shown) to 1.67 to 2.93 on day 9, CI scores during storage at 7°C never exceeded 1.00. On day 9 of storage at 7°C , preharvest applications of 1000 mg/L ABA resulted in significant reductions of chilling-induced damage from 0.65 and 1.00 (untreated plants) to 0.19 in trials 2 and 3.

Leaf electrolyte leakage. LEL assessed 9 d after postharvest storage at 3.5°C was significantly reduced in response to both 1000 mg/L and 1500 mg/L preharvest ABA treatment in trial 2 and in response to 1000 mg/L ABA in trial 3 (Table 3). Percent electrolyte leakage was generally lower in trial 2 (25% to 35%) than in trial 3 (37% to 49%). In trial 2, leaves stored at 7°C for 9 d did not exhibit less LEL when plants were treated with ABA than when plants were untreated, contrary to trial 3, in which LEL was significantly lower after 1000 mg/L ABA treatment. Compared with postharvest storage at 3.5°C which resulted in 25% to 49% electrolyte leakage, leaves exhibited less (12% to 26%) electrolyte leakage at 7°C . LEL was not assessed for trial 1.

Total weight loss. Weight loss of leaves after 9 d of storage at 3.5°C was significantly less in 1500 mg/L ABA-treated plants from trial 1 (Table 4). Weight loss was not significantly reduced in leaves from trials 2 and 3

Table 2. Chilling injury (CI) assessment of greenhouse and field-grown basil (cvs. Di Genova and Nufar) 3, 6, and 9 d after postharvest storage at 3.5 and 7 °C with and without preharvest applications of abscisic acid (ABA). CI was rated on a scale of 0 to 5, with 0 = no visible damage and 5 = severely damaged leaves with necrosis covering most of the leaf surface.

Treatments	Temp	Greenhouse trial			Field trial		
		1	2	3	1	2	3
Day 6							
Water	3.5 °C	2.69 a	0.37	1.43 a	1.88 a	1.72 a	2.63
ABA 1000 mg/L	3.5 °C	2.72 a	0.19	0.62 b	0.89 b	1.29 b	2.43
ABA 1500 mg/L	3.5 °C	2.29 b	0.22	—	0.88 b	1.24 b	2.29
		<i>P</i> = 0.0435	<i>P</i> = 0.3162	<i>P</i> = 0.0247	<i>P</i> = 0.0235	<i>P</i> = 0.0118	<i>P</i> = 0.0774
Water	7 °C	—	0.38	0.00	—	—	2.01
ABA 1000 mg/L	7 °C	—	0.19	0.04	—	—	2.04
ABA 1500 mg/L	7 °C	—	0.22	—	—	—	2.13
			<i>P</i> = 0.6365	<i>P</i> = 0.4047			<i>P</i> = 0.7744
Day 9							
Water	3.5 °C	2.93 a	1.67	1.96 a	2.56 a	2.32 a	2.85
ABA 1000 mg/L	3.5 °C	2.89 a	1.41	0.72 b	1.65 b	1.65 b	2.53
ABA 1500 mg/L	3.5 °C	2.60 b	1.91	—	1.72 b	1.71 b	2.71
		<i>P</i> = 0.0553	<i>P</i> = 0.2930	<i>P</i> = 0.0078	<i>P</i> = 0.0237	<i>P</i> = 0.0067	<i>P</i> = 0.1887
Water	7 °C	—	1.00 a	0.65 a	—	—	2.04
ABA 1000 mg/L	7 °C	—	0.19 b	0.19 b	—	—	2.24
ABA 1500 mg/L	7 °C	—	0.59 ab	—	—	—	2.19
			<i>P</i> = 0.0141	<i>P</i> = 0.0240			<i>P</i> = 0.4544

Different letters within columns at each postharvest temperature and day of data collection indicate significant differences according to multiple comparison of mean ranks ($P < 0.05$).

Table 3. Leaf electrolyte leakage (%) of greenhouse and field-grown basil (cvs. Di Genova and Nufar) 9 d after cold storage at 3.5 and 7 °C with or without preharvest applications of abscisic acid (ABA). Electrolyte leakage is expressed in percent.

Treatments	Greenhouse trial		Field trial	
	2	3	2	3
<i>3.5 °C postharvest storage</i>				
Water	35.42 a	49.16 a	35.53 a	56.06 a
ABA 1000 mg/L	27.28 b	36.92 b	21.50 b	40.89 b
ABA 1500 mg/L	25.46 b	—	19.20 b	35.80 b
	<i>P</i> = 0.0083	<i>P</i> = 0.0377	<i>P</i> = 0.007	<i>P</i> < 0.0001
<i>7 °C postharvest storage</i>				
Water	24.08	15.11 a	—	33.48
ABA 1000 mg/L	21.59	11.79 b	—	29.53
ABA 1500 mg/L	25.67	—	—	30.21
	<i>P</i> = 0.5743	<i>P</i> = 0.0248		<i>P</i> = 0.3370

Different letters within columns at each postharvest temperature indicate significant differences according to Tukey's honestly significant difference test ($P < 0.05$).

when plants received preharvest treatments of 1000 ABA, but significantly more weight loss (19%) was observed in trial 2 for 1500 ABA-treated plants compared with untreated plants (6%). Weight loss of leaves stored at 7 °C postharvest was not reduced in response to 1000 mg/L ABA treatment in trials 2 and 3. In trial 2, similar to postharvest storage at 3.5 °C, weight loss after storage at 7 °C was significantly greater when plants were treated with 1500 mg/L ABA (16%) before harvest than when they were untreated (5%).

Total phenolics content. After 9 d of postharvest storage at 3.5 °C total phenolics content measured for plants from trial 1 was significantly ($P < 0.0001$) greater when plants were treated with 1000 and 1500 mg/L ABA (2.01–2.15 GAE/mg) compared with untreated plants (1.85 GAE/mg). The opposite was observed in trial 3, in which 1000 mg/L ABA significantly ($P = 0.0461$) reduced phenolics content from 2.13 to 1.98. Leaf phenolics content of plants from trial 2 was 1.35 to 1.64 GAE/mg during 3.5 °C storage, but no significant differences were measured in response to ABA treatments (data not shown). During postharvest storage at 7 °C,

leaf phenolics content ranged from 1.55 to 1.79 GAE/mg, but no significant differences were found between untreated and ABA-treated plants in any of the trials (data not shown).

Influence of preharvest ABA applications in open field-grown basil

In trial 1, preharvest applications of 'Di Genova' plants with 1000 mg/L and 1500 mg/L ABA under open-field conditions resulted in lower chilling damage compared with untreated plants after 3.5 °C postharvest storage (Table 2). Although no statistically significant differences were observed after 3 d of postharvest storage (data not shown), on day 6 CI scores of both 1000 mg/L and 1500 mg/L treated plants were significantly lower (0.88–0.89) than untreated plants (1.88). The same was observed on day 9 of storage, when CI scores of ABA-treated plants were 1.65 to 1.72 whereas those of untreated plants were 2.56. Weight loss of plants measured 9 d after postharvest storage at 3.5 °C was nonsignificantly different in ABA-treated and untreated plants (Table 4). Electrolyte leakage and phenolics content were not analyzed in this field trial.

Field trials 2 and 3 were conducted with variety 'Nufar'. In both trials, CI scores assessed on day 3 of postharvest storage at 3.5 °C were not significantly different between ABA-treated plants and untreated plants (data not shown). On day 6 of postharvest storage at 3.5 °C, 'Nufar' plants from trial 2 treated with both 1000 mg/L and 1500 mg/L ABA before harvest exhibited significantly lower CI scores (1.24–1.29) compared with untreated plants (1.72). The same was observed on day 9, when ABA-treated plants exhibited CI scores of 1.65 to 1.71 compared with untreated plants which exhibited CI scores of 2.32. In this field trial, LEL assessed 9 d after postharvest storage was also significantly lower in response to both ABA treatments (19% to 22%) compared with untreated plants (36%), but no significant differences were measured for TWL (Table 3). CI scores of ABA-treated plants from field trial 3 were not significantly reduced compared with untreated plants, but a trend ($P = 0.0774$) for lower chilling damage was observed for plants that received ABA before harvest on day 6 of postharvest storage at 3.5 °C. Compared with plants from field trials 1 and 2, plants from field trial 3 had considerably greater CI scores throughout the duration of the experiment. However, LEL was significantly lower (36% to 41%) in both 1000 and 1500 mg/L ABA-treated plants compared with untreated plants (56%) 9 d after postharvest storage at 3.5 °C (Table 3). Weight loss was not significantly different in ABA-treated and untreated plants from trial 3 (Table 4).

Postharvest storage at 7 °C was only assessed in trial 3 and did not result in any significant differences of CI scores between ABA-treated and untreated plants at any time during postharvest storage. In addition, no differences were observed for LEL or TWL at this storage temperature.

Total leaf phenolics content of plants from field trial 2 and 3 ranged from 1.88 to 2.16 GAE/mg and was not significantly different between untreated and ABA-treated plants stored at 3.5 or 7 °C (data not shown).

Influence of harvesting time and ABA application in greenhouse-grown basil

The effect of preharvest applications of 1000 mg/L ABA and harvesting time on CI after postharvest storage at 3.5 °C or 7 °C, and their interaction was investigated on greenhouse-grown 'Di Genova' plants. CI scores after 3 d of postharvest storage ranged from 0.00 to 0.46, but no significant differences were found in response to ABA pretreatment or time of harvest (data not shown). Six days after storage at 3.5 °C, CI scores were greatest in untreated plants harvested at 1530 hr and lowest in ABA-treated plants harvested 1300 hr and at 1530 hr (Table 5). Whereas treatment effect was significant, harvesting time was not. On day 9 of postharvest storage at 3.5 °C, treatment significantly affected CI scores; greatest scores (1.92–2.08) were measured for leaves from

Table 4. Total weight loss of greenhouse and field-grown basil (cvs. Di Genova and Nufar) 9 d after cold storage at 3.5 and 7 °C with or without preharvest applications of abscisic acid (ABA). Weight loss is expressed as % loss from day 0 to day 9 of cold storage.

Treatments	Greenhouse trial			Field trial		
	1	2	3	1	2	3
<i>3.5 °C postharvest storage</i>						
Water	14.44 a	6.18 b	7.85	14.03	16.28	6.60
ABA 1000 mg/L	10.05 ab	4.29 b	4.08	9.37	15.87	6.71
ABA 1500 mg/L	8.24 b	19.00 a	—	13.45	13.89	7.69
	$P = 0.0084$	$P < 0.0001$	$P = 0.1458$	$P = 0.1911$	$P = 0.4202$	$P = 0.7792$
<i>7 °C postharvest storage</i>						
Water	—	5.54 b	5.63	—	—	6.28
ABA 1000 mg/L	—	6.21 b	4.81	—	—	6.49
ABA 1500 mg/L	—	15.89 a	—	—	—	5.39
		$P < 0.0001$	$P = 0.3942$			$P = 0.5643$

Different letters within columns at each postharvest temperature indicate significant differences according to Tukey's honestly significant difference test ($P < 0.05$).

Table 5. Effect of harvesting time (HT) on chilling injury (CI), total weight loss (TWL), and leaf electrolyte leakage (LEL) of greenhouse-grown basil (cv. Di Genova) 3 to 9 d after postharvest storage at 3.5 and 7 °C with or without preharvest application of ABA. HT is expressed in military time.

Treatment	HT	Day 6	Day 9		
		CI	CI	TWL (%)	LEL (%)
3.5 °C postharvest storage					
Water	1100	1.04 ab	1.38 ab	5.14	49.73 ab
Water	1300	1.50 ab	1.92 a	7.92	41.09 ab
Water	1530	1.75 a	2.08 a	4.80	56.66 a
ABA	1100	0.96 ab	1.08 ab	3.93	45.58 ab
ABA	1300	0.42 b	0.50 b	3.84	32.45 b
ABA	1530	0.50 b	0.67 b	4.87	32.73 b
Treatment		<i>P</i> = 0.0210	<i>P</i> < 0.0010	<i>P</i> = 0.2362	<i>P</i> = 0.0017
HT		<i>P</i> = 0.8958	<i>P</i> = 0.7551	<i>P</i> = 0.8191	<i>P</i> = 0.0480
Treatment × HT		<i>P</i> = 0.1743	<i>P</i> = 0.0752	<i>P</i> = 0.7895	<i>P</i> = 0.0731
7 °C postharvest storage					
Water	1100	0.00	0.54 a	7.75 a	15.88 a
Water	1300	0.29	0.83 a	5.95 ab	13.68 abc
Water	1530	0.04	0.58 a	3.47 b	13.96 ab
ABA	1100	0.00	0.25 a	5.34 ab	11.51 bcd
ABA	1300	0.13	0.21 a	4.30 b	11.13 cd
ABA	1530	0.00	0.13 a	4.78 b	10.41 d
Treatment		<i>P</i> = 0.3253	<i>P</i> = 0.0278	<i>P</i> = 0.1251	<i>P</i> = 0.0050
HT		<i>P</i> = 0.3798	<i>P</i> = 0.7765	<i>P</i> = 0.0024	<i>P</i> = 0.1517
Treatment × HT		<i>P</i> = 0.3798	<i>P</i> = 0.7916	<i>P</i> = 0.0192	<i>P</i> = 0.6795

Different letters within columns at each postharvest temperature indicate significant differences according to Tukey's honestly significant difference test ($P < 0.05$).

untreated plants harvested at 1300 and 1530 HR, and lowest scores (0.50–0.67) were measured for leaves from ABA-treated plants also harvested at 1300 and 1530 HR. Although effect of harvesting time was not significant, there was a trend for interaction of harvesting time and treatment ($P = 0.0752$). No significant differences of CI scores were found at any time during 9 d of storage at 7 °C.

Weight loss of leaves measured on day 9 of postharvest storage at 3.5 °C did not correspond with CI scores, and no significant differences were detected in response to treatment or harvesting time. In contrast, LEL was significantly affected by treatment and harvesting time; LEL was greatest in untreated plants harvested at 1530 HR (57%) and lowest in ABA-treated plants harvested at either 1300 or 1530 HR (32% to 33%). There was a trend for interaction of treatment with harvesting time ($P = 0.0731$). Weight loss of leaves after 9 d of postharvest storage at 7 °C was greatest in untreated basil plants harvested at 1100 HR and lowest in untreated plants harvested at 1530 and ABA-treated plants at 1300 HR and 1530 HR. Whereas

treatment effect was not significant, harvesting time and the interaction of treatment and harvesting time were. LEL after 7 °C postharvest storage was greatest in untreated plants harvested at 1100 HR and lowest in ABA-treated plants harvested at 1300 HR and 1530 HR. Although treatment effect was significant, harvesting time and interaction of treatment and harvesting time were not. In general, LEL was considerably lower after postharvest storage at 7 °C compared with 3.5 °C.

No significant differences of total phenolics content were found in response to treatment or harvesting time no matter the postharvest storage temperature (data not shown).

Discussion

In this study, we evaluated the efficacy of foliar preharvest applications of ABA on alleviating CI in the two basil cultivars Di Genova and Nufar grown in greenhouse and open field under Florida climatic conditions. 'Di Genova' and 'Nufar' are flavorful basil varieties widely grown in basil producing

regions in the United States and worldwide. 'Nufar' is considered a less chilling-sensitive variety (López-Blancas et al., 2014) and is popular because of its resistance to *Fusarium* wilt (Shuler et al., 2008). The beneficial effects of preharvest foliar applications of ABA to alleviate chilling-induced damage were previously observed for cucumber (Rikin and Richmond, 1979). In addition, ABA has been used to alleviate drought stress in crops such as artichoke and muskmelon (Agehara and Leskovar, 2012; Shinohara and Leskovar, 2014). However, most studies were conducted under greenhouse conditions.

In an exploratory experiment, we applied ABA at a concentration of 1000 mg/L 24 h before harvest. This was effective in reducing postharvest CI in greenhouse-grown 'Di Genova' plants. Applying ABA at a concentration of 2000 mg/L did not have any beneficial effects. A series of greenhouse trials and open-field trials was conducted in the following from Nov. 2017 to May 2018 to evaluate efficacy of foliar preharvest applications of ABA at concentrations of 1000 and 1500 mg/L ABA in reducing CI of basil during postharvest storage below 4 °C (3.5 °C). For comparison, postharvest storage at 7 °C also was examined.

None of the greenhouse and field trials yielded significant differences of CI scores on day 3 of postharvest storage at 3.5 °C. In most of the greenhouse and field trials, however, basil plants treated with ABA applied either at 1000 mg/L or at 1500 mg/L exhibited less CI after 6 and 9 d of postharvest storage, suggesting that preharvest applications of ABA are effective in reducing CI damage during postharvest storage at temperatures below 4 °C. Whereas under greenhouse conditions the most effective ABA concentration varied by trial, under open-field conditions, both ABA applied at 1000 mg/L and at 1500 mg/L were similarly effective. The varying results between greenhouse experiments may be associated with different environmental conditions such as daylight and temperature during each month the trials were conducted (November, February, and May). In comparison, field trials were conducted during December to February.

Leaves from greenhouse-grown basil plants stored at a temperature of 7 °C following harvest generally exhibited less chilling-induced damage than those stored at 3.5 °C. This is consistent with other studies suggesting postharvest storage temperatures between 5 °C and 7 °C as optimal for long-term basil storage (Joyce et al., 1986). This reduction of CI damage was not as evident in field trials, possibly because of the additional stress leaves experienced during the time of transport from field to laboratory which generally resulted in higher CI damage at 7 °C compared with the greenhouse trials. More stress imposed on plants grown in a field environment compared with plants grown in a controlled greenhouse environment also may have contributed to these findings.

Leaf damage associated with abiotic stress such as chilling is a manifestation of

cellular damage, particularly loss of cell membrane integrity, and is usually associated with loss of water and electrolytes as well as changes in the oxidative status (Parkin et al., 1989; Verslues et al., 2006). Analysis of LEL conducted in our study showed a significant reduction of ion leakage in response to ABA applications after postharvest storage at 3.5 °C in both greenhouse and field studies, supporting the results of CI score analysis. The correspondence between CI damage and electrolyte leakage was previously reported in basil and in other crops (Liu et al., 2013; Vitor et al., 2018; Wongsheree et al., 2009). LEL was considerably lower during postharvest storage at 7 °C compared with 3.5 °C regardless of the treatment and, except for one greenhouse trial, no significant difference was observed between untreated and ABA-treated plants at 7 °C. This is consistent with the lower CI scores observed at this greater temperature compared with 3.5 °C. These results suggest that preharvest ABA applications are promising for maintaining cell membrane integrity and therefore leaf quality during cold storage at damage-inducing temperatures below 4 °C. Interestingly, despite a significant reduction of electrolyte leakage in greenhouse trial 2, no significant differences of CI scores between ABA-treated and untreated plants were observed at any time point during postharvest storage at 3.5 °C in this trial. This suggests that electrolyte leakage measurement may be useful as a tool for determining the leaf physiological state before noticeable leaf damage. LEL as a marker for CI was previously suggested by Cozzolino et al. (2016). Similar to our findings, this study measured significantly more loss of electrolytes 9 d after postharvest storage of 4 °C compared with 12 °C in the basil cultivars Cammeo and Italino classico. Correspondingly, lemon basil (*Ocimum ×citriodourum*) leaves experienced more loss of electrolytes within 48 h of storage at 4 °C (Wongsheree et al., 2009) than at 12 °C.

Weight loss of leaves was only influenced by 1500 mg/L ABA and only under greenhouse conditions and postharvest storage at 3.5 °C. Surprisingly, whereas ABA reduced weight loss in greenhouse trial 1, it increased weight loss in greenhouse trial 2. The reason for this is unclear but may be associated with the generally low chilling damage observed in this trial. This suggests that under reduced stress conditions high concentrations of ABA may be harmful.

Analysis of total leaf phenolics resulted in opposite responses to ABA in two of the greenhouse trials and postharvest storage at 3.5 °C. No differences between untreated and ABA-treated plants were measured under open-field conditions or postharvest storage at 7 °C. Phenolics are nonenzymatic antioxidants in the cell, and plants usually respond to oxidative stress with greater production of different types of phenolic compounds (Lattanzio, 2003; Sharma et al., 2012). Although our study on 'Di Genova' and 'Nufar' did not measure any consistent differences in

phenolics content, loss of total polyphenol content and antioxidant capacity was observed in other basil cultivars during postharvest storage at 4 °C compared with 12 °C (Fratianni et al., 2017). Cultivar-specific differences are likely one of the reasons for these different findings. This is supported by other studies in which postharvest storage at 6 °C reduced total phenolics content compared with storage at 18 °C in only one of four different basil cultivars tested (Kalisz et al., 2016). Contrary to the observations by Fratianni et al. (2017), this cultivar (lettuce leaf basil) responded with increased phenolics production. It is apparent that cultivars play an important role not only regarding extent but also direction of stress-induced changes in phenolics levels.

Plants store photosynthates as starch during the day and remobilize them during the night to support plant metabolism and growth (Stitt and Zeeman, 2012). Studies have shown that chilling sensitivity of tomato seedlings is greater at the end of the dark period and lower at the start of the dark period, demonstrating the importance of carbohydrates in cold tolerance (King et al., 1982, 1988). In our study, we investigated the effect of harvesting greenhouse-grown basil at 1100, 1300, and 1530 HR on CI after 9 d of postharvest storage. After preharvest applications of 1000 mg/L ABA, we observed significant reductions of CI scores but no significant differences in response to harvesting time. However, a trend for interaction of treatment with harvesting time was observed, suggesting that preharvest applications of ABA may be more effective in reducing CI during 3.5 °C postharvest storage when plants are harvested at 1300 or 1530 HR than when they are harvested at 1100 HR. Similar results were found for LEL, confirming that ABA preharvest applications were more beneficial when combined with afternoon harvesting of basil. At 7 °C postharvest storage weight loss was less in basil plants harvested in the afternoon regardless of the treatment. In another study on basil, harvesting plants between 1800 and 2200 HR increased shelf life by nearly 100% during postharvest storage at 10, 12, and 15 °C compared with harvesting between 0200 and 0600 HR (Lange and Cameron, 1994). Further studies are needed to determine whether evening or nighttime harvest provides a feasible strategy under Florida commercial conditions.

Conclusion

Marketability of produce is highly dependent on the aesthetics and quality of produce. The objective of our study was to evaluate the efficacy of ABA preharvest treatments on postharvest storage of basil at temperatures below and above 4 °C under greenhouse and commercial open-field conditions. We found that foliar application of ABA 24 h before harvest often improved chilling tolerance of the two basil varieties 'Di Genova' and 'Nufar'. Whereas the response of greenhouse-grown basil to 1000 or

1500 mg/L ABA varied by experiment, a concentration of 1000 mg/L ABA was sufficient to suppress CI in field-grown basil in two of three trials. Electrolyte leakage measurements of leaves sometimes supported these findings. Under greenhouse conditions, preharvest ABA applications were most effective in reducing CI during postharvest storage at 3.5 °C when plants were harvested later during the day. Taken together, our results demonstrate a positive influence of ABA on chilling tolerance and suggest that 1000 mg/L foliar preharvest applications of ABA in combination with afternoon harvest are a suitable strategy to alleviate chilling-induced injury during postharvest storage and to extend shelf life of commercially grown basil.

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