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Residual effect of low-pressure stress during simulated air transport on Beit Alpha-type cucumbers: Stomata behavior

É. Laurin [a,*], M.C.N. Nunes [b], Jean-Pierre Émond [c], Jeffrey K. Brecht [b]

[a] *Laval University, FSAA-SGA, Que., Canada*
[b] *Center for Food Distribution and Retailing, Horticultural Sciences Department, University of Florida, IFAS, Gainesville, FL 32611-0690, USA*
[c] *Center for Food Distribution and Retailing, Department of Agricultural and Biological Engineering, Gainesville, FL 32611-0570, USA*

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Abstract

The objective of this work was to elucidate the relationship between low pressure (LP) such as that found aboard cargo aircraft and the residual effect on stomatal aperture of Beit Alpha-type cucumbers during subsequent, simulated after-flight storage. In order to simulate flight conditions, cucumbers previously equilibrated to 4 °C were stored in darkness for 6 h at 71 kPa, 20 °C, and initial relative humidity (RH) of 70% in either airtight or non-airtight (air-flow rate of 0.415 L s^{-1}) containers. Cucumbers in airtight and non-airtight containers at 101 kPa were used as controls. After the flight simulation, the fruit were transferred to 101 kPa cold rooms at 20 °C and 70% RH or 7 °C and 90% RH and stored in darkness for 7 days. Exposure of cucumbers to 71 kPa in either airtight or non-airtight containers increased moisture loss after-flight simulation, and throughout storage compared to 101 kPa. Most stomata were fully or partially open immediately after-flight simulation regardless of treatment, however, cucumbers from the LP treatments retained significantly more open stomata compared to their respective controls after 96 h of subsequent storage at 101 kPa. This suggests that cucumbers exposed to a LP of 71 kPa for only 6 h, may exhibit an indirect stress response that occurs only when the fruit are returned to 101 kPa, preventing stomatal closure. This residual effect may be explained by the possibility that LP enhances outward diffusion of CO_2, reducing intercellular CO_2 concentration (C_i), and causing stomata to open. When the fruit are transferred to 101 kPa, stomata may remain open to restore the C_i.

Keywords: *Cucumis sativus*; Hypobaric; Low pressure; Stress response; Stomata aperture; Weight loss; Intercellular CO_2 concentration; Postharvest storage

1. Introduction

Air cargo transportation of perishables is the fastest growing segment of the air freight market, increasing at a rate of 14% per year (TIACA, 2004). However, the overall loss of produce shipped by air is averaging 25–35% per year, which is attributed primarily to poor control of environmental factors (TIACA, 2004).

Temperature is usually 20 °C or above inside aircraft perishable containers (Downs, 1985; Emond et al., 1999; Stera, 1999) and the cargo hold is pressurized to maintain just 71 kPa (ASHRAE Application Handbook, 1995). Although no data have been reported on the relative humidity (RH) of cargo holds, the same initial RH of 14–19% reported for the passenger cabin (Nagda and Hodgson, 2001) is expected inside the cargo hold because it is part of the same air-conditioned space. During flight, the RH in aircraft cargo compartments can vary widely according to the type of cargo being carried and its tendency to release water vapor. Moreover, inside produce containers, two scenarios for RH can exist, representing the extremes of the range of possible conditions. The first probable scenario is a closed environment, free of ventilation and air movement, since most of the pallets are shrink-wrapped and then placed inside the containers. In this situation, the RH is usually very high (near saturation) due to the water released by the produce transpiration. The water vapor is retained inside containers (Akinaga and Kohda, 1992) and increases the RH. The second scenario consists of an open environment, as the cargo hold is not airtight. In this case, the produce is placed inside a non-airtight container without a shrink-wrap layer, allowing air exchange with the ambient

* Corresponding author. Tel.: +1 352 392 1928x317; fax: +1 352 392 5653.
E-mail address: elaurin@abn-corp.com (É. Laurin).

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environment and therefore, the RH remains very low (around 14–30%). Several studies have addressed the impact of stress conditions such as temperature and RH fluctuations encountered during air shipment on perishables quality (Sharp, 1989; Akinaga and Kohda, 1992; Bollen et al., 1998; Stera, 1999; Emond et al., 1999; Laurin et al., 2003). However, the impact of low pressure (LP) in the range of 71 kPa alone and the combined effects of temperature, RH, and LP occurring during air cargo transportation on postharvest behavior of fresh produce are still unknown. Other studies have been conducted on the effects of LP (i.e., hypobaric) storage on the behavior of harvested commodities (Burg and Burg, 1966; Dilley, 1977; Lougheed et al., 1978; Goszczynska et al., 1988). The general conclusion was that LP in the range of 2–50 kPa have a beneficial effect on produce because of reduced O_2 partial pressure and because it increases outward diffusion of volatiles like ethylene and CO_2, limiting their accumulation inside tissues. However, reduced pressure inside ventilated chambers was also reported to enhance water loss of produce (Burg and Kosson, 1983). For instance, Andersen and Kirk (1986) reported higher water loss for hibiscus (*Hibiscus rosa-sinensis* L.) cuttings stored under hypobaric conditions than cuttings stored under normal atmospheric pressure. McKeown and Lougheed (1980) reported that the highest weight loss of asparagus (*Asparagus officinalis* L.) in hypobaric storage was found while the pressure was decreasing and RH was far from saturation.

Moisture is mainly lost from plant organs by transpiration and is regulated by the properties and actions of stomata, lenticels, cuticles, and epidermal cells (Ben-Yehoshua and Rodov, 2003). Hence, while increased water loss by fresh commodities when exposed to LP is largely a physical response to increased vapor pressure deficit (VPD), it may also be related to altered stomata aperture in photosynthetic organs (Kirk et al., 1986; Veierskov and Kirk, 1986; Collin and Folliot, 1990).

Accordingly, in previous work we have reported that the LP (i.e., 71 kPa) condition found aboard cargo aircraft enhanced water loss of Beit Alpha-type cucumbers (*Cucumis sativus* L.) both during the simulated flight and also after they were transferred back to normal atmospheric pressure (Laurin et al., 2005). The prolonged effect of previous LP exposure over the storage period suggests a stress response. We hypothesized that the increased water loss during the storage period by the cucumbers that had been exposed to simulated flight conditions was due to a low-pressure-induced opening of stomata.

The aim of this work was to elucidate the relationship between LP conditions found aboard aircraft, produce water loss, and stomatal aperture. More specifically, we wanted to study the residual effect of LP in the range of 71 kPa on stomatal function when cucumbers are returned back to normal atmospheric pressure. Finally, we considered whether the benefit of fast delivery provided by air cargo transportation might be overcome by the imbalance of environmental factors such as LP in terms of shelf life.

2. Material and methods

2.1. Plant material

Beit Alpha-type cucumbers (cv. Manar) grown in a greenhouse near Wellborn, Florida were harvested and sorted for uniformity of size and color at the grower facilities. Fruit were held in a cold room at 4 °C for 1 h after harvest then transported by air-conditioned vehicle to University of Florida facilities within 3 h after harvest. Upon arrival, fruit were stored at 4 °C for 6 h to allow preparation of the experimental set-up.

2.2. Flight simulation

In order to simulate the environmental conditions possibly experienced by produce during overseas flights, four treatments were applied to cucumbers. Atmospheric pressures of 71 or 101 kPa were applied under airtight conditions (no air movement) or non-airtight conditions (air flow of $0.415 \pm 0.005\ L\ s^{-1}$). Treatments were applied for 6 h in the dark. For each treatment, approximately $2.97–3.11 \pm 0.001$ kg of fruit were carefully weighed and placed in 7.5-L glass vacuum desiccators in order to provide a ratio of fruit:air volume similar to that in LD3 commercial air cargo containers. Among the 3.00 kg of fruit in each desiccator were six samples consisting of three cucumbers each that were individually weighed and identified. After the flight simulations, the previously weighed samples were transferred to loosely covered plastic containers and stored in darkness either at 20 °C and 70% RH or 7 °C and 90% RH for 7 days. The experiment was repeated using fruit from two different harvests.

2.3. Weight loss

Three samples of three fruit per treatment were weighed before the flight simulation treatments, after the treatments (6 h), and after subsequent storage for 24, 48, 72, 96, 120, and 144 h.

2.4. Scanning electron microscopy (SEM)

Three cucumbers per treatment per day were use for stomata aperture examination. A portion (3 cm^2) of the surface of each cucumber was covered with a thin layer of transparent fingernail polish (Collin and Folliot, 1990) and allowed to dry for 1 h. Imprints were always taken from the same location on the peel using new fruit for each sampling time. Imprints were mounted onto 13-mm aluminium mounting stubs, then sputter coated (model Desk Π, Denton Vacuum, Moorestown, NJ, USA) according to manufacturer instructions with Au/Pd. Peels were viewed and photographed on a field emission scanning electron microscope (model S-400, Hitachi High-Technologies Corp., Tokyo, Japan) for initial condition, after-flight simulation, and after 96 h. The