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Re-evaluating the role of bacteria in gerbera vase life

Rob E. Schouten*, Julian C. Verdonk, Uulke van Meeteren

Horticulture and Product Physiology, Plant Sciences Group, Wageningen University, Droevendaalsesteeg 1, 6708 PD Wageningen, The Netherlands

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ABSTRACT

The relation between bacteria numbers in vase water and vase life of gerbera cut flowers has recently been challenged because of reported negative effects of bactericidal compounds. This relation is investigated using two types of experiments that do not rely on antimicrobial compounds. The first type controls vase water temperature (4, 15, 22 or 28 °C) independently from air temperature (15, 22 or 28 °C) to investigate whether fresh weight behavior for two mini gerbera cultivars ('Okidoki' and 'Kimsey') is affected by bacterial growth and leaking of soluble sugars in the vase water, or by senescence of the flower head. Fresh weight loss, when compared at constant water temperatures, was higher at higher air temperatures. At higher water temperatures and constant air temperatures fresh weight loss was not higher, although bacterial levels were high enough to expect water uptake issues. Also sugar consumption in the vase water depended on water temperature. This indicates that senescence was the main reason for the decline in fresh weight for these flowers, not bacterial growth. The second type of experiments was based on adding predetermined levels of bacteria (0, 10^3 or 10^5 CFU mL^{-1}) and sugars (0.1% glucose or 0.2% sucrose) into vase water of flowers of three large-bloomed ('Carambole', 'Candela' and 'Iceberg') cultivars harvested with closed stem-ends and had their scapes sterilized before the start of vase life. When bacteria were added varying types of responses were observed. 'Carambole' flowers showed lower water uptake and lower transpiration and, early scape bending. Petal wilting was observed for 'Candela' flowers. 'Carambole' flowers showed higher scape sugar leakage levels in the vase water while 'Candela' flowers had higher scape firmness. 'Iceberg' flowers were also affected by bacteria, resulting in early scape bending, although sugar levels in the vase water were low. Furthermore, adding sucrose and/or bacteria in the vase water of one 'Iceberg' and one 'Carambole' flower in the same flask resulted in later scape bending for 'Iceberg' flowers compared to having two 'Carambole' or two 'Iceberg' flowers. The results indicate that bacteria interactions with gerbera flowers depend strongly on genotype.

1. Introduction

Gerbera (*Gerbera jamesonii*) cut flowers are popular because of their variable shapes and colors. However, they often suffer from short vase-life because of bending of the flower scape (stem existing of a long internode without leaves). Bending of a gerbera flower is caused by low turgescence of the flower scape when facing water uptake problems (van Meeteren, 1978a) and/or lack of sclerenchyma development during the elongation of the flower scape (Perik et al., 2012). Bacteria in the vase water might be the most common cause of xylem blockage affecting water uptake (van Meeteren, 1978a; van Doorn, 1997), but bacteria also damage the structure of the flower scape and speed up the senescence by releasing toxic substances (Jones and Hill, 1993). Bacteria in vase water originate from the hairy surface of Gerbera cut flowers (Put, 1990). The introduction of bacteria into vase water, shortened vase life of the gerbera, demonstrating the importance of bacteria for scape blockage (van Doorn and de Witte, 1994). Based on this, antimicrobial compounds such as silver nitrate (van Meeteren, 1978a; Abdel Kader and Rogers, 1986; Nair et al., 2003), 8-hydroxyquinoline citrate (Jones and Hill, 1993), sodium hypochlorite (van Meeteren, 1978a; Prasanth et al., 2009), dichloroisocyanuric acid (DICA) (van Meeteren, 1978a; Jones and Hill, 1993), and essential oils (Solgi et al., 2009) were applied to improve vase-life of cut flowers. However, commonly used antimicrobial compounds were shown to cause a plethora of effects: sometimes they delayed bending, other instances had no effect, and even hastened scape bending in gerbera (de Witte et al., 2014). A low correlation was reported between number of bacteria and time to bend. This was likely related to toxicity on the scape surface and xylem blockage due to dead scape cells. Addition of sucrose counteracted the toxicity which indicated that sugars might serve as alternative source of energy and might help in turgor maintenance (Perik et al., 2012). Therefore, the role of bacteria in the vase

* Corresponding author.
E-mail address: Rob.Schouten@wur.nl (R.E. Schouten).

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water with regard to gerbera vase life is still unclear.

The aim of the research presented here is to re-evaluate the relation between bacterial growth in the vase water and vase life for gerbera cultivars. This is carried out by setting up two types of experiments. The first type controls the vase water temperature independently from the air temperature as to influence bacteria growth without using bactericidal compounds preventing unknown side-effects. Bacterial growth, sugar leakage into the vase water, and flower fresh weight (FW) behavior at varying combinations of air and water temperatures were assessed to study the relation between bacterial growth and vase life limitation for two mini gerbera cultivars. In the second type of experiments, gerbera scapes of three large-bloomed gerbera cultivars were harvested using a sideways pulling motion at the scape base. This resulted in closed scape ends during transport in order to prevent entrance of bacteria into the hollow central part of gerbera scapes. Subsequently, scapes were sterilized. Vase life was assessed with added predetermined amounts of bacteria and sugars. FW behavior, water uptake, transpiration, sugar leakage, bacterial growth and scape characteristics such as firmness and elongation were determined to update existing concepts about the interaction between bacterial growth in the vase water and gerbera vase life.

2. Materials and methods

2.1. Plant material and setup of the temperature experiment

Flowers of two mini gerbera cultivars (pink 'Okidoki and yellow 'Kimsey') were obtained from the Delphy Improvement Centre (Bleiswijk, The Netherlands) in September 2010. Scape length of the harvested flowers was about 50 cm. Flowers were transported to the lab in Wageningen at room temperature in buckets with a low level of tap water containing sodium hypochlorite (0.1%) and stored overnight at room temperature. The next morning, the flowers were re-cut to 45 cm under water to prevent air emboli and the flowers were placed in Erlenmeyer flasks with 200 mL tap water. Flasks were covered with laboratory film (Parafilm®, Bemis, WI, USA) to diminish water evaporation from the flasks and to prevent bacteria entering from the air. Flasks with four flowers each, with three flasks per treatment, were placed in Styrofoam boxes filled with circulating water that was either cooled or heated to 4, 15, 22, or 28 °C. The boxes were placed in temperature and RH (Relative Humidity) controlled cabinets (Weiss Umwelttechnik GmbH, Balingen, Germany) with 200 μmol $m^{-2} s^{-1}$ PAR (Photosynthetically Active Radiation) supplied for 12 h per day. Air temperatures were set to 15, 22, or 28 °C. The air temperature and RH were set in such combinations that VPD (Vapor Pressure Deficit) was 0.92 kPa in all treatments. Bacteria number and sugar content in the vase water, and FW of the flowers were measured at regular intervals. Seven temperature treatments were used: 15–15, 15–22, 15–28, 22–4, 22–22, 22–28, and 28–28 °C (air-water) using four cabinets (Fig. 1A). The choice for the water temperatures is related to the hypothesis that water temperature affects bacterial growth but, not FW behavior, and air temperatures affects senescence. An overview of the light, temperature and RH/VPD conditions for each cabinet achieved during the temperature experiment is shown in Fig. 1B. The difference between set and measured temperatures were small, on average less than 1%, but were larger (about 3%) for most treatments with regard to the RH settings. Settings were disturbed when opening the cabinets for measurements which can be observed by spikes in the RH and VPD data.

2.2. Plant material and setup of the bacteria experiment

Flowers from three gerbera cultivars (red 'Carambole', orange 'Candela' and white 'Iceberg') were obtained from grower Flamma Flora (Waddinxveen, The Netherlands) in July 2012. To prevent contamination of the stems with bacteria during transport, flowers were dry transported to the lab in Wageningen with closed scape ends due to harvesting by twisting the scapes off near the point of attachment to the rhizome without cutting. After arrival, all the flower scapes were recut to 45 cm under water. The complete scapes were sterilized by shortly dipping the scape till its flower head in a 1% sodium hypochlorite with 0.1% Tween 20 solution, wiping the scapes with a tissue to remove air bubbles, and placing the scapes in a 1% sodium hypochlorite solution for two minutes. After rinsing with running tap water, gerbera flowers were put in Erlenmeyer flasks with 200 mL of vase life solutions (made up with tap water), one flower per flask covered with laboratory film (Parafilm®, Bemis, WI, USA). A bacteria vase water stock solution was prepared one week before starting the bacteria experiments by putting a mixed bunch of gerbera flowers from the local flower shop in tap water at room temperature. Treatments are shown in Table 1 with one flower per flask and three flasks per treatment.

2.3. Plant material and setup of the flower interaction experiment

Flowers from two gerbera cultivars ('Carambole' and 'Iceberg') were obtained from Flamma Flora in September 2012. Flowers with closed scapes were recut and sterilized as described above. Gerbera flowers were put in Erlenmeyer flasks with two flowers per flask covered with laboratory film (Parafilm®, Bemis, WI, USA). A bacteria sock solution was prepared as described above. Treatments are shown in Table 1 with four flasks per treatment.

2.4. Flower FW, water uptake and transpiration

FW was measured by recording the difference of flask weight with and without flowers during vas life. FW over time per flower was expressed as percentage of the weight at the start of the experiment. Water uptake rate and transpiration rate (both in mL d^{-1}) were calculated at fixed time intervals by recording the remaining water in flasks without flowers, and the combined weights of the flower and the flask, respectively.

2.5. Bacterial counts in the vase water

Samples of 2 mL were taken at regular time intervals of the vase water to measure bacteria numbers. Samples were divided into two subsamples, and each sub-sample was inoculated on three R2A agar plates (Reasoner and Geldreich, 1985) using a spiral plater (Eddy Jet, IUL Instruments, Barcelona, Spain). Colonies were counted by hand after incubation for 48 h at 28 °C and expressed as CFU mL^{-1}.

2.6. Petal relative water content

During vase life, one petal from the outer layer of each flower at regular time intervals was taken for calculating the relative water content (RWC in %) according to Eq. 1 (Smart and Bingham, 1974). Dry weight was measured after drying for 30 h at 65 °C while the turgid weight was determined after floating the petals in distilled water for 30 h.

$$\mathrm{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid Weight} - \text{Dry Weight}} \times 100 \quad (1)$$

2.7. Carbohydrate scape leakage and vase carbohydrate analysis

The liquid obtained from centrifuging one cm scape pieces from the lower part of the scape at a low centrifugal force ($5939 \times g$) for 5 min was collected in an Eppendorf tube. Carbohydrates were measured by HPLC after adding 1 mL of distilled water to the collected liquid. Carbohydrate scape leakage was obtained for eight scapes per cultivar. Scapes used for carbohydrate scape leakage were not used for vase life