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Effect of antimicrobial compounds on cut *Gerbera* flowers: Poor relation between stem bending and numbers of bacteria in the vase water

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ABSTRACT

Gerbera flowers (*Gerbera jamesonii*) often show stem bending. In four cultivars (Tamara, Liesbeth, Cora, and Mickey), we tested the effects on bending of antimicrobial compounds (chlorine bleach, a slow release chlorine compound, 8-hydroxyquinoline citrate [HQC], silver nitrate, carvacrol and thymol), some combined with sugars. At concentrations used for other cut flowers, inclusion in the vase solution of several of the antimicrobial compounds delayed bending, had no effect, or hastened bending. Hastening of bending was found at higher concentrations. It was accompanied with visible damage on the stem ends. Results with HQC indicated high toxicity as it did not delay bending at any of the concentration tested (100–400 mg L^{-1}). At 200 mg L^{-1} HQC induced growth of bacteria that were not found in the controls. The number of bacteria in the vase water showed a low correlation with bending. Visible toxicity on the stem surface was often associated with a high bacteria count. However, at relatively high concentrations of the antimicrobial compounds stem bending was associated with a low count. This indicated an effect other than bacteria. Water uptake was low in stems that bent early. It is hypothesized that material from dead stem cells resulted in a xylem blockage which led to early bending. Sucrose at 15 g L^{-1} in combination with an antimicrobial compound (slow release chlorine, HQC) resulted in the absence of stem damage and produced much less bending than the same concentration of the antimicrobial compounds alone. Sucrose apparently counteracted the toxic effects of the antimicrobial chemicals.

1. Introduction

Stem bending during early stages of vase life is a major problem in many cultivars of cut *Gerbera* flowers. Inhibiting bacterial growth in the vase water can reduce the incidence of stem bending (Penningfeld and Forchthammer, 1966; van Meeteren, 1978; Jones and Hill, 1993; Liu et al., 2009), suggesting that bacteria are a main cause. The addition of bacteria to the vase water of freshly harvested *Gerbera* stems resulted in rapid stem bending, which further supports the idea that vase water bacteria can be involved. Bending in cv. Liesbeth was induced by water containing more than 10^6 mL^{-1} colony forming units of bacteria (van Doorn and de Witte, 1994).

Currently, the postharvest treatment of *Gerbera* flowers in the Netherlands is limited to dipping the stems, shortly after harvest, for about 24 h at 20 °C in a solution containing 20 mg L^{-1} active ingredient of chlorine bleach (sodium hypochlorite). No advice is given as to any further antimicrobial treatment after harvest, although florists often provide a sachet containing sugars and an antimicrobial compound, which is to be included in the vase water.

Several antimicrobial compounds have been used in tests with *Gerbera* flowers, with or without sugars. These compounds included silver nitrate (Penningfeld and Forchthammer, 1966; van Meeteren, 1978; Steinitz, 1984; Abdel Kader and Rogers, 1986; Nair et al., 2003), 8-hydroxyquinoline citrate (HQC; Jones and Hill, 1993; Nair et al., 2003; Solgi et al., 2009), chlorine bleach (sodium hypochlorite; Prasanth et al., 2009), dichloroisocyanuric acid (DICA; Jones and Hill, 1993), and essential oils such as carvacrol and thymol (Solgi et al., 2009). Other compounds tested in cut *Gerbera* flowers have been described by various authors (van Meeteren, 1978; Jones et al., 1993; Pompodakis et al., 2004; Macnish et al., 2005; Meman and Dabhi, 2006; Liu et al., 2009; Prasanth et al., 2009).

In other cut flowers such as roses, antimicrobial compounds in the vase water applied at adequate concentrations inhibited bacterial growth and delayed both a bacterial blockage in the xylem and flower wilting (which is due to loss of turgor; van Doorn, 1997, 2012). In these experiments sodium hyochlorite was used at 20–80 mg L^{-1}, DICA at 25–400 mg L^{-1}, and HQC at 125–750 mg L^{-1}. The number of bacteria in vase water was inversely related to the

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Table 1
Effects of antimicrobial compounds with and without sucrose in the vase water on cut gerbera flowers (*Gerbera jamesonii*) cvs. Tamara and Liesbeth. Data on cv. Tamara refer to two repeat experiments. Time to stem bending is expressed in days of vase life. Water uptake refers to day 0–3 of vase life, and to the second experiment with cv. Tamara. Vase water bacteria were counted when in the treatment that induced most bending, about half of the stems had bent (day 3 and 4 in the experiment with cv. Tamara and cv. Liesbeth, respectively). Bacterial counts refer to the second experiment of cv. Tamara. a.i.: active ingredient (sodium hypochlorite), cfu: colony forming units.

Treatments		Time to stem bending (d)[a]		Water uptake ($mg\,g^{-1}$ FW h^{-1})[a]	Bacterial count ($cfu\,mL^{-1}$)	Stem damage
Cv. Tamara		Exp. 1	Exp. 2			
Control		6.2 b	6.8 b	10 b	4.9×10^7	
Bleach	10 mg L^{-1} a.i.	11.5 a	7.3 b	12 b	$<10^2$	
	20 mg L^{-1} a.i.	8.2 ab	8.9 ab	13 b	1.8×10^5	Moderate
DICA	50 mg L^{-1}	11.9 a	13.1 a	17 a	$<10^2$	
	100 mg L^{-1}	14.4 a	15.0 a	20 a	$<10^2$	Slight
	200 mg L^{-1}	5.8 b	6.0 b	9 b	6.5×10^6	Moderate
HQC	100 mg L^{-1}	6.0 b	6.1 b	10 b	5.9×10^4	Slight
	200 mg L^{-1}	5.0 c	5.1 c	6 c	1.8×10^5	Moderate
	400 mg L^{-1}	4.9 c	4.6 c	5 c	1.3×10^6	High
$AgNO_3$	0.06 mM	6.7 b	7.1 b	15 ab	5.2×10^5	Slight browning
	0.12 mM	8.9 ab	8.3 b	13 b	–	Fully brown
	0.24 mM	12.2 a	12.8 a	19 a	$<10^2$	Dark brown
Cv. Liesbeth						
Control			7.9 b	14 a	4.5×10^7	
DICA	50 mg L^{-1}		8.9 b	13 a	8.2×10^6	
	100 mg L^{-1}		4.0 c	6 c	1.3×10^5	Slight
HQC	200 mg L^{-1}		4.7 c	7 c	1.8×10^5	Moderate
	400 mg L^{-1}		5.3 c	5 c	$<10^2$	High
DICA	50 mg L^{-1} + sucrose 15 g L^{-1}		14.2 a	9 a	$<10^2$	
	100 mg L^{-1} + sucrose 15 g L^{-1}		16.0 a	10 a	$<10^2$	
HQC	200 mg L^{-1} + sucrose 15 g L^{-1}		12.9 a	9 a	1.1×10^5	
	400 mg L^{-1} + sucrose 15 g L^{-1}		14.9 a	9 a	$<10^2$	

[a] Results are means of ten replications. Data per experiment and column with a different letter are statistically different ($P < 0.05$).

concentration of antimicrobial compound. No stem damage was observed in these experiments (van Doorn et al., 1989, 1990; and unpublished data). Preliminary experiments with *Gerbera* cv. Liesbeth, by contrast, indicated that an inverse relationship between the time to bending (which is due to turgor loss) and the number of bacteria in the vase water was absent.

Sucrose in the vase solution often increases flower life span. It serves as a source of energy, and in *Gerbera* might aid in turgor maintenance (Perik et al., 2012). However, sucrose has the propensity to encourage proliferation of bacteria. It is therefore usually investigated together with an antimicrobial chemical (van Doorn, 1997).

We here tested in more detail the effect of antimicrobial compounds (chlorine bleach, DICA, HQC, silver nitrate, carvacrol and thymol) on the time to stem bending, and compared this with water uptake by the cut flowers and the number of bacteria in the vase solution. We used two *Gerbera* cultivars that in preliminary experiments tended to bend very early (cvs. Tamara and Liesbeth), one that bent somewhat later (cv. Cora), and one that did not show stem bending (cv. Mickey). We also investigated treatments in which some antimicrobial compounds were combined with sucrose. We tested the hypothesis that early stem bending in *Gerbera* cultivars is associated with bacterial numbers in the vase solution that are the same or higher than in the controls.

2. Materials and methods

2.1. Plant material, stem bending

Cut *Gerbera* flowers (*Gerbera jamesonii* Bolus) were obtained from commercial growers. Cultivars used were Tamara, Liesbeth, Mickey, and Cora. Plants were grown in soilless culture. Flowers were harvested at commercial maturity, i.e. when the two outer whorls of flowers in the flower head showed mature stamens. Harvest occurred by hand, using a sideways pulling motion at the stem base. After harvest, the flowers were placed in cardboard boxes and were transported dry to the laboratory in a non-refrigerated car. The time between harvest and arrival in the laboratory was less than 4 h. Upon arrival, the flowers were immediately used for experimentation or were placed dry at 5 °C for at most 4 h before experimentation. Flowers were selected for uniformity, and part of the stem ends (about 5 cm) was re-cut in air, resulting in stems of 45 cm length.

Flowers were individually placed in glass bottles of 20 cm height. The initial angle under which the flower stems are placed in the vase might be important for bending. In these experiments all flowers had an initial stem angle of 15° with respect to vertical. The bottles contained approx. 250 ml of demineralised water, with or without added chemicals (see below). Bottles were in a climate-controlled room at 20 °C and 60% RH. The photosynthetic quantum flux at the floral head was 15 $\mu mol\,m^{-2}\,s^{-1}$. Light was provided by Philips 36W/84 cool white fluorescent tubes, using a 12 h photoperiod (07.00–19.00).

2.2. Evalution of stem bending

Stem bending was defined as in a previous paper (Perik et al., 2012). Briefly, bending was determined by assessing the position of the floral head. Bending had occurred when the upper surface of the floral head moved beyond vertical.

2.3. Chemicals

Chemicals were included in the vase water at the onset of vase life and were not replenished. Chemicals were obtained from Sigma, unless otherwise indicated. Antimicrobial compounds were bleach (sodium hypochlorite), dichloroisocyanuric acid (DICA), 8-hydroxyquinoline citrate (HQC, La Quinoléine, Oissel, France), silver nitrate, carvacrol (Merck) and thymol. All chemicals were directly dissolved in water, except thymol which was first dissolved in ethanol. The applied concentrations are mentioned in Tables 1–3. DICA and HQC were also applied with 1.5 g L^{-1} sucrose. Aminoethoxyvinylglycine (AVG, also indicated as [S]-*trans*-2-amino-4-(2-aminoethoxy)-butenoic acid hydrochloride),