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Bacterial populations related to gerbera (*Gerbera jamesonii* L.) stem break

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Summary. Bacterial distribution, both external (epiphytic) and internal (endophytic), on *Gerbera jamesonii* L. cv. Provence and its relationship to gerbera stem break and ethylene production were investigated. The greatest number of epiphytic bacteria was found at capitulum level and 20 cm below. Three genera of bacteria were identified: *Acinetobacter*, *Bacillus* and *Pantoea***.** A silver-nitrate solution greatly reduced ethylene production in cut flowers. The use of acid fuchsin solution revealed an occlusion of the xylem vessels, probably due to bacterial cells. The bacteria *Acinetobacter*, *Pantoea* and *Bacillus* appeared to be involved in stem break once their populations reached 10^5 cfu g^{-1} of stem tissue.

Key words: epiphytic, endophytic, ethylene production, maintenance solutions.

Introduction

Stem break of gerbera cut flowers is a phenomenon mainly caused by water shortage in the flowers due to the increased difficulty of water flow between the water reservoir and the petals of the flowers (Zieslin *et al*., 1978). There appears to be a competition for the available water between the gerbera flower heads and stems. The increase in flow resistance leads to stem break as a result of microbial activity in the vase water (Aarts, 1957). When the scapes are in a tap water solution they bend and collapse but if silver nitrate (Steinitz, 1983), sodium hypochloride, or dichlorophan (Van Meeteren, 1978) germicides are added to the solution, only the level of scape bending is diminished.

Van Doorn *et al*. (1989) and Bleeksma and van Doorn (2003) found that the wilting of cut roses was related to bacterial levels in the xylem vessels. Van Doorn and De Witte (1994) in gerbera observed an increase in scape curvature with increasing concentration of bacteria in the water, but they concluded that scape curvature was only partially due to the bacterial populations.

Mencarelli *et al*. (1995) suggested that ethylene could be involved in gerbera stem break through a senescence process that was accelerated by several factors, including water stress due to vessel occlusion. The involvement of ethylene in gerbera stem break was subsequently confirmed by using auxins as postharvest treatments (Botondi

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et al., 1998) as well as by etephon treatments in the field (Gerasopoulos *et al.*, 1996).

The purpose of this work was to investigate the relationship between bacterial populations on gerbera cut flowers, ethylene production and gerbera stem break. Previous studies related bacterial populations to stem break but had examined different species of flowers (van Doorn and de Witte, 1994; van Doorn *et al.*, 1998).

Materials and methods

Plant material

Plants of *Gerbera jamesonii* cv. Provence were grown in a glasshouse near Rome. When the flowers were at commercial maturity (the third circle of stamens of the bisexual disc florets visible) they were harvested in the morning by cutting with a sterile scalpel and pulling each stem from the plant. In the laboratory the stems were sorted for straightness, trimmed to a uniform length of 40 cm and placed individually in glass tubes filled either with a tap water (100 ml) or with a silver-nitrate solution (50 ppm). The silver-nitrate tubes were covered with aluminium foil to prevent degradation of the silver nitrate by light; 60 flowers were used in each test. Flowers were kept in a room at 20±2°C and 60±5% RH, in a 12 h day (lighting with Philips TLM 40W/33 RS fluorescent tubes). The cut flowers were inspected daily for bending appearance.

Determination of epiphytic bacteria

The presence of epiphytic bacteria was evaluated on the scapes, petals, and sepals and in the middle of the capitulum. Ten-cm-long portions were excised from six scapes, weighed and then washed for one hour in sterile flasks with 30 ml of sterile distilled water (SDW) on an orbital shaker at 150 rpm. Preliminary tests had shown that parafilm was effective in sealing the extremities of each portion and prevented contamination by endophytic bacteria during orbital washing.

Six capitula were selected and petals and sepals were detached. The petals, sepals, and leftover capitula were weighed separately and washed in 20, 10, and 20 ml of SDW respectively.

After washing, serial dilutions were made of each amount of washing water used, and 0.1 ml aliquots of each dilution were plated, in two repetitions, on nutrient sucrose agar (NSA). The dishes were incubated at 26°C for 2 d. After incubation, bacterial colonies were examined and counted (Meynell and Meynell, 1970). Successively the number of bacterial colonies found was related to the number of epiphytic colony-forming units (cfu) g^{-1} in each flower part.

Determination of endophytic bacteria

Endophytic bacterial populations were isolated from the scapes 0, 1, 2, 3 and 6 d after the cut flowers were placed in the maintenance solutions. On day 0, the scape portions that had been used to isolate the epiphytic bacteria were used. Each stem portion was peeled, weighed and homogenized in 10 ml of SDW with an Ultra Turrax (IKA Labortechnik, Staufen, Germany) for 2 min at 13,500 rpm. On days 1, 2, 3, and 6, three scapes were used from each maintaining solution. Each scape, after surface-disinfection with ethanol, was cut into 4 parts each 10 cm long, peeled, weighed and homogenized. Homogenized tissues were serially diluted and plated on NSA.

Determination of bacteria in maintenance solutions

The two maintenance solutions (tap water and silver-nitrate) were compared to evaluate the growth of the bacterial populations resulting on the gerberas. Bacterial counts were carried out in tap water and in the silver-nitrate solution to evaluate the effect the solutions had on stem longevity.

The maintenance solutions were sampled after 0, 2, 3, and 6 d, and the bacteria in them counted and examined.

Bacterial identification

Bacterial colonies grown on NSA at 26°C for 48–72 h were selected under a stereomicroscope on the basis of their morphological characteristics. Five of the most commonly isolated bacterial colonies were sent to the Central Science Laboratory, Hatching Green, Harpenden (England) for identification by fatty acid profiling.

Ethylene measurement

Every day the flowers in their tubes were placed for 2 h in a larger plastic cylinder, whose opening was sealed with Parafilm (Eze *et al.,* 1986), in order to determine the ethylene concentration. For ethylene analysis, 2-ml air samples were removed

from the head space and injected in a Carlo Erba Fractovap 4200 (Carlo Erba Spa, Milano, Italy) gas chromatograph equipped with a flame ionization detector and a 1-m-long alumina column (80–100 mesh); the detector temperature was 100°C. Ethylene production (μl kg^{-1} h^{-1}) was recorded from the capitulum and from three sections of each gerbera scape (0–10 cm, 10–20 cm, and 20–30 cm from the base of the capitulum). The sections were excised after immersion of the flowers for 1 d in tap water or in the silver-nitrate solution, then placed for 1 h in glass vials capped with a serum stopper. Scape curvature was measured by recording the degree of bending from the initial straight position, and the percentage of scapes that had become bent was recorded daily.

Visualization of vessel obstruction

After 14 d of flower maintenance in water or after the occurrence of stem break, floral scapes were placed in a solution of ethanol (50% v:v) with 0.5% of acid fuchsin. The scapes were cut lengthwise after 5, 10, 15, 30 and 60 min with a sterile scalpel and examined for any obstruction of the xylem vessels leading into the scape, which was shown by a differential colour of the xylem vessels. Stained vessels were expressed as a percentage, from 25 to 100%, of the vessel length of the evaluated stem.

Statistical analysis

The experiments were repeated 3 times. Results were compared by analysis of variance and the T-test, using the least significant difference (LSD) at the 5% probability level.

Results

Flower bending

Flowers maintained in tap water started to bend after 4 d and the bending became clearly evident (more than 40°) after 6 d. Fifty to seventy percent of flowers showed stem bending at the end of the experiments but no collapse was observed. When silver nitrate was used, however, stem bending occurred only after 14 d.

Determination of epiphytic bacteria

In the fresh flowers, the highest levels of epiphytic bacteria were on the capitulum (G) (8.6×10^3 cfu g^{-1}) and on the portion of the scape 20 cm below the capitulum (C) (1×10^4 cfu g^{-1}) (Fig. 1).

Bacterial populations on the petals (F), sepals (E) and basal portions (A) were lower: 9.0×10^2 cfu g^{-1}; 1.6×10^3 cfu g^{-1}; and 1×10^3 cfu g^{-1} respectively. In the rest of the scape (portion B at 30 cm; portion D immediately below the capitulum) the bacterial populations were 3×10^3 cfu g^{-1} (Fig. 1).

Determination of endophytic bacteria

On day 0, the highest bacterial counts (2.3×10^4 cfu g^{-1}) were obtained from the scape portion 20 cm below the capitulum (C); whereas the lowest counts (2.2×10^2 cfu g^{-1}) were in the basal portion

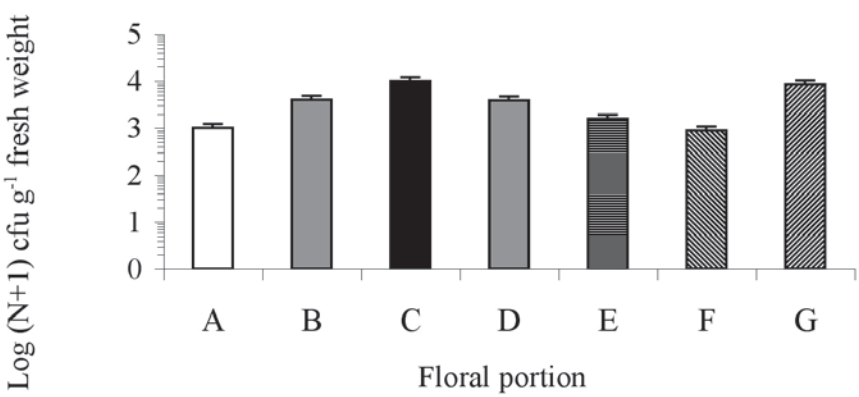

Fig. 1. Epiphytic bacterial populations on gerbera cv. Provence portions after cutting on day 0. A, scape basal portion; B, portion at 30 cm; C, portion at 20 cm; D, portion below the capitulum; E, sepals; F, petals; G, capitulum. Data represent the mean (±SD) of 10 flowers.

(A) and 30 cm below the capitulum (B) (Fig. 2). Subsequently, bacterial populations increased in all floral portions, reaching a maximum of 5×10^6 cfu g^{-1} in the basal portion (A). High bacterial counts were also obtained from the portions 20 and 30 cm below the capitulum (B and C) (3×10^6 cfu g^{-1}). In the scape portion below the capitulum (D), bacterial counts increased during the day assays to 3×10^5 cfu g^{-1} on d 6.

In the gerberas maintained in the $AgNO_3$ solution, the endophytic bacterial population reached a maximum of 4.5×10^4 cfu g^{-1} in the basal portion

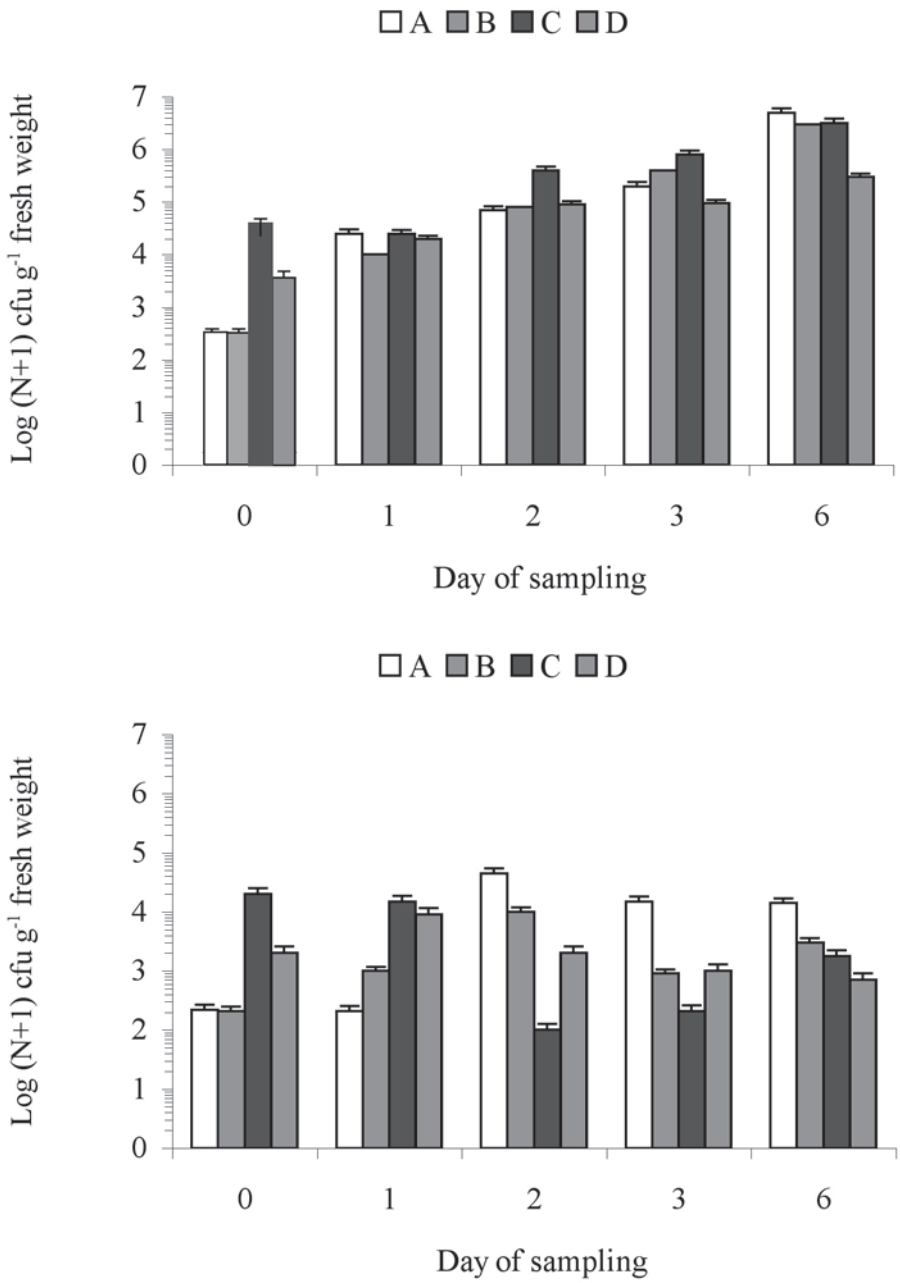

Fig. 2. Endophytic bacterial populations in various gerbera scape portions (see the legend in Fig. 1) using tap water (upper) and $AgNO_3$ solutions (lower). Data represent the means (±SD) of 10 flowers.

of the scape (A) on d 2, and then decreased. In the 30 cm portions (B), the bacterial counts also increased until d 2 and then decreased. In the 20 cm portion (C), bacterial counts were very high on d 0 and 1, declined on d 2, and increased again until d 6 (1.8×10^3 cfu g^{-1}) (Fig. 2). In the portion immediately under the capitulum (D), bacterial counts increased after d 1 and then decreased to 7×10^2 cfu g^{-1} on d 6 (Fig. 2).

Determination of bacteria in maintenance solutions

The bacterial counts in tap water and silver nitrate solution are compared in Fig. 3. In tap water, the number of bacteria was 6.1×10^3 cfu ml^{-1} on d 0 and increased to 7.2×10^6 cfu ml^{-1} from d 3 to the last day of the study. Bacterial populations in the $AgNO_3$ solution were counted only on d 3 and d 6, when they were 1×10^1 and 3×10^1 cfu ml^{-1} respectively.

Bacterial identification

The bacterial colonies differed in their morphological characteristics. The most frequently isolated were of 3 types: type A, circular, yellow, with a frequency of 90%; type B, circular, cream-colored, with a frequency of 70%; and type C, levan, mucoid, light-grey, with a frequency of 35%.

Representative colonies of each type were identified by fatty acid profiling as *Acinetobacter* sp. (type A), *Pantoea agglomerans* (type B) and *Bacillus pumilus* (type C).

All three genera were isolated as epiphytic bacteria from the external parts of the flowers. The *Acinetobacter* sp. and *P. agglomerans* were isolated along the scapes but were more concentrated 20 cm below the capitulum (7.5×10^3 cfu g^{-1} and 1.2×10^3 cfu g^{-1} respectively). The population counts from the other scape portions were lower, and they were not isolated from the capitulum (G). Only the *Acinetobacter* sp. was isolated from the sepals and petals (at 1.6×10^2 cfu g^{-1} and 1×10^2 cfu g^{-1} respectively); and only *B. pumilus* isolates were obtained from the capitulum (9.5×10^3 cfu g^{-1}; Fig. 4).

From the internal parts, and particularly from the scape basal portion of the flowers, only *B. pumilus* was isolated at the beginning of the experiment. Later, *Acinetobacter* sp. and *P. agglomerans* colonies were also obtained.

The *Acinetobacter* sp., *P. agglomerans* and *B. pumilus* were isolated from both maintenance solutions.

Ethylene measurement

Silver nitrate greatly reduced ethylene production which did not rise again during vase life (Fig. 5). By contrast, ethylene from water-treated flowers started to rise when the scape began to bend, sometimes on the same day or one day later. Ethylene production was greater in the portion where the curvature occurred (0–10 cm) than in the other portions (Table 1).

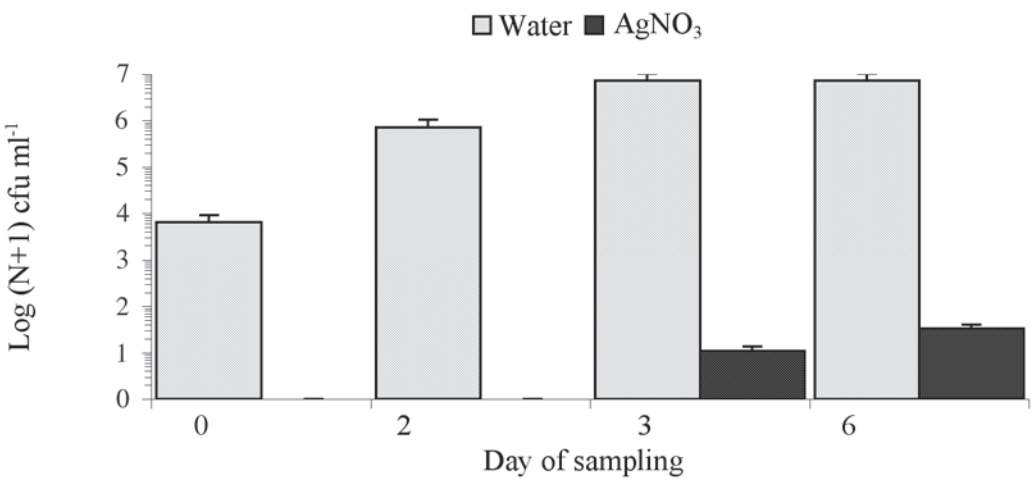

Fig. 3. Cumulative bacterial populations in tap water and $AgNO_3$ solution. Data represent the means (±SD) of 10 flowers.

Visualization of vessel obstruction

After the immersion of the scape into the acid fuchsin solution, fuchsin reached the top of the scape both in the flowers maintained in tap water solution and in those with stem-break, but the length of the red stained vessels changed. In the bent scapes of gerberas maintained in tap water, about 50% of vessels were stained in bent scapes after 30 min, but in the $AgNO_3$ maintained gerberas the percentage of stained vessels was similar to that in the fresh flowers (90–100%) after 15 min.

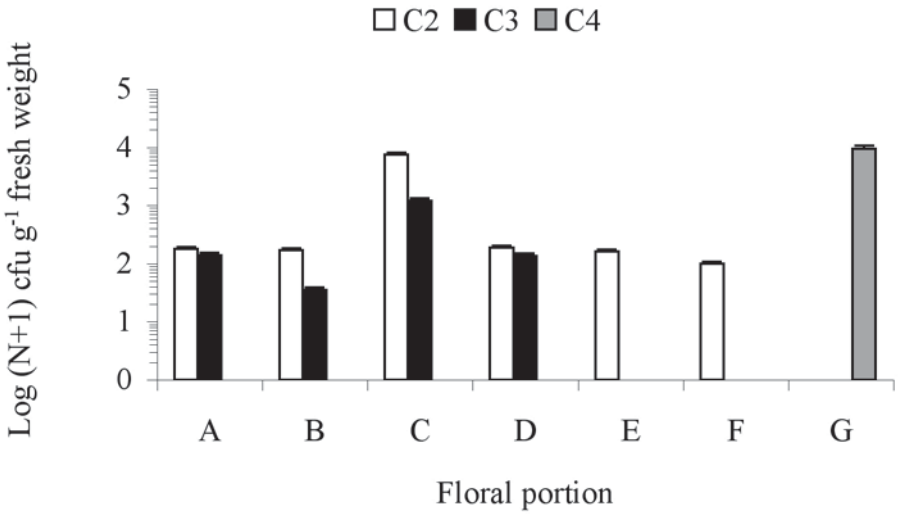

Fig. 4. Different epiphytic bacteria on gerbera cv. Provence portions after 6 d (see legend on Fig. 1): C2, *Acinetobacter* sp.; C3, *Pantoea agglomerans*; C4, *Bacillus pumilus*. Data represent the means (±SD) of 10 flowers.

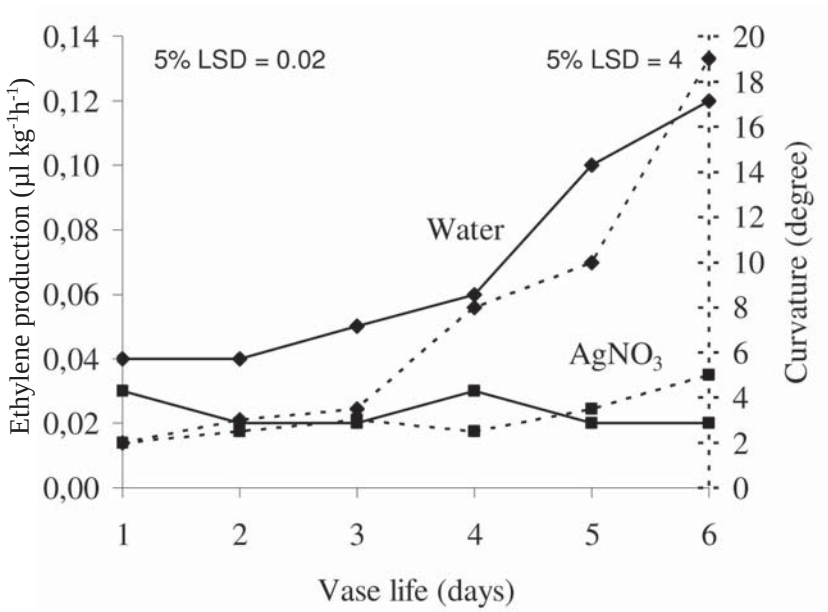

Fig. 5. Trends of ethylene production (continuous line) and degree of curvature (dotted line) of cultivar Provence flowers during vase life.

Table 1. Wound ethylene production (μl kg^{-1} h^{-1}) from the flower (capitulum) and sections of gerbera scape (0–10 cm, 10–20 cm, and 20–30 cm from the base of the capitulum). Sections were excised after the immersion of the flower for 1 day in tap water or in silver nitrate solution. Values are the means (μSD) of sections taken from 5 flowers.

Sample	Wound ethylene production (μl kg^{-1} h^{-1})	
	Water	Silver nitrate
Capitulum	0.42±0.11	0.18±0.01
0–10 cm	2.33±0.30	0.17±0.03
10–20 cm	0.84±0.12	0.14±0.02
20–30 cm	0.63±0.09	0.14±0.01

Discussion

Gerbera curvature of the stem appeared to be related to the endophytic bacterial population levels, which also induced higher ethylene levels, resulting in an evident curvature of the younger portion of the stem (van Dorn and de Witte, 1994; van Doorn, 1998), though this portion may also have been affected by dry storage (van Dorn *et al*., 1994).

With the tap-water solution, the amount of bacteria found below the capitulum was 3.7×10^4 cfu g^{-1}; when $AgNO_3$ was added to this solution, the bacterial count was 2.2×10^2 cfu g^{-1}. In the silver-nitrate solution the gerbera flowers showed an initial stem break only after 14 d compared with 6 d with tap water.

The epiphytic bacteria isolated in this study were mainly *Acinetobacter* sp., *Pantoea agglomerans* and *B. pumilus*.

The highest epiphytic bacterial counts were recorded along the 20 cm scape portions below the capitulum (C). This may have been due to operator handling in commercial environments, but also to the fact that this portion had the most active metabolism in the scape (Mencarelli *et al*., 1995).

Bacillus pumilus occurred as an endophyte inside the flowers. This bacterium is one of the most common in the endophytic microbial community, where it is a contaminant in the symptomless tissue of flowers and other plants (Leifert *et al*., 1994; Isenegger *et al*., 2003; Lacava *et al*., 2004).

All bacteria isolated occurred in both maintenance solutions, with higher counts usually in the tap-water solution but after 3 d sometimes also in the $AgNO_3$ solution. The bacteria probably entered the maintenance solutions from the external parts of the flowers, as also happens with other cut flowers (Teixeira da Silva, 2003).

In tap water, the *Acinetobacter* sp., *B. pumilus* and *P. agglomerans* cells seemed to move from the outside to inside the flower parts, becoming endophytic bacteria with a role in stem break. In particular, it is important to note this role of *B. pumilus* cells here isolated; these cells were previously found in cut-flower water solutions and were also involved in causing disease of plant tissue (Lund, 1986; Ketsa *et al*., 1995).

Only few cells of the three bacterial species were isolated in the $AgNO_3$ solution after 3 d, when the bactericidal activity of $AgNO_3$ started to diminish (Thompson, 1973). The role of the $AgNO_3$ solution in inhibiting ethylene synthesis and as an antimicrobial agent, which was one of the findings of the present study on gerbera cut flowers, has also been reported for other plants (Ketsa *et al*., 1995; Teixeira da Silva, 2003).

The experiments with fuchsin adsorption, in which 50% of vessels became red stained after 30 min, suggested that stem break was related to the partial occlusion of the vessels. Mayak *et al*. (1977) reported that this inhibited the development of endophytic bacteria affecting the vase life of gerbera; moreover, flowers kept in the $AgNO_3$ solution had lower ethylene levels and were without any appearance of stem break.

The absorption of water was clearly reduced by the multiplication of bacteria. A similar finding was reported by van Meeteren (1978).

The bacteria most frequently isolated in this study (*Acinetobacter*, *Bacillus* and *Pantoea*) seem to become involved in stem break whenever their values exceed 10^5 cfu g^{-1}.

Various changes (physical, biochemical) have been related to the occurrence of bacteria in the maintenance solutions of cut flowers. Van Doorn and De Witte (1994) found a relationship between water solution, population density of bacteria, and scape bending.

Hoogerwerf and van Doorn (1992) reported a relationship between the density of bacteria and the type of maintenance solution used in post-harvest cut flowers.

Since van Doorn (1998) related xylem occlusion to endophytic microbial growth, it is reasonable to assume that the gerbera stem break in the present study was related to the xylem occlusion caused by the endophytic bacteria here isolated and the consequent higher ethylene levels.

Ethylene which was here observed in gerbera seems to be involved in the senescence of flowers. Increases in ethylene started initially below the capitulum and positively influenced the growth of bacterial populations that were related to gerbera stem break. This phenomeon was reported earlier for *Achillea filipendulina*, *Celosia argentea*, *Buddleia davidii*, *Cosmos bipinnatus*, *Helianthus maximilianii*, *Narcissus pseudonarcissus*, and *Penstemon digitalis* flowers (van Doorn, 1998; Redman *et al*., 2002), but this is the first time it is reported for cut gerbera as well (Teixeira da Silva, 2003).

Cut-flower species vary in their sensitivity to ethylene; which is involved in flower senescence (Redman *et al*., 2002) but it could also play a secondary role (Spikman, 1989). In the present study it was clear, even without direct tests, that ethylene concentrations changed in response to higher bacterial levels in gerbera cut flowers, and thus contributed to stem break.

The sanitation of cut flowers should be enhanced to protect gerberas against dangerous micro-organisms encountered. Hot-water treatment (Hansen and Hara, 1994) could be one of the methods useful in this regard.

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