# Longevity of *Epidendrum ibaguense* Kunth inflorescences treated with nitric oxide[1]

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**ABSTRACT**

Nitric oxide (NO) acts as anti senescence substance, which may extend the postharvest life of fruits, vegetables and flowers when they are treated with micro molar concentrations of compounds like the donor sodium nitroprusside (SNP). This work aimed to evaluate the effect pulsing or spraying of NO on the longevity of cut *Epidendrum ibaguense* inflorescences. After harvested, the inflorescences were pulsed for 6, 24 or 48 hours with 5, 10, 50, 100 and 500 µM SNP or sprayed until run off with the same mentioned solutions. Controls were treated with distilled water. After the treatment, the flowers were placed in deionized water, which was changed every 2 days. No significant differences were observed on the longevity of flowers treated with 5, 10, 50 or 100 µM SNP, regardless of the mode of application. Inflorescences treated with 500 µM SNP had reduced longevity and increased flower abscission. In inflorescences kept in SNP solution, toxic symptoms such as darkening of the labellum resulting in reduced longevity compared with the control. The longevity of inflorescences sprayed with 500 µM SNP reduced from 6.8±0.57 to 5.1±0.82 days. Collectively, NO treatments were not able to extend the shelf life of *E. ibaguense* inflorescences and high concentrations of the NO donor compound in vase solution or spraying leads to toxicity symptoms on the flower labellum.

**Keywords:** vase life, pulsing, donor sodium nitroprusside.

**RESUMO**

**Longevidade de inflorescências de *Epidendrum ibaguense* Kunth. tratadas com óxido nítrico**

O óxido nítrico (NO) tem propriedades anti-senescência, podendo estender a vida pós-colheita de muitos frutos, hortaliças e flores quando esses são tratados com concentrações micromolares de compostos doadores, como o nitroprussiato de sódio (SNP). O objetivo deste trabalho foi avaliar o efeito do NO aplicado na forma de condicionamento ou via pulverização, sobre a longevidade de inflorescências de *Epidendrum ibaguense*. Após a colheita, as inflorescências foram tratadas por 6, 24 ou 48 horas com solução de condicionamento contendo 5, 10, 50, 100 e 500 µM de SNP ou pulverizadas com SNP nas mesmas concentrações, até o completo molhamento da inflorescência. Os controles foram tratados com água desionizada. Após o tratamento, as hastes foram colocadas em vaso com água desionizada, trocada a cada 2 dias. Não foram observadas diferenças na longevidade das inflorescências quando tratadas com solução de 5, 10, 50 ou 100 µM SNP, independentemente do modo de aplicação. Inflorescências tratadas com 500 µM de SNP tiveram redução na longevidade e aumento na abscisão de flores. Nas inflorescências mantidas em solução de SNP, sintomas de toxidez, como o escurecimento do labelo, resultou na redução da longevidade, comparado ao controle. A longevidade das inflorescências pulverizadas com 500 µM de SNP reduziu de 6,8±0,57 para 5,1±0,82 dias. Coletivamente, os tratamentos com NO não prolongaram a vida de vaso de inflorescências de *E. ibaguense* e altas concentrações de compostos doadores de NO em solução de vaso ou via pulverização induziram o aparecimento de sintomas de toxicidade no labelo das flores.

**Palavras-chave:** vida de vaso, solução de condicionamento, nitroprussiato de sódio.

## 1. INTRODUCTION

The Orchidaceae *Epidendrum ibaguense*, whose flowering occurs almost throughout year in Brazil, exhibits a great potential to be used as a cut flower due to the uniformity of color, exuberance of inflorescence and long flowering stems (MOURA et al., 2010). However, as the species is highly sensitive to ethylene, it observed premature senescence and abscission of the flowers (MAPELI et al., 2009).

Postharvest experiments are necessary to find ways in circumventing this issue and some studies suggest the nitric oxide (NO) potentially acts as an anti-ethylene substance and can extend the shelf-life of ethylene sensitive flowers. Common NO donors such as sodium nitroprusside (SNP) or 2,2'-(hidroxinitrosohydrazino)-bisetanamina (DETA/NO) (ARORA, 2008) are an alternative to others chemical compounds traditionally used, e.g. silver thiossulfate (STS) and 1-methylcyclopropene (1-MCP) (SEYF et al., 2012).

NO plays the role of protecting the cell against oxidative stress (YIN et al., 2012), inhibits the expression of genes involved in the ethylene biosynthetic pathway (MANJUNATHA et al., 2012) and lipid peroxidation (PROCHÁZKOVÁ and WILHELMOVÁ, 2011). However, the promotion or delay the floral senescence by application of NO depends on the concentration and species under study (SANKHALA et al., 2004).

In bamboo shoots, 0.5 mM SNP inhibited the activity of

[1] Received in 08/08/2016 and approved in 03/11/2016

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the enzyme phenylalanine ammonia lyase (PAL), polyfenol oxidase (PPO) and peroxidase (POD) and reduced ethylene biosynthesis and consequently, inhibited browning and increased the shelf-life (YANG et al., 2010). In carnation flowers it was observed a > 4 d increase in vase life when flowers were treated with 0.1 mmol $L^{-1}$ SNP (CHANG-LI et al., 2011). The vase life of rose cut flowers increased from 11 to 13.3 days when treated with 50 µM SNP for 24 hours (SEYF et al., 2012). Additionally, there was increase in soluble solids content, the absorption rate of solution and increase in fresh weight

The use of NO donor compounds used in flowers stems is considered simple technically (BADIYAN et al., 2004) but, Bowyer and Wills (2003a) showed that the use of DETA/NO should not be considered a universal treatment, since not all studied species had increased shelf-life.

In this context, cut flowers can be used as a model to generate important information about the action of NO and clarify how this molecule affects physiological and biochemical processes. Additionally, the increase in shelf-life of several vegetables demonstrates the feasibility of using NO and SNP due to ease of handling and low cost (SEYF et al., 2012). However, more research is needed to evaluate the effectiveness of the SNP on the postharvest life of new cut flower species.

The objective of this work was to evaluate the impact of pulsing and spraying of SNP on the postharvest longevity of cut *Epidendrum ibaguense* inflorescences.

## 2. MATERIAL AND METHODS

The stems of *Epidendrum ibaguense* were harvested between 7 and 8 am, with 10 open flowers, e.g. more than half of buds open. After harvest the stems were placed in water, transported to the lab for standardization to 25 cm and randomly distributed according to the treatments described below.

To evaluate the effect of SNP (Fluka Analytical, Durban, South Africa) as pulse treatment, the flowers were maintained for 6, 24 or 48 hours with the stem base submerged in solutions containing 5, 10, 50, 100 or 500 µM SNP. Controls were treated with deionized water.

To evaluate the effect of SNP as spray treatment the flowers were sprayed with 5, 10, 50, 100 or 500 µM SNP until complete wetting of the inflorescence, i.e. 15 mL per inflorescence. Controls were sprayed with deionized water.

After the treatment the stems were placed in deionized water and kept at 25±2 ºC, relativity humidity of 50-70% with 7-10 µmol $m^{-2}$ $s^{-1}$ of constant illumination provided by white light until the end of vase life. The stems were re-cut in deionized water to 2 cm at the base of the stem every 48 hours and water vase were exchanged. The vase life was evaluated daily and it was considered the end of vase life when more than 50% of the flowers showed wilting or abscised (MORAES et al., 2007).

The experiment was a completely randomized block design with five replications and the experimental unit consists of two stems. Data represent the treatment mean ± standard error.

## 3. RESULTS AND DISCUSSION

The longevity, with an average of 4.6 days, was not affected by 5-100 µM SNP pulsed for 6 hours (Table 1). When supplied for 24 hours, 100 µM SNP reduced longevity of flowers 16%. When supplied for 48 hours, 5 and 10 µM SNP increased the longevity 35 and 25%, respectively. Regardless of the time of pulsing, 500 µM SNP caused a deleterious effect, reducing the average longevity for 2 days.

**Table 1.** Longevity of *Epidendrum ibaguense* inflorescences after pulse with SNP. *Means ± standard error of the mean
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| Pulse duration (hours) | SNP (µM) | Longevity (days)* |
|---|---|---|
| 6 | 0 | 5.0 ± 0.31 |
| | 5 | 5.8 ± 0.20 |
| | 10 | 5.6 ± 0.40 |
| | 50 | 5.4 ± 0.74 |
| | 100 | 5.6 ± 0.24 |
| | 500 | 2.0 ± 0.00 |
| 24 | 0 | 4.8 ± 0.37 |
| | 5 | 4.8 ± 0.80 |
| | 10 | 4.4 ± 0.24 |
| | 50 | 4.4 ± 0.24 |
| | 100 | 4.0 ± 0.00 |
| | 500 | 2.0 ± 0.00 |
| 48 | 0 | 4.0 ± 0.00 |
| | 5 | 5.4 ± 0.74 |
| | 10 | 5.0 ± 0.77 |
| | 50 | 4.0 ± 0.00 |
| | 100 | 4.0 ± 0.00 |
| | 500 | 2.0 ± 0.00 |

Similarly, 100 µM SNP in vase solution decreased longevity of flowers of *Lupinus havardii* (SANKHLA et al., 2005). In roses, the treatment with 100 µM SNP didn't affect the longevity of flowers (SEYF et al., 2012) and the treatment with 40 µM SNP for 24 hours inhibited by about 70% ethylene production and folded the vase life (MORTAZAVI et al., 2011). In carnation flowers, 10 mg $L^{-1}$ DETA/NO increased by 50% the shelf-life (BOWYER et al., 2003b).

*E. ibaguense* flowers treated with 500 µM SNP showed, 48 hours after treatment, darkening of the labellum (Figure 1). High concentrations of NO can cause toxicity by being able to produce cyanide, affecting the cellular metabolism (PROCHÁZKOVÁ and WILHELMOVÁ, 2011), or reacting with superoxide (SEYF et al., 2012). In *Phlox paniculata* L. flowers, 200 µmoL $L^{-1}$ SNP caused yellowing of leaves and promoted senescence (SANKHLA et al., 2004).

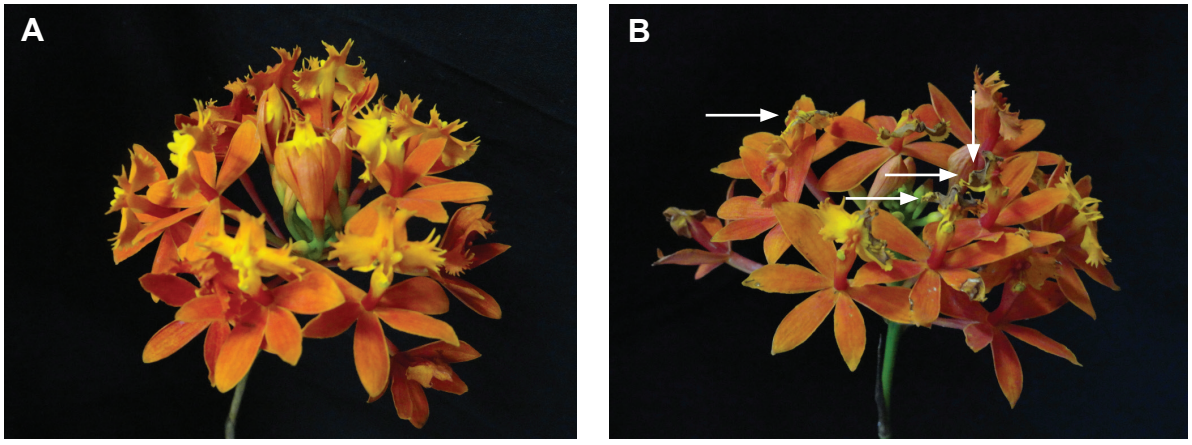


**Figure 1.** *Epidendrum ibaguense* inflorescences 48 horas after pulse treatment with 0 (A) e 500 µM (B) de SNP. The arrows indicate the injury caused by SNP in labellum.

Additionally, the percentage of abscission has increased over the evaluation period in inflorescences treated with 500 µM SNP, regardless of the time of pulsing (data not shown). Similarly, the use of 10 to 200 µmol $L^{-1}$ SNP in *Phlox paniculata* L. promoted abscission (SANKHLA et al., 2004). Different species and cultivars have distinct behaviours when treated with a similar vase solution.

On the 2th day, inflorescences treated with 500 µM SNP, even showing toxicity symptoms, had lower percentages of wilted flowers being 1.2% in flowers treated for 6 hours or showed no wilting in flowers treated for 24 or 48 hours (data not shown), suggesting a possible role of SNP in maintaining water balance in the early stages of senescence. At the end of evaluation period, inflorescences treated with 5 µM SNP for 6 hours showed a reduction in the percentage of wilting by 32% compared to control. Similarly, Chang-Li et al. (2011), observed in carnation flowers treated with 0.1 mmol $L^{-1}$ SNP, delayed wilting petals and maintenance of water balance.

In *E. ibaguense*, the increase in fall flowers and reduced wilting suggests that abscission is related to ethylene and is not related to the reduction in water content of the petals. *E. ibaguense* inflorescences is extremely responsive to inhibitors of ethylene action as 1-MCP or STS. We know that 1-MCP treatment more than doubled the vase-life for this orchid (FINGER et al., 2008). The vase-life of the *E. ibaguense* should be also improved by pulsing with 2 mM STS.

The greater longevity (7.2 days) was observed in flowers sprayed with 10 µM SNP, an increase of 5% compared to control (Table 2). Inflorescences sprayed with 500 µM showed lowest longevity, as observed in pulsed inflorescences, with 25% reduction compared to control. Differently, in carnation flowers, the shelf-life increased about 30% when treated with 1 and 5 µL $L^{-1}$ NO (BOWYER et al., 2003b).

**Table 2.** Longevity of *Epidendrum ibaguense* inflorescences after spray treatment with SNP. *Means ± standard deviation of the mean.

| SNP (µM) | Longevity (days)* |
|---|---|
| 0 | 6.8 ± 0.57 |
| 5 | 6.9 ± 1.14 |
| 10 | 7.2 ± 0.27 |
| 50 | 6.7 ± 0.97 |
| 100 | 6.8 ± 1.25 |
| 500 | 5.1 ± 0.82 |

During the evaluate period, solutions containing 5, 10, 50, e 100 µM SNP inhibited the abscission of flowers, with a reduction of at most 10% (date not shown). Inflorescences sprayed with 500 µM SNP showed an increase in the percentage of abscission during the study period. On the 6$^{th}$ day, these inflorescences showed an increase of 39% from the fallen flowers compared to control. This increase proved the deleterious effect of SNP on *E. ibaguense* and suggests that there was no inhibition on the synthesis or action of ethylene with NO. With the increase in the abscission, the number of open flowers on inflorescences sprayed with 500 µM SNP reduced 23, 30 and 22% in the 4$^{th}$, 5$^{th}$ and 6$^{th}$ days, respectively, compared to control (date not shown).

Thus, the effect of SNP in flowers of *E. ibaguense* was dependent on concentration, regardless of the mode of application. Furthermore, SNP was detrimental to vase when applied in high concentrations, increasing flower abscission and inducing darkening of the labellum.

## 4. CONCLUSIONS

Sodium nitroprusside (SNP, donor sodium nitroprusside), even at low concentrations, were not able to extend the shelf life of *E. ibaguense* inflorescences.

*E. ibaguense* inflorescences treated with 500 mM SNP had reduced longevity and increased flower abscission. When in vase solution, high concentrations of SNP leads to toxicity symptoms on the flower labellum.

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