Europ.J.Hort.Sci., **74** (1). S. 10–15, 2009, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

1-Methylcyclopropene Inhibits Ethylene Effects in Cut Inflorescences and Potted Plants of *Oncidium* and *Odontoglossum* Orchid Species

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Summary

Objectives of the present study were to examine the effects of ethylene and the ethylene perception inhibitor 1-methylcyclopropene (1-MCP) on the senescence of flowers of the orchid genera *Oncidium* and *Odontoglossum*. Cut inflorescences and entire potted plants were treated continuously with 1 μL L^{-1} ethylene and compared to untreated controls. In addition, detached flower stalks and potted plants were pre-treated with 200 nL L^{-1} 1-MCP for 6 h at 20 °C.

The inflorescences and potted plants treated with 1 μL L^{-1} ethylene showed accelerated symptoms of senescence, like wilting of the florets and petals. Other remarkable effects of ethylene were the yellowing of the pedicels and bud dropping on cut inflorescences as well as the yellowing of leaves on potted plants. Control, non-ethylene treated, flowers showed these symptoms 3–8 d later. The plants pre-treated with 1-MCP expressed an increase in shelf life from 2–10 d in absence or presence of ethylene.

In conclusion, genera *Oncidium* and *Odontoglossum* have been proven sensitive to ethylene, and 1-MCP improved their postharvest quality if plants are exposed to exogenous ethylene.

Key words. display life – flower senescence – postharvest quality

Introduction

From the commercial point of view, the two orchid genera *Oncidium* and *Odontoglossum* are currently much less important than the genus *Phalaenopsis* (Winkelmann et al. 2006, www.adivk.de). However, *Oncidium* and *Odontoglossum* and their hybrids bear very beautiful flowers with new and spectacular colours and patterns. The structure and architecture of the flower organs are very attractive and different from other orchid species like *Phalaenopsis* and therefore could be promising ornamental species. Their present minor commercial importance is probably due to the poor display life of the flowers. In order to analyse factors affecting the short display life of the flowers of *Oncidium* and *Odontoglossum* ethylene involvement in flower senescence was investigated in this study.

Ethylene is a natural plant hormone, which is responsible for a number of processes in the plant, including senescence and abscission of organs like buds and flowers. Thus, the exposure of orchid plants to ethylene may result in a reduction of the plant quality, particularly in faster wilting or abscission of florets and petals (Chang et al. 2003; Huang 1998; Ketsa and Rugkong 1999, 2000; Lin et al. 2003; Woltering and van Doorn 1988).

The sensitivity to ethylene of some orchid species like cut flowers of *Cymbidium*, *Phalaenopsis* and *Oncidium* has been demonstrated (Philosoph-Hadas et al. 2005; Porat et al. 1994; Huang et al. 2007). The effect of endogenous ethylene on the senescence of flowers following pollination has been well documented in the orchid species *Phalaenopsis, Dendrobium* and *Oncidium* (Porat et al. 1994; Zhang et al. 1996; Huang et al. 1999; Ketsa and Rugkong 1999, 2000; Huang et al. 2007). Exogenous ethylene can have a dramatic effect, if plants are exposed to it during transport or trading. The problem of plant exposure to exogenous ethylene is well known and many efforts have been reported in preventing early senescence by pretreating plants with ethylene perception inhibitors (Heyes and Johnston 1998; Huang et al. 2003; Lin et al. 2003; Philosoph-Hadas et al. 2005). Well known ethylene biosynthesis and action inhibitors like silver thiosulfate (STS), diazocyclopropene (DACP) or ACC synthase inhibitor aminooxy acetic acid (AOA) have been used for improvement of display life of ornamental plants in general, and also orchids, in particular by delaying wilting and abscission of flowers and buds (Porat et al. 1994; Ketsa and Luangsuwalai 1996; Zhang et al. 1996; Huang et al. 1999; Ketsa and Rugkong 2000; Rattanawisalanon et al. 2003; Uthaichay et al. 2007). DACP and AOA have been shown to be less effective than STS (Mor et al. 1984, Sexton et al. 1995), but STS comprises environmental risks and therefore is not permitted to be used in several countries.

More recently the non-toxic compound 1-MCP has been introduced (Serek et al. 1994, 1995a, b; Porat et al.

1995; Sisler and Serek 1997). It has been proposed that 1-MCP binds to the ethylene receptor and has been demonstrated that is therefore very specific and active at low concentrations (Sisler and Serek 1997; Serek et al. 2006). The success of 1-MCP treatment depends on the genotype, the concentration of 1-MCP, the exposure time, the application temperatures and the maturity of the plant product (Sisler and Serek 1997; Serek et al. 2006).

The effect of 1-MCP in preventing ethylene action in orchids was tested so far on *Phalaenopsis* (Lin et al. 2003; Chang et al. 2003; Porat et al. 1994), *Cymbidium* (Philosoph-Hadas et al. 2005; Heyes and Johnston 1998) and *Oncidium* cut flowers (Huang et al. 2003). So far nothing has been reported on the genus *Odontoglossum*, its sensitivity to ethylene or protection by 1-MCP. Moreover, no information is available on the effect of ethylene and 1-MCP application to the whole orchid plants.

In this study the effect of exogenous ethylene on the display life of cut flowers as well as entire plants of *Oncidium* and *Odontoglossum* was tested. In addition, the effect of 1-MCP on improvement of the post harvest quality of *Oncidium* and *Odontoglossum* in the presence or absence of ethylene was investigated.

Material and Methods

Plant material

Plants of the genus *Oncidium* cv. 'Sweet Sugar' and *Odontoglossum* cvs. 'Stefan Isler Lava Flow', 'Hansueli Isler', 'Cambria Plush' and 'Margarete Holm Larkspur' were provided by a commercial grower (Gartenbau Valtl Raffeiner, Italy). They had been grown in the greenhouse at about 18 °C and a 16/8 h (day/night) photoperiod. The cut inflorescences and the potted plants were used when the first flowers were opened.

Chemicals and treatments

1-MCP was obtained from AgroFresh Inc. (Rohm and Haas, AgroFresh Inc., Philadelphia, USA) in commercially available form, formulated as a powder. The cut inflorescences of *Oncidium* cv. 'Sweet Sugar' and *Odontoglossum* cvs. 'Cambria Plush', 'Stefan Isler Lava Flow' and 'Margarete Holm Larkspur' were placed in 54 L volume glass chambers (aquaria). The inflorescenses were pre-treated with 200 nL L^{-1} 1-MCP in the sealed glass chamber for 6 h at room temperature 20±1 °C. The powder released 1-MCP gas after addition of a few drops of water. Control inflorescenses were incubated in identical glass chambers without 1-MCP for 6 h at room temperature. After the pre-treatment, the chambers were vented for 1 h. Thereafter the cut inflorescences were placed randomly in the glass chambers, sealed and maintained at 20 °C ±1 °C under a 16/8 h (day/night) light from cool white fluorescent lamps providing 20 μmol m^{-2} s^{-1} (Osram, 400 W, Eindhoven, The Netherlands). Ethylene at a concentration of 1±0.2 μL L^{-1} was provided by a simple diffusion system (Saltveit 1978). Control inflorescences were kept in a chamber with ethylene free atmosphere. The concentration of ethylene was determined daily by gas chromatography.

The potted plants of *Oncidium* cv. 'Sweet Sugar' and *Odontoglossum* cvs. 'Cambria Plush' and 'Hansueli Isler' were pre-treated, simlarly to cut inflorescences, with 200 nL L^{-1} 1-MCP in 0.66 m^3 volume growth chambers for 6 h at room temperature. Control plants were kept in a second growth chamber containing air. After ventilation, the potted plants were transferred and placed randomly in growth chambers of 21 m^3. They were incubated under the same conditions as the cut inflorescences. The treatment in these growth chambers was carried out by injection of pure ethylene twice a day to keep the concentration at 1.0±0.2 μL L^{-1}.

In the first experiment, cut inflorescences of one *Oncidium* and three *Odontoglossum* genotypes were treated with 1±0.2 μL L^{-1} ethylene and compared to untreated controls, while in the second experiment the potted plants were tested under the same conditions.

Gas chromatography

The concentration of ethylene in the various chambers was measured using a Perkin-Elmer portable digital gas chromatograph (GC Voyager FFKG312, Ontario, Canada) equipped with a photoionisation detector. The carrier gas was N_2 at 40 mL min^{-1}, the injection and column temperature was 65 °C. Gas samples taken from the chambers were compared to 1 μL L^{-1} ethylene as reference standard.

Measurements and evaluation

The senescence process was monitored visually. The first signs of wilting were considered to be important and to terminate the shelf life. Therefore the time in days till the first petal lost the turgor was determined.

The changes in colour of the leaves of potted plants from green to yellow and brown was also monitored, but not quantified.

Experimental design

The experiment with cut inflorescences was performed in a completely randomized design using two inflorescences per treatment and three replications with the *Oncidium* cultivar 'Sweet Sugar' and two replications with the *Odontoglossum* cultivars 'Stefan Isler Lava Flow', 'Cambria Plush' and 'Margarete Holm Larkspur'. The experiment with potted plants was conducted as a completely randomized design using five plants per treatment and two replications.

The means and the standard deviations were depicted.

Results and Discussion

The untreated controls expressed wilting symptoms 2–10 d later in cut inflorescences (Fig. 1) and 1–4 d later in potted plants than the ethylene-treated ones (Fig. 3). Like in the cut flower experiment more buds developed into flowers when ethylene was not present, just because the senescence began later (Table 1).

Effect of ethylene and 1-MCP treatment on cut inflorescences

The flowers of cut inflorescences treated with 1 μL L^{-1} ethylene wilted 4 to 6 d after the start of the experiment,

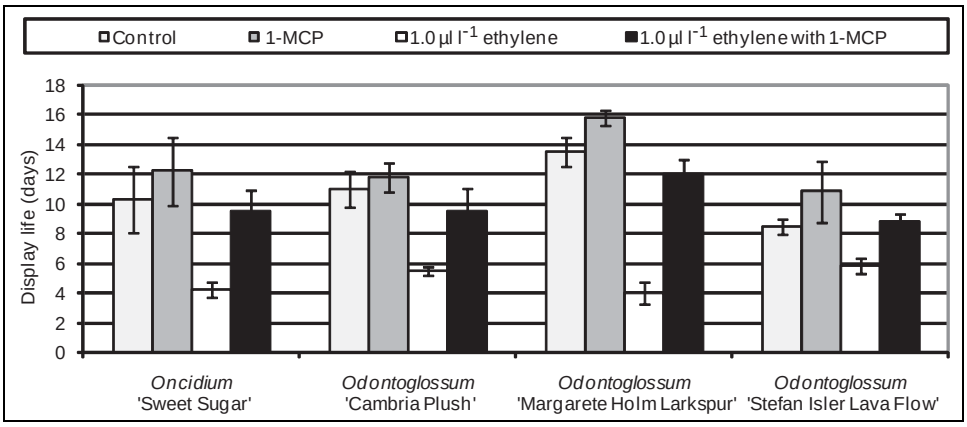


Fig. 1. Display life of cut inflorescences of *Oncidium* and *Odontoglossum* genotypes pre-treated with 0 or 200 nL L^{-1} of 1-MCP at 20 °C for 6 h, and then exposed to 0 or 1 µL L^{-1} ethylene. Bars represent the standard deviation.

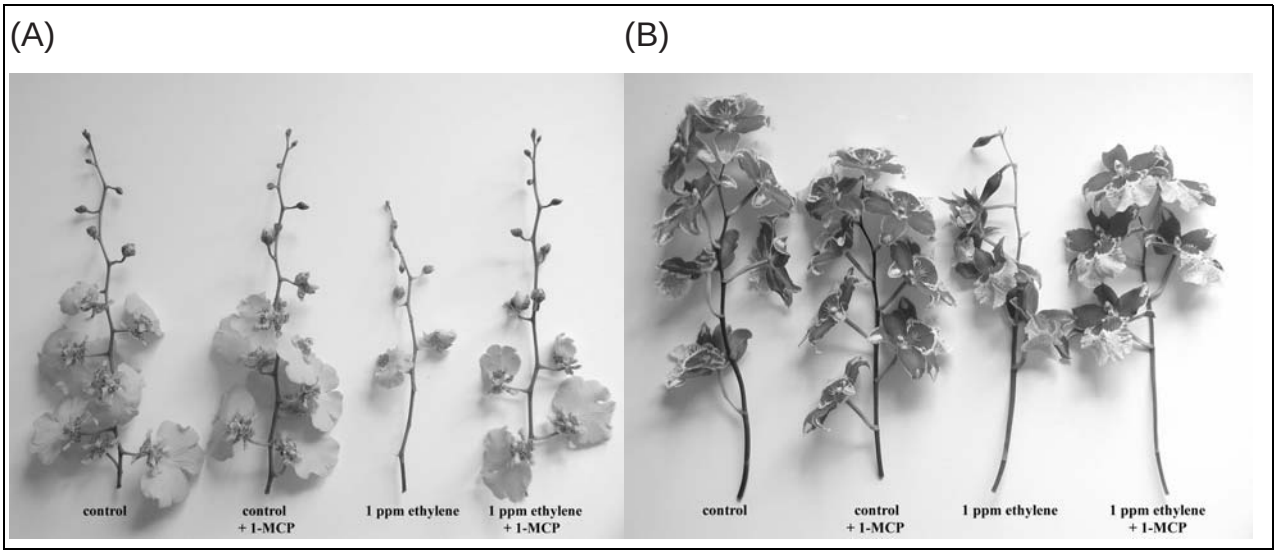


Fig. 2. Cut inflorescences of *Oncidium* cv. 'Sweet Sugar' (Fig. 2A) and *Odontoglossum* cv. 'Margarete Holm Larkspur' (Fig. 2B) pre-treated with 0 or 200 nL L^{-1} of 1-MCP at 20 °C for 6 h, and then exposed to 0 or 1 µL L^{-1} ethylene for 7 d.

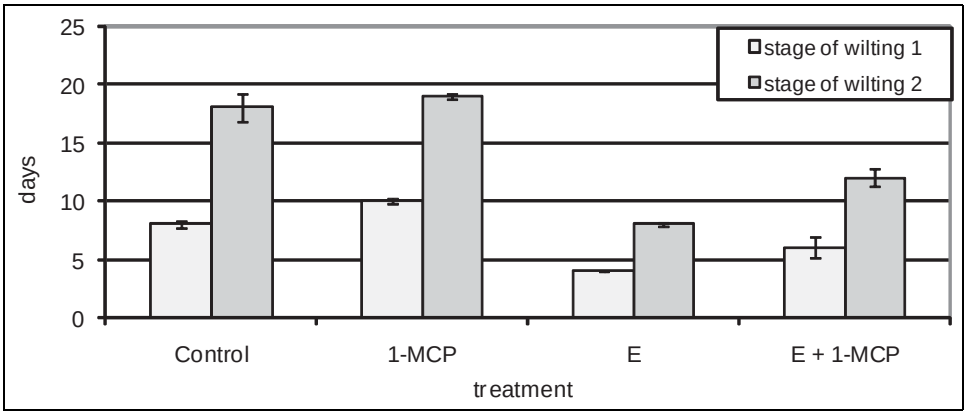


Fig. 3. Display life of potted plants of *Odontoglossum* cv. 'Hansueli Isler' pre-treated with 0 or 200 nL L^{-1} 1-MCP at 20 °C for 6 h and then exposed to 0 or 1 µL L^{-1} ethylene. Treatments: control with and without 1-MCP (1-MCP / Control), 1 µL L^{-1} ethylene with and without 1-MCP (E + 1-MCP / E). Stage of wilting: 1 = first flower wilting and 2 = last flower wilting. Bars represent the standard deviation.

depending on the genotype (Fig. 1). Moreover, bud development stagnated very fast and only fully mature buds did open to flowers. The development of immature buds stopped and most of them abscised in our study. Similar results regarding the fast wilting process were reported by Serek et al. (1995a) on various ornamental crops. The stagnated bud development corresponds with earlier reported behaviour of cut phlox flowers (Porat et al. 1995).

Flowers without 1-MCP pre-treatment initiated wilting 2.5 to 9.5 d later in ethylene-free environment as if exposed to ethylene. Ethylene free-environment was also beneficial for bud development compared to ethylene contaminated room. In 1-MCP pre-treated cut inflorescences the buds continued developing for a longer time into flowers, because this development was not impaired by ethylene-caused senescence. This effect was most visi-

Table 1. Development of buds of *Odontoglossum* cv. 'Margarete Holm Larkspur'

Treatment*[1, 2]	Days												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Control	0	0	1	1	1	1	1	2	3	3	3	3	3
1-MCP	0	0	0	0	1	1	1	2	2	3	3	3	3
E	0	0	0	0	0	0	0	0	0	0	0	0	0
E + 1-MCP	0	0	1	2	3	3	4	5	5	6	7	7	8

*[1]Buds were pre-treated with 0 or 200 nL L^{-1} 1-MCP at 20 °C for 6 h and then exposed to 0 or 1 µL L^{-1} ethylene.
*[2]Treatments: control with or without 1-MCP (1-MCP / Control), 1 µL L^{-1} ethylene with or without 1-MCP (E + 1-MCP / E). Data is given as number of buds per plant that develop into flowers.

Table 2. Abscission of buds and flowers of potted plants of *Oncidium* cv. 'Sweet Sugar'

Treatment*[1, 2]	Days																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Control	0	0	0	0	0	0	0	4	20	49	67	81	94	100				
1-MCP	0	0	0	0	0	0	0	0	0	0	0	1	2	16	38	51	78	100
E	0	0	0	0	0	0	5	46	84	89	100							
E + 1-MCP	0	0	0	0	0	0	0	0	0	3	6	10	26	74	100			

*[1]Buds were pre-treated with 0 or 200 nL L^{-1} 1-MCP at 20 °C for 6 h and then exposed to 0 or 1 µL L^{-1} ethylene.
*[2]Treatments: control with and without 1-MCP (1-MCP / Control), 1 µL L^{-1} ethylene with and without 1-MCP (E + 1-MCP / E). Data is given as percentage of a total number of buds and flowers at day 1.

ble in the pre-treated cut inflorescences exposed to exogenous ethylene (Table 1). The untreated inflorescences exposed to ethylene were not able to develop the buds into flowers, because the senescence process occurred very fast. The results clearly indicate that exogenous ethylene accelerated the senescence of flowers and buds of the investigated genera *Oncidium* and *Odontoglossum*. The senescence of flowers started earlier and showed effects like loss of petal turgor, wilting and the abscission of flowers and buds. Huang et al. (2007) reported that exogenous ethylene induced the gene expression of *OgERS1*, a putative ethylene response sensor, in *Oncidium* 'Gower Ramsey'. This study provided evidence that exogenous ethylene is correlated with a significantly faster senescence as also demonstrated in the results presented in this study.

The cut inflorescences pre-treated with 1-MCP also showed a delayed wilting process; most buds developed to flowers and flower and bud abscission was reduced in comparison to the untreated ones. The display life of the different genotypes of *Oncidium* and *Odontoglossum* were extended by 3 to 8 d. All cultivars reacted in the similar manner, however, the best protection against exogenous ethylene was observed in the cultivar 'Margarete Holm Larkspur', where the wilting process started 8 d later in cut inflorescences pre-treated with 1-MCP than in untreated controls (Fig. 1). Variations in 1-MCP effect among cultivars or species have been also reported in earlier studies; the display life of *Cymbidium* flowers was prolonged by 6 to even 19 d with pre-treatment of 1-MCP (Heyes and Johnston 1998) and in *Dendrobium* by 9 to even 12 d (Uthaichay et al. 2007), whereas in *Oncidium* it increased only with 2 d (Huang et al. 2003). A reduced abscission of flowers and buds pre-treated with 1-MCP has also been shown in earlier studies on Geraldton waxflower (Serek et al. 1995b).

In contrast to our results, where display life was extended also in ethylene-free environment, in 14 different native Australian cut flowers the 1-MCP treatment did not delay the senescence in the absence of exogenous ethylene (MacNish et al. 2000). This leads to the assumption that the genera *Oncidium* and *Odontoglossum* produced endogenous ethylene and the pre-treatment with 1-MCP resulted in protection against such source of ethylene. Similar results were obtained in our experiments with potted plants. The beneficial effect of 1-MCP has been stronger in the inflorescences treated with exogenous ethylene (Fig. 1 and 2), which clearly indicates, that exogenous ethylene is an important factor in the senescence of flowers of *Oncidium* and *Odontoglossum* and use of ethylene receptor blockers eliminate unwished effects of ethylene. Our results support previous reports and could probably be a useful addition to the references reporting the beneficial effect of 1-MCP treatment in preventing ethylene damaging effects on a range of ethylene sensitive cut flowers, summarized by Watkins and Miller on http://www.hort.cornell.edu/department/faculty watkins/ethylene/index.htm, and modified by Serek et al. (2006).

The effect of 1-MCP disappears after a certain period, probably due to the proposed synthesis of new ethylene receptors in plant tissue (Serek and Sisler 2001).

1-MCP untreated cut inflorescences exposed to ethylene showed another important sign of senescence, the yellowing of the stems. This symptom was observed visually after 1–3 d, depending on the genotype (Fig. 2).



Fig. 4. Potted plants of *Odontoglossum* cv. 'Hansueli Isler' pre-treated with 0 or 200 nL L^{-1} of 1-MCP at 20 °C for 6 h, and then exposed to 0 or 1 μL L^{-1} ethylene for 7 d.



Fig. 5. Potted plants of *Oncidium* cv. 'Sweet Sugar' pre-treated with 0 or 200 nL L^{-1} of 1-MCP at 20 °C for 6 h, and then exposed to 0 or 1 μL L^{-1} ethylene for 10 d.

Effect of ethylene and 1-MCP treatment on potted plants

The flowers of plants treated with 1 μL L^{-1} ethylene wilted after 2 to 4 d and thus showed comparable results to cut inflorescences. Similar results were demonstrated on potted *Campanula* and *Schlumbergera* (Serek and Sisler 2001). The reaction of buds and flowers were very similar to buds and flowers of cut inflorescences as well. The untreated plants exposed to ethylene started to abscise buds and flowers first, followed by the untreated control and the pre-treated plants exposed to ethylene (Table 2).

The symptoms of wilting of the plants pre-treated with 1-MCP were delayed (Fig. 3). The mean display life of flowers in the different genotypes was extended by 2 to 3 d. In the experiment with potted plants the wilting symptoms were more uniform throughout the replications. Moreover, the single plants showed wilting over all flowers and buds of an inflorescence at the same time (Fig. 4) whereas in cut inflorescences the bottom flowers wilted first and the buds at the tips at last (Fig. 2).

The prolongation of the display life due to the pre-treatment with 1-MCP was similar in potted plants compared to cut inflorescences. The genotype 'Margarete Holm Larkspur' seemed to be more sensitive to ethylene than the others, therefore it would be interesting to repeat the different treatments also with potted plants. Heyes and Johnston (1998) and Lin et al. (2003) reported a reduction in flower and bud wilting caused by ethylene on potted *Phalaenopsis* due to 1-MCP pre-treatment.

In addition, potted plants showed another interesting symptom of senescence: on ethylene treated plants without 1-MCP pre-treatment yellowing of the leaves was observed after few days (Fig. 5). Chloroses were expressed regularly over the whole leaf.

In conclusion, the genera *Oncidium* and *Odontoglossum* have been proven to be sensitive to exogenous ethylene, and 1-MCP effectively improved their shelf life. As

mentioned above protection by 1-MCP ended after a certain period of time, in average after 3 to 7 d, depending on the genotype. Parameters to be tested in future experiments are the temperature as well as the duration of application and the concentration of 1-MCP. BUANONG et al. (2005) demonstrated with miniature potted roses and KEBENEI et al. (2003) with *Kalanchoe* that the exposure time of 1-octylcyclopropene (1-OCP), an ethylene perception inhibitor similar to 1-MCP, had a significant influence to its effectiveness. In these studies it was also shown that temperature was important. While in miniature potted roses temperatures below 5 °C reduced the performance of 1-OCP, in *Kalanchoe* the effectiveness of 1-OCP decreased already at temperatures below 15 °C.

It will be also interesting to underline the observation in this study with molecular expression studies on genes in the ethylene biosynthesis and perception.

Acknowledgements

The authors would like to thank grower Valtl Raffeiner, Gartenbau Valtl Raffeiner, Italy, for generously supplying the orchid plants.

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Received November 13, 2007 / Accepted September 29, 2008

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