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Postharvest performance of cut rose cv. Lovely Red as affected by osmoprotectant and antitraspirant compounds

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Abstract: In cut flowers, the post-harvest turgor is a critical aspect in a system in which, in the absence of the root system, transpiration water losses must be compensated. Two experiments were conducted in order to elucidate the effect of osmoprotectants (L-Proline) as well as of molecules with antitranspirant behavior (ß-aminobutyric acid - BABA or Pinolene) on the water relations and vase life of rose cut stems. Applications of L-Proline enhanced water fluxes, water conductivity, relative water content and stomatal conductance of rose cut stems in comparison to untreated plants, thus increasing the vase-life of rose cut flowers. BABA treatment reduced the stomatal conductance in rose as well as the daily water consumption, on the other hand senescence phenomena occurred earlier. The water used by pinolene treated stems was lower compared to the control and this was associated with a medium increase of the vase life. Overall, enhanced osmoregulation prolonged the vase life of cut flowers since the improved water status allowed the cut stem to partially continue its metabolic functions. On the other hand, the control of transpirational flux was functional in maintaining cellular turgor in pinolene treated cut stems, whereas with BABA, senescence phenomena occurred probably due to the activation of biochemical pathway of senescence involving abscisic acid. Taking all together, osmoregulation or direct control of transpirational fluxes may provide a promising avenue for improving the post-harvest longevity of cut roses.

1. Introduction

Post-harvest efficiency is a crucial point of the cut flowers commercial value since it is related to growth and storage conditions interacting with the plant genetic background; those aspects will overall contribute to maximize the stems qualitative performance after cutting (Fanourakis *et al.*, 2013). Cut flowers are subjected to a wide range of post-harvest losses as developmental senescence, leaf and petal abscission, leaf discoloration, premature wilting and disease from moulds and fungal pathogens (Scariot *et al.*, 2014). However, among all, water balance is yet a major factor influencing the longevity of cut flowers, in a system in

which water losses must be compensated by water uptake and transport in the absence of the root system (Singh and Moore, 1992; Lu *et al.*, 2010).

In particular, the post-harvest life of cut flowers is strongly dependent on their ability to maintain tissues hydration overtime, and water deficit or wilting mainly occur if the amount of transpiration exceeds the volume of water uptake (Halevy and Mayak, 1981; van Doorn, 2012).

One of the first plant responses to abiotic stress (as for cutting) is the stomatal closure, and this mechanism provides protection against tissues dehydration by reducing transpiration from the leaf surface (Hare *et al.*, 1998). However, in cut stems stomata are not completely closed after cutting, leading to a residual stomatal transpiration that together with cuticular transpiration, determine additional water losses from the stem (van Doorn, 2012). On the other hand, a phenomenon that can severely undermine the cut stems post-harvest performance, is the lowering of water uptake that is mainly due to occlusions located in the basal stem end (He *et al.*, 2006). In rose, one of the main causes of the cut flower wilting is the vascular occlusion determined by bacteria, air emboli and physiological responses to cutting (Fanourakis *et al.*, 2013).

In rose cut flowers, the regulation of water balance has been in the past decades a key aspect in the improvement of the stems vase life (Alaey *et al.*, 2011; Reid and Jiang, 2012). Among several mechanisms suggested that may improve water uptake in response to a stress, hydraulic conductivity variations and accumulation of compatible solutes are the most documented (Chen and Murata, 2002; Ehlert *et al.,* 2009). Compatible solutes or osmolytes are organic compounds which help in raising the osmotic pressure and thereby maintaining both the turgor pressure and the driving gradient for water uptake (Serraj and Sinclair, 2002). Common osmolytes found in plants mainly include proline, trehalose, fructan, mannitol and glycinebetaine and these compounds also help in maintaining the structural integrity of enzymes, membranes and other cellular components during the stress regime (Zhao *et al.,* 2007; Chen and Jiang, 2010). Compatible solutes may be constitutively overproduced in transgenic plants (Zhang *et al.,* 2004) or directly applied on plants to improve stress tolerance under both open-field and protected cultivation (Okuma *et al.,* 2004; Barbieri *et al.,* 2011; Cirillo *et al.,* 2016).

In addition to physiological mechanisms that can

be exploited to improve water uptake, the reduction of transpirational flux has traditionally been one of the main objectives for controlling the cellular turgor after harvesting of fresh-cut vegetables and ornamentals (Prakash and Ramachandran, 2000). The reduction of transpiration can improve the water balance of cut flowers and extend their vase life, whereby artificial closure of the stomata might be an efficient strategy to reduce water losses (Lu *et al.*, 2010; van Doorn, 2012).

One method of limiting water loss involves the use of antitranspirants, which reduce plant transpiration forming a vapour-impermeable film on the leaf surface and, among these, the natural terpene polymer di-1-*p*-menthene (pinolene) is widely applied on different crops (Francini *et al.*, 2011; Abdel-Fattah, 2013). These polymers, also called "Film Forming antitranspirants", sprayed on crops in a form of water emulsion, are generally employed to reduce weathering and extend pesticide efficacy, improving distribution and adherence of agrochemicals and decrease water loss and wilting of young transplants (Gale and Hagan, 1996; Percival and Boyle, 2009).

Research conducted on cut roses revealed that treatments with a Film-forming antitranspirant are able to reduce the degree of fresh weight loss and water loss during transpiration, delay the process of flower opening and slow down the rate of stomatal conductance reduction (Song *et al.*, 2011).

Alternatively to traditional antitranspirants, the use of compounds which may induce a series of stress adaptation mechanisms, such as stomatal closure, could also be considered. Beta-aminobutyric acid (BABA) is a non-proteinogenic amino acid known for its ability to increase plant resistance to biotic (Jakab *et al.,* 2001; Baider and Cohen, 2003) and abiotic stresses (Jakab *et al.,* 2005). Applications of BABA may improve the plant tolerance to stress by activation of defense mechanisms mediated by Abscissic Acid (ABA) and Salicilic Acid (SA) (Zimmerli *et al.,* 2000; Jakab *et al.*, 2005; Baccelli and Mauch-Mani, 2016). Taking this background into consideration, it is clear that an efficient control of water balance is crucial to improve cut flowers vase-life and this can be achieved by using molecules that activate water transport in stem or inhibit transpiration. Therefore, the aim of this study was to assess the influence of exogenous applications of osmolytes such as proline or anti-transpiring solutions like β-aminobutyric acid and Pinolene on the water balance, vase-life and also to shed light on the potential

physiological mechanism(s) involved in cut stems of rose.

2. Materials and Methods

Plant material and growth conditions

Two experiments were carried out in order to assess the effects of exogenous applications of L-Proline (Experiment 1) or antitranspirants [specifically: an active compound film forming (pinolene) and a stomatal closure inducing active compound β-aminobutyric acid ; Experiment 2] on water control in cut stems of rose plants (*Rosa spp.* L.) cv. Lovely Red.

Cut flowers of rose were harvested from two years plants grown in closed soilless system in a heated greenhouse located in Naples, south Italy (40°51'55.5"N 14°20'30.1"E). Rose plants were grown in plastic channels containing pumice and lapillus. The basic nutrient solution was supplied through a drip-irrigation system at a flow rate of 2 L h^{-1}. Irrigation frequency was regularly adjusted during the growing cycle based on the crop water requirements.

At marketable harvest, cut stems were immediately transferred to the laboratory under room conditions, re-cut at the base (2-3 cm) and placed in graduated glass cylinders with 300 ml of deionized water and sodium hypochlorite (50 mg L^{-1}).

Application of compatible solutes and antitranspirants compounds

In the first experiment, two days before harvest, rose plants were treated with 10 mM L^{-1} of L-Proline (Sigma-Aldrich, Saint Louis, Missouri, USA) in 200 ml of deionized water per plant, applied in the growth substrate. Control plants were treated with deionized water only. The treatment was performed at the end of the last daily irrigation and repeated after 24 h.

In the second experiment, two days before harvest and at the end of the last daily irrigation, a substrate treatment was performed on rose plants, with of 0.5 mM L^{-1} of ß-aminobutyric acid (BABA - Sigma-Aldrich, Saint Louis, Missouri, USA) in 200 ml of deionized water per plant, whereas control consisted of plants treated with deionized water.

On a second plot of plants, the Pinolene treatment was performed in post-harvest once transferred to the laboratory. Stems were sprayed with a solution of 50 g L^{-1} of Pinolene (96% poly-1-p-menthene, NU-film, Intrachem bio, Italy) in deionized water. Control stems were sprayed with deionized water only.

Storage and physiological measurements

Part of the stems, weighted and sized based on length and diameters, were placed on ten precision balances (EK-410i, A&D Instruments Ltd, Abingdon, UK) connected via USB to a computer for automatic monitoring of weight through a specific software (RS-com®, Corby, U.K.). These cylinders were sealed with parafilm to avoid water losses through evaporation. RS-com® software was set to record three daily weights in order to determine stems water consumptions over storage. Cut stems were stored for 12 days under room conditions measuring daily mean temperature and relative humidity using a thermo-hygrometer (DO 9847K, Delta OHM Srl, Padova, Italy).

At storage days 2, 4 and 6, water flux measurements were recorded by using a Scholander pressure chamber (3005F01 Plant Water Status Console, Soil mosture Equipment Corp., Santa Barbara, California, USA). Twenty centimeter stem segments (5 cm below the calix after measuring stems diameter) were immersed into a cylinder containing distilled water, placed in the pressure chamber, while the other extremity, out of the chamber, was connected to falcon tubes to collect and weight the water efflux. The system was then subjected to increasing pressure (P= 0.05, 0.1, 0.2, 0.3 MPa) and maintained at each pressure value for 5 minutes up to a constant outflow from the stem.

Water flux (Jv) was expressed as Jv= kg H$_2$O m^{-2} s^{-1} m^{-1}. Water conductivity (Lp) of stems was then expressed by the slope of the regression function of Jv vs. P. Volumes of collected efflux per unit of time (Jv) were normalized to the cutting section surface.

Stomatal conductance (gs) was determined at storage days 2, 4 and 6 using a diffusion porometer (Delta P-4, Delta-T Devices, Cambridge, U.K.) in three daily measurements (h 9:00 AM; h 1:00 PM; h 7:00 PM). Osmotic Potential (Ψπ) was measured using a dew-point psychrometer (WP4, Decagon Devices, Washington) on frozen/thawed leaf samples. Relative Water Content (RWC) value was calculated as: RWC= (leaf fresh weight - leaf dry weight)/(leaf saturated weight - leaf dry weight) (Morgan, 1984). Leaf area was estimated by scanning cut stems leaves and using the *Image J®* software (Abramoff *et al.,* 2004) for image processing. The cut stems vase life was assessed visually using a "quality score" from 0 to 4.

Statistical analysis

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). The RWC data were transformed in

arc-sin before ANOVA analysis.

3. Results

Experiment 1. Effect of L-Proline application on postharvest performance of cut rose

Exogenous applications of L-Proline enhanced significantly water fluxes of rose cut stems in comparison to the untreated control, for all the 3 days of measurements (Fig. 1A). During storage, water flux decreased from day 2 to day 6 in treated stems (Fig. 1A). Moreover, stems water conductivity (Lp) was 3.05 [(kg H_2O m^{-2} s^{-1} m^{-1}) MPa^{-1}] in control and 3.55 [(kg H_2O m^{-2} s^{-1} m^{-1}) MPa^{-1}] in L-Proline treatment (Table 1).

Significant Increase in stomatal conductance (gs) as well as in RWC were observed in L-Proline treatment compared to the control (Table 1). Similarly to the physiological measurements, water consumptions normalized per leaf area were higher in treated stems (Fig. 1B). As a result of cellular osmotic adjustment due to L-Proline application, leaf osmotic potential was lower for treated stems in comparison to untreated control (Table 1). The improved water status of L-Proline treated stems influenced positively cut stems longevity extending their vase life by 2 days compared to the untreated control.
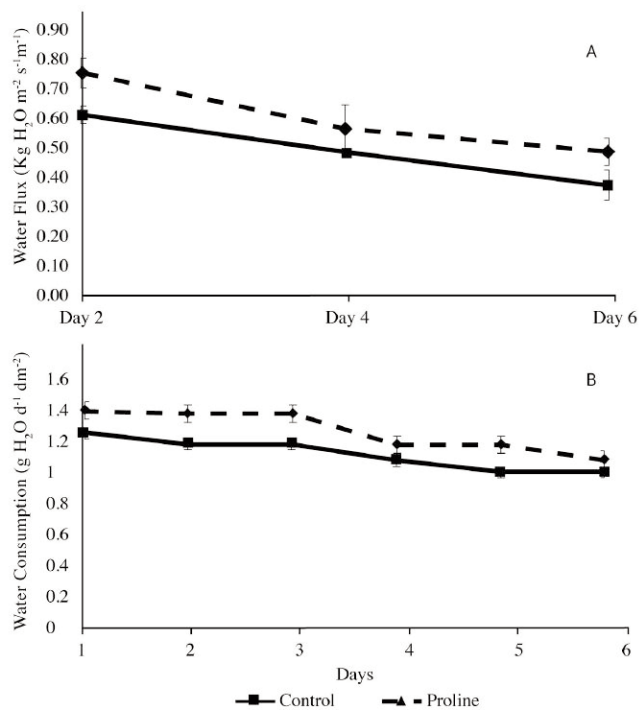

Fig. 1 - Effect of exogenous L-Proline on water flux (A) and water consumptions per leaf area (B) of rose cut stems during storage. Vertical bars indicate ± SE of means.

Table 1 - Effect of exogenous L-Proline on stomatal conductance (gs), relative water content (RWC), osmotic potential ($\Psi\pi$) and water conductivity (Lp) of rose cut stems

Treatment	gs (cm s^{-1})	RWC (%)	$\Psi\pi$ (MPa)	Lp (Kg H_2O $m^{-2}s^{-1}$ m^{-1})
Control	0.38 b	84 b	-0.17 b	3.05 b
Proline	0.42 a	89 a	-0.34 a	3.55 a
Significance	*	*	*	*

NS,*, not significant or significant at P≤0.05 respectively. Within each column, different letters indicate significant differences.

Experiment 2. Effect of antitraspirants application on postharvest performance of cut rose

In our current study, stomatal conductance was reduced by BABA treatment respect to the control (Table 2). Consequently, daily water consumption, normalized per leaf area, was lower for BABA treated stems (Fig. 2B). The application of 0.5 mM of BABA significantly reduced water fluxes during storage (Fig. 2A) as well as the water conductivity (Lp) of rose cut stems (2.6 [(kg H_2O m^{-2} s^{-1} m^{-1}) MPa^{-1}] in control vs. 1.54 [(kg H_2O m^{-2} s^{-1} m^{-1}) MPa^{-1}] in BABA treatment). RWC was significantly higher in the control and it decreased during storage (Table 2). Furthermore, the water potential decreased over time and it was lower for rose stems treated with BABA (Table 2). The vase life, however, was not influenced by the treatment since in BABA treated stems the improvement of the water balance was accompanied by premature yellowing of the leaves. As a consequence of the mechanical stomatal closure, the water use of pinolene treated stems was always lower compared to control (Fig. 3). This was associated with a significant increase of the vase life by 1.5 days (Fig. 4).

Table 2 - Effect of exogenous β-aminobutyric acid (BABA) on stomatal conductance (gs), relative water content (RWC), osmotic potential ($\Psi\pi$) and water conductivity (Lp) of rose cut stems

Treatment	gs (cm s^{-1})	RWC (%)	$\Psi\pi$ (MPa)	Lp (Kg H_2O m^{-2} s^{-1} m^{-1})
Control	0.41 a	79 a	-0,17	2.60
BABA	0.25 b	74 b	-0,27	1.54
Significance	*	*	*	*

NS,*, not significant or significant at P≤0.05 respectively. Within each column, different letters indicate significant differences.

4. Discussion and Conclusions

It is well established that osmotic adjustment contributes to maintain water uptake and cellular turgor (Maggio *et al.*, 2002; Heuer, 2003). Among all the osmolytes involved in this process, it has been sug-

gested that proline, exogenously applied via foliar spraying or through the irrigation water, could localize into the cytoplasm to reduce the cellular osmotic potential and to restore cellular hydration (Gadallah, 1999; Barbieri *et al.*, 2011). In our experiment, L-Proline treatment on rose plants has shown to substantially improve the hydration state of the cut stems, with an observed decrease of the leaf osmotic potential and increased stomatal conductance and leaf RWC. Consequently, the improved hydration state of tissues and stomatal conductance enhanced the water consumption in plants treated with L-Proline.
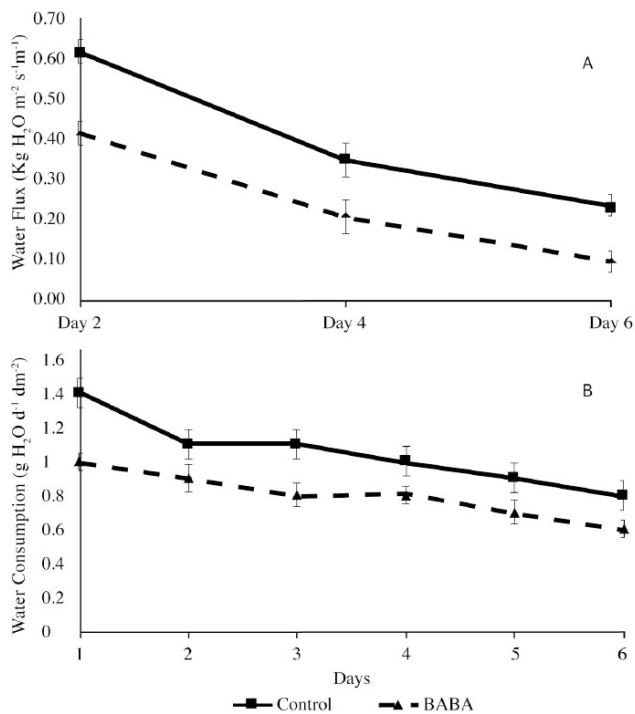
tion of these solutes, such as Proline, is one of the main mechanisms to alleviate the detrimental effects of dehydration (Morgan, 1984; Anjum *et al.*, 2011). In fact, the role of osmolytes includes mainly protection against the deleterious effects of the low water activity, preserving appropriate cellular volume (Csonka and Hanson, 1991).
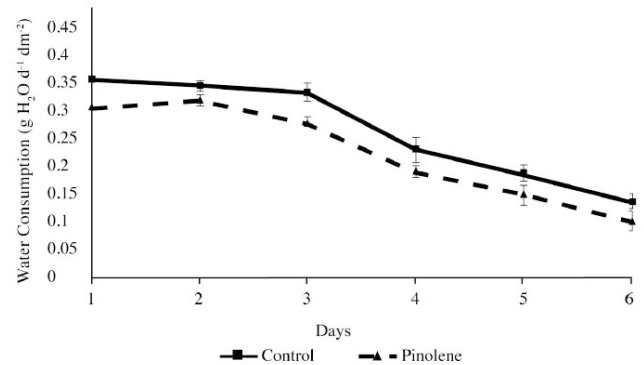

Fig. 3 - Effect of Pinolene on water consumptions per leaf area of rose cut stems during storage. Vertical bars indicate ± SE of means.

Fig. 2 - Effect of exogenous β-aminobutyric acid (BABA) on water flux (A) and water consumptions per leaf area (B) of rose cut stems during storage. Vertical bars indicate ± SE of means.
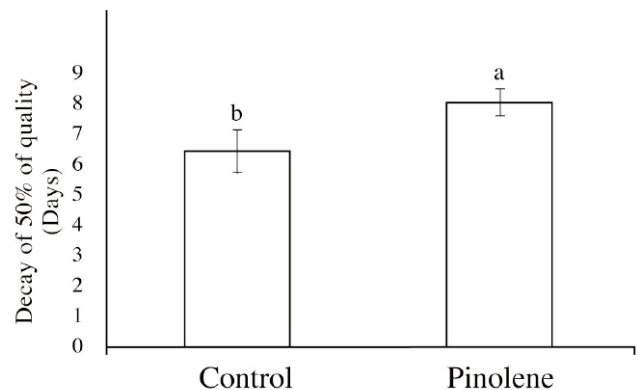

Fig. 4 - Effect of Pinolene on the vase life of rose cut stems expressed as decay of 50% of the stems quality. Different letters indicate significant differences at P≤0.05, vertical bars indicate ± SE of means.

The decline in stem water conductivity, is one of the main reasons for impaired water balance, as well as water stress is the most common reason for reduced cut roses vase life (Halevy, 1976; Joyce and Jones, 1992). The increase in water fluxes and water conductivity for the L-Proline treated flowers was an evidence of improved water status of the plant tissues that probably was the main factor involved in the extended vase life of L-Proline treated stems. It has been demonstrated that, in cut flowers, osmolytes are fundamental compounds in maintaining water balance, a key factor to extend their longevity (Ichimura *et al.*, 1997) as well as accumula-

However, even though it is not yet clear if an extension of the cut flowers vase life may be more related to the ability of stem to maintain sustained water uptake rates or to control water losses, the control of the stomatal conductance is a fundamental determinant of the tissue water balance (Fuchs and Livingston, 1996; Woodward *et al.*, 2002). In nature, it is well known that plants control water losses by regulating transpiration in response to environmental factors (Chaerle *et al.*, 2005). In some respect, cut flowers respond to the same stimuli and the difference between the rate of water uptake and the tran-

spiration rate is one of the parameters that will define their hydration state.

As documented in different cases, BABA acts through potentiation of ABA-dependent signaling pathways (Ton and Mauch-Mani, 2004) and for this reason, we supposed that applications of ß-aminobutyric acid (BABA) may increase the tolerance to water shortage through the induction of functions associated to the synthesis of ABA, such as stomatal closure (Jakab *et al.,* 2001; Desikan *et al.,* 2004).

Applied on rose plants, BABA treatment has induced a decrease in stomatal conductance, with the consequent reduction of the stems water consumptions. Along with this decrease of the transpirational flux, water fluxes and RWC decreased over time and they were generally lower in BABA treated stems. In addition, Lp was lower in BABA-treated stems. The decreased RWC and water potential, together with a reduction of the cut stems hydration state, may be associated to senescence phenomena that occurred with the premature yellowing of the leaves, which is also mediated by ABA (Hunter *et al.,* 2004; Ferrante *et al.,* 2006). Accordingly, Mayak and Halevy (1972) reported that exogenous application of ABA to rose cut flowers accelerate senescence phenomena.

Another strategy to control the plants transpiration and sustain a favorable plant water status is the utilization of antitranspirants compounds (Del Amor and Rubio, 2009). Our results confirmed that film-forming antitranpirants are effective in reducing water losses providing a thin coating on the leaves surfaces leading to an improved tissues water status in cut roses (Moftah and Al-Humaid, 2005; Song *et al.*, 2011; Mikiciuk *et al.*, 2015).

Consistently, this mechanical effect on the transpirational flux regulation was observed on water consumptions normalized per leaf area rose cut stems, that were significantly reduced by pinolene application during the vase life. The reduced water use was correlated to an extended vase life compared to the water-treated control.

In conclusion our results demonstrated that both osmoregulation and direct transpirational control were effective strategies in maintaining an enhanced hydration state of rose cut stems, leading to a prolongation of the stems vase life. Treatment with 10 mM L-Proline has allowed the maintenance of higher stomatal aperture and improved cut stems RWC and Lp during storage. The positive effects on cut stems were measured as decrease of osmotic potential and increased stomatal aperture consequent to osmoreg-

ulation. These physiological conditions are crucial for prolonging the vase life of cut flowers because, despite the absence of the root system, allow the stem to partially continue its metabolic functions.

On the other hand, the reduction of transpiration that is considered a functional target for controlling cellular turgor after harvest thus prolonging cut flowers. Since ABA is involved in the induction of physiological mechanisms that facilitate adaptation to abiotic stress, it has been hypothesized that the administration of BABA, a mediator of ABA functions, may confer a stress protection that could result in enhanced turgor and vase life of cut stems. In fact, our results also demonstrated that applications of 0.5 mM BABA on rose has reduced water consumption by inducing stomatal closure. However, this was associated with a more rapid decay of the cut stems quality probably for earlier oncoming of senescence phenomena. Furthermore, pinolene treatment prolonged the vase life of cut stems, by reducing water losses through transpiration. This was likely due to the formation of a 'film' at the leaf surface that acts as a physical barrier to gas exchanges.

Taking all together, we can conclude that osmoregulation or direct control of traspirational fluxes may provide a promising avenue for improving the post-harvest longevity of cut roses. However, further investigations are required whenever other physio-chemical processes are involved such as the induction senescence phenomena.

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