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Ethylene, 1-MCP and the Anti-Transpirant Effect of Active Compound-Film Forming Blend

Besufkad A*[1] **and Woltering E**[2]

[1]Debre Berhan Agricultural Research center, Crop Research Directorate, Department of Horticulture, 112 Debre Berhan, Ethiopia

[2]Wageningen University and Research center, Department of Plant sciences, Horticulture Production Chains (HPC), 6708 WG Wageningen, The Netherlands

***Corresponding author:** Besufkad A, Debre Berhan Agricultural Research center, Crop Research Directorate, Department of Horticulture, 112 Debre Berhan, Ethiopia, Tel: +251911708180; E-mail: amhabesufkad2@gmail.com

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Abstract

Pot plant production in green house is most of the time under high relative humidity and frequent irrigation. While, during shipping and retailing plants may be exposed to high temperature and infrequent irrigation. These unfavorable conditions often cause water loss, desiccation of plants and short shelf life. To reduce the deteriorating effect of water loss, application of anti-transpirants is one of the integral measures to implement. In the first experiment we evaluated the anti-transpirant effect of two physiologically gaseous active compounds Ethylene and 1-MCP (1-methylcyclopropane) and their interaction with the successful active compound ABA (Abscisic acid). In the second experiment, hoping to discover a more potent anti-transpirant we evaluated the efficacy of an active compound-film forming blend. Excised leaves of *Spathiphyllum* sweet checo were sprayed with different formulations. Weight loss of leaves as gram of water per gram of initial leaf weight and stomatal conductance were measured. On the first experiment, 10 ppm Ethylene and 500 ppb 1-MCP found to reduce water loss conspicuously and negatively interacted with 1.5 mM ABA. On the second experiment, a potent mixture of active compound-film anti-transpirants: ABA 1.5 mM+Leaf shine 1:20 followed by SA (Salicylic acid) 5 mM+Leaf shine 1:20 and SNP (Sodium nitroprusside) 200 μM+Leaf shine 1:20 were discovered. On both experiments significant positive correlations were found between weight loss and stomatal conductance (R=0.975, 0.987, P<0.05).

Keywords: Leaf shine; Abscisic acid; Salicylic acid; Sodium nitroprusside; Stomatal conductance

Introduction

Pot plant production in green house is most of the time under high relative humidity and frequent irrigation. While, during shipping and retailing plants may be exposed to high temperature and infrequent watering. These unfavorable post production environments can cause rapid substrate drying, desiccation of plants and acceleration of senescence and then short shelf life [1].Water loss is the main post production problems that deteriorate the quality of potted plants and make them unsalable [2]. For example, in common potted foliage plants *Monstera deliciosa* and *Philodendron* sp the amount of water loss during shipping and retailing was much higher than their growing environment leading to premature leaf wilting and death [3]. The main cause of water loss from plants is because of transpiration through the stomatal opening. The opening of stomata allows gas exchange between the plant and its environment. In the course of stomatal opening CO_2 can enter into the plant and water can be lost as a vapor. Hence, to avoid desiccation and eventual senescence, it is vital to have a right balance between carbon gain and water loss through stomatal movements [4].

To reduce water loss and its subsequent negative effect on the qualities of pot plants, it is important to develop techniques that can be engaged to reduce transpiration and preserving postharvest quality for extended time [5]. The major techniques that can be used to reduce transpiration could be the use of anti-transpirant agents. Anti-transpirant agents are compounds or provisions capable of reducing water loss from leaves of plants. These agents can be grouped into three groups [6], i) Film-forming types: that can create a physical barrier between the leaf and the surrounding (e.g., glycerol). ii) Reflecting materials: that can reflect the radiation falling on the surface of the leaves to reduce leaf temperature and the light needed for signaling during stomatal opening (e.g., Kaolin), and iii) Physiologically active stomata closing types: such as ABA and NO, Ethylene etc. capable of affecting metabolic processes in leaf tissues. Naturally, plants react to water deficit by closing stomata mainly mediated by endogenous Abscisic acid (ABA). Exogenous application of ABA and other active compounds also proved to induce stomatal closure. In our previous experiments and other related studies ABA is the most potent exogenously applied active compound. However, its efficiency was found to be compromised by the presence of other naturally occurring stomatal closure inducing active compounds like Ethylene [7,8]. The role of Ethylene in stomatal closure is not clear, in some species it mediates auxin induced stomatal opening (Merritt et al. 2001), whereas in other species it induces stomatal closure [9]. A study by Tanaka et al. [7], on Arabidopsis observed Ethylene inhibiting ABA induced stomatal closure. However, Tanaka et al. [7], did not show the sole effects of Ethylene in their system. Therefore, to see the effect Ethylene alone, a study conducted by, Desikan et al. [8], on Arabidopsis intact leaves evidently showed Ethylene induced stomatal closure. Moreover, pre-treatment of leaves with either of Ethylene negative regulators 1-MCP or silver blocked the effect of Ethylene on stomatal closure, indicating Ethylene's effect occurring through its receptors (Desikan et al. 2006). If indeed Ethylene somewhat blocks ABA's effect, theoretically the anti-transpirant effect of Ethylene negative regulator 1-MCP may depend on the presence of Ethylene and its interaction with ABA. Furthermore, a research on the effects of

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1-MCP in well-watered and water-stressed cotton plants discovered lower stomatal conductance in 1-MCP treated water stressed cotton plants than untreated controls. Therefore, the authors concluded that 1-MCP could be used to reduce water loss as a short term solution [10]. Hence, all things considered, the first aim of this study was to evaluate the anti-transpirant effect of Ethylene and 1-MCP and their interaction with ABA.

Film forming anti-transpirants are capable of making a thin glossy layer on the leaf surface so as to physically obstruct stomatal opening. Thus far, Leaf shine is the most widely used commercially available film forming anti-transpirant in Europe. In addition to its anti-transpirant effect it adds an instant natural gloss to leaves of plants and cut foliage in floral bouquets. It removes water spots and calcium deposits and it gives a protective shiny layer which keeps dust away [11]. In our previous experiments there wasn't any active compound treatment that surpassed the efficacy of Leaf shine with their respective concentrations used. Although, our experiment is the first of its kind, hoping to find a more potent anti-transpirant, the second aim of this study was to evaluate the efficiency of different concentrations of Leaf shine amalgamated with successful stomata closing active compounds.

Materials and Methods

Both experiments were done inside the horticultural production chain laboratory of Wageningen University of the Netherlands. Excised leaves of *Spathiphyllum* sweet Checo were used. Leaves were cut from their mother plant using a sharp scissor leaving a 10 cm petiole intact with the leaf and 30 ml of tap water filled in a plastic tube enclosed by a rubber cap was used as a vase solution. Matrix solution (producer unknown) as carrier solution of ABA in the first experiment, film forming anti-transpirant: Chrysal Leaf shine concentrate solution diluted 1:20 and 1:40 with deionised water (Chrysal international B.V,Naarden ,the Netherlands), active compounds: ABA 10% w/v (Valent USA, Walnut Creek, CA, USA), Sodium nitroprusside dehydrate (SNP) and Salicylic acid sodium salt (2 hydroxy benzoic acid) (SA), (from Sigma-Aldrich Corporation, Steinheim Germany) were used. Spraying was done under the flow hood using a very fine airbrush sprayer powered by pressurized gas. The abaxial and adaxial surfaces of excised leaves were sprayed homogenously up until the spraying solution started to flood off the leaf surface. After spraying was done leaves were left to dry outside the flow hood in the laboratory for about 2 hr. For the first experiment, after the respective spray and the necessary data was taken sprayed leaves were transferred to a 70 L enclosed metal tank, the first four treatments in the first tank, leaves to be treated with ethylene in the second tank and leaves to be treated with 1-MCP in the third tank. Ethylene and 1-MCP were injected to the second and third tank using a medical syringe and needle. Aiming to have a better control treatment, leaves to be treated with ethylene and 1-MCP were also sprayed with matrix.After 72 hr in the metal tank, all treatments were taken out and transferred and kept into the flow hood for 10 days. For the second experiment deionised water was used as a carrier solution for active compounds and to compensate for the diluting effect of Leaf shine the concentration of active compounds were raised by 5%. To properly mix active compounds with Leaf shine magnetic stirrers were used for 20 minutes and after leaves were sprayed and dried in the lab for 2 hr they were transferred and kept into the flow hood for 10 days. To ensure maximum transpiration sprayed leaves in the flow hood were exposed to 24 hr light and continuous ventilation. In the course of both experiments, temperature was revolving between 20°C to 22 °C and relative humidity was 45 to 50.

At the start of each experiment initial leaf weight of each leaf was determined. Once leaves were sprayed and dried, the weight of the leaf +tube+vase solution were measured and recorded as a starting point. In the course of the experimental period weight loss from the system was measured and recorded every 24 hr. At the last day of each experiment leaf area was also measured using LI-3100C Area Meter; before the actual leaf area measurement, the area meter was turned on for 15 minutes and calibrated. Daily water loss from the leaf surface was calculated using a simple formula: Daily water loss=(Weight of the leaf+tube+vase solution at day 0, 1, 2…10–weight of leaf+tube+vase solution at day 1, 2, 3….10).The amount of water transpired from the leaf surface each day was then calculated both in terms of amount of water transpired per grams of initial leaf weight and per centimeter square leaf area using the formula: Transpiration 1=((Weight of the leaf +tube+vase solution at day 0, 1, 2…(n)–weight of leaf+tube+vase solution at day 1, 2, 3….(n) / initial leaf weight)) and Transpiration 2=((Weight of the leaf+tube+vase solution at day 0, 1, 2…(n)–weight of leaf+tube+vase solution at day 1, 2, 3….(n)/ leaf area)).Cumulative water loss over time was also calculated by summing up weight loss at a given time points. Stomatal conductance was measured every morning 10:00 to 12:00 hours using SC-1 leaf Porometer for stomatal conductance measurements (Decagon Devices Inc., Pullman, USA).

The design of the experiment was CRD with three replications (leaves as a replication) and data analysis was done using SAS version 9.1. To test differences in weight loss and stomatal conductance one way ANOVA, followed by a Tukey mean separation test was carried out. Correlation analysis was also done to show relationships between the measured parameters.

Results

First experiment (Ethylene and 1-MCP)

The aim of this experiment was to evaluate the anti-transpirant effect of Ethylene and its negative regulator 1-MCP and to explore the possible interaction effect of Ethylene and 1-MCP with ABA. Apart from the control treatments matrix, tap water and Leaf shine 1:20 significant differences were not observed between the tests treatments for either of the parameters measured. However, Ethylene and 1-MCP reduced water loss and stomatal conductance considerably compared to the least effective control treatment 'tap water'. ABA+Ethylene or 1-MCP reduced water loss better than Ethylene or 1-MCP alone and Ethylene reduced water loss more successfully than its negative regulator 1-MCP. Overall, with the respective concentrations tested ABA was a more potent anti-transpirant than Ethylene and 1-MCP and the performance of ABA was reduced either by Ethylene or 1-MCP. 10 ppm Ethylene and 500 ppb 1-MCP reduced the efficacy of 1.5 mM ABA by 6 and 10% respectively (Table 1). Aiming to show the relationship between stomatal conductance and weight loss, a correlation analysis was also carried out and it revealed a significant positive correlation (R=0.975, P<0.05) (Figure 1).

Treatments	**Weight loss (g/g)**	**Stomatal conductance (mmol/m^2/s)**
Leaf Shine 1:20	1.030(a)	0.000(a)
Matrix+ABA 1.5 mM	1.116(ab)	0.800(ab)
Matrix+ABA 1.5 mM+Ethylene 10 ppm	1.178(ab)	1.100(ab)

Matrix+ABA 1.5 mM+1-MCP 500 ppb	1.228(ab)	1.400(ab)
Matrix+Ethylene 10 ppm	1.233(ab)	1.600(b)
Matrix+1-MCP 500 ppb	1.332(b)	1.700(b)
Matrix	1.648(c)	9.767(c)
Tap Water	1.828(c)	12.267(d)
Mean	1.324	3.579
Sig	*	*
CV	7.1	14.0

Table 1: Total weight loss at the 13th day and cumulative stomatal conductance of *Spathiphyllum sweet checo* expressed as (g/g) of initial leaf weight and mmol/m^2/s respectively. Values are based on the three leaves per treatment. Significant differences are denoted by different lower case letters (a,b).

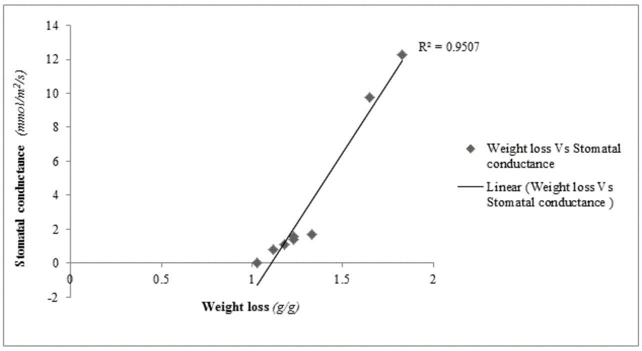

Figure 1: Graph showing the relationship of weight loss (g/g) and stomatal conductance (mmol/m^2/s) on *Spathiphyllum sweet checo*, X axis showing weight loss of all treatments and Y axis represents stomatal conductance of all treatments. Values are averages of the three leaves per treatment.

Second experiment (Active compound-Film forming blend)

This experiment was done to assess the possibility of improving the anti-transpirant effect of a film forming anti-transpirant by incorporating active compounds in it. To assess the possibility of getting a positive combined effect three active compounds with their respective successful concentrations: ABA 1.5mM, SA 5 mM and SNP 200 µM were incorporated into two Leaf shine concentrations LS 1:20 and LS 1:40. Note: Positive combined effect implies when AB>A and B. For both parameters considered, statistically significant differences were displayed mainly between treatments having a Leaf shine component and without a Leaf shine component (Table 2).

Treatments	**Weight loss (g/g)**	**Stomatal conductance (mmol/m^2/s)**
dw+ABA 1.5 mM+LS 1:20	0.581(a)	0.000(a)
dw+SA 5 mM+LS 1:20	0.621(ab)	0.000(a)
dw+SNP 200 µM+LS 1:20	0.636(abc)	0.172(a)
Leaf shine 1:20	0.703(abc)	2.400(b)
Leaf shine 1:40	0.738(abc)	2.800(b)
dw+ABA 1.5 mM+LS 1:40	0.797(bc)	1.100(ab)
dw+SA 5 mM+LS 1:40	0.801(bc)	1.200(ab)
dw+SNP 200 µM+LS 1:40	0.810(c)	0.900(ab)
dw+SA 5 mM	1.947(d)	16.800(c)
dw+SNP 200 µM	1.952(d)	16.167(c)
dw+ABA 1.5 mM	1.985(d)	20.900 (d)
Tap water	2.103(d)	21.100(d)
Mean	1.139	6.961
Sig	*	*
Cv	5.6	10.2

Table 2: Total weight loss at the 10th day and cumulative stomatal conductance of *Spathiphyllum sweet checo* expressed as (g/g) of initial leaf weight and mmol/m^2/s respectively. Values are based on the three leaves per treatment. Significant differences are denoted by different lower case letters (a,b).

A positive combined effect was found between LS 1:20 and active compounds, both in terms of the sole efficacy of Leaf shine 1:20 and active compound treatments. With their respective concentration ABA improves the efficacy of Leaf shine 1:20 better than SA and SNP. In numerical terms, Leaf shine 1:20+1.5 mM ABA, Leaf shine 1:20+5 mM SA and Leaf shine 1:20+200 µM SNP reduced water loss 19,12 and 11% better than sole Leaf shine 1:20 respectively. However, there was no positive combined effect between Leaf shine 1:40 and active compounds. In relation to the sole efficiency of Leaf shine 1:40, there was rather a negative combined effect. SNP reduced its efficiency higher than ABA and SA (Table 2). Similar to the first experiment the relationship between weight loss and stomatal conductance were very strong, with a significant positive correlation ($R=0.987$, $P<0.05$) (Figure 2).

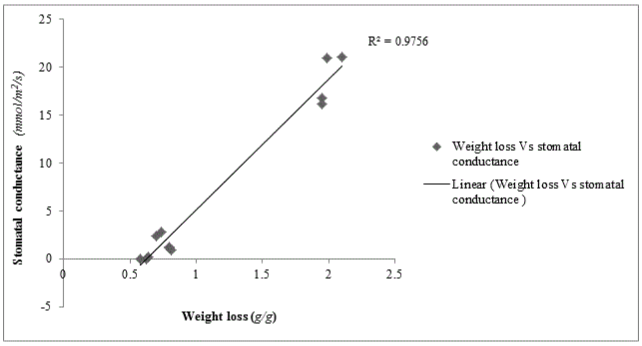

Figure 2: Graph showing the relationship of weight loss (g/g) and stomatal conductance (mmol/m^2/s) on *Spathiphyllum sweet checo*, X axis showing weight loss of all treatments and Y axis represents stomatal conductance of all treatments. Values are averages of the three leaves per treatment.

Discussion

First experiment (Ethylene and 1-MCP)

Parallel to our result, previous observations also confirmed the ability of Ethylene to induce stomatal closure and the subsequent reduction in water loss [9,12]. A detailed study done by Desikan et al. [8] convincingly showed the ability of Ethylene to induce stomatal closure in intact leaves of Arabidopsis. Ethylene supplied either directly as gas or generated from its immediate precursor ACC (1-aminocyclopropane-1-carboxylic acid) initiated stomatal closure 30 minutes after exposure. To further confirm the involvement of Ethylene on stomatal closure they pretreated intact Arabidopsis leaves with Ethylene negative regulator 1-MCP and silver thiosulfate and it inhibited Ethylene induced stomatal closure. As reported by Desikan et al. [8], to cause stomatal closure Ethylene might have induced the generation of H_2O_2 mediated by the NADPH oxidase isoform AtrbohF. Although, detailed information on the sole anti-transpirant effect of 1-MCP could not be found, a study by Eduardo et al. [10] displayed the ability of 1-MCP to reduce stomatal conductance of water stressed cotton plants.

The outcome of our experiment also clearly demonstrated the capacity of Ethylene and 1-MCP to inhibit the anti-transpirant effect of ABA. Likewise, the inhibitory effect of Ethylene has also been reported [7,8,13]. Similar to the observation of Desikan et al. [8] and Chen et al. [13], Ethylene indeed triggered stomatal closure and in the presence of ABA Ethylene might have functioned the other way around. Remarkably, 1-MCP compromised the efficiency of ABA powerfully than ethylene. For the reason that 1-MCP is the so called negative regulator of Ethylene and the inhibitory effect of Ethylene on ABA was already reported, we thought the effect of 1-MCP would be the reverse, but our result confirmed both Ethylene and 1-MCP can reduce transpiration solely and both of them can reduce the anti-transpirant effect of ABA. Hence, at least for concentrations we used, it seems reasonable to conclude that when ABA is used as stomatal closing agent, the presence of both Ethylene and 1-MCP better be avoided and during the need for application of gaseous active compounds aiming to close stomata, both Ethylene and 1-MCP can be used.

Second experiment (Active compound-Film forming blend)

Although, the type of chemical interaction occurred is not yet known, due to the more glue like nature of Leaf shine 1:20 comparable to Leaf shine 1:40, the positive combined effect might have been because of the ability of the less diluted Leaf shine to retain active compounds on the leaf surface for extended period of time than the more diluted form. In our other dissimilar experiments, on the same plant material, when matrix (a glue like carrier solution with no anti-transpirant effect) was used as carrier solution for active compounds, 1.5 mM ABA followed by 5 mM SA and 200 µM SNP were the top three successful active compound treatments and when these active compounds were blended with the less diluted Leaf shine, 1.5 mM ABA followed by 5 mM SA and 200 µM SNP produced a better positive combined effect. Therefore, for the reason that both Leaf shine and matrix are glue like, it looks, as if the effect of the less diluted Leaf shine was retaining these active compounds on the leaf surface while performing its own anti-transpirant effect. On the contrary, these active compounds with their respective concentration compromised the anti-transpirant effect of the more diluted Leaf shine and since we haven't done further analysis and no related studies were found, what might happened is not yet clear and should be the focus of future studies.

Generally, for the concentrations of Leaf shine and active compounds used, their combination may or may not produce a successful blend. Regarding Leaf shine 1:20 and active compounds with their respective concentration, there was a positive combined effect and this specific combination can be used as a powerful anti-transpirant. The appearance of leaves sprayed with active compounds was not appealing as Leaf shine sprayed leaves. Nonetheless, the ability of active compounds to improve the efficacy of a film forming anti-transpirant Leaf shine may open another venue for their future significance.

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