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Application of di-1-p-menthene based anti-transpirant improves the growth of young olive trees

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Abstract: The olive tree undergoes significant stress with high temperatures and irradiance levels that occur during the growing season. Various products are used to mitigate the negative effects of abiotic stress in plants, aiming at different physiological, biochemical and morphological functions, such as the use of plant products that attract considerable interest from the scientific community and commercial enterprises. The aim of the research was to examine the effects of a plant-derived anti-transpirant (Vapor Gard®, V) product on the growth of two year-old olive trees subjected to high temperature in a nursery . (V) is a water emulsifiable organic concentrate of di-1-p-menthene ($C_{20}H_{34}$), a terpenic polymer also known as pinolene. The study was carried out in a greenhouse on trees of a native cultivar of Campania (cv. *Salella*) grown in pot, during the growing season from May to September 2020. The experimental design included an anti-transpirant product (V) applied 5 times at 20 days intervals compared with a Control (C). The following physiological and biometric parameters were evaluated: stomatal conductance, chlorophyll ***a*** fluorescence, SPAD index, RWC, shoots growth, total leaf area per plant, trunk cross-sectional area and dry matter partitioning. The results obtained showed that the application of di-1-p-menthene was able to induce a significant improvement of the shoots growth (+46.6 %) and trunk cross-sectional area (36.54 vs 43.49 mm^2). At same time a significant reduction of the stomatal conductance and an increase of leaf RWC values were recorded. The treatment with the anti-transpirant also favored the increase of SPAD values of leaves (+5.7 %). At the end of the experiment the treated trees had greater total dry matter than the control (+12.1 %).

Keywords: stomatal conductance; chlorophyll ***a*** fluorescence; RWC; growth; dry matter partitioning

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1. Introduction

Olive tree (*Olea europaea* L.) is one of the most important crops in the Mediterranean basin and its growth and development are mainly controlled by atmospheric conditions [1,2]. Olive orchards are strongly exposed to thermal and hydric stresses during the growing season, more noticeable during summer and in the innermost areas of the European country [3], this condition has been highlighted especially in recent years with the scenario of climate change. Climatic variation is indicated by changes in the mean values of the principal weather parameters, such as temperature and rainfall. The atmospheric CO_2 concentration is increasing, and in addition to its direct effects on plant growth, this change is expected to raise the global mean surface temperature and result in an increase in the severity of summer drought [4]. Mearns et al. [5] noted that changes in temperature and precipitation along with a greater frequency of extreme weather (freezing, drought, hail, etc.) have had serious effects on agricultural yield [6]. Temperature affects most plant physiological processes, including photosynthesis and transpiration, both are regulated by stomatal conductance and they mutually affect each other [7]. Photosynthesis is the primary factor driving plant growth, as about 10–40% of the energy goes directly into biomass accumulation under optimal conditions [8] and all stress factors affecting the efficiency of photosynthesis lead to reduced plant biomass and growth [9]. A very powerful tool for studying the photosynthetic metabolism is the measurement of Chlorophyll a (Chl a) fluorescence. This method is based on quick non-destructive measurements which can be conveniently used in vivo in the field [10,11]. Chl a fluorescence measurements provide detailed information about the status and functionality of the photosynthetic apparatus and it gives an insight into the plant physiological response to different environmental stress factors. Based on these considerations, in this research we studied the effect of an anti-transpirant at the physiological level, focusing on photosynhetic metabolism. One neglected agronomic technique that has the potential to significantly contribute to abiotic stress amelioration in food crop production is the use of anti-transpirants. Anti-transpirants are substances that are applied on leaves to reduce transpiration and hence improve plant water potential [12]. The formation of thin films minimizes the escape of water from the plant by decreasing stomatal conductance, thus reducing transpirational losses, improving plant water status and reducing wilting and leaf abscission [13]. To test this hypothesis we intended to evaluate the effects of a plant-derived anti-transpirant on the growth and physiology of young olive trees during and at the end of the growing season. A product based on 1-p-menthene, [pinolene], which is an emulsifiable terpenic polymer distilled from conifer resins was tested; when applied it creates a thin, transparent and flexible coat stopping the water dispersion as a proper physical barrier [14].

2. Materials and Methods

2.1. Biometric and eco-physiological analysis

The trial was conducted at a greenhouse of the Department of Agriculture of the University of Naples Federico II, between the end of May and the end of September 2020, using 2-year-old potted olive trees of the cultivar Salella, the maximum temperature was 47.30 C° while the minimum temperature was 23.04 C° during the vegetative season. Before starting the experiment all the plants were fertilized by spreading 0.5 kg/tree of Nitrophoska Gold. At the start of the trial all plants had homogeneous vegetative characteristics. The study included spray treatments of the anti-transpirant Vapor Gard® (V) and water spray applications (Control, C). (V) is a water emulsifiable organic concentrate of di-1-p-menthene ($C_{20}H_{34}$), a terpenic polymer also known as pinolene, which is prepared as a 2% solution in water. All the leaves of the canopy were sprayed using a portable pump. For each treatment, ten replicates consisting each of a single tree. The anti-transpirant product was applied five times during the growing season. Both treatments were irrigated with water equal to 100% of the evapotranspiration (ET) calculated by weight. During the growing season, the following measurements were carried out: shoots growth with an electronic caliber, trunk cross sectional area (TCSA) by standard formula ($girth^2/4\pi$) and leaf number per plant. On fully developed leaves, the stomatal conductance was measured using a Porometer (Li-1600 Steady State Porometer), leaf SPAD index using a chlorophyll meter SPAD-502 (Konica-Minolta, Osaka Japan). Chlorophyll a fluorescence measurements were recorded in the field on 30 min dark-adapted leaves, randomly sampled among the top 3 fully expanded young leaves of each plant, using a PAR-FluorPen FP 110/D portable fluorimeter (Photon Systems Instruments, Drásov, Czech Republic) equipped with detachable leaf-clips. Ten replicate measurements for each experimental treatment were taken between 09:00–10:00 (Central European Summer Time) for the morning measurements and between 13:30–14:30 for the mid-day measurements, according to the procedure reported by Di Mola et al. [15]. Fluorescence data were analyzed by the FluorPen software ver. 1.1 (Photon Systems Instruments, Drásov, Czech Republic) and the Fv/Fm parameter was calculated as follows: $Fv/Fm = (Fm - F0)/Fm$, where F0 is the basal fluorescence recorded at 40 μs and Fm is the peak of the fast fluorescence rise following illumination of the dark adapted leaves with a saturating flash of light. Plant water status was assessed by calculating the leaf relative water content (RWC) as described by Conti and Smirnoff [16], modified as follows: due to the thick cuticle/epidermal layers, the sampled leaves were rehydrated for 48 h in distilled water at 4 °C in the dark prior to measuring the rehydrated weight. Subsequently, the same leaves were dehydrated for at least 48 hours at 80 °C until constant weight was reached, before their dry weight was measured. RWC was calculated as: RWC (%) = ((fresh weight-dry weight)/(rehydrated weight-dry weight)) x 100. At the end of the growing season the plants were destroyed to determine the dry matter partitioning of the various components of the trees (roots, leaves, wood and total).

2.2. Statistical Analysis

Data are presented as mean ± standard error and were statistically analysed by the analysis of Variance (ANOVA) according to a completely randomized design and the averages of measurements were compared by the Student–Newman–Keuls Test ($p < 0.05$), using the statistical software SPSS for Windows (version 12.0.1, Chicago, IL, USA).

3. Results and discussion

Table 1 shows the effects of V on biometric parameters of treated olive plants compared with the control plants (C), showing significance difference both in shoot growth and in TSCA; also leaf area per plant showed significant differences between the C and V treatment with values of 682.03 and 746.30 cm² respectively. V application has shown positive effects on the shoots growth already 20 days after the first application, showing a significant increase at the end of the growing season compared to C (Figure 1).

Table 1. Effects of di-1-p-menthene (V) applications respect to the Control (C) on shoots growth, TSCA (trunk sectional cross area) and leaf area per plant. Mean ± S.E follow by different letters are significantly different at $p < 0.05$.

	C	**V**
Shoots growth (cm)	14.44 ± 1.55 b	21.17 ± 1.84 a
TCSA (cm²)	1.70 ± 0.54 b	2.30 ± 0.73 a
Leaf area per plant (cm²)	682.03 ± 16 b	746.30 ± 23.88 a

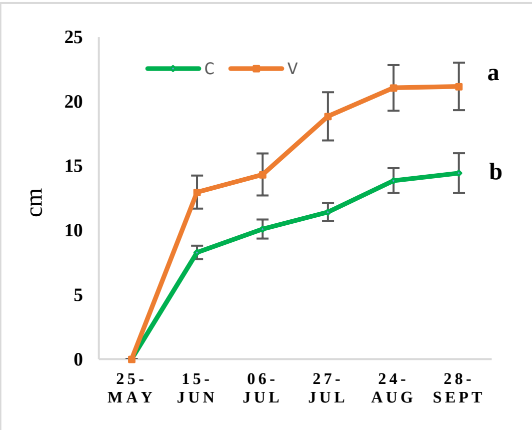

Figure 1. Effects of di-1-p-menthene (V) applications with respect to the Control (C) on shoots growth during the growing season. Bars represent the S.E.Means accompanied by different letters are significantly different the Student–Newman–Keuls Test ($p < 0.05$).

Application of V significantly reduced stomatal conductance with a consequent increase in leaf temperature due to stomatal closure. Leaf temperature showed an increase when the trees were treated with anti-transpirant of about 3.2 % compared to the control plants, while the stomatal conductance showed a significant reduction of 20% following the application of the product at the end of the growing season (Figure 2a). Anti-transpirants are chemical compounds used to limit the transpiration process and to maintain the advantageous parameters of the water balance of plants [17]. Potted trees treated with V product showed greater growth than the control soon after treatment and up to the end of the vegetative season. Generally, this was associated with higher leaf photosynthetic rates and stomatal conductance [18]. That was previously reported by Mikiciuk et al. [19] who found that V application reduced the intensity of strawberry transpiration on average by 24.4%. Ouerghi et al. [20] obtained similar results in wheat and barley cultivated under drought conditions. Similar results have also been observed by Di Vaio et al. [21,22] on "Aglianico" and "Falanghina" (*Vitis Vinifera*), where a product based on 1-p-menthene significantly reduced the stomatal conductance and assimilation rate. Stomatal conductance usually decreases at high temperatures, however, this result is probably due to leaf water deficit or large leaf-to-air water vapour concentration differences generated by high temperature [23,24]. On the other hand, the relative water content index (RWC) is used for the assessment of plants; at the end of the growing season our results showed a 21.8% higher RWC in V treatment compared to C (Figure 2b). Similar results have also been observed by Mikiciuk et al. [19] 2015, where the anti-transpirant used product increased the RWC in leaves of the tested strawberry cultivar by 4.4%. According to Abdel-Fattah [25], anti-transpirants, which form a film on the surface of the plants, increase the RWC in leaves. In this research we followed an experimental protocol based on the measurement of the Chl *a* fluorescence two times during the day. This procedure, not only allowed us to record the amplitude of the daily fluctuations of Fv/Fm, but it also uncovered significant differences between treatments as previously reported [15]. The Fv/Fm ratio represents the maximum quantum yield (or the maximum photochemical efficiency) of PSII in dark-adapted leaves and it indicates the probability that a trapped photon will end up in the reaction center and start a photochemical event. This parameter is widely used as a stress indicator [10,26]. Both C and V plants had an Fv/Fm of 0.81 at 09:00 a.m., corresponding to the optimum value for healthy, non-stressed plants [27]. The Fv/Fm decreased gradually and at the mid-day measurement it was significantly lower than at 09:00 a.m., thus evidencing the daily fluctuation in the maximum quantum yield of PSII photochemistry. At 02:00 p.m. significant differences emerged between C (Fv/Fm = 0.76) and V (Fv/Fm = 0.79) plants. Therefore, the V treatment reduced the mid-day depression in photosynthetic efficiency by narrowing the daily fluctuation in Fv/Fm. Overall, this positive effect on the plant photosynthetic metabolism may explain the higher growth parameters recorded for V treated plants. Our results are consistent with those reported previously by Latocha et al. [28] where anti-transpirant product application increased the chlorophyll content in leaves at the beginning of the experiments while it enhanced the efficiency of the photosynthetic apparatus during almost the whole experimental period on Actinidia arguta. Data on the degradation of the leaf chlorophyll molecule after thermal stress condition were recorded using a Minolta chlorophyll meter SPAD during the vegetative season. Our results showed, at the end of test, positive effects of V product in maintaining higher levels of leaf SPAD than the C (Figure 2d). In fact, V reported an increase in SPAD index equal to 5.7% compared to C. Higher Fv/Fm chlorophyll fluorescence emissions and SPAD index values as a measure of leaf chlorophyll content recorded on anti-transpirant trees compared with C, indicated less damage to the structural integrity of the photosynthetic apparatus and higher leaf chlorophyll content at the cessation of the growing season. Improvements in these two measurements might account for increased yield at harvest in polymer-treated trees as chlorophyll is the major cell of photosynthetic activity within higher plants [29]. Not all studies are in line with these findings, Glynn et al. [30] showed no significant effects of film-forming polymers on leaf Fv/Fm values as a measure of tree vitality and SPAD index values as a measure of leaf chlorophyll content compared to C were recorded on *Aesculus hippocastanum L.* and *Quercus robur L.*

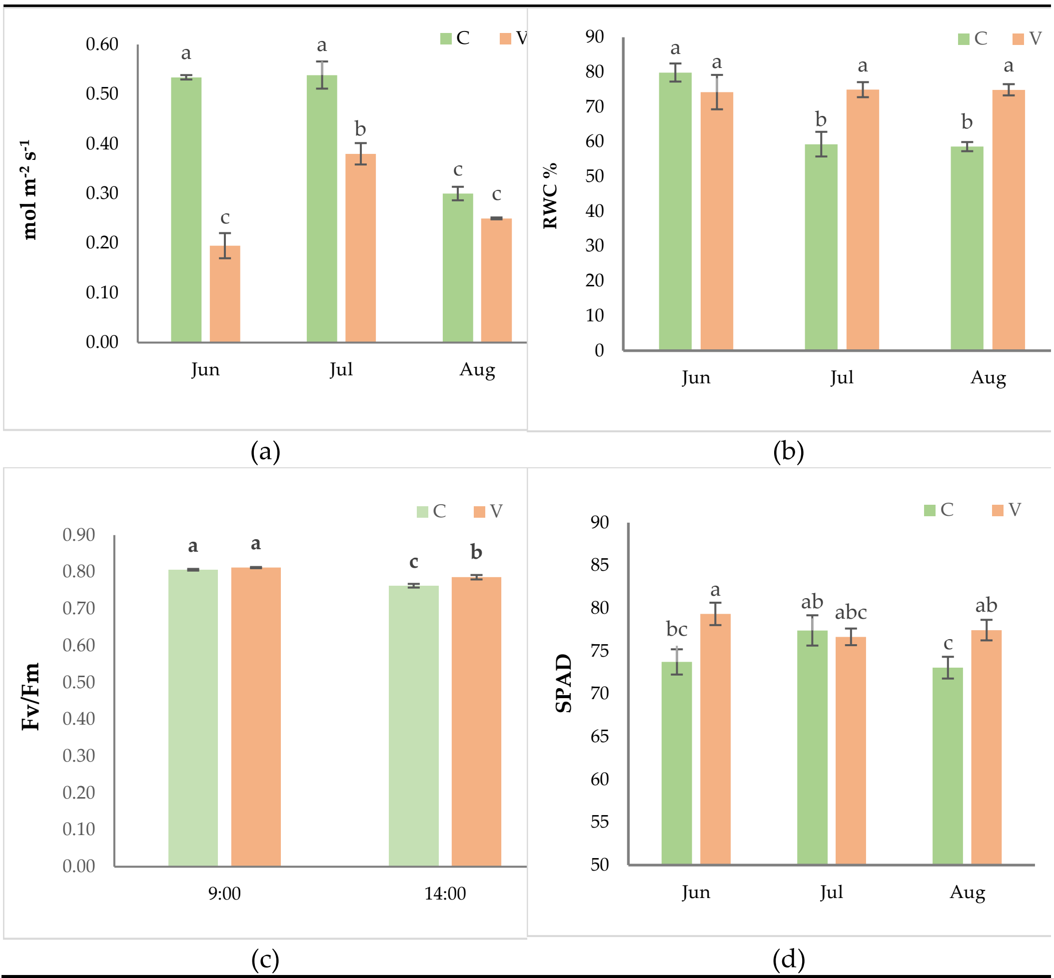

Figure 2. Effects of di-1-p-menthene (V) applications respect to the control (C) on stomatal conductance (a), RWC (b), fluorescence (Fv/Fm) measured at different temperature condition at 9:00 h and at 14:00 h (c) and SPAD (measurement of chlorophyll content) (d) during vegetative season (June, July and August). Bars represent the standard error. Means accompanied by different letters are significantly different the Student–Newman–Keuls Test ($p < 0.05$).

Figure 3 shows the dry matter at the end of the trial, highlighting an increase both in the roots and in the total dry matter with V application, respectively, equal to 15.4% and 10.8%. Our results are consistent with those reported previously on anti-transpirant products that influenced plant height and total dry matter [31-32]. This positive effect of this product based on 1-p-menthene on vegetative growth and development could be due to improved plant water status related to lower transpiration [12].

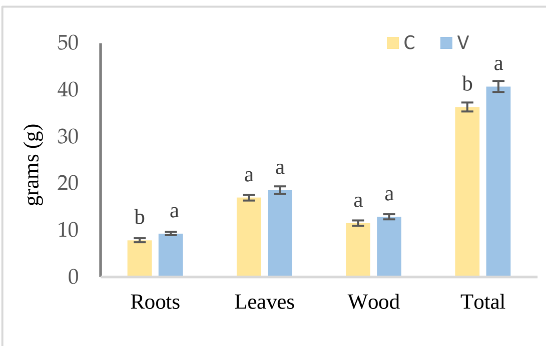

Figure 3. Effects of di-1-p-menthene (V) application in respect to the control (C) on dry matter of roots, leaves, wood and total dry matter. Bars represent the standard error. Means accompanied by different letters are significantly different the Student–Newman–Keuls Test ($p < 0.05$).

4. Conclusions

Foliar application of the Vapor Gard anti-transpirant decreased the transpiration intensity of young olive trees. Plants sprayed with the tested preparation were characterized by a higher relative water content (RWC) in leaves and a higher value of efficiency of photosystem II (PSII) and leaf chlorophyll concentrations (SPAD) at the end of the vegetative season. This positive effect of the product based on 1-p-menthene on vegetative growth and development could be due to improved plant water status related to lower transpiration. This is a preliminary test carried out under greenhouses, therefore further studies are necessary to determine the effect of this product on the physiological and biometric parameters, on the olives and oil quality of mature trees cultivated in open field conditions.

Author Contributions: A.C. wrote the first draft of the manuscript A.C. and G.M. followed the agronomical and physiological measurements. S.C. and C.D.V. provided conceptualization, experimental design, data validation. S.C. formal analysis for RWC and Chl *a* fluorescence; S.C, G.M. and A.T. methodology, data interpretation, writing and review. C.D.V. coordinated the whole project, provided the intellectual input, set up the experiment and corrected the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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