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Application of antiperspirants to improve the condition of ornamental plants subject to medium- and long-distance transport in refrigerated container

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Abstract: The ornamental nursery sector sells and delivers its products not only within the European Union but throughout the world, thus shipping for long distances has become commonplace in the industry. Extended transport times may result in loss of quality and reduced longevity. Consequently, an effective logistics strategy is of competitive importance for nursery production. This research was carried out with the aim of improving long-distance transport conditions (up to 6 weeks) of ornamental plants produced in the nurseries of the Pistoia District. Phenotipic and physiological parameters of plants during transport were studied, testing three biodegradable antiperspirants and a biodegradable microfilm to protect plants on five important pot ornamental species: maple (*Acer palmatum*), cypress (*Cupressocypari leylandii*), privet (*Ligustrum texanum*), nandina (*Nandina domestica*) and viburnum (*Viburnum tinus*). Plant tolerance to storage conditions in refrigerated cell or container (T° = 8-12°C) varied considerably according to the considered species, with cypress resulting extremely tolerant and maple and nandina very sensitive. Treatments with antiperspirants did not exhibit particularly evident effects on the tested species. The use of biodegradable film was inadequate to protect plant quality during long-distance shipments. Even in cases of total or partial loss of leaves by species such as maple and nandina, an optimal recovery of vegetative development was highlighted once these species were relocated in outdoor cultivation. Among physiological parameters, MDA and phenols contents were the most stress-related variables, being negatively correlated to the quality decay of plants transported in dark refrigerated cells for 2-6 weeks.

1. Introduction

The Nursery District of Pistoia (Tuscany) is the heart of Italian ornamental production and leader in Europe. This activity covers over 5200 ha, with about ha 1000 of pottery, 1500 companies, over 5500 direct employees (in addition to the related industries) and a Gross Saleable

Production over 300 M €, of which 160 M € are exported. The distribution of cultivation is as follows: evergreen trees and shrubs 1600 ha, conifers 1350 ha, ornamental deciduous trees 1420 ha, deciduous shrubs 350 ha, creepers and other shrubs 380 ha, roses 100 ha (Marzialetti, 2015).

The ornamental nursery sector sells and delivers its products not only within the European Union but throughout the world, thus shipping, often for long distances, has become commonplace in the industry. Plant transfer, mainly carried by road, rail or sometimes by sea, and in crowded, stifling hot or refrigerated truck, can also be very long. The extended shipping and/or storage times may result in loss of quality. Consequently, an effective logistics strategy is of competitive importance for companies to make sure plants are delivered on time and in the best conditions. Plants travelling long distances are frequently negatively affected by the critical environmental conditions during transport, such as exclusion from light in closed containers, exposure to harmful gases and temperature extremes, poor air ventilation, high relative humidity (RH) and vibration. These conditions can lead to deterioration of even the highest quality plants. Further, the environmental and physical stresses imposed upon plants during transfer are worsened if plants are improperly produced, incorrectly packaged and/or mishandled during shipping or upon receipt. Thus, keeping the quality of potted ornamental plants is an essential condition for their commercial success and for promoting trust in customer relationships.

The main quality parameters for leafy pot plants are the size and the green colour of the leaves (Wang *et al*., 2005). Biotic and abiotic stresses during shipping lead to several physiological disorders with numerous negative effects, such as: leaf yellowing due to decreased photosynthesis (Starman *et al*., 2007), leaf and flower abscission with slowed growth and uptake of water and nutrients, color loss of flowers and leaves, damage to cell membrane phospholipids with increased lipid peroxidase (Mittler, 2002). Phenolic compounds including flavonoids play a role in plant defense against various oxidative stresses, with antioxidant and free radical scavenging activity thus improving plant tolerance to stresses (Trchounian *et al*., 2016; Tohidi *et al*., 2017). The accumulation of these various secondary metabolites has been shown to be influenced by interactions between plant genotype (species, and variety within species) and environmental factors, including cultivation technique, season, abiotic and biotic stress, and nutrient status (Dixon and Paiva, 1995; Vyn *et al*., 2002; Downey *et al*., 2006; Ksouri *et al*., 2007).

This research was carried out within the In.Tra.Viva Project, funded by the Tuscany Region, with the aim of improving long-distance transport conditions (up to 6 weeks) of ornamental plants produced in the nurseries of the Pistoia District and to reduce the die-off of potted plants during transport (up to 30%), mainly caused by the fall of the leaves and the inability to recover the vitality that the same plants had on departure. The commitment of CREA-OF Pescia to the Project includes the following research activities: i) monitoring the phenotypic and physiological behaviour of plants during transport; ii) testing new biodegradable antiperspirant products to increase the resistance duration of plants; iii) testing a biodegradable microfilm to protect plants during transport.

2. Materials and Methods

Five popular pot plant species, commonly grown for outdoor use, were tested for their tolerance to long distance shipping: maple (*Acer palmatum*), cypress (*Cupressocypari leylandii*), privet (*Ligustrum texanum*), nandina (*Nandina domestica*) and viburnum (*Viburnum tinus*). Thirty plants of each species were provided by Giorgio Tesi Group, Pistoia, at the end of March: the plants were 4 years old, grown in 9 L pots Ø 24 cm (maple and viburnum) or 3 L pots Ø 18 cm (cypress, privet and nandina).

Medium and long-distance transport simulation tests were carried out in refrigerated cells (T° = 10°C) at the experimental farm and laboratories of CREA Research Centre for Vegetable and Ornamental Crops (Pescia, PT) during Spring 2021. The spring season is the most important and critical season for the farmers, both from an economic and a physiological point of view since the plants are in full vegetation or yet at the beginning of the flowering stage. Five plants of each species were placed in the nursery, in open air (OA), thus acting as an untreated control not stored in a refrigerated cell. The remaining 25 plants of each species were transferred in the laboratories on April 2nd, measured, treated with antiperspirants and then placed in a refrigerated cell simulating a medium-distance transport (T1 = 2 weeks, from April 14th to April 28th) and a long-distance transport (T2 = 6 weeks, from April 14th to May 26th) at T° = 10°C. The plants were subjected to the following treatments: i) spraying with 'Barzaghi-A 10%' (A) and ii) with 'Barzaghi-B 10%' (B), two experimental and biodegradable antiperspirants based on carboxymethylcellulose; iii) spraying with Vapor Gard® 5% (V), a commercial antiperspirant based on pinolene 96% (di-L-para-menthene); iv) wrapping in a hermetically sealed experimental biodegradable film (P) provided by LaMPo (Department of Chemistry, University of Milan); v) spraying with tap water (C), considered as the stored control treatment. Antiperspirants (A) and (B) were provided by Barzaghi Speciality Chemicals srl, Arluno (MI). The percentages of the active ingredients are not disclosed to the public as these ingredients are protected by patent (N.1428533/15.5.2017). The manufacturer claims about the effectiveness of these products based on specific private research. This study could demonstrate the efficacy of this biodegradable antiperspirant and the manufacturer could use this information to market the product to consumers.

Phenotypical data of all plants (height and diameter) were measured at the start of the trial (April 2nd to 14th), at T1 (April 28th) and T2 (May 26th). Leaf physiological measurements (chlorophyll A, chlorophyll B, phenols, carotenoids and malondialdehyde content) were carried out on leaf samples collected from all plants of the three evergreen species cypress, privet and viburnum at T1 and T2. On the other hand, semi-evergreen nandina and deciduous maple shrubs lost all their leaves during their stay in the cell, hence pigment analysis and estimates of malondialdehyde levels were not performed on these species.

At the end of the cold storage experiment (end of May), all plants were moved to the nursery in open air and placed together with the non-stored control plants (OA).

Malondialdehyde content (MDA), the final product of the lipid peroxidation process, is a widely used marker of oxidative lipid injury caused by environmental stress (Kong *et al.*, 2016). MDA content was measured by 2-thiobarbituric acid (TBA) reaction as reported by Li *et al.* (2010). The absorbance of the aqueous phase was detected at 450, 532 and 600 nm. MDA content was calculated based on the following formula:

$$C\ (\mu mol/g\ weight) = (6.45 \times (A532 - A600) - 0.56 \times A450)\ /\ W\ (sample\ weight\ g)$$

Leaf chlorophyll, carotenoid and phenol contents were analyzed following the method reported by Lichtenthaler and Buschmann (2001) on fresh frozen (-80°C) leaf discs obtained by excising 5-6 fully expanded leaves collected from the middle portion of the plants grown in container at T1 and T2. The absorbances of chlorophyll a and b were assessed spectrophotometrically (Thermo Evolution 300 UV-Visible Spectrophotometer) at 665.2 nm, 652.4 nm, and 470 nm, respectively, while carotenoid and phenol absorbances were read at 260 nm and 530 nm, respectively.

Collected data were subjected to the analysis of variance (ANOVA) to determine the significance level of the different sources of variation: treatment (Tr = 5 levels = A, B, V, C, OA), storage time (ST = 2 levels = T1 and T2) and Tr x ST interaction. Differences between means were tested using Duncan's multiple comparison test with a confidence level of 95%. The statistical analysis packages used for processing were IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp., Armonk, NY).

3. Results and Discussion

No perceptible increments in plant growth (height and diameter) were detected in all refrigerated plant species during the time interval from T0 to T1 and T2, regardless of the type of antiperspirant treatment and biodegradable film used. Conversely, control plants kept in open air (OA) grew and developed as expected (data not reported).

Plant protection with the biodegradable parafilm proved to be ineffective for the purpose. At T1 the biodegradable film resulted perforated in several points by the twigs of the plants, piled up into the refrigerated cell (Fig. 1), while at T2 the film was rotten due to the contact with the humidity of the leaves because of their transpiration. Thus, the parafilm treatment was not considered in the statistical analysis.

After fifteen days of refrigeration, nandina and maple plants lost part of the leaves, whereas at the end of the refrigerated storage, regardless of the treatment, all the leaves had fallen or rotted on the plant (Fig. 2). All nandina plants, when moved outdoors at the end of May, resumed their vegetative activity, reaching development rates comparable to control plants at the end of October (Fig. 3 a). On the contrary, the plants of maple not treated with antiperspirants (C, P and OA) started regularly to veg-

Fig. 1 - The biodegradable parafilm proved to be ineffective for the purpose: at long storage time (T2 = 6 weeks), , relative humidity levels were too high and there was lack of air circulation; plant transpiration caused the humidity around the leaves to be saturated with water vapor and the pellicle to rot.

Fig. 2 - After 6 weeks of refrigerated storage, all the leaves of nandina (2 a) and maple (2 b) had fallen or rotted on the plant.

etate again in the nursery but all those treated with antiperspirants (A, B and V) died (Fig. 3 b). This phenomenon has to be further investigated but it is possible that the antiperspirants reduced transpiration

Fig. 3 - All nandina plants resumed their vegetative activity once they were relocated in the field reaching development rates comparable to control plants after 5 months of cultivation in open air (3 a). Maple plants that were not treated with antiperspirants started regularly to vegetate again in the nursery (3 b, on the left), while those treated with antiperspirants died (3b, on the right).

of maple and thus the plants suffered a too high level of humidity in the pot substrate. It is very important to avoid excessive wetting of pot substrate before loading the plants into the container and to irrigate the plants a couple of days prior to scheduled shipment, so that the excess water can drain completely. In this trial, maybe the humidity level of the soil substrate resulted correct for the control plants but too high for the plants treated with antiperspirants.

No phenotypical differences were observed among cypress plants stored for 15 days and 6 weeks in the refrigerated cells and control plants maintained in the field (Fig. 4) (data not reported). Once moved outdoors in the field, cypresses began to

Fig. 4 - Regardless of the type of spraying treatment used, the cypress plants that were kept in refrigerated cells for 6 weeks did not exhibit any damage.

sprout into new vegetation as the control plants.

Concerning physiological responses of cypress plants during refrigerated storage, ST had a significant effect on all considered parameters, except for carotenoid content, while Tr x ST interaction significantly influenced all parameters of cypresses except for MDA content (Table 1). Only MDA was significantly affected by plant treatment with antiperspirants. More specifically, plants sprayed with tap water only (C) showed significantly higher level of MDA (190.60 µmol/g DW) than those treated with Barzaghi biodegradable antiperspirants (A and B) and Vapor Gard® (V), evidencing a higher level of stress of the untreated plants. MDA values were lowest in B and V treated plants (114.97 and 109.76 µmol/g DW, respectively), highlighting some protective action of these antiperspirants on plants subjected to transport stress, even if the phenotypic analyses did not show significant differences among treatments.

In general, cypress plants kept in open air (OA) showed the highest values of phenols (Fig. 5), carotenoids, chlorophyll a and b compared to stored plants. These plants suffered a late spring frost in mid-April (-0.9°C to -3.3°C from h 4:00 am to h 9:00 am on April 8, 2021). Since plants exposed to various abiotic stress conditions produce many secondary metabolites, including phenolic compounds and carotenoids, in higher concentrations (Yeshi *et al.*, 2022), it can be hypothesized that in our experiment plants may have produced high amounts of phenols and carotenoids in response of spring frost hazard. Moreover, chlorophyll a and b decreased significantly from T1 to T2, indicating a reduction in plant photosynthetic activity during transport over a long period. Indeed, it is well known that stressed plants reduce plant metabolism, especially photosynthetic activity, in order to resist adverse conditions (Starman *et al.*, 2007).

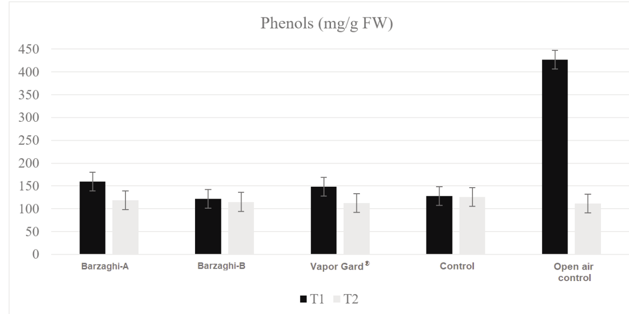

Fig. 5 - Effect of treatment x storage time interaction on phenols content of potted cypress. Error bars indicate the standard error of the mean. T1= medium distance transport, 2 weeks; T2 = long distance transport, 6 weeks.

In privet (Fig. 6 a) and in viburnum (Fig. 6 b), no apparent differences were observed in the growth of plants stored in the refrigerated cell and of control plants maintained in the field (data not reported). About plant development, it was noted that all plants treated with antiperspirants (A, B, V) were characterized by new shoot sprouting, which was absent in all plants not sprayed with antiperspirants (C, P, OA) (Fig. 6 a, b), the meaning of this phenomenon should be furtherly analysed. Moreover, as it was noted also on cypress, all plants of privet and viburnum treated with Vapor Gard® had shinier and brighter green leaves: this was due to the oily matrix of the product which creates this pleasant optical effect. All cold

Table 1 - Effect of storage time (ST) and treatment (Tr) on oxidative stress (MDA), phenols, carotenoids, and chlorophyll contents of potted cypress

Source of variation	MDA µmol/g DW	Phenols mg/g FW	Carotenoids µg/g FW	Chlorophyll a µg/g FW	Chloropyll b µg/g FW
Storage time (ST)	**	**	NS	**	*
T1 = 2 weeks	162.59 a	196.83 a	0.048	0.277 a	0.195 a
T2 = 6 weeks	119.27 b	116.77 b	0.03	0.169 b	0.122 b
Treatment (Tr)	**	NS	NS	NS	NS
Barzaghi-A	141.79 b	139.14	0.025	0.191	0.152
Barzaghi-B	114.97 c	118.5	0.023	0.148	0.114
Vapor Gard ®	109.76 c	130.68	0.026	0.199	0.158
Control	190.60 a	126.64	0.031	0.186	0.123
Open air control	151.31 b	269.05	0.081	0.391	0.248
ST x Tr	NS	**	**	**	*

** significant at p≤001; * significant at p≤005; NS= not significant. Mean values within each column followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

Fig. 6 - In privet (6 a: left, Vapor Gard®; right, Control) and in viburnum (6 b: left, Vapor Gard®; right, Control), it was noted that all plants treated with antiperspirants were characterized by new shoot sprouting; moreover, all plants treated with Vapor Gard® had shinier and brighter green leaves.

stored plants recovered after being transferred to the open field at the end of May, resulting in final growth developmental patterns like control plants at the end of October (data not reported).

In privet, A, B and V antiperspirant treated plants showed a significantly lower phenol content, while storage time significantly affected carotenoids content (Table 2). In addition, a statistically significant interaction between these factors was found for phenols and chlorophyll a. The untreated OA and C plants showed the highest phenol values: privet outdoor plants experienced spring frost disturbance in mid-April, as described for cypress, while the higher phenols content in C plants suggests that untreated plants get stressed by transport conditions more than plants treated with antiperspirants (A, B and V) (Fig. 7). Carotenoid content raised in cold stored privet plants from T1 to T2, indicating that plant stress

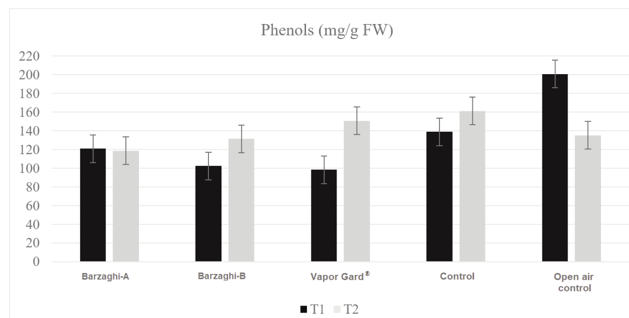

Fig. 7 - Effect of treatment x storage time interaction on phenols content of potted privet. Error bars indicate the standard error of the mean.T1= medium distance transport, 2 weeks; T2 = long distance transport, 6 weeks.

Table 2 - Effect of storage time (ST) and treatment (Tr) on phenols, carotenoids, and chlorophyll contents of potted privet

Source of variation	Phenols mg/g FW	Carotenoids µg/g FW	Chlorophyll a µg/g FW	Chloropyll b µg/g FW
Storage time (ST)	NS	**	NS	NS
T1 = 2 weeks	132.3	0.104 b	0.518	0.324
T2 = 6 weeks	139.5	0.166 a	0.619	0.276
Treatment (Tr)	**	NS	NS	NS
Barzaghi-A	119.78 bc	0.142	0.577	0.282
Barzaghi-B	116.90 c	0.125	0.514	0.275
Vapor Gard ®	124.61 bc	0.11	0.479	0.256
Control	150.17 ab	0.171	0.717	0.366
Open air control	168.06 a	0.128	0.555	0.322
ST x Tr	**	NS	*	NS

** significant at p≤001; * significant at p≤005; NS = not significant. Mean values within each column followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

increased during transport with increasing ST (Table 2). On the contrary, phenols content in OA plants reached the highest value in April (T1) due to late spring frost damages, but thereafter levels were cut down to a normal range within 6 weeks (Fig. 7). Chlorophyll pigment molecules play a key role in photosynthesis; plants use chlorophyll to absorb light and convert it into chemical energy (Bollivar, 2006). In privet plants, chlorophyll content increased from T1 to T2 in both treated (A, B, and V) and untreated (C) plants maintained inside the cold container, while the untreated open-air (OA) plants showed an opposite trend (Fig. 8). In this context, it is probably realistic to assume that the increase in chlorophyll content might be related to water loss occurring in leaves during prolonged storage or transportation rather than to an actual increase in photosynthetic activity (Ferrante *et al.*, 2015). The data regarding MDA analysis were not considered for privet. In fact, the method used to assess MDA was the thiobarbituric acid (TBA) reactive substance assay. This analysis is simple and quick, but it was found to be ineffective for privet species as pointed out by Wang *et al*. (2013). Indeed, it seems that there are substances present in the leaves of this species that interfere with the TBA reagent.

In viburnum species, ST significantly affected carotenoids and chlorophylls contents (Table 3), with highest values (more shiny leaves) found in plants at T1, contrary to what was recorded for privet. MDA levels were influenced by both antiperspirant treatment and Tr x ST interaction. Indeed, MDA values were highest in the control and in plants treated with antiperspirants A and B after 6 weeks of cold storage (T2), indicating that viburnum shrubs get more stress with increasing storage time (Fig. 9). On the other hand, Vapor Gard® (V), seemed to exert some protective action on viburnum plants over time. As expected, plants maintained in open air showed no

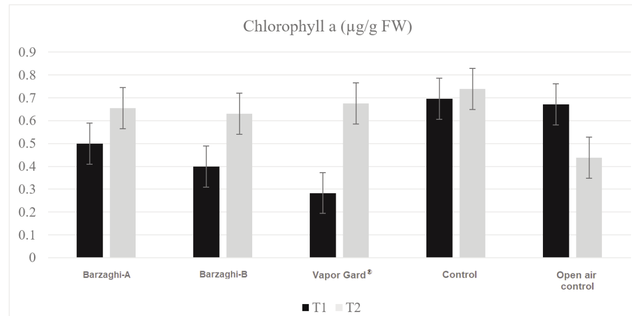

Fig. 8 - Effect of treatment x storage time interaction on chlorophyll a content of potted privet. Error bars indicate the standard error of the mean.T1= medium distance transport, 2 weeks; T2 = long distance transport, 6 weeks.

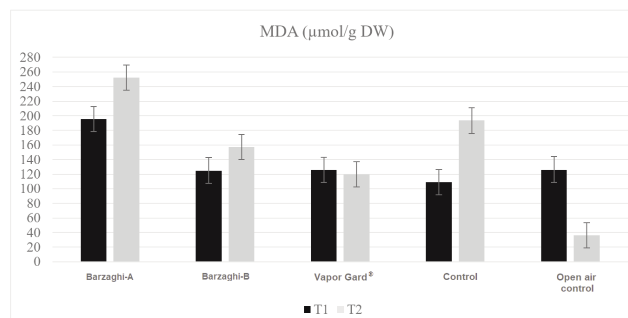

Fig. 9 - Effect of treatment x storage time interaction on malondialdehyde content of potted viburnum. Error bars indicate the standard error of the mean.T1= medium distance transport, 2 weeks; T2 = long distance transport, 6 weeks.

Table 3 - Effect of storage time (ST) and treatment (Tr) on oxidative stress (MDA), phenols, carotenoids, and chlorophyll contents of viburnum privet

Source of variation	MDA µmol/g DW	Carotenoids µg/g FW	Chlorophyll a µg/g FW	Chloropyll b µg/g FW
Storage time (ST)	NS	**	**	**
T1 = 2 weeks	136.22	0.233 a	0.918 a	0.438 a
T2 = 6 weeks	151.77	0.043 b	0.226 b	0.117 b
Treatment (Tr)	**	NS	NS	NS
Barzaghi-A	223.97 a	0.15	0.555	0.261
Barzaghi-B	141.15 b	0.124	0.513	0.242
Vapor Gard ®	122.73 b	0.101	0.501	0.286
Control	150.78 b	0.145	0.626	0.302
Open air control	81.34 c	0.169	0.665	0.296
ST x Tr	**	NS	NS	NS

** significant at p≤001; * significant at p≤005; NS = not significant. Mean values within each column followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

signs of stress over the long term.

It is likely that dark conditions and lack of water over a 6-week period are not limiting factors for the tested species once the optimal conditions of temperature and humidity are met into the container, as it was during our trials. Potted plants must be adequately prepared and carefully handled before long-distance transport to overcome problems of this transitory phase by reducing both the plant's metabolism and normal physiological processes.

In general, even if phenotypical data did not show evident differences for plant growth between long term stored and not stored plants, the physiological analyses on cypressus, privet and viburnum showed interesting significant differences for MDA, phenols and carotenoids: these parameters seem to be correlated to abiotic storage stress of plants and thus could be useful in further studies to monitor the quality of plants before, during and after storage for short, mid and long times in refrigerated cells. This could also help various sectors of the post-harvest ornamentals supply chain to: i) assess the potential quality of plants before shipment; ii) improve plant transport conditions; iii) monitor plant quality throughout the various stages of shipping "from farm to buyer", through the various steps with other components of the supply chain (transporters, wholesalers, markets); iv) understand whether any deterioration in the quality of the plants at the end of the travel was perhaps due to non-maintenance of the optimal conditions envisaged during transport, due to negligence by the operators.

4. Conclusions

Plant tolerance to storage conditions in refrigerated cell or container (T° = 8-12°C) varied considerably according to the considered species. Cypress proved to be extremely tolerant to storage conditions over long periods. Maple and nandina, on the contrary, resulted the most sensitive species to medium- and long-distance transport with a high percentage of fallen or rotten leaves occurring during spring storage in refrigerated cells. Treatments with antiperspirants did not exhibit particularly evident effect on quality value (plant growth and aesthetic appearance of the leaves) in plants kept in the dark in a cold room or container. Only the antiperspirant Vapor Gard® seemed to improve the aesthetic appearance of cypress, viburnum, and privet with shinier and brighter green leaves, probably due to the oily matrix of the product. Furthermore, even in cases of total or partial loss of leaves by species such as maple and nandina, an optimal recovery of vegetative development was highlighted once these species were relocated in outdoor cultivation. The use of the tested biodegradable film was inadequate to protect plant quality during long-distance shipments, thus, further research is needed to improve microfilm performances by changing its thickness and composition. Among physiological parameters, MDA, phenols, and carotenoids contents were the most stress-related variables, being negatively correlated to the quality decay of plants transported in dark refrigerated cells for 2-6 weeks. It is a preliminary study and some uncertainty and/or not complete discussion are due to the lack of some measurement (i.e. leaf colour)., however these parameters could be useful in further studies to monitor the quality of plants before, during and after storage for short-, mid- and long-term transport in refrigerated containers.

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