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THE EFFECTS OF STRESS ON THE QUALITY OF *PELARGONIUM* PROPAGULES DURING SHIPMENT AS MEASURED USING CHLOROPHYLL FLUORESCENCE

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Summary. The potential of slow phase chlorophyll fluorescence (CF) to predict the physiological status of *Pelargonium* propagules (seven varieties in 28 different batches) post shipment or post simulated shipment was evaluated. The rooting ability of the cuttings was recorded and used to confirm the results from the CF data. The use of cardboard versus polystyrene packaging and the inclusion of two treatments (ethylene binding agents included in the packaging or spraying with foliar nutrients) for the alleviation of stress were also evaluated. Chlorophyll fluorescence curves reflecting healthy and stressed *Pelargonium* material were identified and curve shapes were interpreted in conjunction with the ratio Fss:Fmax values which correlated with rooting potential of each batch. The results from the evaluation of the packaging parameters (through varying the amount of 0_2 and using different forms of binding agents) demonstrated that the physiological status of the cuttings improved when stored with $AgNO_3$ and with $KMnO_4$. Cuttings which were stored under anaerobic conditions senesced whereas cuttings stored under normal or reduced 0_2 showed no apparent ill effects. All batches of cuttings showed significant variation in response to various foliar treatments. From the results there was a relationship between CF data and rooting potential of the propagules. Curve types suggest that some batches had exceeded a 'threshold stress level' for certain varieties and consequently would not respond to treatment. The results showed a seasonality effect in the mother stock (reflecting rooting potential) which may have contributed to the variation detected between batches and between different varieties. Overall, the inclusion of ethylene binding agents and O_2 absorbers in the packaging appeared to be

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beneficial to the physiological status of the propagules. The application of chlorophyll fluorescence to the prediction of the physiological status and rooting ability of propagules post shipment is discussed.

Keywords: chlorophyll fluorescence, *Pelargonium*, plant stress

Introduction

Pelargonium is one of the world's most important bedding and pot plants. Recent figures show that the annual sales in Europe and North America are worth in excess of US$ 700 million annually (Mithila *et al*., 2001). According to Cassells (1992) over 90% of the crop is vegetatively propagated. Over the years economic factors have forced growers to raise cuttings in lower cost countries. This can mean that cuttings have to be shipped long distances before they reach the "growing-on" nurseries. As a consequence the quality of the cuttings may be affected by time spent in transit and the subsequent stresses which they may be exposed to. According to Lichtenthaler (1998) any treatment or substance that may have an adverse effect on the plant's natural development processes or on its metabolism can be regarded as stress. Poor cutting quality can result in large monetary losses to the growers. Ethylene is known to have an effect on stored plants at very low concentrations and can result in an alteration of the natural processes of plant development which in some cases can result in plant senescence (Saltveit, 1999). One of the many responses of the plant to gaseous ethylene present in minute concentrations in, for example, packaging is a destruction and yellowing of the chloroplasts. This study was carried out in order to elucidate some of the factors which may affect cutting quality during shipment; firstly, by using chlorophyll fluorescence to monitor incoming batches and secondly, by trying to improve cutting quality through foliar treatments and modified packaging conditions. The light (400–700 nm) which is absorbed by chlorophyll s in the plant and used for photochemical reactions is called photosynthetically active radiation. Various environmental stresses can influence the ability of the plant to perform photochemical reactions. Any excess energy which is not used has to be eliminated and one way to eliminate this excess energy is through re-emission as longer wavelength energy – this is chlorophyll fluorescence. According to Kautsky (1931) there is an inverse relationship between chlorophyll a fluorescence and photosynthesis. The chlorophyll molecule can be compared to a built-in fluorescence indicator embedded in the thylakoid membrane (Schreiber *et al*., 1988). Chlorophyll a fluorescence reflects the primary processes of photosynthesis. Interest has grown in the practical applications of chlorophyll fluorescence as a tool for the determination of photosynthetic activity in plants and their stress limitations. Chlorophyll fluorescence is a sensitive technique and has been used to measure the efficiency of photosynthesis in plants (Lichtenthaler, 1996; Weis and Berry, 1998; Cornic and

Fresneau, 2002) and according to Maxwell and Johnson (2000) it has "become one of the most powerful and widely used techniques available to plant physiologists and ecophysiologists". It has recognized advantages over other techniques in that it is rapid and it is easy to use in that no complicated tests or procedures are involved. Historically the measurement of stress in plants involved destructing sampling (Chaerle and Van Der Straeten, 2001) but chlorophyll fluorescence is a non-destructive method of sampling and if necessary the same material may be sampled many times.

Materials and Methods

Plant Material

Unrooted *Pelargonium* cuttings were received every two weeks by air freight from the Canary Islands. Seven varieties were received in each batch (28 batches): *Pelargonium x hortorum* cvs Rio, Fidelio and Alba and *Pelargonium peltatum* cvs Feuer Cascade, Grenchen, Rigi and Amythest.

Packaging Trials – Conditions of Storage

Batches of *Pelargonium* cuttings arrived in Cork either in sealed polystyrene containers or in cardboard boxes. Ice packs were included in both to depress the temperature. For all of the packaging trials, the cuttings were placed in a sealed bag system using Saranax 116 Barrier Film (Dow Chemical). The cuttings were stored in bags under the following conditions: (1) heat sealed under normal conditions – full O_2; (2) vacuum sealed – minimal O_2 and (3) stored with Ageless FX, an oxygen absorber – reduced O_2. The packaging trials were carried out where cuttings were stored in the presence of: $AgNO_3$ (in both aerobic and reduced O_2 conditions); (2) Solid $AgNO_3$ combined with $KMnO_4$ and Ageless FX; (3) cuttings were sprayed with $AgNO_3$ at different concentrations (under aerobic and reduced O_2 conditions) – 0 ppm (control), 50 ppm, 250 ppm, 400 ppm and 500 ppm and (4) bound $KMnO_4$ – the binding power was increased by attaching $KMnO_4$ to a substrate (vermiculite) – thereby increasing its surface area and thus creating a more efficient ethylene binding agent. The above test was also carried out on cuttings in a reduced O_2 environment and the results were taken after 72 hours. The cuttings were scored using a qualitative colorimetric scale from 0 to 10 where 10 = dark green (apparently healthy) and 0 = all brown (fully senesced).

Foliar Treatments of Cuttings for the Alleviation of Stress

To promote recovery from stress and in order to enhance the quality of rooting and development, the cuttings were sprayed (one application per batch) with the following

constituents either singly or in combination: (1) M+S basal medium (4.4 g.l^{-1}) without sucrose; (2) Sucrose (30 and 15 g.l^{-1}); (3) Benzylaminopurine (BAP) (0.05 to 0.1 mg.l^{-1}); (4) Ammonium Nitrate (NH_4NO_3) (0.0125 M to 0.1 M) and (5) Water. The foliar treatments were applied using a pressurized garden sprayer and each batch was set up as a separate experiment in the form of a replicated randomized block. There were six treatments (including a control and five replicates of each). Each replicate consisted of six cuttings of each variety and the results were taken after a three week interval. The rooting performance was expressed as a weighted rooting index.

Chlorophyll Fluorescence Measurements

To quantitatively measure the level of stress on arrival – slow phase chlorophyll fluorescence induction kinetics were measured using a modulated fluorometer. The modulated fluorometer system was developed by Hansatech (Kings Lynn, UK) and it consisted of the following components, a white light source, fibre optic cable, leaf clamp and a modulated fluorometer. On arrival the cuttings were dark adapted for a one hour period prior to measurement. The data was represented as curve profiles or tabulated values. Curve shape and specific ratios were used to assess the stress levels in the propagule material.

Rooting of Cuttings and Maintenance of Stock Plants

Prior to rooting the cuttings were treated with a fungicide (6 g.l^{-1} Captan 50) (National Agrochemical Distributors Ltd., Blakes Cross, Lusk, Co. Dublin.). The cuttings were rooted in potting compost (3:1 by volume Irish peat moss; sand with Bio-P-Base fertilizer) (Pan Britannica Industries, Herts., UK). After 21 days the cuttings were potted on in compost (as above) with Osmocote (National Agrochemical Distributors Ltd., Blakes Cross, Lusk, Co. Dublin.) a slow release fertilizer. The plants were watered as appropriate and fed every two weeks (Bio Plant Food) (Pan Britannica Industries, Herts., UK). The glasshouse conditions were 15ºC minimum night time temperature and a maximum day temperature of 25ºC. Results

A visual inspection of the cuttings on arrival showed that there was great variability in their condition from batch to batch. Fluorescence emission curves from all of the batches were examined and compared for similarities in both F_{ss}:F_{max} ratio values (see Table 1) and in the subsequent rooting performance of the cuttings. The F_{ss}:F_{max} ratio value was seen to increase with increasing stress levels. However, for many of the varieties in batches 5 to 18, a similar F_{ss}:F_{max} ratio value was recorded. Even though similar F_{ss}:F_{max} ratio values were recorded for different batches, big differences were seen in curve profiles. An examination of the F_{ss}:F_{max} ratio values and subsequent rooting data led the formation of a database of reference curve profiles reflecting healthy (unstressed) and unhealthy (stressed) *Pelargonium* material were identified (see Fig. 1).

Table 1. The F_{ss} (steady state fluorescence):F_{max} (point of maximum fluorescence) ratio value as a measure of batch stress in *Pelargonium* cuttings (for batches 5 to 18). (Stressed values indicated in bold typeface).

Batch No.	Varieties						
	P. x hortorum cv Rio	*P. x hortorum* cv Fidelio	*P. x hortorum* cv Alba	*P. peltatum* cv Feuer Cascade	*P. peltatum* cv Grenchen	*P. peltatum* cv Rigi	*P. peltatum* cv Amythest
5	0.36	0.41	0.36	0.37	0.36	0.34	0.41
6	0.30	0.34	0.34	0.37	0.41	0.42	0.36
7	0.39	0.39	0.30	0.387	0.43	0.41	0.36
8	0.29	0.31	0.30	0.41	0.32	0.36	0.367
9	0.32	0.43	0.29	0.41	0.42	0.42	0.42
10	**0.436**	0.378	0.320	0.369	0.387	0.413	0.419
11*	**0.506**	**0.454**	0.388	**0.535**	**0.433**	**0.433**	**0.426**
12	0.254	0.345	0.316	0.394	**0.407**	0.378	0.276
13	0.347	**0.447**	0.382	0.373	0.374	0.346	0.412
14	0.312	0.377	0.395	0.398	0.386	0.395	–
15*	**0.735**	**0.875**	**0.763**	**0.654**	**0.821**	**0.74**	**0.586**
16*	**0.419**	**0.507**	0.380	0.392	**0.412**	**0.582**	0.294
17*	0.352	0.385	0.331	0.357	**0.422**	**0.557**	0.333
18	0.392	0.346	0.340	0.413	**0.458**	**0.456**	0.407

*Batches delayed during shipping

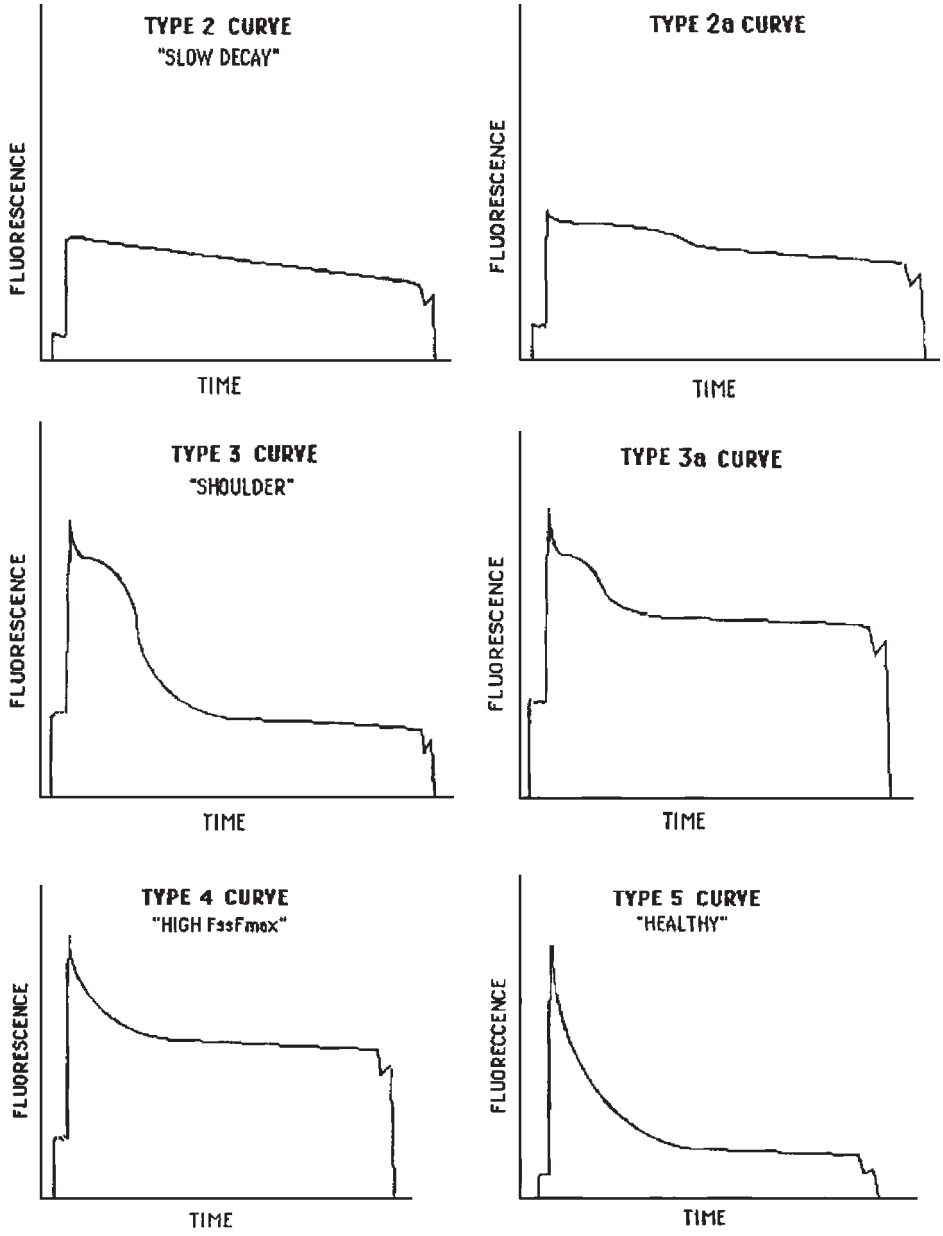

Fig. 1. Chlorophyll fluorescence curves for *Pelargonium* cuttings

Five principle curve shape types were identified and labeled 1 to 5 (see Fig. 1), curve type 1 = most stressed (not shown) and curve type 5 = least stressed with intermediary curve types labeled 1a, 2a etc. produced. Curve shapes from all batches of plants were examined for similarities and compared to rooting performance. Chlorophyll fluor-

escence curves for batch 8 indicated relatively high stress levels. The subsequent rooting performance was poor. The curve types produced from batch 8 indicated that maybe this batch had reached a "threshold stress level" for certain varieties and consequently may not respond to any treatment. The chlorophyll fluorescence curves from batch 9 indicated much lower levels of stress with rooting of the controls much greater than in batch 8. An analysis of variance for batch 9 showed no significance difference between treatments for the batch but large differences between the varieties. For *P. x hortorum* cv Rio and *P. peltatum* cv Alba, the percentage improvement of rooting on the control was 8.3 to 20.8% and 0.00 to 12.5%, respectively. The results from the packaging trials showed that stress was significantly reduced when the cuttings were stored with ethylene binding agents ($KMnO_4$ and $AgNO_3$). Cuttings which were stored under anaerobic conditions were in a bad state physiologically even when ethylene binding agents ($KMnO_4$ and $AgNO_3$) were included in the packaging.

Discussion

The great variability observed in the condition of the cuttings from batch to batch was due to external factors, for example, delays encountered in shipping. The rooting ability of the cuttings was used to confirm the results from the CF data. The F_{ss}:F_{max} ratio value was the first indication that some batches were stressed (see Table 1). All of the batches showed significant variation in response of different varieties to the various foliar treatments. No one beneficial foliar treatment was developed and the overall quality of the cutting appeared to be of great importance. There appeared to be an increase and then subsequent decline in rooting performance overall as the season progressed. The results indicated that the optimal rooting time for cuttings of *P. x hortorum* cv Rio is in April with cuttings of *P. peltatum* cv Alba having an optimal rooting time in May/June. With respect to the packaging treatments, results from the curve shape analysis suggested that when ethylene binding agents were included stress induced by ethylene appeared to be reduced. The results also suggested that cuttings may travel better under reduced oxygen conditions. No beneficial effect was seen when ethylene binding agents were included in the packaging ($KMnO_4$ and $AgNO_3$) under anaerobic conditions. Overall, the results suggest that stress ethylene may be the most significant factor affecting cuttings in shipment given that temperature is controlled in the package.

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