



## Penconazole Induced Heat Tolerance in Scots Pine (*Pinus sylvestris*) and Evergreen Oak (*Quercus ilex*)

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**Abstract.** The ability of penconazole, a triazole fungicide derivative, to protect against and ameliorate heat stress was studied in evergreen oak (*Quercus ilex*) and Scots pine (*Pinus sylvestris*). Under laboratory conditions, heat damage to the leaf photosynthetic system based on the stability of the chlorophyll a/b light-harvesting complex within photosystem II (chlorophyll fluorescence  $F_0$  responses) and leaf photochemical efficiency (chlorophyll fluorescence  $F_v/F_m$  emissions) of detached leaves was constantly less in penconazole treated trees. In both species, greatest protection of the leaf photosynthetic system to heat induced disorders was achieved by application of penconazole at a concentration of 30 g per liter of water compared to penconazole applied at a concentration of 0.15 or 0.45 g per liter of water. Subjecting containerized trees of both species to 10 minutes at 50°C significantly reduced tree vitality with respect to chlorophyll fluorescence  $F_0$  and  $F_v/F_m$  emissions, total foliar chlorophylls, leaf photosynthetic rates ( $P_n$ ) and significantly increased damage to cellular membrane integrity as manifest by higher leaf electrolyte leakage and visual leaf necrosis between stressed and non-heat stressed well-watered trees. The influence of penconazole applied immediately after heat stress on the pattern of recovery over the following twelve weeks demonstrated penconazole treated trees were the most capable of recovery. With respect to chlorophyll fluorescence  $F_0$  and leaf electrolyte leakage values recovery rates of heat damaged trees treated with penconazole ranged from 20%–50% higher than non-triazole treated control trees. In all cases non-penconazole treated control trees had the least capacity for recovery. Regardless of species, height, leaf area, root, shoot, and total plant dry weight were, in virtually all instances, greater than non-penconazole treated controls. The tactical use of the triazole derivative penconazole as an ameliorant against heat damage and recovery from heat stress in Scots pine and evergreen oak would be of benefit to improve tree recovery rates and growth. From a practical point of view penconazole at 30 g a.i. per liter of water is suggested based on the results of this study.

**Key Words.** Chlorophylls; Chlorophyll Fluorescence; Electrolyte Leakage; Fungicides; Growth Inhibitor; Physiogenic Stress; Stress Enzymes.

Higher temperatures in urban environments caused by the lack of transpirational cooling, heat convection, and long wave radiation from nonvegetative surfaces (e.g., buildings, roads) can be detrimental to the biology of trees (Kolb and Robberecht 1996; Ladjal et al. 2000). Under prolonged heat stress, high temperature injury is primarily manifest by leaf and wood desiccation inducing water stress throughout the canopy (Sprugel et al. 1991). With prolonged desiccation, tree limbs and trunks can break and shed prematurely; a phenomenon known as summer branch drop, which appears to be linked to internal water stress (Harris et al. 2004). Such a response is undesirable in highly populated urban areas. Photosynthesis is one of the most heat-sensitive processes in plant cells, leading to numerous changes within the structure and function of the photosynthetic apparatus (Georgieva et al. 2000). Within the photosynthetic system it has been recognized that photosystem II (PSII) is the most thermally liable component of the electron transport chain (Cajane et al. 1998). Among partial reactions of PSII, the oxygen evolving complex is particularly heat sensitive (Georgieva et al. 2000). As higher rates of photosynthesis are recognized as a physiological characteristic conferring robustness during periods of stress, a reduction in leaf photosynthetic rates, or damage to the leaf photosynthetic system can reduce leaf photosynthetic productivity (Percival 2005). Consequently, heat stress can limit the amount of carbohydrates available for growth and maintenance, and subsequently reduce nutri-

ent and water uptake resulting in leaf chlorosis, dieback, and plant death (Georgieva et al. 2000; Ladjal et al. 2000; Percival et al. 2006). Drought conditions recorded in 2003, 2004, and 2006 coupled with hose pipe bans in the South of England, and most climate models predicting hotter drier summers for the UK, have meant heat-related tree disorders may become even more prominent within urban landscapes (Percival et al. 2006). Improving hardiness against heat stress may ensure greater post-planting survival of newly-planted trees and increase longevity of established ones. Consequently, finding exogenous chemicals that enhance tree stress tolerance may prove important under prolonged heat conditions (Still and Pill 2004).

Penconazole forms the active ingredient of a triazole fungicide commonly used within the horticultural, agricultural, and forestry industries for foliar pathogen control (Kenyon et al. 1997; Schnabel and Parisi 1997). Fungicidal properties are via inhibition of the C4-demethylase reactions in fungal sterol biosynthesis (Allingham 2005). However, many triazole based fungicides such as penconazole have been shown to induce a suite of morphological and physiological adaptations that allow plants to tolerate a broad range of environmental stresses to include drought, herbicide, and elevated temperatures (Fletcher et al. 1986; Kraus and Fletcher 1994; Jaleel et al. 2006; Percival and Noviss 2008). Most studies have focused on the growth inhibitor paclobutrazol as a pre-stress treatment to protect plants against drought, herbicide and waterlogging (Asare-Boamah et al. 1986;

Fletcher et al. 2000; Marshall et al. 2000). Paclobutrazol-induced reductions in plant growth are well documented (Watson 2001). Paclobutrazol blocks gibberellic acid biosynthesis, more specifically the pathway at the ent-kaureno oxidation stage. Two to three-year growth over-regulation has been associated with PBZ applications that may be undesirable for landscape planting where aesthetics are a major factor regarding tree selection (Fletcher et al. 2000; Percival 2008). Such long-term over-regulatory growth responses are not associated with penconazole. The influence of penconazole on enhancing the heat tolerance of woody plants has received limited investigation. Consequently, the aims of this study were:

\* To determine the effectiveness of penconazole applied as a foliar spray at a range of concentrations on improving the foliar heat tolerance of evergreen oak (*Quercus ilex*) and Scots pine (*Pinus sylvestris*) under laboratory conditions using detached leaf material.

\* To determine the ability of penconazole to aid whole tree recovery from heat-induced damage.

## MATERIALS AND METHODS

### Plant Material

Evergreen oak and Scots pine were selected for experimental purposes because of their importance in the amenity and forestry industry respectively. Four-year-old cell grown stock *ca.* 103.5 ± 15.4 cm and 110.0 ± 11.3 cm high were used (Alba Trees, Lower Winton, East Lothian, Scotland). Six months prior to experiments trees were potted into 10 liter plastic pots filled with loam soil (24% clay, 45% silt, 31% sand, 3.1% organic carbon, pH 6.2), supplemented with the controlled release N:P:K (29:7:9) fertilizer (Bartlett BOOST, The Doggett Corporation, Lebanon, New Jersey, U.S.) at 1 g per kg of soil. Following potting, trees remained outdoors subject to natural environmental conditions and watered as required. Studies were conducted at Reading University, Earley Gate research site glasshouses (51°43N, -1°08W).

### Triazole Application

Foliar sprays of penconazole (Trade name Topas, United Agri-Products, Alconbury Weston, Huntingdon, UK) were applied until runoff (*ca.* 50 ml per tree) using a hand-held sprayer. The influence of concentration was determined by spraying 10 trees at 0.15, 0.30, or 0.45 g active ingredient per liter of water. Ten trees were sprayed once at each concentration. Non-triazole-treated trees acted as controls. Plants were left for two weeks under glasshouse conditions (22 ± 2°C), supplemented with 400W SON/T high pressure sodium lamps (TLC Electrical, St Philips, Bristol, UK), providing a 16 hour photoperiod and minimum 250 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetically active radiation at the tree crown to promote absorption and uptake of each triazole derivative. During this period, plants were watered until container runoff generally every three days.

### Foliar Tolerance to Heat Stress Under Laboratory Conditions

At week 2 after spraying, five fully expanded nonsenescent leaves per tree were excised at the base of the petiole using a razor blade and placed abaxial surface down, in a petri dish on moist Watmans filter paper sealed with a thin polythene film permeable to air but

not water prior to placing in darkness in a Merck Environmental Growth Chamber for 5, 10, 15, and 20 minutes at a temperature of 50°C. All leaf material was prepared within two hours of collection.

### Recovery of Containerized Trees from Heat Stress

Heat stress was induced by placing containerized stock in a Merck Environmental Growth Chamber for 10 minutes at a temperature of 50°C. At the cessation of the heat period a number of physiological measurements were made on leaf tissue to measure tree vitality. Immediately following these measurements, trees were sprayed until run-off with penconazole at 0.15, 0.30, or 0.45 g active ingredient per liter of water. Ten trees were sprayed once at each concentration. Non-triazole treated trees acted as controls. Trees were then placed outdoors subject to natural weather conditions. Recovery rates were measured at three weekly intervals (June 26, July 17, August 7, August 28) over a 12-week period, and growth recorded at week 12 (August 28). A well-watered group of non-heat stressed trees were used for comparative evaluation of tree vitality and growth under outdoor conditions.

### Tree Vitality Measurements

All vitality measurements were taken on leaf material present on the plant at the initiation of the experiment (existing leaves). During recovery from heat induced damage new leaf formation was observed at *ca.* weeks 6–7 on both nonpenconazole-treated control and penconazole-treated trees. No tree vitality measurements were taken on any newly formed leaf tissue (i.e., new leaves not present at the time of triazole application). If abscission or mortality of tagged leaves occurred the nearest adjacent leaf was tagged for future measurements. At each sampling date, five leaves per tree selected throughout the crown were used for chlorophyll fluorescence and chlorophyll content measurements. Leaves were then tagged to ensure the same leaf was measured throughout the study.

### Chlorophyll Fluorescence

Chlorophyll fluorescence was used as a measure of damage to the leaf photosynthetic system. Leaves were adapted to darkness for 30 minutes by attaching light exclusion clips to the leaf surface and chlorophyll fluorescence was measured using a HandyPEA portable fluorescence spectrometer (Hansatech Instruments Ltd, King's Lynn, UK). Measurements were recorded up to 1 second with a data acquisition rate of 10 μs for the first two milliseconds and of one millisecond thereafter. The fluorescence responses were induced by a red (peak at 650nm) light of 1500 μmol m<sup>-2</sup> s<sup>-1</sup> PAR intensity provided by an array of six light emitting diodes. The ratio of variable (Fv = Fm - Fo) to maximal (Fm) fluorescence (i.e., Fv/Fm where Fo = minimal fluorescence), of dark-adapted leaves was used to quantify the detrimental effects of heat on leaf tissue. Fv/Fm is considered a quantitative measure of the maximal or potential photochemical efficiency or optimal quantum yield of photosystem II (Willits and Peet 2001). Likewise Fv/Fm values are the most popular index used as a measure of plant vitality and early diagnostic of stress (Maxwell and Johnson 2001). Alterations with Fo values in leaf tissue are associated with dissociation of the light-harvesting chlorophyll a/b complexes from the reaction centre complex of photosystem II and/or alterations to the xanthophyll cycle-dependent nonradiative energy dissipation process (Hong and Xu 1999; Yamane et al. 2000).

### Leaf Chlorophyll Concentration

Chlorophyll was extracted from leaf samples by suspending 1 g of fresh laminar tissue in 5 ml 80% v/v aqueous acetone. After centrifugation at 2000 g for two minutes in closed vials, an aliquot of the supernatant was transferred to a 1 cm path glass cuvette and chlorophylls *a* and *b* calculated according to the equations of Lichtenthaler and Wellburn (1983), following measurement of absorbance at 663 and 645 nm in a spectrophotometer (PU8800 Pye Unicam, Portsmouth, UK).

### Photosynthetic CO<sub>2</sub> Fixation (Pn)

Light-induced CO<sub>2</sub> fixation (Pn) was measured in two pre-darkened (20 minutes) fully expanded leaves per tree near the top of the canopy (generally about the fourth leaf from the apex), using an Infra Red Gas Analyser (LCA-2 ADC, BioScientific Ltd, Hoddesdon, Herts, United Kingdom). The irradiance on the leaves was 700–800 μmol m<sup>-2</sup> PAR saturating with respect to Pn; the velocity of the airflow was 1 ml s<sup>-1</sup> cm<sup>-2</sup> leaf area. Calculation of the photosynthetic rates was carried out according to Von Caemmerer and Farquhar (1981). All photosynthetic measurements were taken in the early morning between 8:00–10:00 am on clear or partly cloudy days.

### Leaf Necrosis

Leaf necrosis was assessed visually. Each tree was rated on a 0–5 rating scale, using a visual indexing technique and ratings on the scale: 0 = No necrosis observed; 1 = less than 5% of leaves affected and no aesthetic impact; 2 = 5%–20% of leaves affected with some chlorosis but little or no defoliation; 3 = 21%–50% of leaves affected, significant defoliation and/or leaf chlorosis; 4 = 51%–80% of leaves affected, severe foliar discoloration; 5 = 81%–100% of foliage affected with 90%–100% defoliation.

### Leaf Electrolyte Leakage

Quantitative heat damage to leaf tissue was assessed by measuring electrolyte leakage of entire leaves (two leaves per tree) at each sampling date. Excised leaves were placed in 50 ml Universal bottles containing 30 ml distilled water and gently shaken by hand. Samples were stored at 22°C for 24 hours in darkness prior to conductivity measurements using a Jenway conductivity probe and M4070 meter (BDH, Leicestershire, Loughborough, UK). Total solute leakage was obtained by autoclaving the leaf samples for one hour at 121°C and 0.103 MPa. Results are presented as percent of total solute leakage after 24 hours (McKay 1992).

### Growth

At the cessation of each experiment trees were destructively harvested and leaf, shoot and root dry weight were recorded after oven drying at 85°C for 48 hours. Height was recorded by measuring the distance from the tip of the leading apical shoot to the soil surface. Leaf area size was quantified using a Delta-T leaf area meter (Delta-T Devices Ltd, Cambridge, UK).

### Experimental Design and Statistical Analysis

The experimental design used was a Completely Randomized Block Design (CRBD) in which pots were re-randomized on a weekly basis. Ten trees per treatment were used at 1.5 m spacing to prevent competition for light. Data was analyzed by one-

way ANOVA using the Genstat V program for Windows. Prior to the analysis data were examined for normality and homogeneity of variance (Levene 1960) and data were transformed [ $\log(\gamma+0.5)$ ] when necessary. Significant differences between means were separated by LSD test ( $P < 0.05$ ). Visual leaf necrosis were analyzed after appropriate statistical transformation [ $\arcsin(x^{0.5})$ ].

## RESULTS

### Foliar Tolerance to Heat Stress Under Laboratory Conditions

In both tree species and at all four sampling times (5, 10, 15, 20 minutes) damage to the leaf photosynthetic system based on the stability of the chlorophyll *a/b* light-harvesting complex within photosystem II (chlorophyll fluorescence *F<sub>o</sub>* responses) and leaf photochemical efficiency (chlorophyll fluorescence *F<sub>v</sub>/F<sub>m</sub>* emissions) was constantly less in penconazole-treated trees, indicating a positive influence of penconazole on enhancing foliar tissue tolerance to prolonged heat exposure (Tables 1–4). In the case of Scots pine the heat tolerance of leaf tissue was enhanced 14%–21% (*F<sub>o</sub>*) and 11%–45% (*F<sub>v</sub>/F<sub>m</sub>*) when averaged over a twenty-minute heat exposure period (Tables 1–2). In the case of evergreen oak, the heat tolerance of leaf tissue was enhanced 12%–30% (*F<sub>o</sub>*) and 27–46% (*F<sub>v</sub>/F<sub>m</sub>*) when averaged over a twenty-minute heat exposure period (Tables 3–4) compared to nonpenconazole-treated controls.

**Table 1. The influence of prolonged temperature exposure (50°C) on stability of the chlorophyll *a/b* light-harvesting complex within photosystem II based on chlorophyll fluorescence *F<sub>o</sub>* responses within leaf tissue of Evergreen oak (*Quercus ilex*).**

Treatment	0 min	5 min	10 min	15 min	20 min
Control	238a	589d	645c	633c	688b
0.15g	240a	446bc	564bc	561bc	632ab
0.30g	258a	333ab	451ab	493a	570a
0.45g	229a	427bc	475ab	577bc	610ab

Lower case letters indicate significant differences between means for each evaluation date by LSD ( $P = 0.05$ ).

All values mean of ten trees, five leaves per tree.

**Table 2. The influence of prolonged temperature exposure (50°C) on photochemical efficiency within leaf tissue of Evergreen oak (*Quercus ilex*) based on chlorophyll fluorescence *F<sub>v</sub>/F<sub>m</sub>* responses.**

Treatment	0 min	5 min	10 min	15 min	20 min
Control	0.798a	0.405a	0.251a	0.237a	0.086a
0.15g	0.799a	0.564b	0.378bc	0.335bc	0.102a
0.30g	0.772a	0.682bc	0.483cd	0.433c	0.223c
0.45g	0.785a	0.579b	0.452cd	0.317ab	0.167b

Lower case letters indicate significant differences between means for each evaluation date by LSD ( $P = 0.05$ ).

All values mean of ten trees, five leaves per tree.

In the majority of cases, these increases in heat tolerance were significant at  $P < 0.05$  (Tables 1–2). A significant influence of species and concentration of penconazole applied was recorded with respect to *F<sub>o</sub>* and *F<sub>v</sub>/F<sub>m</sub>* (Table 5). Such a response is reflected in the results where the effects of heat stress had greater detrimental impacts on foliar tissue of evergreen oak compared to



Scots pine for the first 10 minutes. Fo and Fv/Fm values at sampling times 15 and 20 minutes, however, indicated greater detrimental impacts on foliar tissue of Scots pine compared to evergreen oak (Table 1–4). Such a response indicates Scots pine may be able to better cope with heat stress over a short time period (up to 10 minutes), while evergreen oak superior over a prolonged period of intense heat (10–20 minutes). Irrespective of species, greatest protection of the leaf photosynthetic system to heat in-

**Table 3. The influence of prolonged temperature exposure (50°C) on stability of the chlorophyll a/b light-harvesting complex within photosystem II based on chlorophyll fluorescence Fo responses within leaf tissue of Scots pine (*Pinus sylvestris*).**

Treatment	0 min	5 min	10 min	15 min	20 min
Control	90.3a	152.7a	218c	290.4b	314a
0.15g	89.7a	155.7a	205bc	243.4ab	303a
0.30g	82.0a	120.0a	145a	209.6a	298a
0.45g	85.8a	127.6a	156a	236.2ab	299a

Lower case letters indicate significant differences between means for each evaluation date by LSD ( $P = 0.05$ ).

All values mean of ten trees, five leaves per tree.

**Table 4. The influence of prolonged temperature exposure (50°C) on photochemical efficiency within leaf tissue of Scots pine (*Pinus sylvestris*) based on chlorophyll fluorescence Fv/Fm responses.**

Treatment	0 min	5 min	10 min	15 min	20 min
Control	0.825a	0.583a	0.429a	0.158a	0.043a
0.15g	0.816a	0.645abc	0.446a	0.194a	0.067a
0.30g	0.818a	0.702c	0.595b	0.358c	0.100b
0.45g	0.823a	0.680bc	0.507a	0.295b	0.056a

Lower case letters indicate significant differences between means for each evaluation date by LSD ( $P = 0.05$ ).

All values mean of ten trees, five leaves per tree.

**Table 5. P values for chlorophyll fluorescence Fo and Fv/Fm following penconazole treatment.  $P < 0.05$  are considered significant.**

	Fo	Fv/Fm
Species (S)	<0.001	0.008
Concentration (C)	<0.001	<0.001
S x C	<0.001	<0.001

**Table 6. The effects of heat stress (50°C) for 10 minutes on alterations to plant physiology of evergreen oak (*Quercus ilex*) and Scots pine (*Pinus sylvestris*). Measurements were made 24 hours after the cessation of the heat treatment.**

Parameter	<i>Quercus ilex</i>		<i>Pinus sylvestris</i>	
	Nonpenconazole Treated control	Well-Watered	Nonpenconazole Treated control	Well-Watered
% electrolyte leakage	26.7b	5.14a	32.7b	4.56a
Fv/Fm	0.35a	0.83b	0.28a	0.81b
Fo	500.6b	225.3a	221.3b	92.3a
Pn	2.22a	4.21b	1.80a	4.00b
Leaf necrosis	2.5b	0.0a	3.0b	0.0a
Chlorophyll a/b	2.92a	3.00a	2.81a	2.80a
Total chlorophylls	40.5a	82.0b	22.3a	45.6b

% electrolyte leakage, photosynthetic rates (Pn) values mean of 10 trees, two leaves per tree.

Fv/Fm, Fo, total chlorophyll ( $\mu\text{g/g}$  fresh leaf weight), values mean of 10 trees, five leaves per tree.

Leaf necrosis mean of 10 trees.

Lower case letters indicate significant differences in rows between means by LSD at ( $P < 0.05$ ).

duced disorders was achieved by application of penconazole at 30 g per liter of water compared to penconazole applied at 0.15 and 0.45 g per liter of water (Tables 1–4). In this instance all tree vitality measurements were greater than nontriazole-treated controls and penconazole applied at 0.15 and 0.45 g per liter of water.

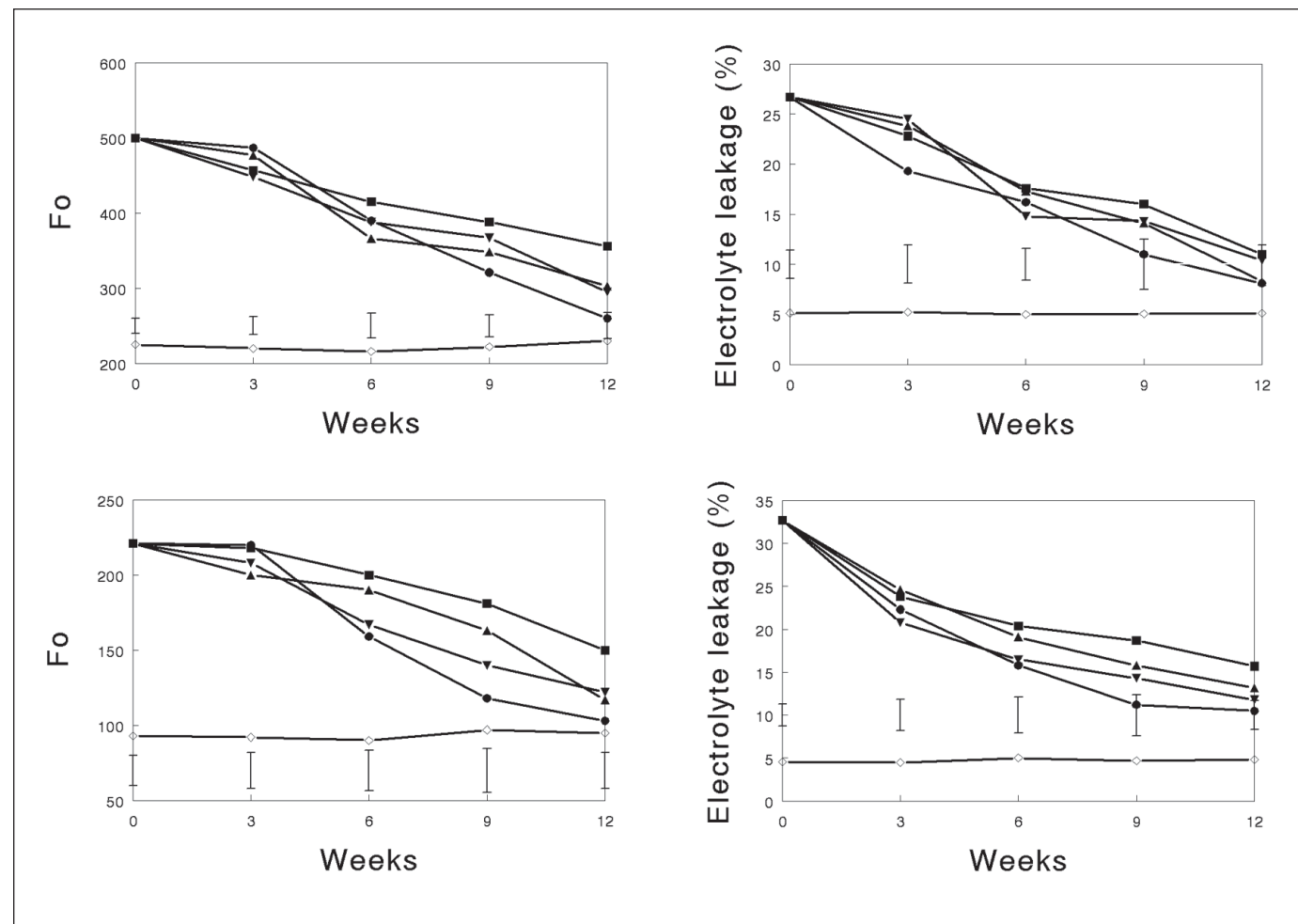
### Recovery of Containerized Trees from Heat Stress

Subjecting containerized trees of both species to 10 minutes at 50°C significantly reduced tree vitality with respect to chlorophyll fluorescence Fo and Fv/Fm emissions, total foliar chlorophylls, leaf photosynthetic rates (Pn) and significantly increased damage to cellular membrane integrity as manifest by higher leaf electrolyte leakage and visual leaf necrosis in comparison with non-heat stressed well watered trees (Table 6). There was, however, no significant effect of heat stress on the ratio of chlorophyll a/b (Table 6). The influence of penconazole (0.15, 0.30, or 0.45 g per liter of water) applied immediately after heat stress on the pattern of recovery over the following twelve weeks is shown diagrammatically for chlorophyll fluorescence Fo and leaf electrolyte leakage values (Figure 1). The pattern of recovery for these two parameters reflects those recorded for chlorophyll fluorescence Fv/Fm emissions, total foliar chlorophylls, leaf photosynthetic rates (Pn) and visual leaf necrosis (data not shown). Namely, irrespective of treatment (with or without penconazole), all parameters began to recover. Penconazole treated trees were the most capable of recovery (Figure 1). With respect to chlorophyll fluorescence Fo and leaf electrolyte leakage values recovery rates of heat damaged trees treated with penconazole ranged from 20%–50% higher than nonpenconazole-treated control trees (Figure 1). In all cases nontriazole-treated control trees had the least capacity for recovery (Figure 1). Foliar application of penconazole at 30 g per liter of water induced greatest recovery rates over the 12-week period. At Week 12, all values of penconazole-treated trees were statistically comparable with well-watered non-heat stressed trees. During the 12 week recovery phase new leaf formation was observed at ca. Weeks 4–6 on both nonpenconazole-treated control and penconazole-treated trees.

Application of penconazole had a marked impact on regeneration from heat stress at the cessation of a 12 week recovery period. Regardless of species, height (evergreen oak only), leaf area, root, shoot, and total plant dry weight were, in virtually all instances, significantly ( $P < 0.05$ ) greater than non-

penconazole-treated controls but not as great as well-watered plants which had the greatest growth (leaf area, root, shoot, total plant dry weight; Tables 7–8). Irrespective of concentration

applied, foliar application of penconazole had no significant influence on the root:shoot ratio compared to nonpenconazole-treated control trees and well-watered plants (Tables 7–8).



**Figure 1.** The influence of penconazole on time course recovery over 12 weeks of chlorophyll fluorescence  $F_o$  values as a measure of stability of the chlorophyll a/b light-harvesting complex within photosystem II and leaf electrolyte leakage as a measure of cellular membrane integrity of evergreen oak (*Quercus ilex*) and Scots pine (*Pinus sylvestris*) following heat stress ( $50^{\circ}\text{C}$ ) for 10 minutes. Error bars = Least Significant Difference at  $P < 0.05$ . ■ = Nontriazole-treated controls, ○ = Well-watered trees, ▲ = penconazole 15 g liter of water, ● = penconazole 30 g liter of water, ▼ = penconazole 45 g liter of water. All values mean of 10 trees.  $F_o$  values mean of five leaves per tree, leaf electrolyte leakage values mean of two leaves per tree.

**Table 7.** The influence of penconazole on growth of containerized evergreen oak (*Quercus ilex*) at the end of a 12-week recovery period following the cessation of heat induced damage ( $50^{\circ}\text{C}$ ) for 10 minutes.

Parameter	Nontriazole Treated control	Penconazole (0.15 g)	Penconazole (0.30 g)	Penconazole (0.45 g)	Well-Watered
Height (cm)	120.4a	125.6ab	126.3ab	130.4ab	135.7b
Leaf Area ( $\text{cm}^2$ )	1109a	1223ab	1435c	1392bc	1488c
Root DW (g)	14.5a	19.6b	18.7b	20.5bc	23.4c
Shoot DW (g)	28.7a	33.4ab	35.8b	38.6bc	44.6c
Total Plant DW	43.2a	53.0b	54.5b	59.1b	68.0c
Root:Shoot Ratio	0.51a	0.58a	0.52a	0.53a	0.52a

All values mean of 10 trees.

Lower case letters indicate significant differences in rows between means by LSD at ( $P < 0.05$ ).

**Table 8. The influence of penconazole on growth of containerized Scots pine (*Pinus sylvestris*) at the end of a 12-week recovery period following the cessation of heat induced damage (50°C) for 10 minutes.**

Parameter	Nontriazole Treated control	Penconazole (0.15g)	Penconazole (0.30g)	Penconazole (0.45g)	Well-Watered
Height (cm)	127.6a	130.5a	144.6a	132.8a	149.3a
Leaf Area (cm <sup>2</sup> )	665.7a	700.4a	803.5ab	792.4ab	855.0b
Root DW (g)	11.8a	15.0ab	15.8bc	18.7c	17.8bc
Shoot DW (g)	24.3a	30.2ab	34.5b	32.9b	33.9b
Total Plant DW	36.1a	45.2ab	50.3b	51.6b	51.7b
Root:Shoot Ratio	0.49a	0.50a	0.46a	0.56a	0.53a

All values mean of 10 trees.

Lower case letters indicate significant differences in rows between means by LSD at ( $P < 0.05$ ).

## DISCUSSION

The fungitoxic effectiveness of penconazole against foliar pathogenic fungi such as apple scab and powdery mildew has been confirmed by several workers under laboratory and field conditions (Kenyon et al. 1997; Schnabel and Parisi 1997; Percival and Boyle 2005). Consequently, penconazole is fully approved for foliar pathogen control under UK pesticide legislation (Anonymous 2010). However, all triazoles, to include penconazole, have been shown to confer stress reducing properties in plants with recorded cases of less drought, freezing, and heat-related plant disorders following pre-treatment with these compounds (Gilley and Fletcher 1997; Jaleel et al. 2007a; Manivannan et al. 2007; Percival and Noviss 2008). Results of this study support previous work. Foliar treatment of both Scots pine and evergreen oak with penconazole at 0.15, 0.30, or 0.45 g a.i per liter of water resulted in significantly less heat induced damage to the leaf photosynthetic system as determined by chlorophyll fluorescence  $F_o$  and  $F_v/F_m$  emissions as a measure of stability of the chlorophyll a/b light-harvesting complex within photosystem II and leaf photochemical activity respectively. This result indicates pre-treatment with the triazole derivative penconazole would allow both tree species to tolerate a longer, high temperature episode compared to nonpenconazole-treated trees. Likewise, application of penconazole immediately after the cessation of 10 minutes 50°C heat stress improved recovery rates of physiological adaptations associated with tree vitality to include chlorophyll fluorescence  $F_o$  and  $F_v/F_m$  emissions, total foliar chlorophylls, leaf photosynthetic rates, cellular membrane integrity and visual leaf necrosis so that by the end of a 12 week recovery period values in some instances were statistically comparable to those of well watered non-heat stressed trees.

Application of triazoles such as penconazole have been shown to induce synthesis of a range of stress protective enzymes and metabolites within plants to include low-molecular mass antioxidants such as carotenoids (carotenes and xanthophylls), reactive oxygen species (ROS), scavenging enzymes, such as superoxide dismutase, catalase,  $\alpha$ -tocopherol, ascorbic acid, and compatible solutes such as proline and glycine betaine, which function as osmoprotectants for proteins (Fletcher et al. 2000; Apel and Hirt 2004; Still and Pill 2004; Fernandez et al. 2006; Jaleel et al. 2007b; Jaleel et al. 2008; Percival and Noviss 2008). High activities or naturally inherent concentrations of these stress protective compounds are regarded as key physiological characteristics conferring plant tolerance to environments where sub-optimal growing conditions prevail (e.g., drought, salinity, root de-oxygenation). As a result, penconazole-enhanced concentrations of these compounds would be important contributors to reducing heat damage to the leaf photosynthetic

structure (Kraus and Fletcher 1994; Jaleel et al. 2006; Jaleel et al. 2008). Improved tolerance to, and recovery from heat therefore may be explained in part, by penconazole-induced alterations to leaf anti-oxidant, metabolite and enzymatic activity.

Chlorophyll fluorescence  $F_o$  and  $F_v/F_m$  emissions as a measure of leaf chloroplast stability and photochemical efficiency provide an indirect measure of tree vitality (Clarke et al. 2000). Alterations to these values are sensitive indicators of damage to the leaf photosynthetic system caused by environmental stress (Percival and Sheriffs 2002). After heat exposure at fixed time periods, damage to the leaf photosynthetic system based on the stability of the chlorophyll a/b light-harvesting complex within photosystem II ( $F_o$  values) and leaf photochemical efficiency ( $F_v/F_m$  emissions) in penconazole-treated trees was significantly less than nonpenconazole-treated trees. The maintenance of relatively high fluorescence values in triazole treated plants under stress has been observed in previous studies (Pinhero and Fletcher 1994) with higher values associated with reduced impairment of leaf photosynthetic activities (Percival et al. 2006). Robustness of the photosynthetic system is an important physiological trait associated with survivability under prolonged environmental stress conditions to ensure carbohydrate production via photosynthesis necessary for the growth and repair of damaged tissue.

Over a 12-week recover period following a 10-minute heat stress period, vitality of penconazole-treated trees as measured by chlorophyll fluorescence  $F_o$  emissions and leaf electrolyte leakage ranged from 20%–50% higher than nonpenconazole-treated trees. Limited studies have recorded the influence of triazoles as an aid toward heat-induced stress recovery. Leaf chlorophyll  $F_o$  and electrolyte leakage values were similar to those of well-watered (i.e., nonstressed trees) by week 12 post-recovery, indicating regeneration and full functioning of the leaf photosynthetic system and cell membrane integrity. The importance of rapid recovery from stress has been shown elsewhere (Gilley and Fletcher 1997; Still and Pill 2004), with genotypes that rebound to original or near original physiological levels most likely to survive and tolerate prolonged stress episodes compared to those that do not or are slower to recover (Aguilera et al. 1997; Bauerle and Dudley 2003). In this study, nonpenconazole-treated control trees had the least capacity for recovery because leaf chlorophyll  $F_o$  and electrolyte leakage were 45%–55% lower at the cessation of the post-heat 12-week recovery period compared to well-watered trees. Improvements in the range of the previously discussed physiological measurements may account for increased growth as measured by height, leaf area, shoot, root, and total plant dry weight in trees treated with penconazole at the end of a post-heat 12-week recovery period (Martin et al. 1987;

McKay 1992; Cameron and Dixon 1997). Besides maintenance of photosynthetic integrity, prevention of leaf abscission has been shown to be a significant contributor to growth under stress as leaves are the major photosynthetic organ responsible for carbohydrate production required for repair and functioning of damaged leaf tissue (Tyree et al. 1993). Likewise, leaf production rates are important variables influencing growth under environmental stresses (Pregitzer et al. 1990; Farrell et al. 1996). Significantly less leaf loss during the recovery period as stimulated by penconazole application enhanced total leaf area and subsequent photosynthetic area necessary for growth (Farrell et al. 1996). Indeed productivity as measured by total tree dry weight has been correlated with net photosynthetic rates (Ort and Boyer 1985).

In conclusion, heat stress is recognized as a problematic factor that indirectly through leaf and wood desiccation may contribute to branch shed and tree decline in urban landscapes (Hitchmough 1994). The tactical use of the triazole derivative penconazole as an ameliorant against heat damage and recovery from heat stress in woody plants would be of benefit to improve tree recovery rates and growth of Scots pine and evergreen oak. From a practical point of view penconazole at 30g a.i. per liter of water is suggested based on the results of this study.

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**Résumé.** La capacité du penconazole – un dérivé du fongicide triazole – à protéger les arbres contre le stress de la chaleur et même à améliorer cette tolérance a été étudiée chez le chêne vert (*Quercus ilex*) et le pin sylvestre (*Pinus sylvestris*). Sous des conditions de laboratoire, les dommages par la chaleur au système photosynthétique foliaire à partir des feuilles des feuilles tombées était constamment inférieur chez les arbres traités au penconazole, et ce en se basant sur le complexe de lumière récoltée par la stabilité de la chlorophylle *a/b* à l'intérieur du photosystème II (réponses à la fluorescence de la chlorophylle *Fo*) et l'efficacité photochimique foliaire (émissions de fluorescence de la chlorophylle *Fv/Fm*). Chez les deux espèces, la plus grande protection du système photosynthétique foliaire face aux désordres par la chaleur a été atteinte par l'application de penconazole à une concentration de 30 g par litre d'eau comparativement à des concentrations de 0,15 ou 0,45 g par litre d'eau. La soumission des deux espèces arbres en contenant durant 10 minutes à des émissions de température de 50°C a significativement diminué la vitalité des arbres selon les émissions de fluorescence de la chlorophylle *Fo* et *Fv/Fm*, la chlorophylle foliaire totale, les taux de photosynthèse foliaire (*Pn*) et a aussi significativement accru les dommages à l'intégrité des membranes cellulaires qui se manifestaient par une perte plus élevée d'électrolytes foliaires ainsi qu'une nécrose foliaire visuelle entre les arbres stressés par rapport aux arbres non stressés par la chaleur et bien arrosés. L'influence du penconazole appliqué immédiatement après un stress de chaleur, et ce en regard du patron de reprise durant les douze semaines qui suivirent démontrèrent que les arbres traités au penconazole étaient ceux qui étaient le plus apte à se remettre. Selon les valeurs de fluorescence de la chlorophylle *Fo* et de perte en électrolytes, les taux de reprise des arbres endommagés par la chaleur et traités avec le penconazole étaient de 20% à 50% plus élevés que les arbres non traités du groupe-témoin. Dans tous les cas, les arbres-témoin non traités au penconazole avaient la plus faible capacité de reprise. Peu importe l'espèce, la hauteur, la surface foliaire ainsi que la masse sèche en racines, pousses et totale de la plante étaient dans la plupart des cas plus élevées chez les arbres traités que ceux non traités. L'utilisation tactique du penconazole, un dérivé du triazole, comme agent pour améliorer la résistance face à la chaleur ainsi que le degré de reprise par la suite chez le chêne vert le pin sylvestre pourrait être bénéfique pour améliorer le taux de reprise et la croissance. D'un point de vue pratique, le penconazole à un taux de 30 g d'ingrédient actif par litre d'eau est suggéré si on se base sur les résultats de cette recherche.

**Zusammenfassung.** Die Fähigkeit von Penconazol, einem Triazol-Fungizid-Derivat zum Schutz gegen Hitzestress wurde bei Immergrünen Eichen (*Quercus ilex*) und Waldkiefer (*Pinus sylvestris*) getestet. Unter Laborbedingungen basierte der Hitzeschaden am Blattphotosynthesesystem auf der Stabilität des Chlorophyll *a/b* Lichternte-Komplexes innerhalb des Photosystems II (Chlorophyll Fluoreszenz *Fo* Resonanz) und die photochemische Effizienz der Blätter (Chlorophyll Fluoreszenz *Fv/Fm* Emissionen) bei abgenommenen Blättern war konstant weniger als in mit Penconazol behandelten Bäumen. Bei beiden Arten war der größte Schutz des Blattphotosynthesesystems durch hitze-induzierte Schäden durch die Applikation von Penconazol in einer Konzentration von 30 g pro Liter Wasser im Vergleich zur Applikation von 0,15 oder 0,45 g/l Wasser erreicht. Wurden Containerpflanzen beider Arten für 10 Minuten 50°C ausgesetzt, reduzierte sich die Vitalität im Hinblick auf Chloro-

phyll Fluoreszenz *Fo* und *Fv/Fm* Emissionen, totalem Blattchlorophyllgehalt und die Blattphotosyntheseraten (*Pn*) deutlich, während verstärkt Schäden auftraten an zellulären Membranen und ihrem Zusammenhalt als Manifest höheren Blattelektrolyt-Austritts und sichtbarer Blattnekrose zwischen gestressten und ungestressten, gut gewässerten Bäumen. Der Einfluss von direkt nach dem Hitzestress applizierten Penconazol auf die Art der Genesung der Bäume in den folgenden zwölf Wochen zeigte, daß Penconazol-behandelte Bäume sich schnell wieder erholen können. Im Hinblick auf die Chlorophyll Fluoreszenz *Fo* und die Blattelektrolyt-Austrittswerte rangierten die Erholungsraten von hitzgeschädigten, mit Penconazol behandelten Bäumen um 20%–50 % höher als die nicht mit Triazol behandelten Kontrollbäume. In allen Fällen hatten die nicht mit Penconazol behandelten Bäume die schlechtesten Erholungsraten. Unabhängig von der Art, war die Höhe, Blattfläche, Wurzeln, Triebe und totales Pflanzentrockengewicht, virtuell in allen Instanzen, größer als bei unbehandelten Kontrollbäumen. Der taktische Einsatz des Triazolderivats Penconazol als ein Verbesserer im Einsatz gegen Hitzeschäden und zur Erholung von Hitzestress bei Waldkiefer und Immergrünen Eichen würde einen positiven Beitrag zur Verbesserung von Erholungsraten und Wachstumsraten von Bäumen leisten.

**Resumen.** Se estudió la habilidad de penconazole, un fungicida derivado del triazol, para proteger contra stress por calor en encinos verdes (*Quercus ilex*) y pinos Scots (*Pinus sylvestris*). Bajo condiciones de laboratorio, el daño por calor al sistema fotosintético de la hoja basado en la estabilidad de la clorofila *a/b* dentro del fotosistema II (respuestas clorofila fluorescente *Fo*) y la eficiencia fotoquímica de la hoja (emisiones *Fv/Fm* clorofila fluorescente) de hojas aisladas fue constantemente menor en árboles tratados con penconazole. En ambas especies, la protección mayor del sistema fotosintético al calor indujo desórdenes que fueron alcanzados por la aplicación de penconazole a una concentración de 30 g por litro de agua comparado a penconazole aplicado a concentración de 0.15 o 0.45 g por litro de agua. Al someter árboles en contenedor de ambas especies 10 minutos a 50°C se redujo significativamente la vitalidad del árbol con respecto a la clorofila fluorescente *Fo* y las emisiones *Fv/Fm*, clorofila total foliar, tasas fotosintéticas de la hoja (*Pn*) e incrementó significativamente el daño a la integridad de la membrana celular como efecto de mayor pérdida de electrolitos foliares y necrosis foliar visual entre estresados y no estresados por calor de árboles bien regados. La influencia de penconazole aplicado inmediatamente después del estrés en patrones de recobramiento en las doce semanas siguientes demostró que los árboles tratados con penconazole fueron más capaces de recobrase. Con respecto a la clorofila fluorescente *Fo* y la pérdida de electrolitos los valores se recobraron a tasas de árboles tratados por daños por el calor con penconazole varió de 20%–50% más altos que árboles de control no tratados. En todos los caso los árboles de control no tratados con penconazole tuvieron la menor capacidad para recobrase. Sin importar la especie, altura, área foliar, raíz, brotes, peso seco total de la planta fueron, virtualmente en todos los casos, mayores que los controles no tratados. El uso táctico de triazol derivado de penconazole como un tratamiento contra el daño por calor y recobramiento del estrés en pino Scots y encino podría ser de beneficio para mejorar los árboles y la tasa de crecimiento. Desde el punto de vista práctico se sugiere el penconazole a una tasa de 30 g por litro de agua con base en los resultados de este estudio.