



## Water-retaining Polymer and Fungicide Combinations Reduce Disease Severity Caused by Horsechestnut Leaf Blotch (*Guignardia aesculi* (Peck) VB Stewart)

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**Abstract.** The influence of six commercially available fungicides incorporated into a water-retaining polymer and applied to the root system of horsechestnut (*Aesculus hippocastanum* L.) as a dip at the time of planting was conducted. Potential increases in resistance against the foliar pathogen *Guignardia* leaf blotch (*Guignardia aesculi*) was then monitored over two growing seasons. Trials were conducted in 2007 and duplicated in 2008. A comparative evaluation of the fungicide penconazole commercially used for *Guignardia* leaf blotch control was studied by spraying trees at the manufacturer's recommended rate of four times during the first growing season but none in the second. None of the treated or control trees died as a result of *Guignardia* leaf blotch attack during the course of the study and none of the fungicide and water-retaining polymer combinations evaluated was phytotoxic to the test trees. Efficacy as *Guignardia* leaf blotch protectant compounds over the first growing season was demonstrated when fungicides were incorporated into a water-retaining polymer. Reductions in *Guignardia* leaf blotch severity were mirrored by increases in leaf chlorophyll fluorescence as a measure of leaf photosynthetic activity and leaf chlorophyll content SPAD values. There were little differences in the magnitude of control efficacy between the fungicides evaluated. Limited efficacy of any of the fungicide and water-retaining polymer combinations as *Guignardia* leaf blotch protectant compounds was, however, demonstrated the following year after application indicating a fungicide and water-retaining polymer root dip provided one growing season protection only. Application of a water-retaining polymer alone had no effect on reducing *Guignardia* leaf blotch severity. Based on visual *Guignardia* leaf blotch severity ratings, greatest protection in both the 2007 and 2008 trial was provided by the synthetic fungicide penconazole applied as a foliar spray four times during the growing season. No efficacy of penconazole foliar sprays as leaf blotch protectant compounds was demonstrated the following year, indicating annual sprays against *Guignardia* leaf blotch are required for control.

**Key Words.** *Aesculus hippocastanum*; Disease Management; Chlorophyll Fluorescence; Leaf Chlorophyll Content; Plant Health Care; Tree Planting.

*Guignardia* leaf blotch of horsechestnut (*Aesculus* spp.) occurs in Europe, North America, and Asia (Pastirčáková et al. 2009). Leaf blotch caused by the fungus *Guignardia aesculi* (Peck) Stewart on *Aesculus* is diagnosed by identifying the conidial state of the pathogen, *Phyllosticta sphaeropsoides* Ellis et Everh (Pastirčáková 2004). The symptoms of leaf blotch disease are brown or reddish-brown lesions that often cover a large portion, or even the entire leaf. Leaves curl and brown, and the tree often appears to be suffering from severe leaf scorch (Percival et al. 2006; Percival 2008). Premature leaf drop normally follows infection. Susceptible species include California, Ohio, red, and yellow buckeyes; several less-known buckeye species; and common, Japanese and red horsechestnuts (Sinclair et al. 1987; Pastirčáková et al. 2009). In terms of *Guignardia* leaf blotch control, frequent fungicide sprays have a known efficacy in reducing severity (Plenk 1996; Pastirčáková 2003).

Creation of amenity woodlands and urban treescapes in the UK are predominantly established using bare-rooted stock of deciduous tree species. Poor growth and survival following out-planting is common during the first few years of establishment (Watson and Himelick 1997). An important cause of poor growth and death in transplanted trees can be attributed to root loss following lifting from the nursery bed, since as little as 5% of a tree's

root system may be moved with a tree (Davies et al. 2002). Following leafing out, the capacity of the roots to supply the leaves with water is severely restricted. This leads to water stress that with time may be characterized by reduced shoot growth, branch dieback, and possibly death; a concept widely described as transplant stress (Fraser and Percival 2003; Percival and Barnes 2004).

Stress is generally recognized as a prerequisite for disease attack with higher rates of attack and/or increased severity of pathogen infection associated with trees of lower vitality. Consequently, trees suffering from transplant stress following out-planting will be especially susceptible to attack. Water-retaining polymers are polymers that absorb large quantities of water and once incorporated into a growing medium are capable of releasing at least 95% to plants. As the polymers dehydrate, discrete non-toxic granules are formed. Consequently, water-retaining polymers are recommended as a means of reducing root desiccation and post-planting mortalities (Johnston and Phipper. 1997; Grazia et al. 2004). The use of a water-retaining polymer in combination with a fungicide at the time of planting has not been investigated. Such a combination may provide a means of increasing the pathogen resistance of trees over a growing season. Aims of this investigation were to determine the influence of i) a range of commercially available fungicides incorporated into a water-retaining

polymer on resistance of horsechestnut (*Aesculus hippocastanum* L.) against the foliar pathogen *Guignardia aesculi* and ii) duration of any resistance conferred by monitoring *Guignardia* leaf blotch severity over two growing seasons under field conditions.

## MATERIALS AND METHODS

Experiments were conducted in 2007 and repeated in 2008. The experiment used bare-rooted stock of horsechestnut obtained from a commercial supplier. To ensure uniformity of stock for experimental purposes, trees were graded and used only if conforming to the physical characteristics specified: height  $100.0 \pm 7.0$  cm, stem diameter  $3.5 \pm 0.30$  cm, root area  $400.0 \pm 50.5$  cm<sup>2</sup>. Trees were then sealed in plastic bags, placed inside larger paper bags, and stored at  $6^\circ\text{C} \pm 0.5^\circ\text{C}$  (a standard storage temperature for trees in the UK) in a refrigerated cold store in darkness. Following six weeks at  $6^\circ\text{C} \pm 0.5^\circ\text{C}$  dark storage, trees were removed from cold store (January 28, 2007; February 1, 2008) prior to fungicide and polymer treatments on the same day as removal.

All fungicides used for experimental purposes (Table 1) were diluted with water to achieve a concentration of 0.15 g and 0.30 g active ingredient (a.i.) per liter of water. Manufacturers generally recommend 0.15 g a.i. per liter of water for plant protection purposes when fungicides are applied as a foliar spray. The water-retaining polymer Aquastore F was hydrated with each fungicide solution at 5 g polymer per liter of solution. Following hydration of each polymer, the root systems of ten trees were dipped for 30 seconds and gently agitated throughout the polymer to ensure maximal contact with the root system. The influence of the water-retaining polymer alone (no fungicide) on *Guignardia* leaf blotch severity was also investigated and bare-rooted stock dipped for 30 seconds in water only (no fungicide treatment or polymer) acted as controls. In addition, a comparative evaluation of the fungicide penconazole, commercially used for *Guignardia* leaf blotch control, was conducted by spraying trees at the manufacturers recommended rate of 1.5 ml l<sup>-1</sup> of water. Penconazole sprays were applied at four growth stages namely: bud break (March 19, 2007; March 23 2008), flower cluster formation

**Table 1. The influence of water-retaining polymer (WRP) and fungicide combinations applied at the time of planting for the control of *Guignardia* leaf blotch on leaves of horsechestnut (*Aesculus hippocastanum* L.) as measured by observed pathogen severity.**

Treatment	Leaf blotch severity	
	2007	2008
Water (control)	4.6a	4.6a
WRP	4.5a	4.4a
WRP + Penconazole (0.15 g)	2.5b	4.4a
WRP + Penconazole (0.30 g)	2.2b	4.2a
WRP + Thiabendazole (0.15 g)	1.8b	4.7a
WRP + Thiabendazole (0.30 g)	2.0b	4.7a
WRP + Propiconazole (0.15 g)	1.8b	4.3a
WRP + Propiconazole (0.30 g)	1.7b	4.5a
WRP + Myclobutanil (0.15 g)	1.6b	5.0a
WRP + Myclobutanil (0.30 g)	2.1b	4.9a
WRP + Epoxiconazole (0.15 g)	2.5b	4.2a
WRP + Epoxiconazole (0.30 g)	1.6b	4.0a
WRP + Potassium Phosphite (0.15 g)	2.4b	4.4a
WRP + Potassium Phosphite (0.30 g)	2.5b	4.6a
Penconazole (spray)	0.0c	4.1a

Note: All values mean of ten trees. Also, lowercase letters indicate significant differences between means for each evaluation date ( $P = 0.05$ ).

(April 21, 2007; April 27, 2008), 90% petal fall (May 18, 2007; May 22, 2008), and full leaf expansion (June 21, 2007; June 25 2008). Prior to the first penconazole spray application, trees were inspected and no visible symptoms of *Guignardia* leaf blotch were apparent. Following dipping, trees were immediately planted out into field trial plots at the University of Reading, Shinfield Experimental Station, Reading ( $51^\circ43\text{N}$ ,  $-1^\circ08\text{W}$ ) at 1.5 m spacing. A randomized complete design was used. There were fifteen treatments; 6 fungicide  $\times$  2 concentrations, 1 water-retaining polymer, 1 control, and 1 penconazole foliar sprayed comparative analysis with ten trees per treatment to provide a total of 150 trees used in each experimental year. The soil was a sandy loam, containing 5%–7% organic matter with a pH of 6.4. Weeds were controlled chemically using glyphosate (Roundup®) prior to planting and by hand during the trial. No irrigation was required and no fertilizer was applied to trees during each experiment.

## Tree Vitality

Five leaves per tree were randomly selected throughout the crown and used for chlorophyll fluorescence and chlorophyll content measurements. Leaves were then tagged to ensure only the same leaf was measured throughout. Each five fluorescence and chlorophyll content values per tree were pooled to provide one value per tree for statistical analysis purposes.

## Chlorophyll Fluorescence

Chlorophyll fluorescence was used as a measure of damage to the leaf photosynthetic system and to identify potential phytotoxicity effects. Leaves were adapted to darkness for 10 minutes by attaching light exclusion clips to the leaf surface and chlorophyll fluorescence was measured using a Handy PEA portable fluorescence spectrometer (Hansatech Instruments Ltd, King's Lynn, UK). Measurements were recorded up to 1 second with a data acquisition rate of 10 $\mu\text{s}$  for the first 2 milliseconds and of 1 millisecond thereafter. The fluorescence responses were induced by a red (peak at 650 nm) light of 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  Photosynthetically Active Radiation intensity provided by an array of six light emitting diodes. The ratio of variable ( $F_v = F_m - F_o$ ) to maximal ( $F_m$ ) fluorescence—i.e.,  $F_v/F_m$  where  $F_o$  = minimal fluorescence, of dark-adapted leaves was used to quantify the detrimental effects of *Guignardia* leaf blotch infection on leaf tissue.  $F_v/F_m$  is considered a quantitative measure of the maximal or potential photochemical efficiency or optimal quantum yield of photosystem II (Willits and Peet 2001). Likewise  $F_v/F_m$  values are the most popular index used as a measure of plant vitality (Maxwell and Johnson 2001; Percival 2004).

## Leaf Chlorophyll Concentration

Data on degradation of the leaf chlorophyll molecule as a result of *Guignardia* leaf blotch infection were recorded using a Minolta chlorophyll meter SPAD-502. Chlorophyll was measured at the midpoint of the leaf next to the main leaf vein. Calibration was obtained by measurement of absorbance at 663 and 645 nm in a spectrophotometer (PU8800 Pye Unicam, Portsmouth, UK) after extraction with 80% v/v aqueous acetone (regression equation =  $6.00 + 0.058x$ ;  $r^2 \text{ adj} = 0.88$ ,  $P \leq 0.001$ ) (Lichtenthaler and Wellburn 1983).

### Guignardia Leaf Blotch Severity

*Guignardia* leaf blotch severity was assessed visually in September 2007, 2008, and 2009. Each tree was rated on a 0 to 5 rating scale, using a visual indexing technique and ratings on the scale: 0 = no leaf blotch observed; 1 = less than 5% of leaves affected and no aesthetic impact; 2 = 5%–20% of leaves affected with some yellowing but little or no defoliation; 3 = 21%–50% of leaves affected, significant defoliation (30%–50%) and/or leaf yellowing; 4 = 51%–80% of leaves affected, severe foliar discoloration and defoliation (51%–90%); 5 = 81%–100% of foliage affected with 91%–100% defoliation.

The individual ratings for each tree in each treatment were used as a *Guignardia* leaf blotch severity index for statistical analysis.

Mean *Guignardia* leaf blotch severity values for treatments were transformed using the arcsine<sup>-1</sup> transformation. All data were analyzed using ANOVA after checks for homoscedasticity were met using an Anderson-Darling test and the differences between means were separated by the Least Significance Difference (LSD) at the 95% confidence level ( $P = 0.05$ ) using the Genstat for Windows 14th Edition program. Back transformed *Guignardia* leaf blotch severity values are presented here to ease interpretation of data (Blaedow et al. 2006).

### RESULTS

Damaging outbreaks of *Guignardia* leaf blotch were recorded on control trees in both the 2007 and 2008 trials as indicated by leaf blotch severity ratings of 4.6 on leaves of horsechestnut at the cessation of each growing season, respectively (Table 1; Table 2). None of the treated or control trees died as a result of leaf blotch attack during the course of the study, and none of the fungicide and water-retaining polymer combinations evaluated was phytotoxic to the test trees (data not shown). Efficacy as leaf blotch protectant compounds was demonstrated when fungicides were incorporated into a water-retaining polymer over the first growing season—i.e., observed pathogen severity was, in all cases, significantly lower ( $P < 0.05$ ) compared to water dipped controls. In these cases, leaf blotch severity was reduced by 46%–65% (2007 growing season) and by 41%–67% (2008 growing season), respectively. Significant reductions in leaf blotch severity were mirrored by significant increases in leaf chlorophyll fluorescence as a measure of leaf photosynthetic activity (20%–29%, 2007 growing season; 32%–43%, 2008 growing season) and leaf chlorophyll content SPAD values (40%–91%, 2007 growing season; 25%–58%, 2008 growing season); (Table 3; Table 4; Table 5; Table 6). There were little differences in the magnitude of efficacy between fungicides and concentration applied where leaf blotch severity, leaf chlorophyll fluorescence, and leaf chlorophyll content were statistically similar between treatments in both the 2007 and 2008 studies (Table 3; Table 4; Table 5; Table 6). Limited efficacy of any of the fungicide and water-retaining polymer combinations as leaf blotch protectant compounds was demonstrated the following year after application. In most cases, observed leaf blotch severity, leaf chlorophyll fluorescence Fv/Fm values, and leaf chlorophyll content were statistically comparable to water-treated controls. This indicates a fungicide and water-retaining polymer combination applied as a root dip at the time of planting provides one growing season's protection. Application of

a water-retaining polymer root dip alone (i.e., no fungicide) had no effect on reducing leaf blotch severity at the cessation of both the 2007 and 2008 study. In all cases, observed leaf blotch severity, leaf chlorophyll fluorescence Fv/Fm values, and leaf chlorophyll content were statistically comparable to water-treated controls. Based on visual observation of leaf blotch severity, the greatest protection in both the 2007 and 2008 trials was provided by the synthetic fungicide penconazole applied as a foliar spray four times during the growing season. In both the 2007 and 2008 studies, leaf blotch severity was reduced by 100% (Table 1; Table 2). In addition, the highest chlorophyll fluorescence Fv/Fm and SPAD values as measures of leaf photosynthetic activity and chlorophyll content respectively were recorded in penconazole foliar spray treated trees compared to water-treated controls and fungicide and water-retaining

**Table 2. The influence of water-retaining polymer (WRP) and fungicide combinations applied at the time of planting for the control of *Guignardia* leaf blotch on leaves of horsechestnut (*Aesculus hippocastanum* L.) as measured by observed pathogen severity.**

Treatment	Leaf blotch severity	
	2008	2009
Water (control)	4.6a	4.1a
WRP	4.5a	4.0a
WRP + Penconazole (0.15 g)	2.5bc	4.3a
WRP + Penconazole (0.30 g)	2.2bc	4.4a
WRP + Thiabendazole (0.15 g)	1.8bc	4.2a
WRP + Thiabendazole (0.30 g)	2.0bc	4.1a
WRP + Propiconazole (0.15 g)	1.8bc	4.4a
WRP + Propiconazole (0.30 g)	1.7bc	4.7a
WRP + Myclobutanil (0.15 g)	1.5c	4.0a
WRP + Myclobutanil (0.30 g)	2.1bc	4.5a
WRP + Epoxiconazole (0.15 g)	2.5bc	4.9a
WRP + Epoxiconazole (0.30 g)	1.6bc	4.3a
WRP + Potassium Phosphite (0.15 g)	2.4bc	4.7a
WRP + Potassium Phosphite (0.30 g)	2.7b	4.1a
Penconazole (spray)	0.0d	4.2a

Note: All values mean of ten trees. Also, lowercase letters indicate significant differences between means for each evaluation date ( $P = 0.05$ ).

**Table 3. The influence of water-retaining polymer (WRP) and fungicide combinations applied at the time of planting for the control of *Guignardia* leaf blotch on leaves of horsechestnut (*Aesculus hippocastanum* L.) as measured by leaf chlorophyll fluorescence Fv/Fm values.**

Treatment	Fv/Fm	
	2007	2008
Water (control)	0.557a	0.506a
WRP	0.569ab	0.511a
WRP + Penconazole (0.15 g)	0.669ac	0.497a
WRP + Penconazole (0.30 g)	0.721c	0.488a
WRP + Thiabendazole (0.15 g)	0.661abc	0.522a
WRP + Thiabendazole (0.30 g)	0.659abc	0.481a
WRP + Propiconazole (0.15 g)	0.709bc	0.505a
WRP + Propiconazole (0.30 g)	0.700abc	0.526a
WRP + Myclobutanil (0.15 g)	0.684abc	0.501a
WRP + Myclobutanil (0.30 g)	0.692abc	0.482a
WRP + Epoxiconazole (0.15 g)	0.727c	0.490a
WRP + Epoxiconazole (0.30 g)	0.714bc	0.513a
WRP + Potassium Phosphite (0.15 g)	0.667abc	0.518a
WRP + Potassium Phosphite (0.30 g)	0.688abc	0.511a
Penconazole (spray)	0.807c	0.516a

Note: All values mean of ten trees. Also, lowercase letters indicate significant differences between means for each evaluation date ( $P = 0.05$ ).

polymer combinations (Table 3; Table 4; Table 5; Table 6). No efficacy of penconazole foliar sprays as leaf blotch protectant compounds was, however, demonstrated the following year after application. In all cases, observed leaf blotch severity, leaf chlorophyll fluorescence Fv/Fm values, and leaf chlorophyll content were statistically comparable to water-treated controls.

## DISCUSSION

Results of this study show that use of a fungicide and water-retaining polymer combination applied to bare-rooted stock of horsechestnut (*Aesculus hippocastanum* L.) at the time of planting can result in significant reductions in outbreaks of *Guignardia aesculi*, the causal organism of *Guignardia* leaf blotch, over a single growing season. However, no significant degree of

**Table 4. The influence of water-retaining polymer (WRP) and fungicide combinations applied at the time of planting for the control of *Guignardia* leaf blotch on leaves of horsechestnut (*Aesculus hippocastanum* L.) as measured by leaf chlorophyll fluorescence Fv/Fm values.**

Treatment	Fv/Fm	
	2008	2009
Water (control)	0.515a	0.548a
WRP	0.536a	0.555a
WRP + Penconazole (0.15 g)	0.699b	0.529a
WRP + Penconazole (0.30 g)	0.714bc	0.567a
WRP + Thiabendazole (0.15 g)	0.683b	0.580a
WRP + Thiabendazole (0.30 g)	0.724bc	0.536a
WRP + Propiconazole (0.15 g)	0.739bc	0.545a
WRP + Propiconazole (0.30 g)	0.689b	0.566a
WRP + Myclobutanil (0.15 g)	0.717bc	0.584a
WRP + Myclobutanil (0.30 g)	0.722bc	0.532a
WRP + Epoxiconazole (0.15 g)	0.701b	0.498a
WRP + Epoxiconazole (0.30 g)	0.681b	0.504a
WRP + Potassium Phosphite (0.15 g)	0.688b	0.522a
WRP + Potassium Phosphite (0.30 g)	0.705b	0.519a
Penconazole (spray)	0.813c	0.550a

Note: All values mean of ten trees. Also, lowercase letters indicate significant differences between means for each evaluation date ( $P = 0.05$ ).

**Table 5. The influence of water-retaining polymer (WRP) and fungicide combinations applied at the time of planting for the control of *Guignardia* leaf blotch on leaves of horsechestnut (*Aesculus hippocastanum* L.) as measured by leaf chlorophyll content (SPAD) values.**

Treatment	SPAD	
	2007	2008
Water (control)	21.0a	22.4abc
WRP	18.7a	24.5abc
WRP + Penconazole (0.15 g)	35.8cd	25.0bc
WRP + Penconazole (0.30 g)	36.0cd	19.8ab
WRP + Thiabendazole (0.15 g)	33.1bc	19.6ab
WRP + Thiabendazole (0.30 g)	34.0bc	23.5abc
WRP + Propiconazole (0.15 g)	40.2de	24.2abc
WRP + Propiconazole (0.30 g)	38.8d	21.5abc
WRP + Myclobutanil (0.15 g)	33.1bc	20.4ab
WRP + Myclobutanil (0.30 g)	31.9bc	19.7a
WRP + Epoxiconazole (0.15 g)	32.8bc	20.5ab
WRP + Epoxiconazole (0.30 g)	35.0c	22.9abc
WRP + Potassium Phosphite (0.15 g)	29.9b	23.1abc
WRP + Potassium Phosphite (0.30 g)	29.4b	26.3c
Penconazole (spray)	43.8e	20.5a

Note: All values mean of ten trees. Also, lowercase letters indicate significant differences between means for each evaluation date ( $P = 0.05$ ).

**Table 6. The influence of water-retaining polymer (WRP) and fungicide combinations applied at the time of planting for the control of *Guignardia* leaf blotch on leaves of horsechestnut (*Aesculus hippocastanum* L.) as measured by leaf chlorophyll content (SPAD) values.**

Treatment	SPAD	
	2008	2009
Water (control)	25.9a	26.8b
WRP	26.0a	23.9ab
WRP + Penconazole (0.15 g)	39.9def	25.0ab
WRP + Penconazole (0.30 g)	37.5bcde	24.5ab
WRP + Thiabendazole (0.15 g)	41.0ef	20.9a
WRP + Thiabendazole (0.30 g)	40.0def	23.7ab
WRP + Propiconazole (0.15 g)	40.8ef	26.1b
WRP + Propiconazole (0.30 g)	34.1bc	23.5a
WRP + Myclobutanil (0.15 g)	35.2bcd	24.4ab
WRP + Myclobutanil (0.30 g)	37.1bcde	20.6a
WRP + Epoxiconazole (0.15 g)	33.9bc	24.7ab
WRP + Epoxiconazole (0.30 g)	38.2cde	25.2ab
WRP + Potassium Phosphite (0.15 g)	42.4b	28.9b
WRP + Potassium Phosphite (0.30 g)	33.6bc	27.7b
Penconazole (spray)	44.2f	26.0a

Note: All values mean of ten trees. Also, lowercase letters indicate significant differences between means for each evaluation date ( $P = 0.05$ ).

control was manifest the following year after planting, indicating that foliar sprays of an appropriate fungicide would be required to keep *Guignardia* leaf blotch severity to acceptable levels.

Little differences in the magnitude of efficacy between fungicides and concentration applied were recorded. This indicates that where reductions in leaf blotch severity are required for the first year after planting, several commercially available fungicides exist for this purpose. The range of fungicides evaluated for test purposes in this study are extensively used to control key fungal pathogens of woody plants, agricultural and horticultural crops, and orchard and forest trees. Results presented here, however, are the first to show efficacy of these fungicides against *Guignardia* leaf blotch. Although this study showed no significant influence of the type of fungicide used, previous evaluation of fungicides against *Guignardia* leaf blotch under *in vitro* and *in situ* conditions demonstrated marked sensitivity to individual products and concentration (Plenk 1996; Zimmermannová-Pastirčáková 2003). Under *in vitro* conditions fungicide efficacy was in the order mancozeb > fenarimol > benomyl > dodine > iprodione. Under *in situ* conditions (i.e., foliar sprays of each fungicide), efficacy was in the order fenarimol > benomyl > mancozeb > dodine > iprodione. Previous research has demonstrated that the efficacy of injected pesticides is related to uptake and translocation from injection site to target, which in turn is dependent on pesticide solubility, health of transport tissues within the vascular system, and tree species (Tattar et al. 1998; Young, 2002; Doccola et al. 2003). Although the mode of application differed in this study (root polymer dip versus injection), results demonstrate that irrespective of the fungicide used, sufficient quantities are translocated to foliar tissue to confer *Guignardia* leaf blotch protectant properties throughout the canopy over an entire growing season.

The impact of plant pathogen infection on leaf photosynthetic structure and processes can be monitored through changes in chlorophyll fluorescence kinetics to provide an insight into plant responses to pathogen invasion (Berger et al. 2007a; Berger et al. 2007b; Chaerle et al. 2007); in some circumstances, changes in fluorescence can be detected before symptoms of pathogen infection become visibly apparent (Bonfig et al. 2006; Berger et

al. 2007a). Chlorophyll fluorescence Fv/Fm ratios are regarded as a highly sensitive measure of damage to photosystem II and therefore, indirectly the leaf photosynthetic apparatus. Consequently, Fv/Fm ratios have been used to quantify damage and/or impairment of the leaf photosynthetic apparatus following fungal invasion and colonization of the leaf surface (Percival and Fraser 2002). *Guignardia* leaf blotch is regarded as a foliar blight with enzymatic degradation damage of the leaf, chlorophyll molecule, and photosynthetic system as a consequence of infection (Pastirčáková 2003). Fv/Fm ratios  $\geq 0.75$  are associated with healthy plants. Only trees sprayed with penconazole four times throughout the growing season had values higher than 0.75 at the cessation of each experiment. Affected leaves of controls with heavy infection and fungicide/water-retaining polymer treated trees displaying milder infection symptoms were characterized by decreased Fv/Fm, below the 0.75 threshold. The brown or reddish-brown lesions that cover a large portion or even the entire leaf indicate *Guignardia* leaf blotch exerts its influence over the host via vascular connections (Pastirčáková 2003). It is known that *Guignardia* leaf blotch produces hydrolytic enzymes that degrade the epidermis, cell wall and necrosis symptoms develop as intercellular mycelium spreads rapidly within the leaf (Schlösser 1983; Pastirčáková 2004). The consequence of such interaction distresses the photosynthetic mechanism directly or indirectly and impairs its ability to quench excitation energy. In normal situations, light capture is accompanied by photochemical and non-photochemical quenching mechanisms that balance photon utilization for electron transport purposes and repair of oxidative damage or heat dissipation (Anderson et al. 1997; Cruz et al. 2004). When absorbed light energy exceeds the leaf's capacity to use trapped energy through photosynthesis or dissipate it by heat, damage to PSII occurs. Results of this study indicate that symptom development in *Guignardia* leaf blotch infected foliage is associated with increased excitation pressure at PSII centers, followed by oxidative damage and irreversible destruction of centers (Baker et al. 2007; Horton and Ruban 2005; Muller-Moule et al. 2004). Such action leads to loss of chlorophyll and chlorosis (visible *Guignardia* blotch infection severity) as observed in this study.

Penconazole, when applied four times during the growing season, proved 100% effective for *Guignardia* leaf blotch control. The effectiveness of penconazole against several other fungal pathogens under laboratory and field conditions has been confirmed by other authors (Kenyon et al. 1997; Mmbaga and Sauve 2004; Percival and Boyle 2005; Schnabel and Parisi 1997). Results of this study support these conclusions with repeat penconazole sprays proving to be the optimal treatment in terms of reduced *Guignardia* leaf blotch severity, improved photosynthetic efficiency (Fv/Fm), and higher leaf chlorophyll content (SPAD).

Results regarding the use of a water-retaining polymer alone on reducing *Guignardia* leaf blotch development conclude no significant benefit as observed *Guignardia* leaf blotch severity, leaf chlorophyll fluorescence Fv/Fm values, and leaf chlorophyll content SPAD values were in both the 2007 and 2008 trials comparable to water treated controls. Consequently, use of water-retaining polymer alone as *Guignardia* leaf blotch protectant compound appears limited based on results of this study and are not recommended.

In conclusion, results provide evidence that use of commercially available fungicides products in combination with a water-retaining polymer applied at the time of planting as a root dip to bare rooted stock can be used to reduce *Guignardia* leaf blotch

severity over a single growing season. However, foliar sprays of an appropriate fungicide would be required the year after planting to keep *Guignardia* leaf blotch severity to acceptable levels. Further research is ongoing, evaluating fungicide and water-retaining polymer root dips against several other key fungal pathogens of trees.

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**Résumé.** Une étude a été faite à propos de l'influence de six fongicides commercialement utilisés et incorporés dans un mélange d'eau et de cristaux de gel-polymère appliqués par trempage lors de la plantation de marronniers d'Inde (*Aesculus hippocastanum* L.). L'accroissement potentiel en résistance face à la rouille des feuilles du marronnier (*Guignardia aesculi*) a par la suite été documenté au cours de deux saisons de croissance. Les essais ont été menés en 2007 et par la suite répétés en 2008. Une évaluation comparative du penconazole, un fongicide commercialement employé pour le contrôle de la rouille des feuilles du marronnier, a été faite en vaporisant des arbres selon les recommandations du manufacturier à quatre reprises durant la première saison seulement (aucune vaporisation durant la seconde année). Aucun des arbres traités ou ceux du groupe témoin n'est mort des suites de la rouille des feuilles du marronnier durant la période d'étude et aucun des arbres traités avec la combinaison fongicide et polymère n'a eu à subir de phytotoxicité par cette méthode. L'efficacité de la protection obtenue par la combinaison fongicide et polymère pour la première saison de croissance a été démontrée. La diminution des dommages par la rouille des feuilles du marronnier se voyait également via une augmentation de la fluorescence de la chlorophylle en tant que mesure de l'activité photosynthétique foliaire et des valeurs de SPAD de contenu en chlorophylle foliaire. Il y avait peu de différence dans la magnitude de l'efficacité du contrôle entre les fongicides évalués. L'efficacité limitée de n'importe laquelle des combinaisons fongicide et polymère en tant que mélange protecteur contre la rouille des feuilles du marronnier a été néanmoins démontrée l'année suivant l'application, ce qui indiquait que le traitement par trempage avec une combinaison fongicide et polymère ne fournissait une protection que durant une seule saison de croissance. L'utilisation de polymère seulement (sans fongicide) n'a eu aucun effet sur la diminution de la sévérité par la rouille des feuilles du marronnier. En se basant sur des degrés visuels de sévérité de dommages par la rouille des feuilles du marronnier, la meilleure protection en 2007 et 2008 a été obtenue avec le fongicide synthétique penconazole appliqué par vaporisation foliaire à quatre reprises durant la saison de croissance. Aucune efficacité des vaporisations foliaires de penconazole en tant que protecteur contre la rouille des feuilles du marronnier n'a été démontrée l'année suivante, ce qui indiquait qu'une vaporisation annuelle était requise pour assurer une protection.

**Zusammenfassung.** Der Einfluss von sechs kommerziell erhältlichen Fungiziden, die in ein Wasser enthaltendes Polymer eingearbeitet waren und auf das Wurzelsystem vom Roßkastanie (*Aesculus hippocastanum* L.) als ein Dip während der Pflanzung appliziert wurden, wurde durchgeführt. Mögliche Zunahmen an Resistenz gegenüber dem blattschädigenden Erreger *Guignardia* Blattfleckenkrankheit (*Guignardia aesculi*) wurde dann für die kommenden zwei Perioden überwacht. Die Versuche wurden 2007 durchgeführt und 2008 wiederholt. Eine vergleichende Bewertung des Fungizids Penconazol, welches kommerziell bei *Guignardia* Blattfleckenkrankheit verwendet wird, wurde während des Besprühens von Bäumen entsprechend der Empfehlungen des Herstellers von viermaliger Anwendung während der ersten Saison studiert, aber nicht in der zweiten Saison. Keiner der behandelten Bäume oder aus der Kontrollgruppe starb während der Dauer der Behandlung aufgrund

der Blattfleckenkrankheit und keines der verwendeten und bewerteten Fungizide und der wasserenthaltenden Polymer-Kombinationen zeigte phytotoxische Reaktionen bei den Testbäumen. Die Reduzierung des Befalls spiegelte sich in dem Anstieg der Blattchlorophyll-Fluoreszenz als ein Maß für Blattphotosyntheseleistung und Blattchlorophyllgehalt. Es gab wenig Differenzen bei dem Ausmaß der Wirksamkeit zwischen den bewerteten Fungiziden. Eine begrenzte Wirksamkeit irgendeines der Fungizide oder der wasserenthaltenden Polymer-Kombinationen zeigte sich in dem darauf folgenden Jahr darin, dass der Schutz eines Fungizids oder wasserenthaltenden Polymer-Wurzel Dips nur für eine Saison anhielt. Die Applikation eines wasserenthaltenden Polymers allein hatte keinen Effekt in der Reduktion des Befalls mit der *Guignardia* Blattfleckenkrankheit. Basierend auf visueller Kontrolle des Befalls wurde der größte Schutz in beiden aufeinander folgenden Versuchen in 2007 und 2008 mit dem synthetischen Fungizid Penconazol erreicht, welches als Blattspray viermal pro Jahr in der Saison appliziert wurde. Es gab keine Wirksamkeit des Penconazol-Blattsprays im Folgejahr, was bedeutet, dass jährliche Anwendungen gegen diesen Erreger zur wirksamen Kontrolle erforderlich sind.

**Resumen.** Se estudió la influencia de seis fungicidas comercialmente disponibles incorporados en un polímero de retención de agua, aplicados al sistema de raíces de castaño de Indias (*Aesculus hippocastanum* L.) con un baño en el momento de la plantación. Fueron monitoreados los aumentos potenciales en la resistencia contra el patógeno *Guignardia* del tizón foliar (*Guignardia aesculi*) después de dos temporadas de crecimiento. Los ensayos se llevaron a cabo en 2007 y se duplicaron en 2008. Una evaluación comparativa del fungicida penconazol utilizado comercialmente para el control de la mancha foliar *Guignardia* se estudió rociando los árboles a la tasa recomendada por el fabricante cuatro veces durante la primera temporada de crecimiento, pero no en la segunda. Ninguno de los árboles tratados o de control murió como resultado del ataque de *Guignardia* durante el estudio y ninguna de las combinaciones de fungicida y polímero evaluados fue fitotóxica para los árboles de prueba. La eficacia de los compuestos como protección contra *Guignardia* durante el primer ciclo de cultivo se demostró cuando los fungicidas se incorporaron a un polímero de retención de agua. Las reducciones en la severidad del ataque de la mancha foliar *Guignardia* se reflejó en aumentos en fluorescencia de la clorofila como una medida de la actividad fotosintética de las hojas y los valores del contenido de clorofila SPAD en la hoja. Hubo pequeñas diferencias en la magnitud de la eficacia de control entre los fungicidas evaluados. La limitada eficacia de cualquiera de las combinaciones de fungicida y polímeros de retención de agua fue sin embargo demostrada el año siguiente después de la aplicación, lo que indica protección solamente en una temporada. La aplicación del polímero de retención de agua por sí sola no tuvo efecto en la reducción de la severidad de la mancha foliar *Guignardia*. Con bases visuales las tasas de reducción de la mancha foliar *Guignardia* dieron mayor protección, en los ensayos de 2007 y 2008 con el fungicida penconazol aplicado como spray foliar cuatro veces durante la temporada de crecimiento. No se demostró la eficacia de las pulverizaciones foliares de Penconazol como protector del tizón foliar el año siguiente, indicando que las aspersiones anuales contra el tizón foliar *Guignardia* son necesarios para su control.