Effects of Copper-Based Fungicides on Foliar Gas Exchange in Tart Cherry

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ABSTRACT

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In the Great Lakes region of the United States, cherry growers are poised to re-adopt copper-based fungicides to manage cherry leaf spot disease (CLS), caused by *Blumeriella jaapii*. However, application of copper is often associated with leaf bronzing. In growth chamber experiments, bronzing was observed on foliage of tart cherry (*Prunus cerasus* 'Montmorency') seedlings 1 week following application of a copper-based fungicide, only when leaves were also exposed to nightly dew. In potted, 1-year-old trees outdoors, light-saturated rates of net CO₂ assimilation (*A*) and stomatal conductance (*g_s*) were not affected by treatment with copper sulfate, chlorothalonil, tebuconazole, or trifloxystrobin compared to a nonsprayed control. In 2005 and 2006, *A* and *g_s* were measured during late summer on leaves of mature trees in an orchard subjected to the following fungicide programs: synthetic fungicides only; synthetic fungicides integrated with copper-based fungicides; or not sprayed. Bronzing symptoms were observed on trees sprayed with copper. Regression analysis revealed that neither *A* nor *g_s* decreased as leaf surface area affected by bronzing increased ($R^2 = 0.004$, $P = 0.80$ and $R^2 = 0.006$, $P = 0.74$, respectively). Leaf bronzing associated with application of copper-based fungicides may therefore be inconsequential to foliar gas exchange in tart cherry during late summer.

Cherry leaf spot (CLS), caused by the fungal pathogen *Blumeriella jaapii* (Rehm) Arx, is an important disease of tart cherry (*Prunus cerasus* L.) in the Great Lakes region of the United States (12) and in moist, temperate climates of southern and eastern Europe (1,19). Symptoms and signs of CLS on leaves include necrotic lesions, chlorosis, and white- or cream-colored acervuli on the abaxial leaf surface (12,28). Left unmanaged, CLS can impair photosynthesis (24) and can cause premature defoliation that leads to a reduction in fruit quality and yield, and predisposes fruit buds and the vascular cambium to low-temperature injury or mortality during winter (10).

In the United States, CLS was managed historically by foliar applications of copper-based fungicides (14). By 1950, synthetic fungicides had replaced copper in CLS management programs because the former were easier to apply and did not result in leaf bronzing that occurred with repeated copper applications. By the late 1970s, however, strains of *B. jaapii* resistant to one class of synthetic CLS fungi-

cides, the benzimidazoles, were discovered (13). Resistance to the sterol demethylation inhibitors (DMI) also has been reported (20). The failure of synthetic fungicides has revived interest in copper-based fungicides (21), due to their nonspecific mode of action (29), which has not been compromised by resistant fungal pathogens despite use on diverse crops for more than a century. McManus et al. (21) reported that integrating copper-based fungicides after bloom into cherry disease management programs was effective in controlling *B. jaapii* presumed to be DMI-resistant. These practices sometimes resulted in bronze-colored lesions on leaf veins and diffuse bronzing between veins, particularly on the abaxial leaf surface (21). These observations are not unique to *B. jaapii* management programs. Copper-based fungicides used during bloom to manage *Monilinia laxa* (Aderhold & Ruhland) Honey in tart cherry resulted in significantly more leaf injury compared to synthetic fungicides (9). Similarly, Lalanette and McFarland (15) observed leaf discoloration in peach and nectarine when using copper-based bactericides to manage *Xanthomonas arboricola* pv. *pruni*.

The effects of fungicides on plant physiology, in general, have been reviewed (8,22). More specifically, the DMI fungicide epoxiconazole was found to reduce photosynthetic oxygen evolution in *Galium aparine* L. (3). Likewise, the strobilurin class of fungicides reduced photo-

synthesis in greenhouse-grown wheat, barley, and soybean (23). Palmer et al. (25) observed that application of a copper hydroxide and sulfur mixture reduced net CO₂ assimilation in apple leaves, relative to a program that relied exclusively on synthetic fungicides. However, those workers did not observe any significant reductions in leaf photosynthesis when copper hydroxide was applied alone (25). It is not clear if the fungicides currently used to manage CLS, including copper, are detrimental to tart cherry leaf physiology. Thus, while resuming the use of copper-based fungicides may be an asset to programs aimed at managing CLS, especially where DMI-resistant strains of *B. jaapii* are present, research is needed to determine if treatment with copper is detrimental to foliar gas exchange in tart cherry. The aims of this study were the following: (i) to determine if synthetic and copper-based fungicides affect leaf gas exchange in tart cherry; (ii) to explore the effect of nightly dew (leaf wetness) exposure on the development of copper-associated bronzing; and (iii) to investigate relationships between the extent of leaf surface area with copper bronzing and rates of net CO₂ assimilation (*A*) and stomatal conductance (*g_s*).

MATERIALS AND METHODS

Effect of fungicides on photosynthesis.

The effects of various fungicides on *A* and *g_s* in the absence of CLS were assessed using potted trees in a cold frame on the University of Wisconsin campus (UW) in Madison (south-central WI, USA, 43°N, 89°W) in 2005 and 2008. The site was isolated from known sources of *B. jaapii* (e.g., ornamental or wild cherry trees). One-year-old (1.6 cm caliper diameter) tart cherry trees (*Prunus cerasus* L. 'Montmorency' on *Prunus mahaleb* L. rootstock) were planted in 8-liter plastic pots with three parts potting mix (Metro-Mix 300 series, Sun Gro Hort., Bellevue, WA) and one part field soil. 'Montmorency' was used because it is the most widely grown cultivar of tart cherry in North America. Trees were planted in May (2005 and 2008), and all trees received one slow release fertilizer tablet (15:8:11, 7.5 g, Osmocote-Plus, Scotts-Sierra Co., Marysville, OH) embedded in the soil mix. Trees were watered as needed during both years.

Groups of seven (2005) and five (2008) potted trees were sprayed with one of four

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fungicides or distilled water on 27 July, 3 August, 10 August, and 19 August 2005, and on 24 July, 31 July, 8 August, and 16 August 2008. Except as indicated below, fungicides were diluted or dissolved in 1 liter of distilled water and then applied to the point of runoff using a hand-held spray bottle. In 2005, fungicide, amount of active ingredient per 1 liter of distilled water, brand name, and manufacturer were the following: chlorothalonil (1.24 g, Bravo WeatherStik, Syngenta Crop Protection, Inc., Greensboro, NC); copper sulfate (0.60 g metallic copper, Cuprofix Disperss 20DF, Cerexagri, Inc., King of Prussia, PA); tebuconazole (0.07 g, Elite 45DF, Bayer CropScience, Research Triangle Park, NC); trifloxystrobin (0.04 g, Flint 50WDG, Syngenta). Control trees were treated with distilled water. In 2005, all fungicides were applied at rates within the ranges recommended for application to cherry in the field. In 2008, fungicide application rates were the same except for chlorothalonil, which was applied at a 10× concentrated rate of 12.4 g of chlorothalonil per liter of distilled water, and was sprayed on 24 July only. Trees were arranged in a completely randomized design, but those treated similarly were temporarily grouped together for application of fungicide or water. Application was performed under calm conditions to minimize drift. After drying, trees were returned to their original location.

Instantaneous rates of photosynthetic gas exchange were measured using a portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE). Rates of net CO₂ assimilation (*A*, μmol CO₂ m⁻² s⁻¹) and stomatal conductance (*g_s*, mol H₂O m⁻² s⁻¹) were simultaneously measured on cloudless days between 0900 h and 1300 h. On 27 August 2005 and 22 August 2008, one fully expanded leaf from each potted tree was placed in the cuvette and measured in situ. Conditions in the cuvette sample chamber (with an area of 6 cm²) were: [CO₂] = 380 μmol mol air⁻¹; photosynthetic photon flux density (PPFD, provided by a red-blue LED array) = 1,800 μmol m⁻² s⁻¹. Leaf temperature within the cuvette was not regulated and ranged from 22 to 36°C. Treatments were blocked by the time of day to account for changes in ambient air temperature when gas exchange was measured.

Effect of dew on copper-induced lesions. Two trials were conducted in a growth chamber. Tart cherry ‘Montmorency’ seedlings were grown from seed in three parts sterilized field soil and one part potting mix (Metro-Mix 300 series). The seedlings were watered and fertilized (15:30:15) as needed and grown in a growth chamber at 25°C with a 14 h light:10 h dark photoperiod provided by fluorescent lamps (PPFD = 700 μmol m⁻² s⁻¹). Relative humidity ranged from 30 to 50% with transient increases to 80 to 90%

shortly after seedlings were watered. After 30 days of growth, and with leaves fully expanded, seedlings (groups of five for the first trial and groups of four for the second trial) were randomly assigned to one of the following copper-based fungicide programs: copper application once weekly for 4 weeks, copper application once, or no copper application; and each of these was present in each of two dew exposure regimens: nightly for 4 weeks, or no exposure to dew. Fungicide applications for all treatments occurred on 28 February, 7 March, 14 March, and 21 March 2005 for the first trial, and on 13 May, 20 May, 27 May, and 3 June 2005 for the second trial. Seedlings assigned to treatments that incorporated dew exposure were exposed nightly from 28 February to 27 March and from 13 May to 9 June, for the first and second trials, respectively. A copper sulfate-based fungicide (Cuprofix Disperss, 20DF, Cerexagri) was mixed in distilled water at a rate of 0.6 g of metallic copper per liter of distilled water, a rate similar to the rate recommended for application to cherry in the field. The fungicide was applied to the seedlings using a hand-held spray bottle to the point of runoff and allowed to air dry in a ventilated hood. Seedlings that were also assigned to the dew-exposure regime were placed for 12 h overnight in a dew chamber that was set to 24, 10, and 39°C for the air, wall, and water temperatures, respectively. Dew formation on leaves was verified visually. Seedlings were arranged in a completely randomized two-factor design, but those treated similarly were temporarily grouped together for fungicide application. After drying, from either fungicide application or dew-exposure, seedlings were returned to their original locations in the growth chamber.

Severity of foliar copper bronzing was assessed 1 week following the final copper-based fungicide application (28 March 2005 for the first trial and 10 June 2005 for the second trial). For the first trial, one fully expanded leaf was arbitrarily selected from each seedling and was visually scored for the percentage of leaf area exhibiting veinal and interveinal bronzing on the abaxial surface. The scoring system used was: 1 = no symptoms, 2 = 1 to 10%, 3 = 11 to 20%, 4 = 21 to 50%, and 5 = more than 50% of the leaf area affected. For the second trial, three fully expanded leaves were arbitrarily selected from each seedling and harvested for digital leaf image analysis (described below). The seedling averages of digital leaf images were then used in data analysis.

Effects of fungicide programs and copper-associated bronzing on photosynthesis in an orchard. Experiments were conducted in a mature orchard at the University of Wisconsin Peninsular Agricultural Research Station (UWPARS) in Sturgeon Bay (northeastern WI, USA, 44°N, 87°W) during 2005 and 2006. The orchard block of 65 tart cherry trees (‘Montmorency’ on *P. mahaleb* rootstock) was established in 1995 with tree spacing 7.7 m within row and 7.7 m between rows. Trees were irrigated through a drip irrigation system as needed during both years.

UWPARS orchard spray programs are summarized in Table 1. The orchard block at UWPARS was subdivided into three sections, and each section was subjected to one of three following disease management programs: (i) standard program, which relied exclusively on synthetic fungicides; (ii) integrated copper program, which integrated the use of synthetic fungicides with copper hydroxide fungicide;

Table 1. Fungicide programs applied to ‘Montmorency’ tart cherry trees at Sturgeon Bay, WI^x

Application date ^y	Conventional synthetic program		Integrated copper program	
	Active ingred. ^z	Rate (g ha ⁻¹)	Active ingred. ^z	Rate (g ha ⁻¹)
2005				
25 May	Chlorothalonil	2,780	Chlorothalonil	2,780
3 June	Trifloxystrobin	140	Tebuconazole	189
18 June	Tebuconazole	189	Copper hydroxide	1,965
28 June	Trifloxystrobin	140	Copper hydroxide	1,965
13 July	Tebuconazole	189	Tebuconazole	189
24 August	Chlorothalonil	2,780	Chlorothalonil	2,780
2006				
25 May	Chlorothalonil	2,780	Chlorothalonil	2,780
8 June	Trifloxystrobin	140	Copper hydroxide	1,965
19 June	Tebuconazole	189	Copper hydroxide	1,965
28 June	Trifloxystrobin	140	Copper hydroxide	1,965
10 July	Tebuconazole	189	Tebuconazole	189
18 July	Tebuconazole	189	Tebuconazole	189
18 August	Chlorothalonil	2,780	Chlorothalonil	2,780

^x In addition to synthetic and integrated copper fungicide programs, a nonsprayed control was included.

^y Only one active ingredient per fungicide program was applied on each date.

^z Active ingredient. Fungicide common names, brand names, formulations used, and manufacturer: chlorothalonil (Bravo WeatherStik, Syngenta Crop Protection); copper hydroxide (Kocide 2000, Griffin L.L.C., in 2005 and Champion, Nufarm Americas, in 2006); tebuconazole (Elite 45DF, Bayer CropScience LP); and trifloxystrobin (Flint 50WDG, Syngenta Crop Protection). Amounts of copper refer to metallic copper equivalent applied.

and (iii) a nontreated control. Treatments were randomly assigned to each of the three sections in 2005 and then rerandomized in 2006. Fungicides were applied in a volume of 560 liters per hectare with an airblast sprayer, which is a typical practice in commercial tart cherry production. The first fungicide application for each program was made during petal fall (25 May in 2005 and 2006), with subsequent applications made 7 to 15 days thereafter. The spray programs were similar in 2005 and 2006, with the following exceptions: application dates differed; the integrated copper program included two sprays of copper hydroxide in 2005 but three sprays in 2006; and the brand of copper hydroxide differed in the 2 years, although the actual amount of copper hydroxide and metallic copper were the same.

Conditions and instrument settings under which A and g_s were measured in the orchard were the same as those used in the cold frame, except that the temperature within the cuvette was regulated and ranged from 25 to 28°C. Five trees from each of the three treatment groups were arbitrarily selected for gas exchange meas-

urements on 17 August 2005. From each tree, two shoots bearing “sun-exposed” leaves (i.e., leaves from the periphery of the canopy that were not excessively shaded by other leaves) that were fully expanded were arbitrarily selected. One leaf per shoot was measured, tagged, then brought back to the laboratory for leaf image analysis (described below). The orchard experiment was repeated on 17 August 2006.

The percentage of leaf area exhibiting copper-associated bronzing in growth chamber and orchard experiments was quantified as described previously (7) and as follows. Copper-associated bronzing was present only on the abaxial surfaces of leaves that had been treated with copper-based fungicides. The abaxial surfaces of copper-sprayed leaves were scanned using a flatbed scanner (Epson Perfection 2480 PHOTO, Epson America, Inc., Long Beach, CA) at 400 dpi resolution. The images were then individually analyzed for percentage of area affected by bronzing using ASSESS image analysis software (American Phytopathological Society, St. Paul, MN). Leaf damage was defined as

bronzing of the leaf veins and tissue between veins.

Statistical analyses. Residuals from each analysis were tested for normality using the Anderson-Darling test and, unless stated otherwise, did not deviate from the properties of a normal distribution ($P \geq 0.23$), or violate homoscedasticity ($P \geq 0.51$). Values of Cook’s D -statistic were evaluated according to the methods of Cohen et al. (6) and Jobson (11) and were small enough to warrant not deleting any points as outliers. All analyses were completed using SAS (v. 9.1.3, SAS Institute Inc., Cary, NC).

Means of A and g_s from the cold frame experiments were analyzed using a completely randomized design (CRD) one-way analysis of variance (ANOVA) with time of day as a blocking effect (seven blocks in 2005 and five blocks in 2008) and fungicide as the single fixed effect. Trees were the experimental units ($n = 7$ in 2005 and $n = 5$ in 2008). The two cold frame experiments were analyzed separately. ANOVA was performed with PROC GLM.

Data from the two growth chamber trials were analyzed separately using CRD two-

Fig. 1. Effect of fungicide application on net CO₂ assimilation and stomatal conductance from cold frame trials in 2005 and 2008. For each treatment, $n = 7$ in 2005 and $n = 5$ in 2008. Treatments: Con (control sprayed with distilled water), CuS (copper sulfate), Teb (tebuconazole), Tri (trifloxystrobin), Chl (chlorothalonil). Bars represent $\pm$ one standard error of the mean.

way ANOVA with number of copper-based fungicide applications (0, 1, or 4) and dew-exposure (dew or no dew) as fixed effects. Seedlings were the experimental unit ($n = 5$ and $n = 4$ in the first and second trials, respectively). The data from both trials violated the assumption of normality ($P = 0.02$ for trial 1 and $P < 0.005$ for trial 2). Common variable transformations were not corrective, so these data were analyzed in a one-way nonparametric Kruskal-Wallis rank test using PROC NPAR1WAY. The levels of copper-based fungicide application and dew exposure were combined to form a single, combinatorial independent variable.

Means of A and g_s from the orchard experiments were analyzed using a CRD one-way ANOVA, with year as a blocking effect (two blocks, one each for 2005 and 2006) and fungicide program as a fixed effect. The group of trees allocated to each fungicide program was the experimental unit ($n = 1$ in 2005 and 2006 each). Blocking by year has been used previously by researchers utilizing large groups of trees (16). ANOVA was performed using PROC GLM. When appropriate, Tukey-adjusted multiple pairwise-comparisons were used to separate means.

Multiple linear regression (MLR) was used to analyze the relationship between percent leaf area exhibiting bronzing, year, and their interaction and A or g_s , separately. MLR models were derived using data from the integrated copper fungicide program of the orchard study with individual leaves as the experimental unit ($n = 10$ in 2005 and 2006 each), and backward elimination was used to simplify MLR models. The "year" variable was also added as a repeated effect to MLR models to quantify leaf-to-leaf correlation within the integrated copper fungicide program for each year. Assessing this correlation aided in establishing independence between individual leaves. In all cases, this repeated effect did not affect values of Akaike's Information Criterion (5) to an extent that justified specification of leaf-to-leaf correlation in any model, so it was removed from all models. All regression models were selected using PROC MIXED, and simplified models were rerun in PROC REG to obtain coefficient of determination values.

RESULTS AND DISCUSSION

Effect of fungicides on leaf gas exchange in potted tart cherry. Net CO_2 assimilation (A) and stomatal conductance (g_s) were not affected by fungicide treatments when compared with a control (Fig. 1). In general, treatments with a higher mean A also had higher mean g_s . Neither leaf bronzing nor CLS were observed on potted trees in either 2005 or 2008. Contrary to our findings, synthetic fungicides similar to those used for CLS management have been found to alter leaf gas exchange

in other crops. For instance, epoxiconazole, a DMI fungicide, reduced photosynthetic oxygen evolution in *Galium aparine* L. by almost 50% relative to controls (3). Similarly, A and g_s were reduced in wheat after being sprayed with picoxystrobin, a strobilurin fungicide (23). A rotation of dodine, mancozeb, and captan fungicides applied to mature apple trees, however, did not affect leaf values of A (25). These results illustrate that a consistent understanding of how fungicides affect leaf photosynthesis has not yet been achieved. The absence of detectable fungicide effects on A and g_s of potted tart cherry trees was particularly important in this system, because the nonsprayed trees represented a

true negative control group (i.e., trees that were neither treated with a fungicide nor infected with *B. jaapii*).

Effect of a copper-based fungicide and dew on leaf bronzing. Application of a copper-based fungicide and exposure to dew led to increased leaf bronzing severity (Fig. 2). Results for both trials were similar. In the first trial, mean bronzing severity was estimated using a visual scoring system and ranged from 1.0 (no symptoms) to 3.6 (11 to 20% of the leaf surface showing symptoms) and differed among treatments ($P = 0.0003$, Fig. 2A). In the second trial, mean bronzing severity was estimated using digital image analysis and ranged from none to 7.7% of the leaf sur-

Fig. 2. Effect of copper-based fungicide application and exposure to dew on the severity of leaf bronzing. Results presented are from the first (A) and second (B) trials. Data are original means and standard errors ($n = 5$ and $n = 4$ in A and B, respectively). P values correspond to the nonparametric Kruskal-Wallis rank test. Treatments: Cu4:D (copper sulfate weekly for 4 weeks, dew exposure nightly), Cu4 (copper sulfate weekly for 4 weeks, no dew exposure), Cu1:D (copper sulfate once, dew exposure nightly), Cu1 (copper sulfate once, no dew exposure), Cu0:D (no copper sulfate, dew exposure nightly), and Cu0 (no copper sulfate, no dew exposure). Bars represent $\pm$ one standard error of the mean.

face and differed among treatments ($P = 0.003$, Fig. 2B). In both trials, seedlings receiving at least one copper-based fungicide application and nightly dew exhibited the most severe bronzing (Fig. 2). Seedlings that received only copper-based fungicides, only dew, or neither treatment exhibited little or no bronzing (Fig. 2).

Earlier studies have documented that application of copper-based pesticides is associated with leaf injury in *Prunus* species (9,14,15,21). In tart cherry, injury to spur leaves was more severe in a wet year than a dry year (9). Results from the current investigation also indicate that leaf wetness, in this case dew, influences leaf bronzing. After application of a copper-based fungicide to leaf surfaces, small amounts of toxic copper ions are released from insoluble, or “fixed,” residues in the presence of water (29). The formation of

dew and perhaps other sources of moisture (e.g., rain) might affect the development of copper-associated leaf discoloration. A possible explanation for the lack of leaf bronzing on the potted trees may be that the cold frames were surrounded on three sides by greenhouses. This relatively protected environment may have retained heat and therefore reduced the duration of time that temperatures were below the dew point. A revision of copper-based fungicide application recommendations based upon environmental monitoring might be justified if field studies on the role of leaf wetness duration on copper-associated bronzing corroborated our studies done in a growth chamber.

Effect of fungicide program on leaf gas exchange in an orchard. Bronzing symptoms were visible in both years only on leaves treated with the integrated cop-

per program. Mean A for the synthetic treatment was higher than mean A for the nonsprayed treatment ($P = 0.05$, Fig. 3A), but did not differ from mean A for the integrated copper treatment (Fig. 3A). Mean A likely was reduced in nonsprayed trees due to leaf damage caused by CLS (data not shown). Means of g_s did not differ among treatments ($P = 0.10$, Fig. 3B). A true negative control group was impossible to establish at the UWPARS orchard where *B. jaapii* is endemic. Therefore, in addition to utilizing trees that were not sprayed to test the effects of copper-based fungicides on leaf photosynthesis in an orchard, this experiment also included a conventional program that included only synthetic fungicides to control CLS. A similar approach was taken by Palmer et al. (25), who used apple trees that were treated with synthetic fungicides as a practical control group when evaluating the effects of sulfur- and copper-based fungicides on apple leaf photosynthesis.

Leaves sampled from the integrated copper fungicide program in 2005 and 2006 exhibited percentages of surface bronzing that ranged from 5 to 45%, with most leaves showing less than 20% of leaf surface area affected (Fig. 4). Multiple linear regression (MLR) was used to analyze the relationship between A or g_s and percent leaf area visibly affected by copper-associated bronzing. For both of the MLR models, “year” and the “year $\times$ percent leaf bronzing” independent variables did not have effects ($P \geq 0.10$) and thus were removed during model selection. Therefore, the final models were simple linear regression models with percent leaf bronzing as the effect (Figs. 4A and 4B). Increases in copper-associated bronzing on the abaxial leaf surface did not have an effect on A ($P = 0.80$, Fig. 4A) or g_s ($P = 0.74$, Fig. 4B). It has been previously reported that copper accumulation may negatively influence photosynthesis, possibly by eroding the leaf cuticle (18) or by disrupting photosynthetic electron transport (4,22,26). However, the lack of correlation between leaf bronzing and photosynthetic gas exchange in this study corroborates the findings of Palmer et al. (25), who reported that applications of copper-hydroxide to apple leaves did not result in a significant decline in A .

To the best of the authors’ knowledge, these findings represent the first attempt to relate the severity of leaf injury from a fungicide to leaf photosynthetic processes. These findings are not necessarily definitive because alternative modeling approaches and other variables important to gas exchange exist. For example, Erickson et al. (7) and Roloff et al. (27) employed a nonlinear model first described by Bastiaans (2) to observe photosynthetic impairment of the apparently healthy leaf tissue surrounding visible lesions caused by fungi. This study did not consider the

Fig. 3. Effect of fungicide application on net CO₂ assimilation (A) and stomatal conductance (B) in a mature orchard. Data are from 2005 and 2006 combined in a randomized complete block design, with year as a blocking effect. For each fungicide program, $n = 2$. Means followed by different letters in A are significantly different, and were separated by Tukey-adjusted multiple pairwise-comparisons ($\alpha = 0.05$). Bars represent $\pm$ one standard error of the mean.

possibility that visibly healthy leaf tissue could be affected by copper-associated bronzing or that remaining healthy tissue might compensate for tissue lost to bronzing. The possibility of compensation is supported by experiments in which foliar rates of gas exchange increased in remaining tart cherry leaf tissue following mechanical removal of portions of leaves (17).

In summary, fungicides used to manage CLS did not significantly affect net CO₂ assimilation or stomatal conductance in tart cherry in the absence of disease and copper-bronzing symptoms. In a mature orchard, photosynthesis was not negatively affected in trees subjected to programs based on synthetic and copper-based fungicides, even though leaves in the latter program typically showed 10 to 20% of their area affected by copper bronzing.

These findings suggest that discoloration of tart cherry leaves associated with copper fungicide application may be inconsequential to the photosynthetic physiology of the leaf in late summer. This is significant considering the necessity of leaves in acclimation to cold winter temperatures and long-term survival of trees (10). However, the effects of copper-based fungicides on fruit quality and yield would require measuring photosynthesis earlier in the growing season and directly measuring fruit weight and sugar content.

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LITERATURE CITED

1. Annesi, T., Motta, E., and Forti, E. 1997. First report of *Blumeriella jaapii* teleomorph on wild cherry in Italy. *Plant Dis.* 81:1214.
2. Bastiaans, L. 1991. Ratio between virtual and visual lesion size as a measure to describe reduction in leaf photosynthesis of rice due to leaf blast. *Phytopathology* 81:611-615.
3. Benton, J. M., and Cobb, A. H. 1997. The modification of phytosterol profiles and *in vitro* photosynthetic electron transport of *Galium aparine* L. (cleavers) treated with the fungicide, epoxiconazole. *Plant Growth Regul.* 22:93-100.
4. Burda, K., Kruk, J., Schmid, G. H., and Strzalka, K. 2003. Inhibition of oxygen evolution in Photosystem II by Cu(II) ions is associated with oxidation of cytochrome b₅₅₉. *Biochem. J.* 371:597-601.
5. Burnham, K. P., and Anderson, D. 2002. AIC differences, Δ_i . Page 70 in: *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer-Verlag, New York.
6. Cohen, J., Cohen, P., West, S. G., and Aiken, L. S. 2003. Influence on the regression estimates. Page 404 in: *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences*. Lawrence Erlbaum Associates, Mahwah, NJ.
7. Erickson, J. E., Stanosz, G. R., and Kruger, E. L. 2003. Photosynthetic consequences of *Marssonina* leaf spot differ between two poplar hybrids. *New Phytol.* 161:577-583.
8. Garcia, P. C., Rivero, R. M., Ruiz, J. M., and Romero, L. 2003. The role of fungicides in the physiology of higher plants: Implications for defense responses. *Bot. Rev.* 69:162-172.
9. Holb, I. J., and Schnabel, G. 2005. Effect of fungicide treatments and sanitation practices on brown rot blossom blight incidence, phytotoxicity, and yield for organic sour cherry production. *Plant Dis.* 89:1164-1170.
10. Howell, G. S., and Stackhouse, S. S. 1973. The effect of defoliation time on acclimation and dehardening in tart cherry (*Prunus cerasus* L.). *J. Am. Soc. Hort. Sci.* 98:132-136.
11. Jobson, J. D. 1991. Influence, outliers and leverage. Page 159 in: *Applied Multivariate Data Analysis*. Springer-Verlag, New York.
12. Jones, A. L. 1995. Cherry leaf spot. Pages 21-22 in: *Compendium of Stone Fruit Diseases*. J. M. Ogawa, E. I. Zehr, G. W. Bird, D. F. Ritchie, K. Uriu, and J. K. Uyemoto, eds. American Phytopathological Society, St. Paul, MN.
13. Jones, A. L., and Ehret, G. R. 1980. Resistance of *Coccomyces hiemalis* to benzimidazole fungicides. *Plant Dis.* 64:767-769.
14. Keitt, G. W., Blodgett, E. G., Wilson, E. E., and Magie, R. O. 1937. The Epidemiology and Control of Cherry Leaf Spot. *Res. Bull.* 132. Wisconsin Agricultural Experiment Station, Madison.
15. Lalancette, N., and McFarland, K. A. 2007. Phytotoxicity of copper-based bactericides to peach and nectarine. *Plant Dis.* 91:1122-1130.
16. Lan, Z., Scherm, H., and Horton, D. L. 2003. Reduced midseason pesticide program for control of scab and plum curculio in peach. *Plant Dis.* 87:699-706.
17. Layne, D. R., and Flore, J. A. 1992. Photosynthetic compensation to partial leaf area reduction in sour cherry. *J. Am. Soc. Hort. Sci.* 117:279-286.
18. Lichton, J. E., and de Godoy, S. A. P. 2006.

Fig. 4. Effect of bronzing on net CO₂ assimilation (A) and stomatal conductance (B) of mature tart cherry leaves sampled from the integrated copper fungicide programs from 2005 and 2006. Each point represents one leaf ($n = 10$ for each year). Data were analyzed using linear regression. P values correspond to slopes only.

- Morphology and epicuticular wax content of coffee leaves after fungicide application. *Pes. Agro. Bras.* 41:919-926.
19. Linhard-Pedersen, H., and Hockenhull, J. 1996. Effects of urea on the formation of apothecia and winter conidia of *Blumeriella jaapii* (cherry leaf spot) and associated micro-fungi on overwintered cherry leaves. *Gartenbauwissenschaft* 61:257-261.
 20. Ma, Z., Proffer, T. J., Jacobs, J. L., and Sundin, G. W. 2006. Overexpression of the 14 α -demethylase target gene (*CYP51*) mediates fungicide resistance in *Blumeriella jaapii*. *Appl. Environ. Microbiol.* 72:2581-2585.
 21. McManus, P. S., Proffer, T. J., Berardi, R., Gruber, B. R., Nugent, J. E., Ehret, G. R., Ma, Z., and Sundin, G. W. 2007. Integration of copper-based and reduced-risk fungicides for control of *Blumeriella jaapii* on sour cherry. *Plant Dis.* 91:294-300.
 22. Murthy, C. S. H. N. 1983. Effects of pesticides on photosynthesis. *Residue Rev.* 86:107-129.
 23. Nason, M. A., Farrar, J., and Bartlett, D. 2007. Strobilurin fungicides induce changes in photosynthetic gas exchange that do not improve water use efficiency of plants grown under conditions of water stress. *Pest Manag. Sci.* 63:1191-1200.
 24. Neiderleitner, S., and Knoppik, D. 1997. Effects of the cherry leaf spot pathogen *Blumeriella jaapii* on gas exchange before and after expression of symptoms on cherry leaves. *Physiol. Mol. Plant Pathol.* 51:145-153.
 25. Palmer, J. W., Davies, S. B., Shaw, P. W., and Wunsche, J. N. 2003. Growth and fruit quality of 'Braeburn' apple (*Malus domestica*) trees as influenced by fungicide programmes suitable for organic production. *N.Z. J. Crop Hortic. Sci.* 31:169-177.
 26. Patsikka, E., Aro, E. A., and Tyystjarvi, E. 1998. Increase in the quantum yield of photoinhibition contributes to copper toxicity in vivo. *Plant Physiol.* 117:619-627.
 27. Roloff, I., Scherm, H., and van Iersel, M. W. 2004. Photosynthesis of blueberry leaves as affected by Septoria leaf spot and abiotic leaf damage. *Plant Dis.* 88:397-401.
 28. Stanosz, G. R. 1992. Effect of cherry leaf spot on nursery black cherry seedlings and potential benefits from control. *Plant Dis.* 76:602-604.
 29. Ware, G. W., and Whitacre, D. M. 2004. Copper. Pages 158-159 in: *The Pesticide Book*. MeisterPro Press, Willoughby, OH.