Effectiveness of Selected Fungicides in Controlling Foliar Diseases of Common Periwinkle (*Vinca minor* L.)

Mary C. Koelsch¹ and Janet C. Cole²

Department of Horticulture and Landscape Architecture, Oklahoma State University, Stillwater, OK 74078

Sharon L. von Broembsen²

Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078

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Abstract. Common periwinkle and 'Bowles' periwinkle production has declined in the southern United States due to foliar diseases caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. and *Phoma exigua* Desmaz. var. *inoxydabilis* Boerema & Vegh in Vegh et al. Our study determined whether several labeled and experimental fungicides could control pathogens causing foliar diseases in common periwinkle in vitro and outdoors during two consecutive summers. Five concentrations of each of eight fungicides were used to test inhibition of mycelial growth of *P. exigua* var. *inoxydabilis* and two isolates of *C. gloeosporioides* on fungicide-amended agar. All concentrations of propiconazole inhibited growth of *P. exigua* var. *inoxydabilis* (100%) and both isolates of *C. gloeosporioides* (>96%). Cyproconazole completely inhibited mycelial growth of *P. exigua* var. *inoxydabilis*. Thiophanate methyl/mancozeb partially inhibited growth of *C. gloeosporioides* (50%). In outdoor trials, plants were sprayed weekly with the following fungicides and rates (in g a.i./liter): thiophanate methyl/mancozeb, 1.35; propiconazole, 0.14; thiophanate methyl, 0.84; triforine, 0.27; cyproconazole, 0.08; triforine-CC 17461, 0.27; or CGA 173506, 0.90. Thiophanate methyl/mancozeb was most effective at reducing foliar necrosis during both seasons. Shoot dry weights of plants treated with thiophanate methyl/mancozeb were significantly higher at the end of each growing season than those of plants treated with the other fungicides or the nontreated control plants. Chemical names used: dimethyl [(1,2-phenylene)-bis (iminocarbonothioyl)] bis [carbamate] and a combination of zinc ion and manganese ethylenebisdithiocarbamate (thiophanate methyl/mancozeb); 1-[2-(2',4'-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl]-1*H*-1,2,4-triazole (propiconazole); dimethyl [(1,2-phenylene)-bis (iminocarbonothioyl)] bis [carbamate] (thiophanate methyl); *N,N'*-[1,4-piperazinediylbis (2,2,2-trichloroethylidene)] bis [formamide] (triforine); 2-(4-chlorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-butan-2-ol (cyproconazole); *N,N'*-[1,4-piperazinediylbis (2,2,2-trichloroethylidene)] bis [formamide] with micro emulsion (triforine-CC 17461); 4-(2,2-difluoro-1,3-benzodioxol-4-yl) pyrrole-3-carbonitrile (CGA 173506).

Once established within a landscape, common periwinkle is a relatively low-maintenance shade plant; however, the number of common

periwinkle plants propagated and produced by growers has declined, and common periwinkle has even been eliminated from some nursery inventories. This decline has been due to the difficulty of implementing effective control methods for foliar diseases during production. Nursery growers have estimated plant losses ranging from 10% to 60% of total production for each season (M. Andrews, Greenleaf Nursery, Tahlequah, Okla., and G. Percefull, Arrowhead Growers, Tulsa, Okla., personal communication). In addition, growers have indicated that common periwinkle has more disease problems than cultivars such as 'Bowles' periwinkle.

Disease symptoms have included leaf and stem lesions and stem death. Two fungal pathogens, *Colletotrichum gloeosporioides* and *Phoma exigua* var. *inoxydabilis* recently have been identified as the cause of these disease symptoms (Koelsch, 1993).

Previously, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) was used in spray programs for disease outbreaks on common periwinkle; however, the removal of ornamental plant uses from its label necessitates finding a suitable substitute. Our objective was to determine the effectiveness of several fungicides in controlling foliar diseases of common periwinkle and 'Bowles' periwinkle.

Materials and Methods

Inhibition of fungal mycelial growth by fungicides in vitro. One isolate of *C. gloeosporioides* from common periwinkle (K1), one isolate from 'Emerald Gaiety' evergreen wintercreeper [*Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Emerald Gaiety'] (K2), and one isolate of *P. exigua* var. *inoxydabilis* from common periwinkle (K4) were isolated from leaf or stem lesions. Leaf and stem pieces were plated onto plastic petri dishes (9 cm in diameter) containing potato-dextrose agar (PDA) consisting of 39 g PDA/liter of sterilized, distilled, deionized water and were autoclaved at 120°C for 20 min. Boerema (1976) described techniques to identify *P. exigua* var. *inoxydabilis* spores cultured from small leaf spots. Large, sunken, necrotic lesions on *Vinca minor* leaves and stems were the source of the K1 isolate of *C. gloeosporioides* (Koelsch, 1993).

Each isolate was transferred to PDA amended with the following fungicides and rates (g a.i./liter): thiophanate methyl/mancozeb (TPMM) (Grace-Sierra, Milpitas, Calif.), 0, 0.80, 1.60, 2.40, or 4.80; propiconazole (PROP) (Ciba Geigy, Greensboro, N.C.), 0, 0.03, 0.07, 0.28, or 0.56; thiophanate methyl (TPML) (Grace-Sierra), 0, 0.21, 0.42, 0.84, or 1.68; triforine (Ortho, Chevron Chemical Co., San Ramon, Calif.), 0, 0.14, 0.27, 0.40, or 0.54; cyproconazole (CYPC) (Sandoz Crop Protection, Des Plaines, Ill.), 0, 0.04, 0.08, 0.16, or 0.32; tetrachloroisophthalonitrile [chlorothalonil (CLTL)] (Fermenta, Mentor, Ohio), 0, 0.94, 1.88, 3.75, or 7.50; cupric hydroxide (CUOH) (Griffin Chemical Corp., Valdosta, Ga.), 0, 0.28, 0.56, 6.25, and 12.50; or a coordination product of zinc ion and manganese ethylene bisdithiocarbamate [mancozeb (MANC)] (Rohm and Haas, Philadelphia), 0, 0.74, 1.49, 2.98, or 5.96. These rates corresponded to 0, half the lowest recommended rate, the lowest recommended rate, the highest recommended rate, and twice the highest recommended rate for each fungicide. Cultures were grown in plastic petri dishes (9 cm in diameter) that were wrapped in foil and placed in a dark incubator at 27 ± 1°C for 6 days. Mycelial growth was measured every 2 days. A completely randomized design with three plates for each isolate and fungicide was used, and the study was repeated once. Analysis of variance procedures and regression analysis were used to determine treatment differences.

Field studies of fungicidal control (year 1). Rooted cuttings of 'Bowles' periwinkle were planted on 24 Mar. 1992 in 3.8-liter pots containing a medium consisting of 3 pine bark

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¹Graduate Research Assistant.

²Associate Professor.

: 1 peat : 1 sand (by volume) amended with (in $\text{kg}\cdot\text{m}^{-3}$) 4.7 17N-3.6P-10K slow-release fertilizer (Osmocote, Grace-Sierra), 3.0 gypsum, 3.0 dolomite, and 0.9 micronutrients (Micro-max, Grace-Sierra). Plants were grown outdoors under full sun with a maximum photosynthetic photon flux (PPF) of $1275\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ on a black groundcover fabric. The plants received $\approx 13\ \text{mm}$ water daily by overhead sprinkler irrigation. Beginning 5 May 1992, plants were sprayed to runoff weekly using a CO_2 -pressurized backpack sprayer that delivered $0.2\ \text{liter}\cdot\text{m}^{-2}$. Fungicides evaluated were (in g a.i./liter) TPMM, 1.35; PROP, 0.14; TPML, 0.84; triforine, 0.27; CYPC, 0.08; triforine-CC 17461 (Ortho, Chevron Chemical Co.), 0.27; and CGA 173506 (Ciba Geigy), 0.90. These fungicides were recommended by manufacturers as having a high potential for

controlling the foliar diseases that we tested. No treatment was applied to the control plants. Plants were rated every 2 weeks throughout the growing season by three independent raters on a scale from 1 to 5 [1, no foliar symptoms; 2, $\geq 1\%$ and $\leq 33\%$ necrotic plant tissue; 3, $\geq 33\%$ and $\leq 66\%$ necrotic plant tissue; 4, $\geq 66\%$ and $\leq 99\%$ necrotic plant tissue; and 5, 100% necrotic tissue (plants were dead)]. The experiment was terminated 12 Oct. 1992, and roots and shoots were harvested and oven-dried at 44°C for 7 days before weighing.

Field studies of fungicidal control (year 2). 'Bowles' periwinkle and common periwinkle plants were planted on 31 Mar. 1993 and treated with fungicides starting 13 May 1993 as previously described, except that the plants were placed in a shadehouse with a maximum PPF of $945\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. An additional fungi-

cide treatment consisted of alternating weekly applications of TPMM with PROP. The experiment was terminated on 21 Sept. 1993, at which time shoot dry weights were determined.

In both years, all plants were healthy at the time of planting, with no foliar disease symptoms. Plants were not artificially inoculated with fungal spores but received natural inoculation through the growing environment. During each growing season, *C. gloeosporioides* and *P. exigua* var. *inoxydabilis* were isolated from infected areas of plants as previously described.

A completely randomized design was used for each outdoor experiment, with 20 'Bowles' periwinkle plants per fungicide treatment in year 1 and 15 plants each of common periwinkle and 'Bowles' periwinkle per fungicide treatment in year 2. Analysis of variance procedures and least significant difference tests were used to determine differences between fungicide treatments in both years and cultivar differences in the second trial.

Results

Inhibition of fungal mycelial growth by fungicides in vitro. All concentrations of PROP inhibited growth of *P. exigua* var. *inoxydabilis* (100%) and both isolates of *C. gloeosporioides* ($>96\%$) (Fig. 1, Table 1). CYPC produced the second highest percent inhibition at all rates for all fungal isolates. Higher rates of the combination of TPMM gradually increased percent inhibition for each isolate. The compounds TPML, MANC, CUOH, and CLTL had the lowest percentage of inhibition against all isolates.

Field studies of fungicidal control. During year 1, disease symptoms were first noted on plants in all treatments beginning 4 June 1992 (Fig. 2), and the number of lesions increased

Table 1. Trend analysis of growth inhibition of *Colletotrichum gloeosporioides* isolated from 'Bowles' periwinkle (K1) or 'Emerald Gaiety' wintercreeper (K2) and *Phoma exigua* var. *inoxydabilis* (K4) grown on fungicide-amended agar.

Fungicide	Active ingredient (g a.i./liter)	K1	K2	K4
Thiophanate methyl/mancozeb	Linear	*	*	*
	Quadratic	*	*	*
Propiconazole	Linear	**	**	**
	Quadratic	**	**	**
Thiophanate methyl	Linear	NS	NS	NS
	Quadratic	NS	NS	NS
Triforine	Linear	**	**	**
	Quadratic	**	**	**
Cyproconazole	Linear	**	**	**
	Quadratic	**	**	**
Chlorothalonil	Linear	**	**	**
	Quadratic	**	**	**
Cupric hydroxide	Linear	**	**	**
	Quadratic	**	**	**
Mancozeb	Linear	NS	NS	NS
	Quadratic	NS	NS	NS

NS, *, **Nonsignificant or significant in respect to linear or quadratic responses at $P \leq 0.05$ or 0.01 , respectively.

Fig. 1. Growth inhibition of *Colletotrichum gloeosporioides* isolated from (●) 'Bowles' periwinkle (K1) or (▲) 'Emerald Gaiety' wintercreeper (K2) and (■) *Phoma exigua* var. *inoxydabilis* (K4) grown on fungicide-amended agar. Fungicides tested were thiophanate methyl/mancozeb (TPMM), cyproconazole (CYPC), propiconazole (PROP), chlorothalonil (CLTL), thiophanate methyl (TPML), cupric hydroxide (CUOH), triforine (TRIF), and mancozeb (MANC).

*Data truncated because change was minimal at $>6\ \text{g a.i./liter}$.

throughout the growing season. From 4 June through 29 July 1992, TPMM suppressed foliar symptoms more than the other fungicides tested. At harvest, shoot dry weights of the TPMM-treated 'Bowles' periwinkle plants were higher than those of plants receiving any other fungicide treatment or the nontreated control plants (Table 2). Root dry weights were not affected by fungicide treatment.

During year 2, there were more lesions on plants receiving PROP and TPMM in alternate weeks than on plants receiving TPMM only. However, beginning 28 June 1993, plants receiving both treatments were rated lower (fewer symptoms) than plants from all other fungicide treatments and control plants for both cultivars (Fig. 3, Table 3). Beginning 10 June for both cultivars, plants treated with CYPC received higher severity ratings than those of any other treatment. At harvest, plants sprayed with TPMM had the highest shoot dry weight (Table 2), and plants treated with CYPC had the lowest.

The common periwinkle was rated the same as or lower than 'Bowles' periwinkle during May of year 2 in all fungicide treatments, except TPMM. During June, there were no differences in ratings between cultivars; then 'Bowles' was generally rated lower (fewer symptoms) than common periwinkle for the remainder of the study.

Discussion

With the loss of benomyl as a commercially available fungicide, the activity of several fungicides against the two major foliar pathogens of common periwinkle (*C.*

gloeosporioides and *P. exigua* var. *inoxydabilis*) is encouraging. In the in vitro study, PROP, CYPC, and TPMM all inhibited mycelial growth, with PROP causing the greatest inhibition. However, efficacy of fungicides in the field did not always correspond with the in vitro study. TPMM and TPMM alternated with PROP were the best overall field treatments; however, none of the treatments provided complete disease control under field conditions.

There were symptoms of *C. gloeosporioides* more frequently than *P. exigua* var. *inoxydabilis* in both field studies. During the 1992 trial, plants rapidly declined after mid-June. The first half of the summer was more mild and wet than the second half. These conditions could have influenced fungal sporulation, growth, and disease severity on the plants. Chakraborty et al. (1990) reported that, after inoculation of *C. gloeosporioides* on *Stylosanthes scabra* Vog., optimal infection occurred when plant leaves were wet for 16 h, temperatures were between 20 and 30°C, and relative humidity was high for 12 h after inoculation. In addition, growers have stated that while disease symptoms first occur in spring, the plants flourish and appear healthy as new growth becomes vigorous and hides the diseased areas. The infected leaves present at the time of this growth flush later provide inoculum for infection when conditions are favorable for disease development. This ready supply of inoculum may quickly reinfect plants, making a stringent fungicide application schedule critical.

Disease susceptibility of common periwinkle and 'Bowles' periwinkle were com-

pared in year 2. Disease severity of the 'Bowles' periwinkle was lower regardless of fungicide treatment, indicating that common periwinkle is more susceptible to disease infection. Similarly, common periwinkle growers have noted that cultivated lines seem more resistant to foliar diseases than the native species (*G. Percefull*, Arrowhead Growers, Tulsa, Okla., and *P. Warren*, Warren & Son Nursery, Oklahoma City, Okla., personal communication). The less severe disease symptoms in cultivated lines may be due to more stringent sanitation during the propagation of cultivated lines or the selection of more resistant lines along with the selection of other desirable characteristics. Propagation material for native lines is taken directly from the wild and already may be infected with pathogens. Using clean stock or resistant common periwinkle cultivars would reduce the disease severity in common periwinkle plantings.

The fungicides PROP, CYPC, and TPML were systemic; all others were protectant fungicides, except TPMM, which is a combination of both types. Plants treated with TPMM had lower disease severity ratings than plants treated with the other fungicides in both field studies. This combination of systemic and protectant action was apparently more effective at suppressing disease symptoms throughout each growing season than the individual fungicides. Although the systemic fungicides PROP and CYPC inhibited mycelial growth most in vitro, these systemics alone did not prove the most effective in the field studies. The TPMM combination was more effective in inhibiting mycelial growth in vitro than either fungicide alone at the same concentrations.

CYPC and PROP are sterol-inhibitors. The visual ratings of CYPC-treated plants in the second summer were higher (less desirable) than those of plants treated with any other fungicide due to phytotoxicity rather than foliar disease symptoms. Phytotoxicity was not as apparent during the first summer, possibly due to the different growing environments of the 2 years. The amended agar study showed

Table 2. Dry weight of shoots and roots of 'Bowles' periwinkle (1992, year 1) and shoot dry weight of common periwinkle and 'Bowles' periwinkle (1993, year 2) receiving weekly fungicide applications. Data for the two cultivars were combined in 1993.

Fungicide	Dry wt (g)		
	1992		1993
Control	Shoot	Root	Shoot
	0.6	1.2	18.3
Thiophanate methyl/mancozeb	1.9	1.3	24.3
Propiconazole	0.5	1.1	14.7
Thiophanate methyl	0.3	0.8	17.2
Triforine	0.8	1.6	15.3
Cyproconazole	0.8	1.9	8.0
Triforine (CC 17461)	0.8	1.0	18.3
CGA 173506	0.7	1.1	18.1
Thiophanate methyl/mancozeb + propiconazole	---	---	21.9
Significance (LSD _{0.05})	0.93	NS	2.48

Fig. 2. Visual ratings of 'Bowles' periwinkle receiving weekly fungicide applications throughout the 1992 growing season (year 1). Fungicides tested were (■) thiophanate methyl/mancozeb, (▲) propiconazole, (▼) thiophanate methyl, (◆) triforine, (○) cyproconazole, (□) triforine-CC 17461, (Δ) CGA 173506, and (●) no fungicide. Rating scale: 1 = no foliar symptoms, 2 = $\geq 1\%$ and $\leq 33\%$ necrotic plant tissue, 3 = $\geq 33\%$ and $\leq 66\%$ necrotic plant tissue, 4 = $\geq 66\%$ and $\leq 99\%$ necrotic plant tissue, and 5 = 100% necrotic tissue. Vertical bars represent least significant difference at $P \leq 0.05$.

complete mycelium growth inhibition with *P. exigua* var. *inoxydabilis* and increasing inhibition with *C. gloeosporioides* as CYPC concentrations increased. The difference between the high percentage of mycelial inhibition by PROP compared to the disease severity ratings in the field studies may have resulted

from continuous exposure of pathogens to the fungicide medium in vitro. Water from irrigation or rainfall may have decreased plant exposure to the fungicides in the field by physically removing the fungicides from the plants.

The fungicides MANC, CLTL, and TPML are commonly used in fungicide rotations.

Fig. 3. Visual ratings of (A) common periwinkle and (B) 'Bowles' periwinkle receiving weekly fungicide applications throughout the 1993 growing season (year 2). Fungicides tested were (■) thiophanate methyl/mancozeb, (▲) propiconazole, (▼) thiophanate methyl, (◆) triforine, (○) cyproconazole, (□) triforine-CC 17461, (Δ) CGA 173506, (▽) thiophanate methyl/mancozeb alternated with propiconazole weekly, and (●) no fungicide. Rating scale: 1 = no foliar symptoms, 2 = $\geq 1\%$ and $\leq 33\%$ necrotic plant tissue, 3 = $\geq 33\%$ and $\leq 66\%$ necrotic plant tissue, 4 = $\geq 66\%$ and $\leq 99\%$ necrotic plant tissue, and 5 = 100% necrotic tissue.

Table 3. Significance (least significance difference at $P \leq 0.05$) of main effects and interactions between cultivar and fungicide in visual ratings during the 1993 growing season (year 2).

Date	Cultivar (CV)	Fungicide (F)	CV $\times$ F
13 May	0.02	NS	0.01
24 May	0.05	NS	0.08
10 June	NS	0.12	NS
28 June	NS	0.16	NS
15 July	0.08	0.16	0.21
28 July	0.69	0.15	NS
12 Aug.	0.79	0.17	NS
23 Aug.	NS	0.17	NS
9 Sept.	0.08	0.17	NS
20 Sept.	0.08	0.17	0.15

NS=Nonsignificant.

None of these fungicides satisfactorily inhibited mycelial growth of the fungi in in vitro studies; thus, they were not compared in the outdoor screening trials. MANC has been used to control diseases caused by *Colletotrichum Corda* in *Sturm. sp.* on alfalfa (*Medicago sativa* L.) (Broschous and Kirby, 1988); CLTL has been used to control numerous diseases on ornamentals, turf, and vegetables. The conidia and sclerotia of tomato anthracnose [*Colletotrichum coccoides* (Wallr.) S.J. Hughes] were shown to be sensitive in vitro to CLTL (Dillard, 1988).

Although some of the fungicides tested suppressed disease symptoms, no fungicide eliminated the foliar diseases in actively growing plants. TPMM and PROP used in rotation with TPMM as preventative treatments were the most effective in reducing disease symptoms. In addition, using 'Bowles' and other more resistant cultivars also may reduce disease severity and result in less need for fungicide use in periwinkle production.

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