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Señor Director

División de Nuevas Creaciones

Superintendencia de Industria y Comercio

Ministerio de Comercio, Industria y Turismo

E. S. D.

Ref.: Expediente No. 09 044463

Solicitud de Patente de invención para: **"METODOS PARA REDUCIR LA INCIDENCIA DE BOTRYTIS Y PARA MEJORAR LA DURACION EN ALMACENAMIENTO DE LAS FLORES"**.

Solicitante: **SYNGENTA PARTICIPATIONS AG**

RESPUESTA AL EXAMEN DE BUSQUEDA DE ANTERIORIDADES

Yo, **MARGARITA CASTELLANOS ABONDANO**, mayor de edad, identificada y domiciliada como aparece al pie de mi firma, en atención a su oficio No. 12794 notificado el **25 de julio de 2012**, atentamente me permito dar repuesta al mismo de la siguiente manera:

Presento copia completa de los artículos requeridos por ese Despacho como resultado de la búsqueda de anterioridades:

- [X] MINUTO A ET AL: "CHEMICAL CONTROL OF GREY MOULD OF CYCLAMEN": MEDEDELINGEN-FACULTEIT LANDBOUWKUNDIGE EN TOEGEPASTEBIOLOGISCHE WETENSCHAPPEN, UNIVERSITEIT GENT,

GENT, BE, vol. 61, no. 2A, 1 January 1996 (1996-01-01), pages 453-459, XP001118975 ISSN:1373-7503".

- CHASTAGNER, G.A. ET AL: "Efficacy of fungicides in controlling leaf spot on bulbous iris" ACTA HORTICULTURAE, 673, 509-512 CODEN: AHORA2; ISSN: 0567-7672, vol.2, 2005, XP00911249.

Cumplido así con lo dispuesto, atentamente solicito a Ud. se continúe con el trámite correspondiente a esta solicitud de Patente de Invención.

Del Señor Director,


Margarita Castellanos Abondano

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18 SET. 2012

CHEMICAL CONTROL OF GREY MOULD OF CYCLAMEN

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SUMMARY

Grey mould, incited by *Botrytis cinerea*, can cause serious damages on cyclamen in the presence of conditions highly favourable to the pathogen. Its control relies, beside the adoption of appropriate cultural techniques, on the application of chemicals. However, chemical control is complicated by the widespread presence of populations of *B. cinerea* resistant to benzimidazoles and dicarboximides. Several experimental trials have been carried out during the past 5 years in order to test the efficacy of different fungicides, chosen among those already available and some new ones at present under development. Among the already available fungicides, the mixtures of carbendazim+diethofencarb, procymidone+thiram and chlozolinat+thiram performed well in all trials. Among the new fungicides, fludioxonil, alone and in mixture with cyprodinil were highly effective. Also pyrimethanil provided good disease control. By considering the phytotoxicity, among the tested fungicides and under our experimental conditions, only anilazine, chlorothalonil, fluazinam and tebuconazole caused unacceptable flower injury.

INTRODUCTION

Botrytis cinerea Pers. causes a petal spot and rot of developing flowers and leaves on cyclamen which can be very serious under conditions very favourable to grey mould development. The petal spots are at first mere flecks, then enlarge to 1 to 4 mm and often coalesce. On colored petals, an outer band of living tissues has more intense than the normal colour. The center of the spots are at first water soaked, and finally become tan and necrotic. Under high relative humidity, the blossom is decayed and intense sporulation of the pathogen takes place on it. When this decaying tissue contact leaves or developing flowers, they are also decayed. Under severe disease conditions, symptoms are so severe to make the plant not suitable for sale (McCain, 1985).

Control measures against grey mould involve sanitation to reduce the spore inoculum, and relative humidity management, mainly through ventilation.

Several fungicides are helpful in reducing the amount of damage on leaves and developing flowers. However, chemical control is often complicated by the presence of resistant populations of the pathogen, a phenomenon quite widespread in Italy on ornamental crops (Pappas, 1982; Garibaldi and Gullino, 1990; Gullino, 1992). Moreover, not all fungicides effective against *B. cinerea* on most crops can be applied on cyclamen due to their possible phytotoxicity and to the presence of visible residue on the plant, particularly on the flowers.

During the past 4 years, several experimental trials have been carried out on varieties of cyclamen commonly grown in Italy with the aim of evaluating the efficacy of new fungicides, recently developed for their efficacy against grey mould, compared to older ones, as well as their selectivity on the crop. The most interesting results obtained are here reported.

MATERIALS AND METHODS

All the experimental trials have been carried out at the Centro Regionale di Sperimentazione e Assistenza Agricola di Albenga (Northern Italy) belonging to the Chamber of Commerce of Savona.

Cyclamen plants, belonging to the cvs Romeo, Rosamunda, Scarlet, Finlandia, Fidelio, Sylvia, as indicated under tables 2-7, were grown in terracotta pots, 14 cm diameter, 2 l of soil capacity and maintained at a density of 10 pots/m², under greenhouse conditions. A soil mix normally adopted by

local growers was used. Fertilization was carried out at 10-12 day interval by using N:P₂O₅:K₂O ratio of 3:1:1 during the vegetative phase and 2:1:3 before and during blooming. Sprinkle irrigation was adopted and conditions very favourable to grey mould development (i.e. high RH) were maintained through the trials.

Plants were artificially inoculated, immediately after the first treatment, with a suspensions of conidia (10⁴ conidia/ml) of a mixture of 4 strains of *B. cinerea*, with a 70:30 ratio between conidia sensitive (S) and resistant to benzimidazoles and dicarboximides (RBD). Such a composition of sensitive and resistant populations of the pathogen is similar to that normally observed in the practice in the area where our investigation took place (Gullino, 1992)

Chemical treatments were carried out, with the fungicides listed under table 1, by using a knapsack sprayer and applying 800 l/ha. Plants were sprayed until runoff. The number of sprays carried out in each trial are reported under tables 2-7.

Disease severity was evaluated at regular intervals, by evaluating the rotted surface on 20-40 flowers/replicate, by using an index from 0 (healthy flower) to 100 (rotted flower). The presence of phytotoxicity symptoms was also recorded: particularly, fungicide deposit, spots on petals and tip necrosis were considered and rated. All experiments have been carried out with a randomized block design, with three replicates, each replicate being represented by 6 pots.

The data collected were statistically analyzed, according to Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Grey mould incidence has been very high in some trials (i.e. trial 1, 1992, table 2, trials I and II 1995, table 6) and medium in the other trials. All the tested fungicides provided a satisfactory control of grey mould. Some of them gave a very good control of grey mould, with very slight variability within the different trials.

Among the fungicides already marketed, the mixtures carbendazim + diethofencarb, procymidone + thiram and chlozolinate + thiram did perform well in all trials. Among the new fungicides, fludioxonil, alone and in mixture with cyprodinil, and fluazinam were highly effective. Also pyrimethanil provided good disease control.

By considering the phytotoxicity, among the tested fungicides and under our experimental conditions, only anilazine, chlorothalonil, fluazinam and tebuconazole caused unacceptable flower injury (table 1).

In conclusion, the present arsenal of fungicides for grey mould control looks quite satisfactory. Several of the registered fungicides provide a good control of grey mould. Since they belong to different chemical groups, a rotation of different active ingredients is possible and highly recommended.

The future availability of new, specific botryticides will further improve the arsenal, thus helping the grower making the most technically rational choice.

ACKNOWLEDGEMENTS

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Table 1 - List of fungicides used and evaluation of their phytotoxicity

ACTIVE INGREDIENT	FORMULATION	% A.I.	PROVIDED BY	PHYTO-TOXICITY
Anylazine	Dyrene, SC	38.4	Bayer	**
Benomyl	Benlate, WP	50.0	Du Pont	*
Carbendazim	Bavistin, FL	41.7	BASF	*
Carbendazim	Bavistin, WP	50.0	BASF	*
Carbendazim + diethofencarb	Sumico, WP	25.0+25.0	Cyanamid Italia	*
Chlorothalonil	Daconil, WG	75.0	CIBA	**
Chlozolate + thiram	Serinal T, WP	20.0+40.0	Isagro Italia	*
Cyprodinil + fludioxonil	A 9219 B, WG	62.5	CIBA	*
Dichlofluanid	Euparen, WP	50.0	Bayer Italia	*
Fluazinam	F 686, SC	50.0	S.I.A.P.A.	**
Fludioxonil	CGA 173506, WG	50.0	CIBA	*
Iprodione	Rovral, FL	25.0	Rhone-Poulenc	*
Iprodione	Rovral, WP	50.0	Rhone-Poulenc	*
Prochloraz	Octave, WP	46.1	Agrevo	*
Prochloraz	Sportak, EC	46.1	Agrevo	*
Procymidone	Sialex, WP	50.0	S.I.A.P.A.	*
Procymidone+thiram	Sialex T, WP	12.3+49.0	S.I.A.P.A.	*
Tebuconazole	Folicur, EW	25.0	Bayer	**
Thiophanate methyl	Enovit, FL	38.3	SIPCAM	*
Thiram	Pomarsol, WG	50.0	BAYER	*
Vinchlozolin	Ronilan, FL	41.3	Basf	*

* very low damage carried out on flowers

** phytotoxic symptoms causing commercially unacceptable flower injuries

Table 2 - Effectiveness of different fungicides against grey mould on cyclamen (cv Romeo and Rosamunda, trial 1/1992).

FUNGICIDE *	g a.i./100 l	DISEASE INDEX (0-100) ON CV **	
		Romeo	Rosamunda
--	--	54 b ***	48 b
carbendazim	100	18 a	17 a
carbendazim+diethofencarb	25+25	11 a	13 a
chlorothalonil	195	16 a	12 a
dichlofluanid	100	8 a	4 a
iprodione	100	12 a	13 a
iprodione	75	11 a	16 a
prochloraz	80	19 a	12 a
procymidone	30	17 a	16 a
procymidone+thiram	25+98	10 a	14 a
thiram	150	18 a	12 a

* Date of sprays: 25/1/92; 2/2; 9/2

** Evaluated on 17/2

*** The value of the same column followed by the same letter do not statistically differ following Duncan's Multiple Range Test (P = 0.05)

Table 3 - Effect of different fungicides against grey mould of cyclamen (cv Romeo and Rosamunda, trial 2/1992).

FUNGICIDE *	g a.i./100 l	DISEASE INDEX (0-100) ON CV °	
		Romeo	Rosamunda
--	--	24 b **	32 c
benomyl	100	22 ab	25 bc
carbendazim	100	15 ab	17 ab
carbendazim+diethofencarb	25+25	19 ab	14 b
chlorothalonil	195	19 ab	20 ab
dichlofluanid	100	14 ab	18 ab
iprodione (wetable powder)	100	15 ab	21 ab
iprodione (wetable powder)	75	12 a	17 ab
iprodione (flowable)	100	14 ab	14 a
prochloraz	80	18 ab	23 bc
procymidone	30	19 ab	17 ab
procymidone+thiram	25+98	17 ab	16 ab
thiram	150	16 ab	17 ab

* Date of spray: 20/2; 27/2; 7/3.

° Evaluated on 9/3

** See table 2

Table 4 - Efficacy of different fungicides against grey mould of cyclamen (cv Scarlet, 1993).

FUNGICIDE *	g a.i./100 l	DISEASE INDEX (0-100) **
--	--	15 d ***
benomyl	100	7 a-d
carbendazim	100	5 abc
carbendazim+diethofencarb	25+25	3 ab
chlorothalonil	195	4 abc
chlozolate+thiram	70+140	4 abc
dichlofluanid	100	13 cd
iprodione (wetable powder)	100	5 abc
iprodione (flowable)	100	7a-d
methyl-thiophanate	50	5 abc
prochloraz	80	8 a-d
procymidone	30	10 a-d
procymidone+thiram	25+98	12 bcd
thiram	150	2 a
tolyfluanid	50	3 ab
vinchlozolin	37.5	5 abc

* Date of sprays: 27/1; 3/2 ** Evaluated on 8/2 *** See table 2

Table 5 - Effectiveness of different treatments against grey mould on cyclamen (cv Finlandia, 1994).

FUNGICIDE *	g a.i./100 l	DISEASE INDEX (0-100) **
--	--	15 b ***
benomyl	100	9 ab
carbendazim	100	9 ab
carbendazim+diethofencarb	25+25	5 a
chlorothalonil	195	8 ab
chlozolate+thiram	70+140	8 ab
dichlofluanid	100	9 ab
iprodione (wetable powder)	100	7 ab
iprodione (flowable)	100	8 ab
methyl-thiophanate	50	6 a
prochloraz	80	11 ab
procymidone	30	7 ab
procymidone+thiram	25+98	11 ab
vinchlozolin	37,5	8 ab
tebuconazole	37.5	7 ab
thiram	150	10 ab

* Date of spray: 23/12/93 ** Evaluated on 11/01/94. *** See table 2

Table 6 - Efficacy of different fungicides against grey mould on cv Finlandia, Fidelio and Sylvia (1995).

FUNGICIDE *	g a.i./100 l	DISEASE INDEX (0-100) ON CV			
		Finlandia		Fidelio & Sylvia	
		Trial 1/95	Trial 2/95	Trial 1/95	Trial 2/95
		2/2	27/2	28/2	3/4
--	--	28 f**	35 c	40 c	33 c
anilazine	115.2	11 d	9 ab	6 ab	n.t.
benomyl	40	11 d	11 ab	14 ab	5 a
carbendazim+diethofencarb	25+25	4 ab	10 ab	9 ab	5 a
chlorothalonil	112.5	18 e	8 ab	15 ab	17 b
cyprodinil+fludioxonil	62.5	n.t.	6 a	6 ab	7 a
dichlofluanid	100	8 a-d	12 ab	6 ab	4 a
fluazinam	50	4 a	2 a	5 a	n.t.
fludioxonil	50	6 a-d	8 ab	15 ab	n.t.
iprodione (flowable)	75	7 a-d	4 a	11 ab	3 a
procymidone+thiram	12.3+49	11 cd	8 ab	13 ab	15 b
pyrimethanil	80	6 abc	10 ab	10 ab	7 a
tebuconazole	50	5 ab	17 b	6 a	n.t.
thiram	100	10 bcd	9 ab	12 ab	7 a

* Date of sprays: Finlandia: trial 1: 25/1; trial 2: 13/2; 21/2 ** See table 2
Fidelio & Sylvia: trial 1: 21/2; trial 2: 23/3

Table 7 - Efficacy of different fungicides against grey mould of cyclamen (cv Fidelio, 1996).

FUNGICIDE *	g a.i./100 l	DISEASE INDEX (0-100) **
--	--	20 cd ***
benomyl	40	20 cd
carbendazim	100	26 d
carbendazim+diethofencarb	25+25	10 abc
cyprodinil+fludioxonil	62.5	12 abc
chlozolate+thiram	50+100	11 abc
dichlofluanid	100	6 a
fludioxonil	50	15 a-d
iprodione (flowable)	75	19 b-d
pyrimethanil	60	18 b-d
tebuconazole	50	18 bc
thiram	100	19 b-d

* Date of sprays: 20/2; 27/2; 7/3 ** Evaluated on: 9/3 *** See table 2

Efficacy of Fungicides in Controlling Leaf Spot on Bulbous Iris

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Keywords: *Iris x hollandica*, bulbous iris, leaf spot, *Mycosphaerella macrospora*, disease management

Abstract

Annually, about 130 ha of field-grown bulbous iris (*Iris x hollandica*) are produced in western Washington. The production of iris bulbs and cut flowers has an estimated value of \$3.4 to 5.6 million. Leaf spot or "fire", caused by *Mycosphaerella macrospora*, is the most important foliar disease on this crop. Two trials were conducted to compare the efficacy of several standard and newer "reduced risk" fungicides for their ability to control leaf spot on two-year-old plantings of 'Saturnus' and 'Apollo' iris. Applications of Compass 50W (trifloxystrobin), Daconil Ultrex 82.5WP (chlorothalonil), Decree 50WDG (fenhexamid), Heritage 50WG (azoxystrobin), Insignia 20WP (pyraclostrobin), Medallion 50WP (fludioxonil), Phyton-27 (copper sulfate), Switch 62.5WG (cyprodinil + fludioxonil), and 26GT 23.3 (iprodione) were applied to plants at approximately 14 day intervals from 12 February through 21 May, 2003. The plot design was a randomized complete block with three blocks per cultivar. Treatments were applied to 1.5 m of row in each block. Disease pressure was high during late May and early June. On 'Apollo' iris, applications of Insignia, Heritage and 26GT provided excellent disease control and reduced the percentage of foliage that was killed. Insignia and Heritage also provided excellent disease control on 'Saturnus' iris. Applications of Medallion, Daconil Ultrex, and 26GT also reduced the level of disease compared to the unsprayed check. The results of these trials indicate that Insignia and Heritage have the potential to provide control of iris leaf spot that is equal to or better than that achieved by the standard materials used by growers.

INTRODUCTION

The mild, maritime climate along the coastal areas of western Washington provides optimal growing conditions for spring flowering bulbs (Gould, 1993). Annually, about 130 ha of field-grown bulbous iris (*Iris x hollandica*) are produced in western Washington. The production of iris bulbs and cut flowers has an estimated value of \$3.4 to 5.6 million.

Leaf spot or "fire", caused by the fungus *Mycosphaerella macrospora* (Kleb.) Jorstad, is the most important foliar disease on this crop (Ellis and Waller, 1974; Moore et al., 1979; Chastagner and Byther, 1990). Initial symptoms of this disease can appear on newly-emerged foliage, but limited disease development does not generally occur until after bloom. Symptoms include small tan to brown spots on leaves with a water-soaked edge that later becomes yellow. Spores produced on these spots serve to spread the disease during the growing season. Following bloom, infections can become so numerous that they coalesce and kill the foliage. The premature death of foliage can significantly reduce bulb yields (Gould, 1959). Warm, wet weather, acid soils, and high planting densities favor this disease. In addition to bulbous iris, it can also be a serious problem on rhizomatous iris.

Growers commonly utilize a combination of cultural practices and fungicide applications to manage leaf spot (Byther and Chastagner, 1993; Grebus et al., 2002a, b; Moore et al., 1979). Cultural practices include the removal and destruction of old foliage residues, annual harvest, reduced planting densities to promote good air circulation, maintenance of soil pH at or above 6, and crop rotation.

There are a number of fungicides that are currently labeled for controlling leaf spot and growers in the Pacific Northwest (PNW) typically make 2 to 6 applications of these materials during the growing season. Each year, a number of field and greenhouse trials are conducted at WSU Puyallup to identify potential new products or approaches that could be used to manage economically important diseases of ornamental bulb crops. This paper reports the results of two trials that were conducted to compare the effectiveness of several newer fungicides, such as Heritage, that are classified as "reduced risk" materials by the U.S. Environmental Protection Agency to some of the more commonly utilized materials.

MATERIALS AND METHODS

During spring 2003, two leaf spot trials were established in a commercial growers field of bulbous iris. One trial was established in a two-year-old planting of 'Saturnus' iris and one was in a field of two-year-old 'Apollo' iris. A total of nine fungicides were included in these tests (Table 1). Applications of a single rate of each fungicide were applied to a 1.5 m long section of row within three blocks in each plot. The plot design was a randomized complete block.

All applications were done with a Solo backpack sprayer equipped with an 8003LP Tee-Jet nozzle at 1.05 kg/cm². Applications were applied at approximately 14-day intervals between 12 February and 21 May 2003. All treatments were applied in the equivalent of 935.4 L of water/ha. Capsil was added to the Phyton-25 sprays as an adjuvant at 0.32 ml/L.

The effectiveness of each treatment in controlling the development of leaf spot was determined by periodically rating disease severity on the foliage during the growing season. Severity was rated on a scale of 0 to 10, where 0 = none, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, ..., and 10 = 91-100% of the foliage killed by the disease. Data were subjected to analysis of variance, and Duncan's Multiple Range Test was used to separate treatment means.

RESULTS AND DISCUSSION

Limited leaf spot was present on the iris in these trials until late April and early May. Disease severity increased through early June, at which time foliage on most of the unsprayed check plants had been killed. There were significant differences in the effectiveness of the various treatments in controlling leaf spot. On 'Apollo' iris, applications of Insignia, Heritage and 26GT provided excellent disease control and reduced the percentage of foliage killed by leaf spot (Table 2).

Insignia and Heritage also provided excellent disease control on 'Saturnus' iris (Table 3). By early June, the 'Saturnus' iris plants that had been sprayed with Medallion, Daconil Ultrex, and 26GT also had reduced levels of disease compared to the unsprayed check (Table 3).

CONCLUSION

There are 11 different types of fungicides that are listed for leaf spot control on bulbous iris in the 2004 edition of the Pacific Northwest Disease Control Handbook (Pscheidt and Ocamb, 2004). In 1998, a pesticide use survey of commercial growers in the PNW showed that chlorothalonil was the most common fungicide used to control leaf spot on field-grown bulbous iris (G.A. Chastagner, unpublished results). Various formulations of chlorothalonil were applied to over 90% of the iris grown in western Washington. Other fungicides, such as iprodione, vinclozolin, and mancozeb were also often incorporated into the growers' leaf spot disease management spray programs and were applied one or more times to at least 10% of the acreage.

The results from these trials indicate that Insignia and Heritage have the potential to provide control of leaf spot that is equal to or better than currently utilized fungicides. In a limited test involving only two fungicides, Holcomb and Moorman (2000) also showed that two applications of Heritage during late April and mid May provided signif-

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icant reduction in leaf spot development on rhizomatous iris in a landscape situation.

Only a single rate and application timing of Insignia and Heritage were tested in our studies. Studies are currently underway to determine the optimal rates and timing of application for Heritage and Insignia. Given the limited disease development during the early part of the growing season, it may be possible to reduce the number of applications of these materials that are needed. Studies are also needed to determine their effectiveness in controlling ink spot [*Drechslera iridis* (Oud.) Ellis], which can also be a problem on bulbous iris foliage in some fields.

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Tables

Table 1. Products tested.

Trade name and formulation	Active ingredient
26 GT 23.3	iprodione
Capsil (surfactant)	organosilicone
Compass 50W	trifloxystrobin
Daconil Ultrex 82.5WP	chlorothalonil
Decree 50WDG	fenhexamid
Heritage 50WG	azoxystrobin
Insignia 20WP	pyraclostrobin
Medallion 50WP	fludioxonil
Phyton-27	copper sulfate
Switch 62.5WG	cyprodinil (37.5%) + fludioxonil (25%)

Table 2. Severity of leaf spot on 'Apollo' iris foliage.

Treatment	Product/L	Severity ¹		
		15 May	28 May	9 June
Check	-	3.7 a ²	7.6 a	9.0 a
Phyton-27	1.2 ml	3.3 ab	7.6 a	9.0 a
Decree 50DWG	1.2 g	3.7 a	7.6 a	8.6 a
Switch 62.5W	0.45 g	2.6 bc	7.3 a	8.3 a
Phyton-27	2.0 ml	4.0 a	8.0 a	8.0 a
Daconil Ultrex 82.5 WP	1.68 g	1.3 de	6.3 a	7.6 a
Medallion 60WP	0.3 g	2.0 cd	6.3 a	5.6 b
26GT 23.3	2.56 ml	1.0 e	3.6 b	1.6 c
Heritage 50WG	0.3 g	1.0 e	3.3 b	1.6 c
Insignia 20WP	0.3 g	1.0 e	2.0 b	1.0 c

¹ Rated on a scale of 0 to 10 where 0 = none and 10 = 91-100% of the foliage killed.

² Numbers in columns followed by the same letter are not significantly different, P=0.05, Duncan's multiple range test.

Table 3. Severity of leaf spot on 'Saturnus' iris foliage.

Treatment	Product/L	Severity ¹		
		15 May	28 May	9 June
Check	-	3.7 a ²	8.0 a	9.3 a
Phyton-27	1.2 ml	4.0 a	8.0 a	9.0 a
Phyton-27	2.0 ml	3.0 ab	7.6 a	9.0 a
Switch 62.5W	0.45 g	3.7 a	7.6 a	8.7 a
Decree 50DWG	1.2 g	2.3 bc	6.3 ab	7.7 a
Medallion 60WP	0.3 g	1.3 cd	3.6 cd	5.0 b
Daconil Ultrex 82.5WP	1.68 g	1.0 d	3.3 cd	4.7 b
26GT 23.3	2.56 ml	1.3 cd	4.6 bc	3.7 bc
Heritage 50WG	0.3 g	1.0 d	2.0 d	1.3 c
Insignia 20WP	0.3 g	1.0 d	1.7 d	1.0 c

¹ Rated on a scale of 0 to 10 where 0 = none and 10 = 91-100% of the foliage killed.

² Numbers in columns followed by the same letter are not significantly different, P=0.05, Duncan's multiple range test.