

## THE USE OF SERENADE BIOFUNGICIDE TO CONTROL FOLIAR FUNGAL DISEASES OF FLORIDA CITRUS

H. BRETT HIGHLAND<sup>1</sup>  
*AgraQuest, Inc.*  
211 Roberts Rd.  
Nokomis, FL 34275

L. W. TIMMER  
*University of Florida*  
Citrus Research and Education Center  
700 Experiment Station Rd.  
Lake Alfred, FL 33850

*Additional index words.* alternaria brown spot, fruit drop, greasy spot, melanose, postbloom, scab

*Abstract.* Serenade® *Bacillus subtilis* biofungicide (QRD 137, QRD 131, QRD 132, QRD 141) is a new biologically based fungicide/bactericide registered for use against a variety of fruit pathogens, including fire blight, scab and powdery mildew of apples and pears, mummy berry of blueberry, and gray mold (sour rot), powdery mildew and downy mildew of grapes. Serenade has been shown to inhibit plant pathogens by stopping spore germination, disrupting germ tube and mycelial growth and producing a zone of inhibition through secondary metabolites to restrict pathogen growth. Field trials were conducted in Florida citrus demonstrating the activity of Serenade against postbloom fruit drop (*Colletotrichum acutatum*), Alternaria brown spot (*Alternaria alternata*), citrus scab (*Elsinoe fawcetti*), and greasy spot (*Mycosphaerella citri*). Serenade biofungicide can be viewed as a viable, effective, safe, and IPM acceptable alternative for foliar disease control in Florida citrus.

---

<sup>1</sup>Corresponding author.

Serenade WP (wettable powder) (QRD 132, QRD 137, QRD 141) and Serenade AS (aqueous suspension) (QRD 131, QRD 143) are two new biologically based fungicides that will soon be registered for use against fungal pathogens in *Citrus*. Serenade from AgraQuest, Inc. (Nokomis, Fla.), is based on a proprietary strain of a bacteria, *Bacillus subtilis*, and is the only broad spectrum foliar biofungicide with efficacy as good or better than conventional pesticides for certain diseases. It has unique modes of action, is environmentally safe, safe for beneficial insects, and has no pre-harvest interval and a four hour re-entry interval. The signal word on the labels is caution. The WP and AS formulations have shelf life stabilities of approximately 2-3 years. Serenade is compatible as a tank mix with many common citrus fungicides including oils and coppers, and should be applied in conjunction with other preventative controls. Serenade works by production of secondary metabolites that disrupt germ tube formation of pathogens, restrict pathogen attachment by creating a zone of inhibition where applied, and prevent pathogen spores from germinating.

The spectrum of diseases activity with Serenade in citrus includes citrus scab caused by *Elsinoe fawcettii*, Alternaria brown spot caused by *Alternaria alternata*, postbloom fruit drop caused by *Colletotrichum acutatum*, greasy spot caused by *Mycosphaerella citri*, and citrus melanose caused by *Diaporthe citri*. Postbloom fruit drop attacks citrus flowers during the bloom causing water soaked brown patches on the bloom and later cause the blighted petals to remain firmly attached to the basal disk (Bhatia et al., 2003). After flowering, fruitlets abscise at the base of the ovary leaving a basal disk or so called "button", which leads to decreased yield. Postbloom fruit drop affects most citrus but is most severe on Navel and Valencia oranges in Florida (Agostini et al., 2003). Alternaria brown spot (or leaf spot) attacks from spring flush to summer and causes large necrotic to small circular leaf spots and blighted areas on the leaves. Lesions can have an extensive yellow halo. This disease affects mostly tangerines and tangerine hybrids. Citrus scab causes pronounced protuberances on the undersides of leaves, and conical or warty outgrowths on the fruit rind. Scab also attacks during the spring flush period and effects mostly tangerines and their hybrids and grapefruit. Greasy spot attacks during the spring and summer flush period. Affected areas on leaves become dark brown to black with a greasy appearance. Greasy spot rind blotch appears as necrotic flecks in the epidermis between the oil glands. The disease attacks all citrus but tends to be more severe on grapefruit and lemons. Melanose appears as small brown discrete sunken spots on the leaves. Pustules also occur on the fruit which can coalesce to form extensive diseased areas. Melanose is most serious on grapefruit produced for the fresh market.

This paper examines the effect of Serenade in programs to control these diseases in Florida citrus. Particular emphasis will be placed on common fungicide programs (mixtures or alternations of fungicides) that will be used in citrus production.

### Materials and Methods

Screen house trials were conducted by using potted citrus seedlings and inoculated with conidial suspensions of the organisms causing postbloom fruit drop, scab, and melanose. Treatments were applied with a hand sprayer prior to inoculation with pathogens.

Field trials were conducted in small plots (2-8 trees per treatment replication) with 4-6 replications per treatment. Plots were maintained under common commercial growing

Table 1. Efficacy of Serenade WP against postbloom fruit drop, screen house evaluation, buttons per cluster (L. W. Timmer, Lake Alfred, Fla., 2002).

Treatment	% Symptomatic flowers	Buttons/cluster
Untreated	58 a	14.3 a
Serenade WP 0.03 lb/gal	6 bc	5.8 ab
Serenade WP 0.06 lb/gal	33 ab	2.7 b
Serenade WP 0.03 lb/gal plus Ferbam 0.04 lb/gal	0 c	4.3 ab
Benlate 0.015 lb/gal	0 c	5 ab

One application. Mean separation by the Waller-Duncan k-ratio t-test, P = 0.05.

conditions in Florida. Treatments were applied during the commonly accepted treatment windows for the particular disease targeted (Timmer, 2004). Applications were made using common high volume air blast commercial equipment (Highland, Yonce experiments) or handgun sprayer (Timmer experiments). The number of applications that were applied fell under the commonly accepted commercial application protocols for the particular diseases in question. All diseases in field trials were naturally occurring epidemics.

### Results

*Postbloom fruit drop.* Initial screen house trials indicated that Serenade was having an effect on the post bloom fruit drop severity, however no rate response was noted. The addition of Ferbam resulted in slightly better control (Table 1).

In this field trial Serenade used alone or mixed with Ferbam numerically reduced the incidence of PFD and significantly increased fruit yield compared to the untreated check. Topsin M worked well in this trial, but Gem was mostly ineffective (Table 2).

*Citrus Scab.* In these screen house trials Serenade reduced the incidence of citrus scab, although Benlate was the best treatment in the trials (Table 3).

In this trial Serenade used alone or mixed with copper significantly reduced disease severity and increased marketable fruit compared to the untreated control. Serenade plus copper at 2 lb was more effective than copper alone at 2 lb. Gem provided better control than Serenade in this trial (Table 4).

*Alternaria brown spot.* In this trial Serenade used alone or mixed with copper significantly reduced disease severity and increased marketable fruit compared to the untreated control. The addition of copper seemed to improve the activity of

Table 2. Efficacy of Serenade WP against postbloom fruit drop, field trial (buttons per 2 ft<sup>2</sup> area) and yield (fruit/2 ft<sup>2</sup> area). Trial conducted by H. Yonce at KAC Agricultural Research, St. Cloud, Fla., 2003.

Treatment	Buttons/2 ft <sup>2</sup> area	Fruit/2 ft <sup>2</sup> area
Untreated	17.7 a	3.4 b
Serenade WP 6 lb/a	11.6 a	6.8 a
Serenade WP 6 lb plus Ferbam 5 lb/a	12.4 a	6.1 a
Ferbam 5 lb/a	12.8 a	5.6 ab
Topsin M 5 lb/a	9.2 a	6.3 a
Gem 8 oz/a	16 a	4.7 ab

One application. Mean separation by the Student-Newman-Keuls test, P = 0.05.

Table 3. Efficacy of Serenade WP against citrus scab, screen house evaluation on rough lemon, lesions per leaf (L. W. Timmer, Lake Alfred, Fla., 2001).

Treatment	Experiment 1 Lesions/leaf	Experiment 2 Lesions/leaf
Untreated	5.9 a	49.8 a
Serenade WP 0.11 lb/gal	1.7 a	22 ab
Serenade WP 0.22 lb/gal	2.9 a	35.8 ab
Benlate 0.15 lb/gal	0.4 b	13.1 b

Mean separation by the Waller-Duncan k-ratio t-test, P = 0.05. Applications 3 or 5 and 13 or 15 d prior to budbreak.

Serenade in this trial. Gem provided better control than Serenade in this trial (Table 5).

*Greasy spot.* In this trial Serenade used alone or mixed with oil significantly reduced disease severity on the leaves but only slightly improved marketable fruit compared to the untreated control. The addition of oil seemed to improve the activity of Serenade in this trial. Abound plus Trilogy provided excellent control in this trial (Table 6).

In this trial Serenade used alone or mixed with oil or copper reduced disease severity on the leaves compared to the untreated control. The addition of oil or copper seemed to improve the activity of Serenade in this trial (Table 7).

*Melanose.* In this screen house trial Serenade significantly reduced the incidence of citrus melanose, although Abound was the best treatment in the trial (Table 8).

In this trial Serenade alone or mixed with oil or copper significantly reduced disease severity on the leaves. The addition of oil or copper seemed to improve the activity of Serenade in this trial (Table 9).

Table 4. Efficacy of Serenade WP against citrus scab, field trial on Marsh grapefruit, disease severity (L. W. Timmer, Lake Wales, Fla., 2003; Johnston and Timmer, 2004).

Treatment	Severity rating 0-5	Marketable fruit (%)
Untreated	1.98 a	30 d
Serenade WP 4 lb	1.29 b	67 c
Serenade WP 2 lb plus Kocide 2000 1 lb	1.24 b	67 c
Serenade WP 4 lb plus Kocide 2000 2 lb	1.07 b	80 b
Kocide 2000 2 lb	1.26 b	69 bc
Gem 4 oz.	0.31 de	98 a

Mean separation by the Waller-Duncan k-ratio t-test, P = 0.05. Three applications, first growth flush, petal fall, marble-size fruit.

Table 5. Efficacy of Serenade WP against citrus Alternaria brown spot, field trial, disease severity (leaves) and marketable fruit (L. W. Timmer, Bereah, Fla., 2003).

Treatment	Severity rating 0-5	Marketable fruit (%)
Untreated	1.29 a	61.8 c
Serenade WP 4 lb	0.92 b	75.0 b
Serenade WP 2 lb plus Kocide 2000 1 lb	0.35 c	93.2 a
Serenade WP 4 lb plus Kocide 2000 2 lb	0.29 c	93.8 a
Ziram 7 lb	0.33 c	93.6 a
Gem 8 oz.	0.22 c	97.2 a

Mean separation by the Waller-Duncan k-ratio t-test, P = 0.05. Three applications, first growth flush, petal fall, marble-size fruit.

Table 6. Efficacy of Serenade WP against citrus greasy spot, field trial on Marsh grapefruit, disease severity (leaves) and marketable fruit (L. W. Timmer, Lake Alfred, Fla., 2001; Bhatia et al., 2003).

Treatment	Severity rating 0-5	Marketable fruit (%)
Untreated	1.5 a	74 c
Serenade AS 2%	1.1 b	80 b
Serenade AS 2% plus Sunspray 9E 5 gal	0.6 c	79 bc
Abound EC 12.4 oz plus Trilogy 70% 1.25 gal	0.7 c	92 a

Mean separation by the Waller-Duncan k-ratio t-test, P = 0.05. Three applications.

## Discussion

Serenade biofungicide has been shown in field and screen house trials conducted since 2000 to have activity on a wide assortment of foliar citrus plant pathogens. Controlled screen house experiments plants were inoculated whereas those conducted under field conditions were exposed to natural epidemics. Serenade is crop safe and can easily and routinely be used in citrus production in conjunction with more traditional fungicides such as coppers and oils. Serenade has a unique mode of action for resistance management to aid in decreasing the probability of resistance among the targeted diseases. In citrus disease management, proper and timely applications are essential in order to achieve maximum control (Agostini et al., 2003). Serenade, acting in a preventative manner, fits into the normal disease control protocols used by growers.

Serenade can be applied to control early season citrus diseases such as postbloom fruit drop (PFD), scab, and Alternaria leafspot in preventative applications. Multiple applications during the bloom period for PFD control may be necessary. PFD resistance to Benlate (Topsin M) type fungi-

Table 7. Efficacy of Serenade WP against citrus greasy spot, field trial, percent control on foliage (H. B. Highland, Felda, Fla., 2000).

Treatment	Percent control
Untreated	0.0 a
Serenade WP 4 lb	30 a
Serenade WP 4 lb plus paraffinic spray oil 5 gal	64 a
Serenade WP 4 lb plus oil 5 gal fb Serenade WP 4 lb plus Kocide 2000 3 lb	66 a
Kocide 2000 3 lb fb paraffinic spray oil 5 gal	56 a

Mean separation by the Student-Newman-Keuls test, P = 0.05. Three applications. Percent control calculated based on untreated.

Table 8. Efficacy of Serenade WP against citrus melanose, screen house evaluation on Duncan grapefruit seedlings, lesions per leaf (L. W. Timmer, Lake Alfred, Fla., 2001; Agostini et al., 2003).

Treatment	Experiment 1 No. lesions/leaf
Untreated	2.1 a
Serenade WP 0.11 lb/gal	1.5 b
Serenade WP 0.22 lb/gal	1.4 bc
Abound 0.13 oz/gal	0.8 de

Mean separation by the Waller-Duncan k-ratio t-test, P = 0.05. Applications 5 and 11 d prior to bud break.

Table 9. Efficacy of Serenade WP against citrus melanose, field trial, percent control on foliage (H. B. Highland, Felda, Fla., 2001).

Treatment	Percent control
Untreated	0.0 c
Serenade AS 2%	51 ab
Serenade AS 2% plus Kocide 2000 4 lb fb Serenade AS 2% plus paraffinic spray oil 5 gal	75 a
Kocide 2000 3 lb fb paraffinic spray oil 5 gal	75 a

Mean separation by the Student-Newman-Keuls test, P = 0.05. Four applications. Percent control calculated based on untreated.

cides has not been detected to date, however it continues to be of concern. Scab disease may develop resistance to the Benlate and strobilurin class fungicides, and these fungicides should not be applied more than once per season (Agostini et al., 2003). *Alternaria* leafspot may need to be treated multiple times (2 applications during flush, every 10 days thereafter), and Serenade, therefore, is an alternative for this disease if used in rotation with other fungicides or if mixed with copper.

Mid to late season diseases such as greasy spot and melanose are usually controlled with either coppers in late spring and early summer, and oil sprays mid summer to late summer. Serenade can be used as an alternative to oils and coppers or as a tank mix to improve control for these diseases.

In general Serenade should be used as a preventative fungicide, with applications at the appropriate time cycle for the particular diseases targeted. For best results under heavy disease pressure, Serenade should be applied as a tank mix with oils or copper based fungicides. This product can also be alternated or tank mixed with strobilurin chemistry. Serenade is not systemic, and the addition of a surfactant such as Biotune adjuvant by AgraQuest, Inc., or other spreading surfactant may improve results by aiding in coverage.

#### Literature Cited

- Agostini, J. P., P. M. Bushong, and L. W. Timmer. 2003. Greenhouse evaluation of products that induce host resistance for control of scab, melanose, and *Alternaria* brown spot of citrus. *Plant Dis.* 87:69-74.
- Bhatia, Alka and L.W. Timmer. 2003. Evaluation of products for control of citrus greasy spot on grapefruit and oranges, 2001-02. Fungicide and Nematicide Tests (on line) Report 58: MO07.DOI.10.1094/FN58. The American Phytopathological Society, St. Paul, MN
- Johnston, T. and L. W. Timmer. 2004. Evaluation of products for control of citrus scab on Marsh grapefruit, 2003. Fungicide and Nematicide Tests Report 59: V028.DOI.10.1094/FN59. The American Phytopathological Society, St. Paul, MN.
- Timmer, L. W. (ed). 2004. Florida Citrus Pest management Guide. Univ. of Florida, Inst. of Food & Agric. Sci., Publ. No. SP-43
- Timmer, L. W., S. M. Garnsey, and J. H. Graham (eds.). 2000. Compendium of Citrus Diseases, 2nd Ed. APS Press, Inc., St. Paul, MN.