Relationships of Environmental Factors and Inoculum Levels to the Incidence of Postbloom Fruit Drop of Citrus

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ABSTRACT

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Postbloom fruit drop, caused by *Colletotrichum gloeosporioides,* and environmental variables were monitored in three citrus (*Citrus* species) orchards from 1989 to 1991. In a navel orange orchard near Arcadia, the incidence of blossom blight was up to 30% in 1989 and up to 75% in 1991, but was less than 10% in other years and at other sites. The area under the curve for blossom blight was related positively to the number of persistent buttons (calyxes and floral disks) and related negatively to fruit counts. Persistent buttons and fruit counts were related negatively. A stepwise multiple regression equation was developed from the 1989 and 1991 data from the navel orange orchard near Arcadia. Inoculum level, measured as the number of diseased flowers 3–4 days before the target date, and the total rainfall from 4 to 8 days before the target date explained 50–65% of the variability in disease incidence. Leaf wetness 4–8 days before the target date was a significant factor, but it explained only a small percentage of the variability. Temperature and relative humidity were not important factors in disease development under these conditions. The equation developed should, with further refinement and validation, provide short-term predictions of disease development and assist growers in the timing of fungicide applications.

Additional keyword: epidemiology

Postbloom fruit drop of citrus (*Citrus* species) was first described in Belize in 1979 (5), although characteristic symptoms were observed as early as 1956 or 1957. The disease is caused by the slow-growing orange strain of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. (1,5), which is distinguishable from the cosmopolitan, saprophytic strain associated with dead and senescent citrus tissue (1,3,6,12). The fungus infects citrus flowers and produces orange- to peach-colored spots on the petals or affects entire flower clusters when conditions are favorable for disease development. Subsequently, fruitlets abscise and leave a persistent calyx (button) surrounded by distorted leaves with enlarged veins. The buttons persist indefinitely, and the tissues become enlarged with time.

Conidia of *C. gloeosporioides* are produced abundantly in acervuli on infected petals. The optimum temperature for spore germination is ≈23 C, with infection occurring in 12–18 hr (3). Symptoms develop 3–4 days after inoculation. Flowers are resistant in the pinhead to round bud stage, become susceptible when they begin to elongate, and are highly susceptible once open (4,5). In Belize, disease development is associated with high rainfall followed by prolonged wetting (4) during flowering, but little else is known about the conditions that favor disease development. Dispersal of the pathogen appears to be primarily by rain splash and to a lesser extent by windblown rain (1). The fungus apparently survives as appressoria on vegetative plant surfaces between flowering periods (1). The highest numbers of conidia of *C. gloeosporioides* were recovered in bottle traps used to collect runoff water during the bloom period, but conidia of pathogenic and saprophytic strains were indistinguishable (7).

After its discovery in Belize, postbloom fruit drop was reported in Brazil, Argentina, Dominica, and Colombia (3). It has been observed in most other humid, tropical citrus-growing areas in Central and South America and the Caribbean (L. W. Timmer, *unpublished*). The disease first appeared in 1983 on Tahiti limes in southern Florida (8). Subsequently, postbloom fruit drop appeared on sweet oranges and other citrus, primarily in southwest Florida and in some areas on the east coast. It has been observed only occasionally in the northern and central Ridge growing areas (10). Disease severity has varied greatly with location and year, but occasionally growers have reported yield losses of 50–90%.

This study was undertaken to determine the environmental conditions favorable for disease development under field conditions, to determine factors related to the disease's sporadic distribution in orchards, and to develop a predictive equation useful in making decisions on fungicide applications.

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MATERIALS AND METHODS

Study sites. Three bearing orchards in southwest Florida with a history of postbloom fruit drop were selected for study. Two orchards, one of navel oranges (*Citrus sinensis* (L.) Osbeck) propagated on sour orange (*C. aurantium* L.) rootstock, and the other of Valencia sweet orange on the same rootstock, were located near Arcadia, Florida. The orchards were about 300 m apart. The third orchard was navel orange on Swingle citrumelo (*Poncirus trifoliata* (L.) Raf. × *C. paradisi* Macf.) rootstock near LaBelle, Florida. In each orchard, five blocks of four trees each were selected at random in a 1-ha portion of the orchard.

When these studies were initiated, there was some doubt whether the fruit drop problem in Florida was due to the slow-growing orange strain of *C. gloeosporioides* found in Belize, to damage by the widespread saprophytic strain of *C. gloeosporioides* under especially favorable conditions, or even to thrips (2). Thus, flowers were monitored at the Citrus Research and Education Center in Lake Alfred where the postbloom fruit drop problem had never been observed and only the saprophytic strain had been isolated. At that location, 10 single trees in an orchard of Pineapple sweet orange on rough lemon rootstock (*C. jambhiri* Lush.) were monitored. To provide information throughout the bloom period, 10 Valencia sweet orange trees on the same rootstock in a nearby orchard were selected and monitored after flowering was complete in the first orchard.

Evaluation of flowering and of disease severity. The incidence of postbloom fruit drop was determined by inspecting all flowers on each tree which had opened recently or were about to open, up to 100 per tree. Flowers were selected arbitrarily around the periphery of the tree. To determine the degree of flowering on each date, the number of open and nearly open flowers was counted on each tree and averaged across the four trees in each plot, then across the five plots. The proportion of flowers affected by the disease was determined twice weekly in most cases and is reported for each date as the percent affected averaged across the four trees in each plot and across the five plots. Because petals drop quickly after the flowers open, disease assessments were conducted on newly opened flowers on each date. Graphs of the disease-incidence data plotted against days

during bloom were cut out, and the area was determined by passing them through a leaf area meter to calculate the area under the curve (AUC). The AUC (in %-days) presented is the average of the AUC for the five four-tree plots.

The number of persistent buttons and fruit on 12 branches, three chosen randomly in each quadrant of the tree, was counted in June of 1989, 1990, and 1991, after normal physiological drop had occurred, to evaluate relative disease incidence.

Climatic data. Rainfall, temperature, relative humidity, and leaf wetness were measured with a micrologger at the location in Arcadia. Temperature was recorded in a continuously shaded location at about midcanopy height. The leaf-wetness sensor was mounted on the micrologger stand at a 30° angle from the horizontal. It was exposed to full sun because most of the flowers are on the outside of the tree canopy.

At the site in LaBelle, only rainfall data were collected. A post-mounted rain gauge was placed near the center of the experimental area, and rainfall was recorded daily.

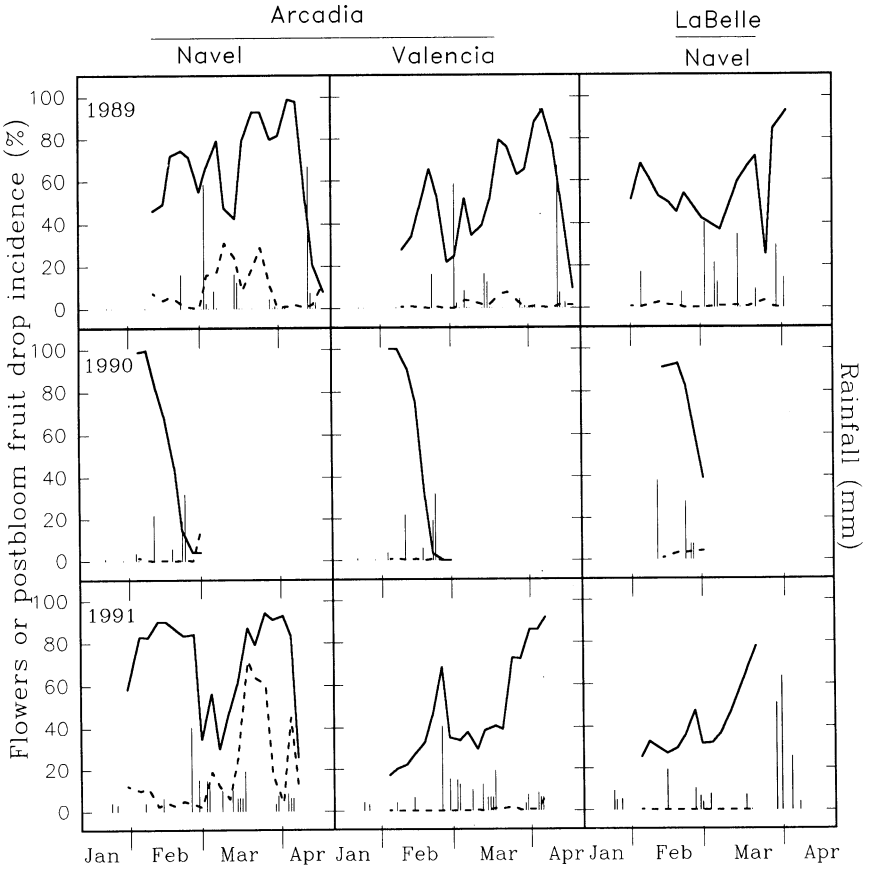

Fig. 1. Occurrence of postbloom fruit drop in three orchards from 1989 to 1991. Solid line = degree of flowering; broken line = percentage of flowers affected by postbloom fruit drop; and vertical bars = rainfall in mm.

Table 1. Disease incidence as measured by area under the curve (for blossom blight), button count, and fruit count in three orchards from 1989 to 1991

Disease incidence Orchard	1989	1990	1991
AUC[a]			
Arcadia–navel	28.0	0.4	86.1
Arcadia–Valencia	5.3	2.8	1.8
LaBelle–navel	3.1	2.5	0.2
No. of buttons[b]			
Arcadia–navel	67.8	2.8	258.2
Arcadia–Valencia	4.6	2.0	49.1
LaBelle–navel	7.0	5.6	3.1
No. of fruit[b]			
Arcadia–navel	12.7	74.3	1.9
Arcadia–Valencia	21.6	66.8	24.1
LaBelle–navel	13.4	32.2	ND[c]

[a] Area under the curve for % of blossom blight, based on twice-weekly counts of affected blossoms (in %-days).
[b] Average number of persistent buttons or fruit counted on 12 branches per tree (three per quadrant).
[c] Not determined.

Equation development. Data for 1989 and 1991 from the navel orange orchard near Arcadia were used to develop an equation to predict disease incidence. In these two cases, disease incidence was greater than 20%, and a complete set of weather and disease-incidence data was available.

Stepwise multiple regression analysis was used to develop equations to predict disease incidence on a target date. Factors considered in the analysis were the following: 1) previous disease—total number of flowers affected on 20 trees 3–4 and 7 days before the target date; 2) rainfall—total rainfall for the period (mm); 3) leaf wetness—number of hours with greater than 20% leaf wetness; 4) temperature (degrees C)—average for the period; and 5) relative humidity (%)—average for the period. We used the total number of diseased flowers rather than the percentage because inoculum production is related to the absolute number of flowers affected, and not necessarily to the percentage. Environmental parameters were considered for all possible 2-day periods or more, from 3 to 9 days before the target date; that is, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, 3–5, 4–6, 5–7, 6–8, 7–9, 3–6, 4–7, 5–8, 6–9, 3–7, 4–8, 5–9, 3–8, 4–9, and 3–9 days before the target date. Best-fit equations were developed with the stepwise multiple regression procedure of SAS Version 6.0 (9) for 1989 and 1991 data. Equations developed with the 1989 data were used to predict values obtained for the 1991 season, and vice versa. In all cases, coefficients of determination adjusted for degrees of freedom were used to evaluate the correlation between predicted and observed values.

RESULTS

Disease occurrence. In 1989, in the navel orange orchard near Arcadia, some flowers and low levels of disease were present when observations began in early February. Moderate levels of disease with up to 30% of the flowers affected occurred at some points during bloom (Fig. 1). Despite severe losses in 1988, little disease developed in 1989 in the Valencia orchard near Arcadia or in the navel orchard near LaBelle.

In 1990, little disease developed at any site. A freeze in December 1989 caused some defoliation of trees, and warm temperatures in January 1990 induced a profuse flowering of short duration. Very little rainfall occurred during the flowering period (Fig. 1). Only a few buttons were produced, and large numbers of fruit were set and remained to maturity (Table 1).

In 1991, rainfall was high and disease was severe in the navel orchard near Arcadia. Nevertheless, little disease devel-

oped in the Valencia orchard located nearby, which received similar amounts and durations of rainfall. Little rain occurred in the orchard near LaBelle during the bloom periods, and only low levels of disease were detected. No blossom blight was detected, and no buttons were formed at the Lake Alfred site.

In the navel orchard near Arcadia in 1989 and 1991, where significant disease developed, some flower infection was noted in January, and about 5–10% of the flowers were blighted by the first evaluation in early February (Fig. 1). Peaks in blossom blight incidence occurred shortly after rain events, and incidence declined following rain-free periods.

Regression analyses were conducted with all 3 yr of data to determine the relationships between the AUC for blossom blight, the number of buttons formed, and the number of fruit set. Where substantial disease occurred in the navel orchard near Arcadia, there was a strong positive relationship between AUC and the number of buttons formed (Table 2). There was a significant negative relationship between the AUC vs. fruit set in the navel orchard near Arcadia. There were also significant negative relationships between buttons and fruit set in all three orchards.

Equation development. Separate analyses were conducted with the 1989 and 1991 data from the navel orchard near Arcadia to develop equations. In both years, disease measured 3–4 days before the target date as the number of affected flowers per 20 trees explained a greater amount of the variability than disease measured 7 days before.

Total rainfall (as mm × 100) was evaluated over all possible 2-, 3-, 4-, and 5-day periods before the target date. Most periods tested had significant R^2 values. Although many of the periods tested yielded similar R^2 values, the period from 4 to 8 days before the target date most consistently explained a higher percentage of the variability in the data from both years and was consistent with the biology of the organism.

Leaf wetness, measured as the total number of hours with greater than 20% leaf wetness, was a significant factor in many regression analyses, but it explained only a small proportion of the variability. Because leaf wetness occurred nearly every night, 10 hr per day were subtracted from the total number of hours during the period, with the value not allowed to go below zero. When the regression was repeated, this measure resulted in R^2 values two to five percentage points higher than when leaf wetness was excluded.

Inclusion of temperature and/or relative humidity in the stepwise regressions did not greatly increase R^2 values. Temperature explained up to 12% of the variability in some 1989 equations tested, but it was not significant in any 1991 equations. Relative humidity was not a significant factor in any of the equations tested. Hence, temperature and relative humidity were excluded from further consideration.

Square root transformation of the values included in all equations usually increased R^2 values when applied to the individual factors or all factors used in an equation. For example, if previous disease, rainfall, and leaf wetness were included in the 1989 model without transformation, the R^2 was 70.2%; with transformation of all factors, the R^2 was 75.3%. In 1991, the R^2 was 54.9% if the above three factors were included as nontransformed values, whereas the R^2 was 65.7% when all values were transformed.

The following equations provided the best fit for the 1989 and 1991 data: for 1989, $y = -2.28 + 0.84\ (TD)^{½} + 0.26\ (R)^{½} \times 100 - 0.9\ (LW)^{½}$; for 1991, $y = -59.7 + 1.34\ (TD)^{½} + 0.91\ (R)^{½} \times 100 + 7.33\ (LW)^{½}$; where y = predicted % of flowers affected, TD = number of diseased flowers on 20 trees 3–4 days before target date, R = total rainfall in mm 4–8 days inclusive before target date, and LW = number of hours of leaf wetness − 50 hr (10 hr per day) for the period 4–8 days inclusive before target date.

In 1989, the previous disease factor explained 22.0% of the variability, addition of the rain variable increased the R^2 to 73.9%, and addition of leaf wetness increased it to 75.5%. In 1991, previous disease explained 32.1% of the variability in percent disease, and addition of rainfall and leaf wetness increased the R^2 to 53.2% and 62.7%, respectively.

A variety of "if, then" statements were tested with previous disease, rainfall, and leaf wetness. Because low previous disease resulted in a low percentage of infection even when rainfall was high, the statement, "if TD ≤ 75, then TD = 0," was included in the models. When included, the overall R^2 for 1989 was 75.3% ($y = -0.44 + 0.62\ (TD)^{½} + 0.26\ (R)^{½} \times 100 - 0.57\ (LW)^{½}$, and for 1991 was 65.7% ($y = -53.9 + 1.23\ (TD)^{½} + 0.94\ (R)^{½} \times 100 + 6.78\ (LW)^{½}$. No other statements tested provided any greater increase in overall R^2 values.

When regression analysis was used to compare the 1991 observed values with those predicted by the 1989 equation, disease incidence was greatly underpredicted, especially at high levels of disease. When regression analysis was used to compare 1989 observed values with those predicted using the 1991 equation, the model underpredicted low values and overpredicted high values. Thus, all data from 1989 and 1991 were combined and analyzed by stepwise multiple regression to select a final equation for use in the field. Because leaf wetness explained only a low percentage of the variability and is information not readily available to growers, it was dropped from the equation. The statement "if TD ≤ 75, then TD = 0" was retained. Thus, the final equation was $y = -7.15 + 1.28\ (TD)^{½} + 0.44\ (R)^{½} \times 100$. The observed values for 1989 and 1991 versus those predicted by this equation are presented in Fig. 2. The R^2 for the fit of the predicted values to those of a perfect prediction (predicted = observed) was 57.8% (Fig. 2). The predicted values for various hypothetical levels of disease and rainfall are given in Table 3.

DISCUSSION

An important factor determining the development of postbloom fruit drop is the availability of inoculum. Of the three orchards studied, only one developed significant disease levels, and then in only 2 of the 3 yr studied. In those cases, between 5 and 10% of the flowers were already affected when initial counts were made in early February (Fig. 1). Some disease developed in all orchards in all years, indicating the presence of the pathogen. However, in cases where inoculum levels were low at the outset, disease losses were insignificant. Direct comparisons can be made in the navel and Valencia orchards in Arcadia, which were located in close proximity and where environmental differences were minimal. The navel orchard had many declining trees and young replants, which have a tendency to bloom out-of-season. Thus, whenever warm weather occurred in winter, sufficient off-bloom developed to provide susceptible tissue even in the colder months. Although measurements were made only on healthy, mature trees, the fungus could easily have spread to trees used for evaluation. In contrast, there were few declining trees in the Valencia orchard, and there was virtually no winter bloom to carry over inoculum. Thus, even when rainfall occurred during flowering, disease developed slowly and

Table 2. Correlation coefficients (*r*) and the probability level (*P*) of the area under the curve (AUC) for blossom blight, button count, and fruit count in three orchards affected by postbloom fruit drop

	Navel-Arcadia		Valencia-Arcadia		Navel-LaBelle	
Comparisons	***r***	***P***	***r***	***P***	***r***	***P***
AUC vs. buttons	+0.94	<0.001	−0.26	0.34	+0.45	0.08
AUC vs. fruit	−0.77	<0.001	−0.26	0.31	ND[a]	ND
Buttons vs. fruit	−0.75	<0.001	−0.40	<0.001	−0.89	<0.001

[a] ND = not determined.

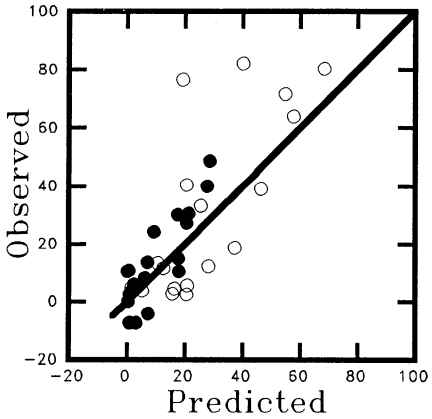

Fig. 2. The observed percentages of flowers affected in 1989 and 1991 in the navel orange orchard near Arcadia and those predicted by the equation $y = -7.15 + 1.28 (TD)^{½} + 0.44 (R)^{½} \times 100$; where TD = total number of diseased flowers per 20 trees, and R = rainfall in mm. The solid dots represent 1989 data, and the open circles represent 1991 data. The R^2 for fit to a perfect prediction (represented by the solid line) is 57.8%.

Table 3. Predicted percentages of blossoms affected using the equation developed, $y = -7.15 + 1.28 (TD)^{½} \times 100$[a], for various hypothetical levels of disease and rainfall

No. of diseased blossoms/tree	Predicted % of blossoms affected at rainfall amounts for previous 5 days					
	0 mm	5 mm	10 mm	20 mm	50 mm	100 mm
1	−1	8	12	18	29	43
5	6	15	19	25	37	49
10	11	21	25	30	42	54
20	18	28	32	38	49	62
40	29	39	43	49	60	73
80	44	54	58	63	75	88

[a] TD = total number of affected flowers on 20 trees; R = total rainfall in mm for the past 5 days.

late in the bloom season. In Lake Alfred, where the slow-growing orange form of *C. gloeosporioides* has never been isolated, no disease developed in any of the 3 yr.

Of the environmental variables studied, only rainfall amounts were highly important in determining disease severity. Temperature and relative humidity were not major factors in explaining disease incidence. The optimum temperature for spore germination is 23 C, but nearly 50% germination occurred over the range of 10–30 C (5). Thus, except for the cool, dry days following the passage of cold fronts, temperatures were probably near the optimum range for much of the flowering period. Leaf wetness explained a significant, but low, amount of the variability in different stepwise multiple regression analyses of the 1989 and 1991 data in the navel orange orchard. Flower infection occurs in 18 hr at 19–30 C (5), and in our study, 10–14 hr of leaf wetness occurred almost nightly. Thus, any heavy dew or fog would provide sufficient leaf wetness to allow infection. However, no large increases in disease were noted following extended periods of leaf wetness. Therefore, the importance of rainfall appears to be primarily in dispersing conidia and secondarily in providing moisture for spore germination.

The final equation developed with stepwise multiple regression explained a substantial amount of the variability, considering that only two variables were included. Thus, with simple counts of the number of affected flowers and the measurement of rainfall for the past 5 days, which is routinely maintained by most growers, a fairly accurate, short-term prediction of disease can be made (Table 3).

Even with an accurate prediction, several questions remain for a grower making decisions on spray applications. First, at what predicted percentage of affected flowers should fungicide applications be initiated? In fungicide trials in these same orchards, we observed up to 30% of blossoms affected (Fig. 1) with no yield loss (11). On the other hand, with that amount of inoculum present, the disease could have increased rapidly with additional rainfall and resulted in substantial crop loss. In 1991 (11), this occurred, and spray programs utilizing one or two applications of benomyl failed to control the disease, and yield loss was great. Five applications of benomyl during bloom controlled blossom blight and increased yield by 300% over the unsprayed control (11). We suggest tentatively that any prediction of 20% disease or greater should trigger a fungicide application.

The second problem is that the model does not take into consideration the amount of bloom on the tree. Early or late in the bloom period, even high percentages of flowers affected would not result in great yield loss. Thus, predictions of high disease levels late in the season can be ignored, because much of the fruit has already been set. However, early in the season such predictions should not be ignored, even though fruit loss would be low. Inoculum buildup can be substantial on the early bloom and can result in severe disease if rainfall follows.

The equation developed in our studies represents a preliminary attempt to predict future disease incidence and could possibly be refined with additional years of data. However, more basic information is needed to develop a more refined model. In this study, we have assumed that all infected flowers produce similar amounts of inoculum. The time required for infected petals to produce conidia and the duration of inoculum production under various conditions needs to be investigated. The relative importance of rainfall rates and durations on conidial dispersal and inoculum production must be determined. Meanwhile, the current equation can help guide growers in determining whether or when to make fungicide applications.

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