

Review Article

## Where have all the flowers gone? Postbloom fruit drop of citrus in the Americas.

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### Abstract

Postbloom fruit drop (PFD), caused primarily by *Colletotrichum acutatum*, is a serious disease annually in the humid tropical citrus areas of the Americas and occurs more sporadically in the humid subtropics. The fungus infects flowers of all citrus species producing orange-brown lesions on petals that result in abscission of the fruitlets leaving the persistent calyx and floral disk attached to the twigs. *C. acutatum* also causes Key lime anthracnose and is morphologically identical to PFD, but the strains can be differentiated by molecular means and pathogenicity tests. *C. acutatum* produces abundant conidia on infected petals that are dispersed primarily by rain splash. After the bloom season, the fungus persists as appressoria on persistent calyces and other vegetative plant parts. Those appressoria are stimulated to germinate by flower extracts and produce secondary conidia to initiate a new disease cycle. Some cultural measures are useful in reducing disease severity, but control is based principally on application of fungicides. Benzimidazole fungicides and captafol were the best materials in the past but are no longer available. The QoI fungicides, folpet and tebuconazole + trifloxystrobin are the most effective among currently available materials. Forecasting systems based primarily on the availability of inoculum and recent rainfall were developed to aid growers on timing fungicide applications. The expert system, PFD-FAD, based on environmental conditions, inoculum, disease history, and varietal susceptibility is the most effective means currently available for scheduling fungicide applications.

### Introduction

Postbloom fruit drop (PFD) was first formally described in Belize by Fagan (1979). However, there are previous reports of a disease that had all of the symptoms of PFD in various areas of Central and South America in the 1950s and 60s (Timmer et al. 1994). The disease is now known to occur in all humid citrus areas from Florida (USA) in the north to Uruguay in the south. It is endemic in many humid tropical areas such as southern Mexico, Belize, Costa Rica, and many islands of the Caribbean. In those areas, it occurs every year and it is one of the main diseases that affect yield. In other areas, it is a more sporadic problem, but when conditions are favorable, the crop losses can exceed 50%. The disease has never been reported outside of the Americas.

### Symptoms

The disease is usually first observed as necrotic peach to orange-colored lesions on open flowers (Fig. 1). Although unopened and even pinhead flowers may be affected, petals on open flowers are the most susceptible to infection. The pistils and stamens of the flowers as well

as the pollen can also be infected (Lin et al. 2001; Marques, Amorim, Spósito, Marin, et al. 2013). Whole flower clusters may be attacked, leaving entire branches with orange to brown petals clinging to inflorescences. After petal fall, the calyces and floral disks which normally abscise if no fruit is set, remain attached to the twig (Fig. 2). These persistent calyces, commonly called buttons, survive for the life of the twig. Thus, signs of the disease are usually present year round even though the infection occurs only during bloom. The buttons are characteristic of the disease and are rarely associated with any other disorder. However, similar buttons of unknown cause have been observed on some pummelos in Florida (Timmer, personal observations). Leaves surrounding infected flowers are often distorted with twisted laminae and enlarged veins.

### Causal Agents

Fagan (1979) reported *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz as the causal agent of PFD based on morphology of the pathogen.



**Fig. 1.** Flowers affected by postbloom fruit drop, caused by *Colletotrichum acutatum*. Note the orange-brown lesions on the petals and concentric rings of acervuli formed on the older lesions. Photos courtesy of MM Dewdney.



**Fig. 2.** Persistent calyces on inflorescences produced following infection of flowers by *Colletotrichum acutatum*. Note that many of the fruitlets have abscised revealing enlarged and swollen floral discs. Some fruitlets may remain attached, but never develop. Photos courtesy of MM Dewdney.

Agostini et al. (1992) differentiated the strains of *Colletotrichum* occurring in Florida and designated them as slow-growing orange (SGO) for the pathogen of PFD, KLA for isolates causing anthracnose on Key lime, and fast-growing gray for the saprophyte and postharvest pathogen. They found that the KLA isolates produced all the symptoms of PFD when inoculated on sweet orange flowers, but SGO did not produce typical lime anthracnose symptoms on Key lime leaves. On the basis of morphology and mitochondrial RFLP analysis, Brown et al. (1996) determined that the causal agents of PFD and KLA were *C. acutatum* J.H. Simmonds and differentiated it from the common saprophyte and postharvest pathogen, *C. gloeosporioides*. However, Lima et al. (2011) subsequently found that in some cases in Brazil PFD could be caused by *C. gloeosporioides*, but it was not as aggressive as *C. acutatum*. McGovern et al. (2012) also reported that the disease in Bermuda was caused by *C. gloeosporioides*. Nevertheless, the primary pathogen in almost all areas including Brazil is *C. acutatum*. Brown et al. (1996) speculated that PFD may have developed in Florida and other areas after *C. acutatum* moved from Key lime to sweet orange.

To test that hypothesis, Peres et al. (2008) collected isolates in the United States (Florida), Brazil (São Paulo),

Mexico, Belize, Costa Rica, and the Dominican Republic to determine if there were consistent genetic differences between PFD and KLA isolates over the geographic area where these diseases occur. The internal transcribed spacers 1 and 2 and the gene encoding the 5.8S ribosomal RNA subunit within the nuclear ribosomal cluster (ITS) and the intron 2 of the glyceraldehyde-3-phosphate dehydrogenase gene were sequenced for isolates from PFD-affected sweet orange and KLA-affected Key limes. Based on the sequence data, almost all isolates clustered into 2 well-supported clades, a KLA and a PFD clade, with little or no sequence variation among isolates within clades over all the areas sampled. In greenhouse inoculations with PFD and KLA isolates from Florida, isolates from both clades produced PFD symptoms on Orlando tangelo flowers, although KLA-clade isolates produced significantly less severe symptoms. PFD-clade isolates were not pathogenic to Key lime foliage, confirming previous studies by Agostini et al. (1992). These studies indicated that PFD and KLA are caused by distinct phylogenetic lineages of *C. acutatum* that are also biologically distinct. Thus, it is highly unlikely that PFD originated from the KLA strain in recent history.

In Brazil, the genetic structure of *C. acutatum* populations collected from sweet orange orchards

showing PFD was determined using 9 microsatellite markers, which enables differentiation of haplotypes and inferences on the predominant mode of reproduction (Ciampi-Guillard et al. 2013). *C. acutatum* populations exhibited a nearly panmictic genetic structure and a high degree of admixture, indicating either ongoing contemporary gene flow at a regional scale or recent introduction from a common source. Shared haplotypes among orchards separated by 400 km suggested natural dispersal of the pathogen, possibly by pollinators. The authors believed that *C. acutatum* was recently introduced into Brazil, but found surprising levels of haplotypic diversity and gametic equilibrium suggesting recombination by parasexual or other means and found a high degree of variability considering such a recent introduction.

Thus, in citrus, we have 3 diseases caused by *Colletotrichum* spp. – PFD, caused mostly by *C. acutatum* and occasionally by *C. gloeosporioides*; KLA, caused by a unique strain of *C. acutatum*; and postharvest anthracnose, caused by *C. gloeosporioides* (Timmer and Brown 2000). PFD has been reported only in the Americas and there are no reports from other continents even though citrus originated in Southeast Asia. KLA has been reported in the Americas, but there is one report from Zanzibar (Wheeler 1963). Thus, the sudden emergence of PFD as an important problem within the last 50 years remains a mystery.

### Disease Cycle and Epidemiology

*C. acutatum* infects flowers readily, producing extensive infection in 48 hours and acervuli in 5 days (Agostini et al. 1992, Zulfiqar et al. 1996). In petals, penetration occurs intra and intercellularly and through the stomata, with intercellular penetration being the most frequent (Marques, Amorim, Spósito, Appezzato-da-Glória 2013). Abundant conidia formed in acervuli on the surface of infected flowers are readily splash-dispersed to healthy flowers (Timmer et al. 1994) (Fig. 3). During the season, the disease continues to spread in a planting as long as flowers are available and environmental conditions are favorable. Conidia deposited on vegetative surfaces form appressoria and quiescent infections that serve as survival structures during periods when flowers are absent. These appressoria will germinate to produce secondary conidia when exposed to petal extracts under moist conditions (Agostini and Timmer 1994; Zulfiqar et al. 1996; MacKenzie et al. 2010). Thus, the first petals produced in the spring probably stimulate the germination of appressoria and the formation of conidia to initiate a new infection cycle. Application of sucrose to leaves inoculated with PFD isolates increased the number of propagules recovered from leaves, suggesting that the response to flower extracts is nutritional (MacKenzie et al. 2010). Thus, the *C. acutatum* on sweet orange functions as a necrotroph on flowers and as a biotroph on leaves (Zulfiqar et al. 1996; Peres et al. 2005).

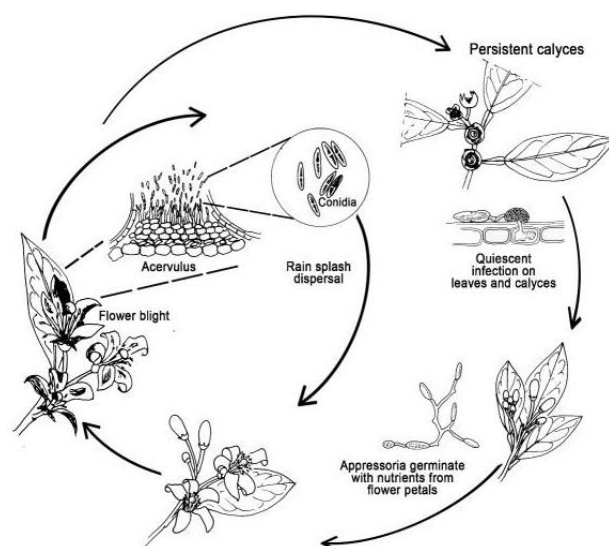


Fig. 3. Life cycle of *Colletotrichum acutatum* on citrus trees (courtesy of LE Chandler; reproduced, by permission, from Peres et al. 2005).

The fungus reproduces primarily on flower petals and does not readily colonize and form acervuli on other tissues (Zulfiqar et al. 1996). Acervuli only form on other tissues when the tissue is killed artificially, and the fungus does not reproduce in the absence of flowers (Zulfiqar et al. 1996; Timmer et al. 1998; Peres et al. 2005). MacKenzie et al. (2010) compared isolates of *C. acutatum* from sweet orange, Key lime, strawberry, blueberry, and leatherleaf fern and found little evidence of cross pathogenicity. Sequences of 3 genes indicated that isolates from the same host were identical or very similar to each other and distinct from those isolated from other hosts.

Conidia are splash-dispersed to healthy flowers within the same tree. Infection is more severe in the lower than in the upper canopy, presumably because of washing of conidia downward. The disease is usually initiated in a season by infection of early flowers produced prior to the primary bloom. Timmer and Zitko (1995) found that the number of persistent calyces on trees was a good indicator of PFD incidence the following season. Those calyces carry large numbers of appressoria from the previous season. Disease spread between trees is probably due mainly to rain splash and wind-blown rain, although equipment and humans can probably spread the disease under moist conditions. Agostini et al. (1993) in Florida found that tree-to-tree spread in plantings was uniform in all directions except when a significant weather event occurred with wind-blown rain. In contrast, Silva-Junior et al. (2010) in Brazil found that initial distribution of infected trees was random and the pattern became aggregated as incidence increased. The difference may be that in Florida, spread was followed in very young plantings with a single source of inoculum, whereas in Brazil, quiescent infections may already have existed on some trees. Long-distance dispersal could conceivably be by insects such as bees that visit flowers and are known to carry conidia of the fungus (Timmer et al. 1994). Many

other insects such as fruit flies can be contaminated with conidia of *C. acutatum* (Peña and Duncan 1989). Pollen can also be infected making the spread by bees and other insects even more likely (Marques, Amorim, Spósito, Marin, et al. 2013). Spread of *C. acutatum* by bees and hives has been confirmed in Brazil (Amorim, personal communication).

The disease is most severe when the bloom extends over a long period of time and rainfall during bloom is extensive. The optimum temperature for growth of *C. acutatum* is 24 to 27 °C (Agostini et al. 1992; Fagan 1979). It is doubtful that temperature has a great impact on disease epidemics (Timmer and Zitko 1993). The pathogen can grow at temperatures as low as 15 °C. While cool temperatures slow down the infection process and conidial production, they also slow down the progress of the bloom offering more opportunities for rain events and infection to occur during the bloom period.

## Control

### *Cultural methods*

The key to avoiding PFD is to have a very compact bloom which starts and is completed in less than a month. With a very short bloom period, there is insufficient time for inoculum to build up and cause a serious epidemic in a planting. This is very difficult to achieve under most circumstances. However, there are some practices which may help restrict the bloom period and reduce disease severity. In some areas, it may be possible to limit irrigation in winter to prevent earlier flowering in the spring. Eliminating declining trees from plantings is also helpful. Trees in decline tend to flower off cycle and build up inoculum for the main bloom. PFD in Florida has resurged after many years of minimal problems in the state, and is probably attributable to increased tree decline and off-season flowering caused by huanglongbing (greening) that is now common in the state. In some areas, overhead irrigation was used which greatly extended the wetting periods and splash dispersed inoculum within trees and to nearby trees. Switching to micro sprinkler or drip irrigation has helped reduce PFD severity.

Virtually all citrus species and cultivars are susceptible to PFD. There is no concrete evidence of differences in the susceptibility of the petals of different citrus species to infection. Nevertheless, disease severity differs greatly between species. Those species or cultivars that flower erratically have more significant damage than are those that have compact blooms. Thus, lemons, limes, Navel oranges, and other cultivars that flower with relatively little stress and produce frequent off-season flowers tend to maintain inoculum levels and have more problems with PFD. In most cases, growers cannot choose more tolerant cultivars since other considerations greatly outweigh their susceptibility to PFD.

### *Effective fungicides*

In the early days in Belize, Fagan (1984) found that benomyl and captafol were the most effective fungicides

for PFD control. Captan, maneb, and other contact fungicides also provided some control of the disease. Timmer and Zitko (1992) in Florida also found that benomyl and captafol were effective for PFD control. The degree of control appeared to be related to the number rather than the timing of the applications based on bloom stage. Where disease was severe, applications at 10-day intervals increased yields 3-fold indicating the potential losses to PFD. Benomyl effects on *C. acutatum* are interesting in that the fungicide does not greatly inhibit the growth of the fungus nor does it inhibit conidial germination or affect appressoria on the leaf surface (Peres et al. 2002). It appears to act by inhibiting infection and early development of the fungus. Surprisingly, *C. acutatum* did not develop resistance to benomyl even after repeated exposure in culture or citrus plantings (Peres, Souza, Peever, et al. 2004). In contrast, *C. gloeosporioides* is highly sensitive to benomyl and resistant strains can be selected in culture or in the field relatively easily.

Although both benomyl and captafol provide excellent control of PFD when properly applied, neither is readily available in most citrus areas. Both have been removed from the market for toxicological or market considerations. Other benzimidazole fungicides such as carbendazim are also effective against the disease (de Goes et al. 2008; Silva-Junior et al. 2014) and would be reasonable substitutes for benomyl. However, in recent years, the use of the benzimidazole fungicides thiabendazole, carbendazim, and thiophanate-methyl have also been restricted. Folpet, a member of the same fungicide group as captan, has also proven quite effective for control of PFD in Brazil (Feichtenberger and Spósito 2000; de Goes et al. 2008). In Florida, Timmer and Zitko (1998) found that QoI inhibitors, such as trifloxystrobin and azoxystrobin, provided some control of the disease, but were not as effective as benomyl. Silva-Junior et al. (2014) found that the mixture of trifloxystrobin + tebuconazole was the most effective product in studies in Brazil. Although many products on the market provide some degree of control of PFD, the fact is that growers have been left with no highly effective materials. Thus, control of a very difficult disease has been made even more problematic.

### *Predictive models*

The incidence of PFD in Florida was best predicted based on the number of infected flowers 3 to 4 days prior to any target date and the rainfall 4 to 8 days before that date (Timmer and Zitko 1993). Temperature and relative humidity did not explain any of the variability in disease incidence, but leaf wetness had a low but significant effect. Thus, it appeared that inoculum availability and rainfall were the main factors in determining disease incidence. Based on this information, a predictive equation was developed:  $y = -7.15 + 1.2 \sqrt{TD} + 0.44 \sqrt{R} \times 100$ , where  $y$  = the percentage of affected flowers,  $TD$  = total number of infected flowers on 20 trees, and  $R$  = rainfall in the last 5 days in inches. They then used this

equation to forecast disease and make fungicide applications (Timmer and Zitko 1996). The predicted and observed incidences were significantly related in 7 of 9 cases. Sprays based on this model reduced PFD incidence and increased fruit counts from 23% to over 500%, depending on the disease severity, while minimizing the number of fungicide sprays.

Because the predictive model developed in Florida to time fungicide applications was difficult to implement by growers and inadequate for use in many other regions, an expert system (PFD-FAD) with broader applicability was developed (Peres et al. 2002). The PFD-FAD system considers previous history of PFD in the grove, susceptibility of the citrus species, the stage of the bloom, rainfall, duration of leaf wetness following the rain, and the current inoculum levels in the grove. It predicts the need for fungicide application based on these factors and the time since the last application. The PFD-FAD system is easy to use and minimizes the need for precise scouting of groves and acquisition of exact weather information, and is more widely applicable to other regions. The PFD-FAD system was compared to the PFD model, a grower's program, and to a non-sprayed control in Brazil in 2001 and 2002 (Peres, Souza, Timmer 2004). The PFD model and the PFD-FAD were equally effective and reduced counts of persistent calyces by about 50% and increased fruit counts. The PFD-FAD is available in English, Spanish, and Portuguese at <http://pfd.ifas.ufl.edu/> for the best available recommendation on timing of spray applications. However, as mentioned above, the fungicides available currently in most citrus areas are not as effective as previously available products.

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