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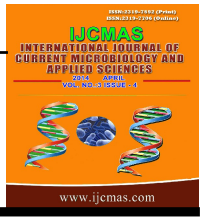


Review Article

Citrus Melanose (*Diaporthe citri* Wolf): A Review

K.Gopal*, L. Mukunda Lakshmi, G. Sarada, T. Nagalakshmi, T. Gouri Sankar, V. Gopi and K.T.V. Ramana

Dr. Y.S.R. Horticultural University, Citrus Research Station, Tirupati-517502, Andhra Pradesh, India
Corresponding author

A B S T R A C T

Keywords

Citrus Melanose; *Diaporthe citri;* post-harvest disease

Citrus Melanose disease caused by *Diaporthe citri* Wolf is a fungus that causes two distinct diseases on Citrus species viz, the perfect stage of the fungus causes melanose, disease characterized by lesions on fruit and foliage and in the imperfect stage; it causes Phomopsis stem-end rot, a post-harvest disease. It is one of the most commonly observed diseases of citrus worldwide. As the disease is occurring in larger proportions and reducing marketable fruit yield hence, updated information on its history of occurrence, disease distribution and its impact, pathogen and its morphology, disease symptoms, epidemiology and management are briefly reviewed in this paper.

Introduction

Citrus Melanose occurs in many citrus growing regions of the world and infects many citrus species. It affects young leaves and fruits of certain citrus species or varieties when the tissues grow and expand during extended periods of rainy or humid weather conditions. The symptoms of this widely distributed fungal disease vary from small spots or scab-like lesions to patterns of damage referred to as tear-drop, mudcake, and star melanose. This is one of the most commonly observed diseases of citrus fruits worldwide. Citrus melanose is caused by the plant-pathogenic fungus *Diaporthe citri* (anamorph = *Phomopsis citri*). It can create severe fruit rind blemishes, but the fungus does not normally affect the pulp. On leaves, the small, black, raised lesions are often surrounded by yellow halos and can cause leaf distortion. On the fruit, the disease produces a superficial blemish which is unlikely to affect the overall yield of processing fruit, but causes external blemishes which reduce the value of fruit intended for the fresh market. This disease is generally of minor economic importance on foliage. All commercial citrus varieties grown in Florida are susceptible to melanose. Pustules are larger on grapefruit, so blemishes tend to be more serious on this species. The disease typically attacks sweet orange (*Citrus sinensis*), grapefruit (*C. paradisi*) and

pummelo (*C. grandis*). The disease reduces the aesthetic quality of fresh fruits for the market, although it does not affect edibility.

History, Taxonomy and Phylogeny

Diaporthe citri Wolf (anamorph: *Phomopsis citri* Fawc.) is a fungus that causes two distinct diseases on Citrus species. In the perfect stage, this fungus causes melanose, disease characterized by superficial fruit and foliar lesions, and in the imperfect stage, it causes Phomopsis stem-end rot, a post-harvest disease (Whiteside, 1988). While melanose was first found and described by Swingle and Webber in 1892 near Citra, Florida (Wolf, 1926), it wasn't until 1928 that Koch's postulates were completed and *Diaporthe citri* was confirmed as the causal agent of melanose on citrus (Bach and Wolf, 1928). In 1912, Fawcett proposed the causal agent of stem-end rot as *P. citri*. Descriptions of two pathogens belonging to the genus *Phomopsis* that caused stem-end rot on citrus were published in 1922, and they were given different species names because of differences in growth characteristics in vitro and pathogenicity in planta. The first was discovered in the Isle of Pines, West Indies, in 1917, on grape fruit, and the name *P. caribaea* was proposed by the author (Horne, 1922). The second was found in 1919 in California on lemons. The fungus was tentatively named *P. californica* (Fawcett, 1922).The first evidence that these two diseases–melanose and stem-end rot - were caused by the same fungus was published by Floyd and Stevens in 1912. The authors were unable to isolate any fungus from melanose infected leaves, but could reproduce classic symptoms of melanose by using washings of stem-end rot affected twigs as inoculum, and from pure cultures of *P. citri* both melanose and stem-end rot could be reproduced on citrus plants. Fawett (1932) compared isolates of *P. citri* and *D. citri* from across the globe as well as *P. californica* and found the teleomorphs indistinguishable. Eight years later Ruehle and Kuntz (1940) showed that the spores of both *P. citri* and *D. citri* caused melanose on citrus leaves.

Because the name *Diaporthe citri* has been applied to melanose and stem rot disease of *Citrus* for decades and Fawcett (l.c.) explicitly stated that he was unaware of any previous *Diaporthe* or *Phomopsis* on *Citrus*, it is proposed that *Phomopsis citri* H.S. Fawc. be conserved against its earlier homonym *P. citri* (Sacc.) Traverso & Spessa.This will make the former name available as a basionym for *Diaporthe citri* (H.S. Fawc.) F.A. Wolf, giving this name priority over what would otherwise be the earlier taxonomic synonyms *D. citrincola* and *Phomopsis caribaea*, both of which predate the currently first legitimate publication as *Diaporthe citri* F.A. Wolf. As no type material of *P. citri* was found at either BPI or FLAS, leaving only an illustration (Fawcett, l.c.) as a potential, but unsatisfactory, iconotype, a recent specimen from diseased *Citrus* sp. Hence, it is proposed as a conserved type for *Phomopsis citri* (Amy Rossman *et al* 2013).

Another synonymous species of *D. citri* is *D. medusaea* Nitschke, and because the latter species was described first, Fisher proposed in 1972 that the name *D. medusaea* should take precedence over *D. citri*. In the same article, Fisher proposed that because *P. cytosporella* was described prior to *P. citri*, the former name should be used. This nomenclature was never adopted. The genus Diaporthe/ Phomopsis

belongs to the phylum Ascomycota, class Sordariomycetes, order Diaporthales, and family Diaporthaceae (James *et al.* 2006; Rossman *et al*. 2007). Combining morphological, cultural and molecular data, four species were distinguished from *F. vulgare*. *Diaporthe angelicae* is shown to be the most common pathogen of this host in Portugal. *Diaporthe lusitanicae* is newly described, whereas the teleomorph of *Phomopsis theicola* was revealed to be distinct from *Diaporthe theicola*, and is described as *Diaporthe neotheicola* (Santos and Phillips, 2009). To date, several molecular phylogenetic studies on genera within the Diaporthales have been published (Zhang and Blackwell, 2001; Castlebury *et al*. 2002; Udayanga *et al.* 2011; Udayanga *et al*. 2012) but none have included D. citri/P. citri. Among the eight described families within the Diaporthales, the family most related to Diaporthaceae is likely Valsaceae (Castlebury *et al*. 2002). Although species of *Diaporthe* have in the past chiefly been distinguished based on host association, Gomes *et al* (2013) studies confirm several taxa to have wide host ranges, suggesting that they move freely among hosts, frequently co-colonising diseased or dead tissue. In contrast, some plant pathogenic and endophytic taxa appear to be strictly host specific. Given this diverse ecological behaviour among members of *Diaporthe*, future species descriptions lacking molecular data should be strongly discouraged (Gomes *et al*. 2013). Multi-locus phylogeny based on combined sequences of rDNA ITS, and partial sequences from the translation elongation factor 1-α, β tubulin and calmodulin genes, reveal three new species, *Diaporthe siamensis, D. thunbergii* and *D. pterocarpicola* from Thailand (Dhanushka Udayanga *et al*; 2012). Multi-gene phylogenetic analysis based on ITS rDNA, tef1-α, β tubulin and calmodulin grouped eight most discrepant strains into three distinctive clusters, cluster 1 (Rc001, Eu004 and Eu009), cluster 2 (ZJWCF252, Sjm001 and Ac001) and cluster 3 (Pcs013 and Sfp005) respectively with high support values. This cluster above represent three potentially novel species provides strong evidence of high biodiversity and novelty of *Diaporthe* endophytes from southeast China (Jiaying Wang *et al* 2014). Six new species of *Diaporthe, D. beilharziae* on *Indigofera australis, D. fraxini-angustifoliae* on *Fraxinus angustifolia* subsp. *oxycarpa, D. litchicola* on *Litchi chinensis, D. nothofagi* on *Nothofagus cunninghamii, D. pascoei* on *Persea americana* and *D. salicicola* on *Salix purpurea* from Australia are described and illustrated based on morphological characteristics and molecular analyses (Tan *et al;* 2013).

Symptoms

Melanose on Leaves: Symptoms on foliage begin as tiny water-soaked specks that become depressed in the center and surrounded by a translucent yellow area which is not depressed. Later, the leaf cuticle ruptures and a gummy substance is exuded which turns brown and hardens. The yellowish margin disappears and the hardened gummed areas will have a sandpaper-like texture. Infected areas on the leaf may be scattered, aggregated, or in streaks, depending on where water transported the inoculums prior to infection. Severely infected leaves become pale green to yellow, can be distorted and fall from the tree. Melanose is seldom severe on the spring growth flush. When it does occur on this flush, the pustules are usually few and little or no leaf drop occurs (Fig. 1). On the summer growth

flushes, melanose can be severe enough to cause defoliation, particularly in years following freeze-induced twig die-back. On fruit there is a tendency for the diseased areas to form tear-streak and water droplet patterns. Young green twigs can be infected. In most cases, it is difficult and not economical to try to control melanose on the foliage.

Melanose on Fruit

Light infestation produces scattered specks on the fruit. Fruit infected when young may remain small and abscise prematurely. Late infection produces flatter pustules. Under severe condition solid patches of blemish are produced, the fruit surface can crack producing a roughened condition called mudcake melanose. Mudcake melanose occurs if infection takes place soon after petal fall (Fig. 1). Melanose on fruit can be distinguished from rust mite injury by the presence of a roughened surface with melanose and a smooth rind blemish with rust mite injury.

Star Melanose

"Star melanose" occurs when copper is applied late during hot, dry weather and is due to copper damage to leaves. It has no relationship to melanose but may resemble symptoms of that disease. Copper causes the developing tissues to become more corky and darker than normal and the shape of the lesion often resembles a star (Fig. 1).

Distribution and impact

D. citri affects all citrus cultivars, although it is most severe in grapefruit and lemons (Mondal *et al.* 2007). The disease is present in most citrus growing areas in about 80 countries. Further, seven of the ten highest citrus producing countries are reported to have *D. citri* (FAOSTAT, 2010). Fruit severely affected by melanose is not marketable as fresh fruit and therefore end up being processed for juicing. This severely reduces market value to 1/5 of the value relative to that of non-blemished fruit. Slightly affected fruit can still be sold in the fresh fruit market, but the value is ¼ that of non-blemished fruit (Kuhara, 1999). This loss of value can cause significant impacts on farmers and fruit exporters. Melanose affects all citrus species, but grapefruit and lemons are the most susceptible. The disease occurs in most citrus-growing areas of the world. It is most severe in moist, subtropical regions, less severe in the humid tropics, and unimportant in arid areas. It usually does not affect tree growth or fruit yield, but can reduce profitability of the fresh-market fruit. In Florida, Timmer *et al*. (1998) reported that a reduction of 10% in the percentage of fresh-market grapefruit by melanose caused a loss of $866 per hectare.

The pathogen and morphology

The fungal pathogen *Diaporthe citri* Wolf is the teleomorph or sexual stage, and the anamorph is *Phomopsis citri* Fawc. A synonym for *D. citri* is *D. medusaea* Nits. The pathogen mostly infects leaves and fruits, but it may also cause a stem-end rot of citrus, and it infests dead twigs as a saprophyte.

D. citri produces two-celled hyaline ascospores, and within each cell are two oil droplets, or guttulae. Ascospores may be slightly constricted at the septum and range in size from 11.5 to 14.2 microns by 3.2 to 4.5 microns (Fig. 2). Ascospores are borne inside the perithecium, a flask

shaped structure. The perithecia are circular, flattened at the base, with long black beaks that taper out. The beaks range from 200 to 800 microns long by 40 to 60 microns wide, and the base of the perithecium typical size ranges from 125 to 160 microns in diameter (Whiteside, 1988; Wolf, 1926). The perithecial stromata stay within the bark of the plant, but the perithecia protrude to the outer surface of the stem and are plainly visible with a dissecting microscope. Because ascospores are forcibly ejected from the asci, they become windborne and are responsible for the long distance spread of the pathogen (Wolf, 1926). Upon finding a suitable substrate, one or both spores may germinate, producing hyphae that quickly become septate mycelium (Whiteside, 1988). In culture, the mycelium is fan-shaped and white in color (Timmer *et al*.2004).

The conidial state of the fungus is the most important for the disease cycle. *P. citri* produces copious in pycnidia. The pycnidia are scattered on the substratum and are dark in color, ovoid, thick-walled and erumpent. Pycnidia form on dead wood and decaying fruit. The fungus produces two types of conidia: alpha and beta. The alpha conidia are functional, single-celled, hyaline, and contain two guttulae. They are the primary means by which the fungus is disseminated short distances, and they range in size from 5 to 9 microns by 2.5 to 4 microns. The beta conidia tend to be produced in older pycnidia than the alpha conidia, and are long, slender rod shaped structures that do not germinate and are hooked at one end. Because the conidia are embedded in a slimy matrix within the erumpent pycnidium, rainy conditions spread the conidia to nearby substrates with great efficiency. The conidia then germinate, forming mycelium on a new substrate and starting the disease cycle over again (Whiteside, 1988; Wolf, 1926).

The fungal mycelia are never abundant in melanose lesions. The production of gum, sparse amounts of mycelia in melanose lesions and the ubiquitous presence of *Colletotrichum gloeosporioides* on the surface of most Citrus leaves and secondary fungal invaders in the fissures created by *D. citri,* caused significant delays in fulfilling Koch's postulates early in the course of the research on *D. citri* (Bach and Wolf, 1928; Whiteside, 1988). Fifteen new species are described, namely *Diaporthe arengae*, *D. brasiliensis*, *D. endophytica*, *D. hongkongensis*, *D. inconspicua*, *D. infecunda*, *D. mayteni*, *D. neoarctii*, *D. oxe*, *D. paranensis*, *D. pseudomangiferae*, *D. pseudophoenicicola*, *D. raonikayaporum*, *D. schini* and *D. terebinthifolii*. A further 14 new combinations are introduced in *Diaporthe*, and *D. anacardii* is epitypified (Gomes *et al.* 2013).

Epidemiology

Susceptibility of citrus tissue

Melanose attacks the foliage, fruit and twigs when they are immature. As the tissues mature they become more resistant to infection. Immediately after petal-fall the young developing fruit are very susceptible to infection and if wet conditions prevail for several days at this stage, serious blemishes on fruit will occur. Fruit rind becomes more resistant to infection eight or nine weeks after petal fall, no further infection of fruit. The symptoms of melanose on fruit can differ according to the time and level of infection. 'Mud cake' melanose develops

when the rind is heavily infected shortly after petal fall, mostly on late bloom fruit. Infection towards the end of the period of susceptibility produces small spots referred to as flyspeck melanose (Whiteside, 1976). A leaf remains susceptible to melanose infection for only about a week after unfolding from the bud. Although immature leaf growth can be infected in the early spring it is not usual for melanose to be active in August to September. However, the late summer leaf flush is often badly infected. Serious defoliation and death of shoots can adversely affect the productivity of trees. This applies particularly to Washington navel oranges. Disease severity is greatly affected by tree vigour. Young vigorous trees need precautionary spraying in early years.

D. citri is primarily a saprophyte that survives on and derives energy from living and dead wood (Kucharek *et al.* 2000; Mondal *et al*. 2007). Perithecia and pycnidia are only produced on dead and dying twigs and on fruit affected by stem-end rot, and because perithecia are rarely formed, the conidia produced by pycnidia are the principal source of inoculum (Bach and Wolf, 1928; Kuhara, 1999). Ascospores are forcibly discharged and are important for long distance dispersal (Kucharek *et al*.2000).

Infection of the disease is initiated when ascospores of the Diaporthe stage or conidia of the Phomopsis stage land on leaf tissue. In dry conditions, or at temperatures below 17°C or above 35°C, the spores die and no infection occurs (Kuhara, 1999). It has been found that the pathogen needs 10 to 24 hours of moisture for the spores to germinate, depending on the temperature (Agostini *et al.* 2003; Kucharek *et al*. 2000). Given sufficient ambient temperature and moisture on the surface, in approximately 36 to 48 hours the spores will germinate, and a germ tube will directly penetrate the cuticle layer (Bach and Wolf, 1928). The hyphae branch out and travel between epidermal cells. The cells are degraded by enzymatic action, and hemicelluloses gum surrounds the infected tissue and forces parenchyma cells apart. At this point, typically 4-5 days post inoculation, the degradation of cells becomes visible as a sunken area on the leaf surface. As the infection progresses, the plants wound response catalyzes the formation of a suberized layer of corky tissue, which grows until it reaches 7 to 12 layers of individual cells. The gum darkens while the normal cells beneath continue growing. This corky layer is pushed out, finally bursting the cuticle, creating small, brown pustules that have the feel of sandpaper.

Disease Cycle

Recently killed young twigs in the tree canopy supports spore production for occurrence of fungal infection. Also, the host tissue must be in a susceptible state. Temperature and rainfall conditions during the period of leaf expansion and during the first 12 weeks after petal fall regulate disease severity. After a spore lands on susceptible tissue, a period of 10-12 hours of moisture is required for infection at 25°C whereas at 15°C 18-24 hours of wetness are necessary for infection. Thus extended wet periods resulting from afternoon rain showers plus dew periods in May and June coupled with warmer temperatures during these months create favorable weather for infection. In contrast, rainfall prior to May in central and south Florida often is associated with fast-moving cold fronts that are quickly followed by temperatures that are too cool

Fig.1 A: Symptoms of Melanose pustules on grapefruit leaf and fruit; B: Speck melanose on grapefruit; C: Mudcake and tear-staining symptoms on grape fruit; D: Star Melanose; E: Tear-Stain melanose; F: Melanose symptoms around the stem end of fruit

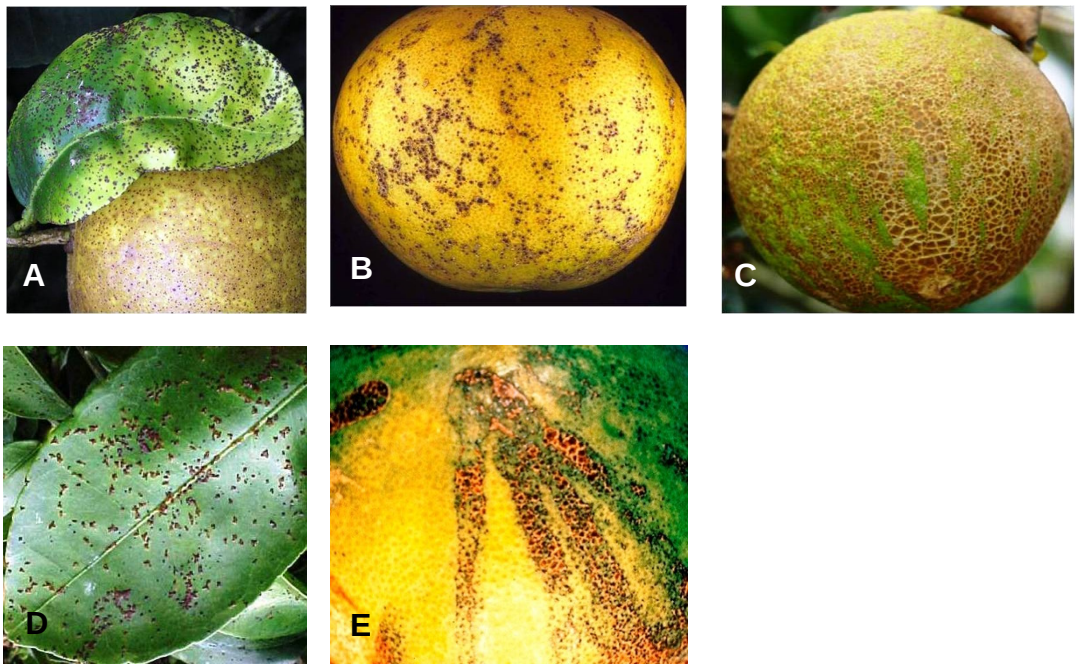
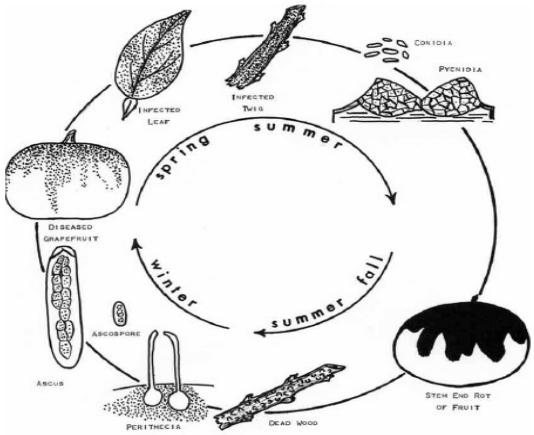


Fig.2A Culture on PDA; B: Conidiophores; C: Conidia

Fig.3 Seasonal development and life cycle of melanose fungus, *Diaporthe citri* (Harry C. Burnett, 1962).



for infection. Also, winds behind the front quickly dry surface moisture on foliage. At temperatures between 24-28°C initial symptoms appear 4 to 7 days after infection.

The fungus *Diaporthe citri* produces ascospores and pycnidiospores and the latter spore is the part of the life cycle that provides most of the inoculums for disease (Fig. 3). Ascospores are formed within perithecia on decaying wood on the soil or on dead branches remaining on the tree. These spores are produced in relatively small numbers and contribute slightly to disease potential in a grove. Their main contribution to disease development relates to spread of the fungus over long distances because ascospores are windborne. Pycnidiospores, on the other hand, are produced abundantly on dead branches within pycnidia. They provide for short distance spread within a tree or from one tree to an adjacent tree by rain-splash. Spores can be washed down over infected branches by rain or irrigation water and spreading them to lower leaves, fruit, or live branch tissue of the tree canopy (Fig. 3).

Therefore, freeze-damaged citrus trees, older groves, and poorly maintained groves with much recently killed wood should be considered high melanose incidence areas. Leaves become resistant to infection once they are fully expanded. Fruit rind becomes resistant about 12 weeks after petal fall, but the later the infection occurs during that 12- week period the smaller will be the resulting pustules. Thus, even though suitable weather conditions exist for infection after late June or early July, melanose will not infect the fruit rind after that time except in years when the bloom is later than normal.

Disease Control

Prevention is better than cure, hence sanitation out and removal of dead wood to remove inoculum of the melanose fungus is important, especially in older trees. Protectant copper sprays are the only product registered for melanose control. Timing of spray applications is very important. With Washington navel and Valencia oranges the spray should be applied at full petal fall. With lemons where lemon scab is also a problem, the initial application should be made at half petal fall. In wet weather, especially if melanose is serious, a follow-up spray should be applied 6–8 weeks after the initial spray. Whiteside (1985) considered it impossible for one treatment to protect the fruit for the whole 9–12 week period of fruit susceptibility. If a treatment is applied at petal fall, the fruit will be too small to retain much fungicide and what little material is retained will soon be dissipated through fruit enlargement, if not by rainfall or irrigation. With the petal fall treatment, only 3–4 weeks protection against melanose can be expected in wet conditions, leaving another 6 weeks for the fruit to be attacked. The copper sprays act as protectants, preventing infection of the young developing fruit. The melanose fungus harbored in the dead wood throughout the tree is little affected by these sprays and they do not reduce the inoculums available from the dead wood. Where melanose leaf infections are likely to be serious on foliage in late summer, a further protectant copper spray should be applied.

Grapefruit is susceptible to melanose infection from fruit set until it reaches 2.5-3 inches in diameter, normally in late June or early July. Fungicides are effective for only short periods when applied to rapidly

expanding fruit or leaves. Since April is usually a low rainfall month and fruit is small and growing rapidly, the first spray for melanose control is not usually applied until mid to late April. One or two applications are sufficient for control on oranges and most tangerines unless the trees have abundant dead wood, like in a year after a freeze. For fresh market grapefruit, the first application should be made when the fruit reaches a diameter of 1/4 to ½ inch. With average quality copper products, usually about 2 lb/acre of metallic copper are needed for each 3-week period. Rates can be decreased if sprayings are given more frequently or increased if sprayings are made less often. Additional sprayings should be given at 3-week intervals until the fruit becomes resistant. For melanose control on large trees, no more than 8-12 lb metallic copper is needed per year even if copper is also used for the control of scab and/or greasy spot. Copper residues are reduced with fruit expansion and as a result of rainfall. It is based on fruit growth models, the rate and time of the last application, and rainfall since the last spray. It has proven helpful for timing of sprays for melanose control. An early June spray of copper to control late melanose damage will serve as the first greasy spot spray. If copper fungicides are applied from May to September, they should be applied when temperatures are moderate (<94°F), at rates no more than 2 lb of metallic copper per acre, without petroleum oil, and using spray volumes of at least 125 gal/acre (Dewdney and Timmer, 2011). Applications of pyraclostrobin to the spring flush growth of citrus trees are much more likely to provide control of melanose, scab, and Alternaria brown spot than those of famoxadone or copper hydroxide (Mondal ,2007). Mancozeb (CHEN Guo-qing *et al* 2010, JIANG Li-ying *et al* 2012) and 24% Fenbuconazole SC (QIN Shao-yuan *et al*; 2012) were the best one for controlling citrus melanose in China.

The strobilurin fungicides, Abound, Gem, Headline, or Quadris Top, are also effective for melanose control and can be used at any time for disease control. Copper fungicides are more economical and are most important for melanose control. However, since copper fungicides applied in hot weather can damage fruit, use of strobilurins at that time will avoid this damage and control greasy spot as well as melanose. Strobilurins appear to have lower residual activity for melanose control than do copper fungicides (Dewdney and Timmer, 2011). Thus, applications may have to be made at shorter intervals, especially when rainfall is high. Further, fungi may develop resistance to strobilurin fungicides. These materials should never be used more than twice in a row, and no more than two strobilurin applications should be used for melanose control.

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