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Citrus Scab (*Elsinoe fawcettii*): A Review.

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Review Article

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ABSTRACT

Elsinoë fawcettii Bitancourt and Jenkins is the causal agent of citrus scab. It is widely distributed, occurring in many citrus growing areas in the world where rainfall conditions are conducive for infection. It affects all varieties of citrus, resulting in serious fruit blemishes and economic losses world-wide. Conidia are produced from the imperfect stage of the fungus, *Sphaceloma fawcettii* Jenkins, and serve as the primary source for inoculation in the field. *E. australis* causing sweet orange scab differs from *E. fawcettii* in host range and is limited to southern areas in South America. *E. fawcettii* rarely causes lesions on sweet orange, whereas *E. australis* attacks all sweet oranges as well as some tangerines and their hybrids. Unlike *E. fawcettii* that induces lesions on all parts of citrus, *E. australis* appears to affect only fruit. In addition, *E. australis* can be distinguished from *E. fawcettii* based on the sizes of ascospores (12- 20 x 15-30 µm in *E. australis*). Furthermore, *E. australis* does not produce spindle-shaped conidia in scab lesions that are often associated with *E. fawcettii. Elsinoë* spp. produces two kinds of conidia: hyaline conidia and spindle conidia. Hyaline conidia of *Elsinoë* spp. are one celled, elliptical, and 2-4 x 4-8 µm and are the primary source for inoculation. Spores are produced on diseased tissue after only one to two hours of wetting. Spores are then dispersed by water splash to healthy young leaves and twigs where the infection takes place. Three to four hours of wetness is required for infection. Citrus is most susceptible at fruit developing stage. Citrus scab affects a variety of citrus species while Sweet orange scab affects primarily orange and mandarines. The best management measure is avoiding entry of contaminated material. Once established, the disease can only be managed by two chemical sprays, the first one when 25 % of the flowers are open, followed by a second spray 7-10 days after. Copper-based fungicides, benomyl, thiram, captafol and chlorothalonil, carbendazim, thiophanate methyl, trifloxystrobin, ferbam, azoxystrobin, trifloxystrobin, pyraclostrobin plus oil are the best chemicals for scab control.

INTRODUCTION

Citrus scab *(Elsinoë fawcettii* Bitancourt & Jenkins) is widely distributed, occurring in many citrus growing countries in the world where weather conditions are favourable for infection. Citrus is one of the

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largest fruit industries in the world. In India, it is the second largest after mango. Over the last 30 years, the area and production under citrus cultivation has increased at the rate of 11 and 9%, respectively, which shows that the expansion of citrus industry was quite sustainable. It is one of the choicest fruits having highest consumer's preference both as fresh fruits as well as for its refreshing processed juice. Collectively citrus fruit in India have an estimated production of 7.47 million tonnes from an area of 0.85 million hectors [1].

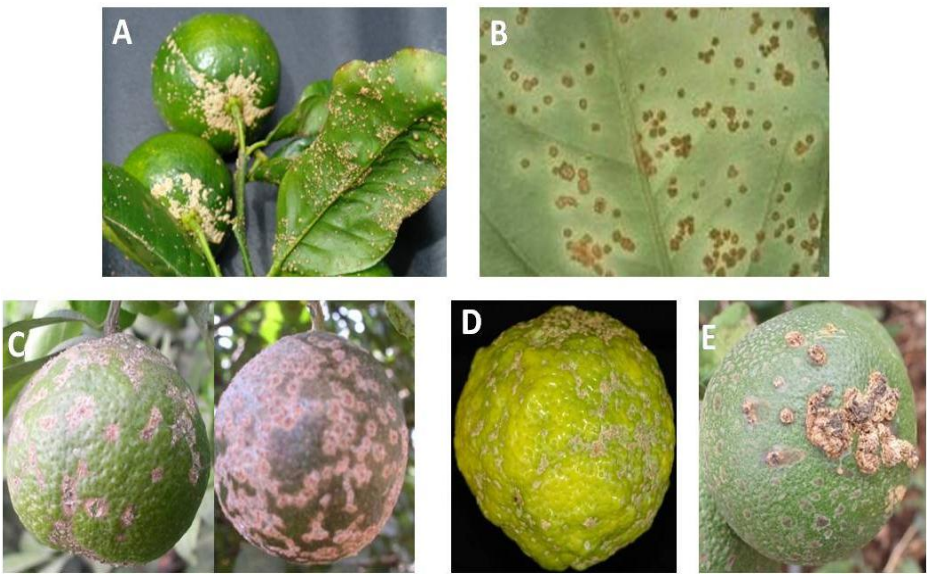
Citrus cultivation in India is plagued with various problems due to limiting growing conditions, scarce water resources and high incidence of pests and diseases warranting great care from planting till the plants come to bearing in order to sustain a productive life of a minimum of 15-20 years.

Citrus cultivation facing plethora of production constraints viz., non-availability of disease free planting material, occurrence of bud wood transmissible diseases and insect pests, general neglect, irregular bearing, scarcity of water and poor management practices, cultivation in unsuitable soils [2,3]. Besides this, citrus species are prone to attack by more than 150 diseases and disorders caused by fungal, viral and few bacterial pathogens right from nursery level to bearing stage resulting in considerable losses [4,5]. In citrus orchards, *E. fawcettii* affects mostly sour oranges and susceptible cultivars of lemons, mandarins, tangelos and grapefruits, whereas most cultivars of oranges and limes are less or not affected. The disease is particularly serious in the nursery on susceptible rootstocks such as sour oranges, rough lemons, *Poncirus trifoliate* and *Citrus limonia*. It may stunt seedlings or make them bushy and difficult to bud. Scabs lesions are present particularly on the young growth. Severely infected fruits are scarred and distorted and consequently unmarketable. *S. fawcettii* var. *scabiosa* has a very similar impact, but on a different host range. *E. australis* differs in more typically causing fruit scab, mainly on oranges.

Citrus scab is widespread in areas where suitable conditions of temperature and rainfall or high humidity prevail. Elsewhere, it occurs when new flush and fruit setting coincide with spells of relatively warm, humid weather. It is also favoured by local conditions such as damp, low-lying soils and dense, shaded citrus groves. Severe outbreaks of citrus scab occur only in areas where susceptible species or cultivars of citrus fruit are grown for the fresh market and where young plants or new growth develop under favourable conditions. Losses largely depend on seasonal and local variations in weather. The disease is not a problem in areas with a limited annual rainfall (less than 1300 mm). In the Mediterranean region and, more generally, in citrus-growing areas with a dry climate, scab even if present, is rare or unimportant. Citrus scab pathogen biology, pathotypes, metabolites of fungi, taxonomy and phylogeny, disease distribution, hosts and host resistance, epidemiology and management are briefly reviewed in this paper.

Symptoms

Figure 1: Scab symptoms on (A) Leaves and fruits of Tangerine (B) Close view of scab pustule on lower side of leaf (C) Scab pustule (late infection) on Rangpurlime matured fruit (D) scab pustule on Roughlemon (E) scab pustule on Sweet orange cv. Sathgudi.



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On many host species and cultivars, *Elsinoë fawcettii* appears as warty pustules that are raised on citrus leaves and fruit (Fig. 1). The lesions typically form along the main veins and change appearance as they age. Lesions on leaf are initially semi-translucent dots that become small nipple-like projections with a cream to a pale yellow color. Lesions can increase up to 3 mm in diameter and develop a scab-like appearance. Older scab lesions are typically cinnamon to honey in color, warty, deeply cracked and will split as they age. Fruit and young shoots can develop similar symptoms, but symptoms on young shoots are not observed as often. The stromatic portion of the pustules are the site of the fungal spore production [6,7].

On grapefruit and sweet orange, the lesions appear to be flattened scabby sheets. The age of the tissues at the time of infection also affects the elevation and size of lesions. The lesions formed on young tissues tend to be raised and those on more mature tissues are flatter. Due to variations among cultivars and infected-tissue age, it is difficult to differentiate citrus scab from other citrus canker on the basis of symptoms alone.

Biology of *Elsinoë fawcettii* causing Citrus Scab

Citrus scab is caused by the pathogenic fungus, *Elsinoë fawcettii* Bitancourt & Jenkins (Anamorph: *Sphaceloma fawcettii* Jenkins) that belongs to the kingdom Fungi, phylum Ascomycota, class Loculoascomycetes, order Myriangiales, and family Elsinoaceae [8]. The teleomorph of *Elsinoë fawcettii* is very rare and has been reported only in Brazil [9,10]. *E. fawcettii* forms stromata that contain numerous spherical asci within the pseudothecial locules. Each ascus harbors eight filamentous ascospores that are hyaline and oblong elliptical with the size of 5-6 x 10-12 µm [11]. *Elsinoë* spp. produces two kinds of conidia: hyaline conidia and spindle conidia. Hyaline conidia of *Elsinoë* spp. are one celled, elliptical, and 2-4 x 4-8 µm and are the primary source for inoculation [6]. *E. fawcettii* is slow-growing on PDA (potato dextrose agar), but it forms characteristic, raised colonies from single spores or small mycelia fragments after about 4-6 days [12]. The organism can be cultivated on a selective media that consists of PDA, dodine, streptomycin sulfate, and tetracycline hydrochloride [6]. On PDA, the colors of the colonies vary among isolates ranging from pale ochraceous to dark vinaceous in the center [12]. Conidia are produced within the acervulus which is typically a flat or saucer-shaped bed of conidiophores growing side by side and arising from a stromatic mass of hyphae [8] and capable of reproducing by formation of a new germ tube [6,11]. In contrast, the colored, spindle-shaped conidia that are produced mainly on scab lesions can germinate to produce hyaline conidia. In culture, *E. fawcettii* produces raised, slow-glowing colonies that are usually beige to tan or vinaceous to black. Isolates of *E. fawcettii* grow slowly in axenic culture, forming < 10-mm colony size in 30 days. Most strains of *E. fawcettii* secrete red pigments after 10-15 day incubation in the light. Identification of citrus scab pathogens is primarily based on their host ranges, because it is difficult to differentiate based on their morphologies. *Elsinoë fawcettii* causing common scab was found in many citrus producing areas worldwide.

Pathotypes

Whiteside [13,14] described two pathotypes, designated as the “Florida broad host range (FBHR)” and the “Florida narrow host range (FNHR)” [15]. The FBHR pathotype mainly attacks the leaves and fruits of lemon (*C. limon* (L.) Burm. F.), sour orange (*C. aurantium* L.), grapefruit (*C. paradisi* Macf.), and Temple/Murcott tangors (*C. sinensis* (L.) Osbeck x *C. reticulata* Blanco), and the fruit of sweet orange (*C. sinensis*). The FNHR pathotype fail to infect sour orange, Temple tangor, and sweet orange fruit [15,16]. All pathotypes of citrus scab attack rough lemon (*Citrus jambhiri* Lush). *E. australis* Bitancourt & Jenkins (anamorph *S. australis* Bitancourt & Jenkins.), causing sweet orange scab differs from *E. fawcettii* in host ranges and is limited to southern South America. *E. fawcettii* rarely causes lesions on sweet orange, whereas *E. australis* attacks all sweet oranges as well as some tangerines and their hybrids [17]. Unlike *E. fawcettii* that induces lesions on all parts of citrus, *E. australis* appears to affect only fruit. *E. fawcettii* produces smaller ascospores compared with those that are produced by *E. australis* (5–6 mm X 10–12 mm vs. 12–20 mm X 15–30 mm, respectively). Only *E. fawcettii* produces dark pigmented and spindle-shaped conidia on scab lesions [18]. The host range and symptoms are considered as the best distinguishing factors between *E. fawcettii* and *E. australis*. Only *E. fawcettii* can cause raised scab lesions on leaves which appear as the papillate pustules described earlier [19]. Furthermore, *E. australis* does not produce spindle-shaped conidia in scab lesions that are often associated with *E. fawcettii.* Molecular studies have been employed recently for identification and differentiation of different species and isolates of *Elsinoë* [15,16,20]. For example: *E. fawcettii* and *E. australis* are differentiated by endonuclease restriction analysis of the amplified internal transcribed spacers (ITSs) of ribosomal DNA. *E. fawcettii* isolated from Florida and Australia could be separated by random amplified DNA polymorphism (RAPD) analysis [16].

Isolates from Florida, Australia, Argentina, and Jeju Island (Korea) were genetically differentiated using random amplified polymorphic DNA markers. *E. fawcettii* from Korea, Florida, and Australia, *E. australis* from Argentina, and Natsudaidai isolates clustered closely within groups, but were clearly distinguishable among groups [20].

Two scab pathogens of citrus, *Elsinoë fawcettii* and *E. australis,* cause citrus scab and sweet orange scab, respectively, and pathotypes of each species have been described. Random Amplified Polymorphic DNA (RAPD) assays clearly distinguished *E. fawcettii* and *E. australis*, and the sweet orange and Natsudaidai pathotypes within *E. australis* also could be differentiated. Specific primer sets, Efaw-1 for *E. fawcettii*; Eaut-1, Eaut-2, Eaut-3, and Eaut-4 for *E. australis*; and EaNat-1 and EaNat-2 for the Natsudaidai pathotype within *E. australis* using RAPD products unique to each species or pathotype were developed. Other primer sets, Efaw-2 and Eaut-5, which were specific for *E. fawcettii* and *E. australis,* respectively, were designed from previously, determined ITS sequences. The Efaw-1 and Efaw-2 primer sets successfully identified *E. fawcettii* isolates from Korea, Australia, and the United States and the Eaut-1 to Eaut-5 primer sets identified both the sweet orange pathotype isolates of *E. australis* from Argentina and the natsudaidai pathotype isolates from Korea. The EaNat-1 and EaNat-2 primer sets were specific for isolates of the natsudaidai pathotype. The Efaw-1 and Efaw-2 primer sets successfully detected *E. fawcettii* from lesions on diseased leaves and fruit from Korea and primer pairs Eaut-1, Eaut-2, Eaut-3, Eaut-4, and Eaut-5 detected *E. australis* from lesions on sweet orange fruit from Brazil [21].

Four pathotypes of *E. fawcettii* and two of *E. australis* have been described to date based on host range. The host specificity and genetic relationships among 76 isolates from Argentina, Australia, Brazil, Korea, New Zealand, and the United States were investigated. Based on pathogenicity tests on eight differential hosts, 61 isolates were identified as *E. fawcettii* and 15 as *E. australis*. Of 61 isolates of *E. fawcettii*, 24 isolates were identified as the FBHR pathotype, 7 as the FNHR pathotype, 10 as the Tryon's pathotype, and 3 as the "Lemon" pathotype. Two new pathotypes, the "Jingeul" and the satsuma, rough lemon, grapefruit, clementine (SRGC), are described, and four isolates did not fit into any of the known pathotypes of *E. fawcettii*. Of the 15 isolates of *E. australis* from Argentina and Brazil, 9 belonged to the sweet orange pathotype and 6 from Korea to the natsudaidai pathotype. *E. fawcettii* and *E. australis* were clearly distinguishable among groups by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) assays and the *E. fawcettii* group was divided into three subgroups, A-1, A-2, and A-3. The A-1 group was composed of the FBHR, FNHR, and SRGC pathotypes; some Lemon pathotypes; and the uncertain isolates. The A-2 subgroup included all of the Tryon's pathotype isolates and one of the three Lemon pathotype isolates and the A-3 group contained the Jingeul pathotype isolates. *E. australis* was differentiated into two groups: B-1, the natsudaidai pathotype isolates, and B-2, the sweet orange pathotype isolates. Isolates of *E. fawcettii* and *E. australis* were clearly distinguishable by sequence analysis of the internal transcribed spacer (ITS) region and the translation elongation factor 1 α (TEF) gene. There were also fixed nucleotide differences in the ITS and TEF genes that distinguished subgroups separated by RAPD-PCR within species. Two species of *Elsinoë*, two pathotypes of *E. australis*, and at least six pathotypes of *E. fawcettii* were confirmed and described their distribution [22]. Li-Yuan Wang *et al.* [23] reported the existence of novel pathogenic variant or cryptic subspecies in *E. fawcettii* that were not previously described in Florida. A new disease of jojoba in Australia was caused by *Elsinoë australis,* a pathogen which is normally associated with citrus. The isolates from jojoba represent a new pathotype of *E. australis* (Ash, 2012).

Taxonomy and Phylogeny

Elsinoë fawcettii was first discovered in 1935 on the ripe rind of a Satsuma orange, *citrus nobilis unshiu* Sw., in M'Boy, São Paulo, Brazil. Bitancourt and Jenkins [9,10] described the disease, it is associated with its anamorph *Sphaceloma fawcettii* var. *fawcettii*. The disease known by many common names in various languages: Citrus scab, common citrus scab, sour orange scab, Gale commune des agrumes, Zitrusschorf, Verrugose dor citros, and Costra o roña de los ágrios. This fungus belongs to the phylum Ascomycota, the Class Dothideomycetes, the order Myriangiales, the Family Elsinoacea, and the genus *Elsinoë*. *E. fawcettii* has not yet been included in a phylogenetic study on a genus or species level. However, the class Dothideomycetes and the order Myriangiales have been included in a few phylogenetic studies [25,26].

The Dothideomycetes class contains a heterogeneous group of fungi that can exist in almost every fungal niche around the world [26]. They are most typically found as pathogens, endophytes, or epiphytes of

living plants. Additionally, they have also been found as saprobes in dead or partially degraded plant matter in leaf litter or in dung degrading cellulose. Many species are described as being lichenized, but others in the class occur as parasites on fungi or on animals [25]. The order Myriangiales has also been included in these studies; it is reported to be most closely related to the order Dothideales. In these studies, the node that supports the Dothideales, Capnodiales, Myriangiales, and Mycosphaerellaceae is heavily supported. The order Myriangiales includes organisms that are saprobes, epiphytes and biotrophs [25,26]. The anamorphs in this order, like the anamorph of *Elsinoë sp*., are typically acervular coelomycetes which are asexual fungi that form conidia in pycnidia or acervuli [25,27]. Phylogenetic analysis based on the ITS1 region clearly differentiated isolate Ef41 from the other *E. fawcettii* isolates. Ef41 also displays very distinct extracellular activities of cell wall degrading enzymes and proteases, indicating the presence of novel pathogenic variant or cryptic subspecies in *E. fawcettii* [23].

Citrus scab hosts and disease distribution

Hosts of scab pathogens: List of citrus hosts of different scab pathogens is given in Table 1.

Distribution of Scab disease pathogens: The disease has been reported from many citrus growing regions in the world (Table 2).

Host resistance

Out of 11 Rangpurlime strains, Rangpurelime Florida, and Rangpurlime Texas were found moderately resistant and Shomyndong, Milam, Khatazamir, Brazilliam Rough lemon and Chase Rough lemon among 18 rough lemon types were found free from leaf and fruit infection under south Indian conditions [28]. Highly susceptible citrus types are some tangerine (*C. reticulata*) varieties such as Fremont, Clementine and Murcott, Rough lemon (*C. jambhiri*), Tangelo (*C. reticulata* x *C. paradisi*) (variety Orlando), Tahitian lime (Persian lime, *C. latifolia*), Rangpur lime (Mandarin lime, *C. limonia*), Frost Satsuma mandarin (*C. unshiu*). Not susceptible or immune citrus types are sweet orange, navel orange (*C. sinensis*), Pummelo, shaddock (*C. grandis*), Grapefruit, pomelo (*C. paradisi*). Some lime and tangerine varieties at Waiakea are not symptomatic. Citrus scab resistance was observed in *Citrus deliciosa, C. tangerina, C. nobilis*; a mandarin hybrid (*C. nobilis x C. deliciosa*) and a Satsuma hybrid (*C. unshiu x C. sinensis*) [29].

Table 1: List of citrus hosts plants of scab pathogen

Scab Pathogen	Citrus Hosts
Elsinoë fawcettii	Sour oranges (*Citrus aurantium*), grapefruits (*C. paradisi*), lemons (*C.limon*), mandarins (*C. reticulata*), oranges (*C. sinensis*) and tangelos (*C. paradisi* x *C. reticulata*), calamondins (*C. madurensis*), *C. hystrix*, *C. limonia*, *C. nobilis*, *Poncirus trifoliata*, rough lemons (*C. jambhiri*) and satsumas (*C. unshiu*), *C. latifolia*, *Fortunella margarita*, oranges and pummelos (*C. maxima*).
Elsinoë australis	Oranges (*C. sinensis*), sour oranges (*C. aurantium*), lemons (*C. limon*), mandarins (*C. reticulata*), satsumas (*C. unshiu*), limes (*C. aurantiifolia*), grapefruits (*C. paradisi*) and *Fortunella*.
Sphaceloma fawcettii var. *Scabiosa*	Lemons (*C. limon*) and rough lemons (*C. jambhiri*), and sour oranges (*C. aurantium*).

Epidemiology

Source of inoculums and spread: Fungal spores are readily produced on the surface of scab lesions on young fruits and leaves throughout the year (Fig. 2). The Rough lemon and Rangpurlime rootstock are also highly susceptible to scab and can act as a source of inoculums. Spores of the fungus are spread in the orchard by rain, overhead irrigation and spraying drift [30]. Dew may also cause the spores tobe liberated from the lesions but due to the limited splashing action, there would only be localised dispersal [30]. Some dry spores can also be spread by winds in excess of 2 m/s [30,31].

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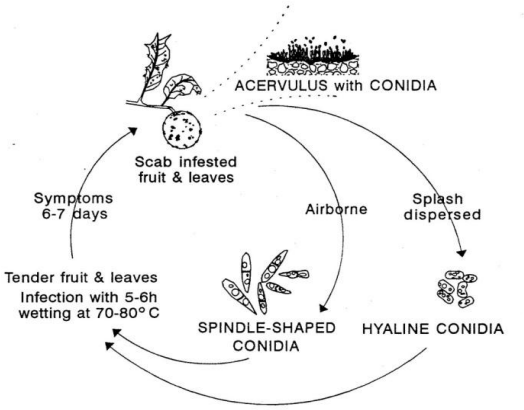

Figure 2: Life cycle of citrus scab (*Elsinoe fawcetti)* disease (Courtesy APS).

Infection periods: Leaves are most susceptible to infection just as they emerge from the bud up until they are 25% expanded and become tolerant before reaching full size. Small immature lemon fruits are susceptible to infection from half petal-fall to about three months later. Spore germination and to cause infection the scab spores need a wetting period from rain, overhead irrigation or spraying of at least 4 hours continuously or 1–2 hours followed by 3–4 hours within the next 24 hours [12]. Short period of wetting of foliage by non-fungicidal spray treatments has also been found to promote infection [30]. Temperature does not appear to have a greater role on the disease but spores germinate quicker at higher temperatures. The severity of infection by citrus scab depends on the inoculum load in the trees and the amount and frequency of wetting periods, whilst the growth flush and fruit rind are more susceptible to attack [30]. The extended bloom period in lemons makes accurate timing of sprays difficult.

Table 2: Distribution of Scab pathogen in different citrus growing regions in the world.

Region/continent	Scab Pathogen		
	Elsinoë fawcettii	*Elsinoë australis*	*Sphaceloma fawcettii* var. *Scabiosa*
EPPO region	Spain (Canary Islands only), all records of this pathogen in the region (Greece, Italy, Lebanon, Morocco, Russia) are dubious.	Italy	Absent.
Asia:	Bangladesh, Brunei Darussalam, Cambodia, China, Georgia, Hong Kong, India, Indonesia, Japan, Korea Democratic People's Republic, Korea Republic, Lao, Lebanon, Maldives, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Viet Nam, Yemen.	India (Tamil Nadu; dubious)	Hong Kong, Indonesia, Malaysia, Sri Lanka
Africa	Ethiopia, Gabon, Ghana, Kenya, Madagascar, Malawi, Morocco, Mozambique, Nigeria, Sierra Leone, Somalia, South Africa, Tanzania, Uganda, Zaire, Zambia, Zimbabwe.	Ethiopia	Comoros, Madagascar, Malawi, Zambia, Zimbabwe.
South America	Argentina, Bolivia, Brazil (Bahia, Ceará, Espiritu, Santo, Minas Gerais, Rio de Janeiro, São Paulo), Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, Uruguay, Venezuela.	Argentina, Bolivia, Brazil, Ecuador, Paraguay, Uruguay	Argentina, Brazil
Oceania	American Samoa, Australia, Cook Islands, Fiji, French Polynesia, Guam, Micronesia, New Caledonia, New Zealand, Papua New Guinea, Samoa, Solomon Islands, Vanuatu.	Cook Islands, Fiji, New Caledonia, Niue, Samoa	Australia, Fiji, New Caledonia, Papua New Guinea, Solomon Islands.
EU	Present.	Absent	Absent.
Central America and Caribbean	Barbados, Belize, Cayman Islands, Costa Rica, Cuba, Dominica, Dominican Republic, El Salvador, Grenada, Guadeloupe, Guatemala, Haiti, Honduras, Jamaica, Martinique, Nicaragua, Panama, Puerto Rico, St. Lucia, Trinidad and Tobago.	Dominica	Absent.
North America	Bermuda, Mexico, USA (Alabama, Florida, Georgia, Hawaii, Louisiana, Mississippi, Texas).	Absent.	Absent.

Young tissue is susceptible, regardless of tree age. Scab pustules produced on spring flush serve as inoculums for infection of fruit [32]. Spores of *E. australis* may be dispersed over short distances by air [17]. *E. australis* overwinters in the tree canopy on fruit and other plant organs that were infected during the previous season. Inocula consist of conidia, and presumably ascospores, from scabs formed on leaves, twigs and fruit. In laboratory studies, scab was most severe at temperatures from 23.5 to 27 °C and much less severe at 17, 20, 30, or 32 °C. A leaf wetness duration of 4 h was sufficient for some infection, but 12

h of leaf wetness were needed for maximum infection. In field studies, grapefruit seedlings with new shoots were placed beneath trees weekly, and disease severity was evaluated in relation to environmental factors [33,34].

Relationships between diseases incidences measured at two levels in a spatial hierarchy are derived. These relationships are based on the properties of the binomial distribution, the beta-binomial distribution, and an empirical power-law relationship that relates observed variance to theoretical binomial variance of disease incidence. And it is shown that the effect of aggregation of incidence at the lower of the two scales is to reduce the rate of increase of disease incidence at the higher scale [35].

Disease Control

It is a more prevalent disease in humid citrus-growing areas in many countries. Although the damage produced by scab is superficial and does not affect internal fruit quality, it reduces acceptability for the fresh-fruit market. Prophylactic protective copper sprays are the only products registered to control scab in citrus. Since copper is a protecting fungicide the entire fruit surface needs to have a continuous coating of copper in order to be protected from infection by the scab spores. The protective copper coating does not expand as the fruit grow so unprotected gaps on the fruit surface will occur and these areas will be susceptible to infection. So, the protective copper layer may need to be reapplied to the growing fruit during the susceptible stage. It is endemic in Florida where climatic conditions are highly favourable for the pathogen cycle and disease. The disease predominantly affects summer growth flushes as summer rain showers frequently occur in Florida and produce sufficient wetness for conidial germination to cause infection. The most effective strategy recommended for scab management is to remove and destroy the inoculums sources. Frequent applications of fungicides are absolutely needed to manage the disease if the fruit is intended for the fresh market. Several fungicides such as thiophanate methyl, azoxystrobin, trifloxystrobin, pyraclostrobin, ferbam, and copper fungicides can be used for control of citrus scab [32]. The disease was effectively controlled by spraying Macuprax (0.3%) or Burcop (0.3%). The fungicides left a longer residual effect for 30-40 days on the foliage and enabled a reduction in the frequency of sprays [36]. Two sprays of hexaconozole (0.01%) + streptomycine sulphate (100 pp) one at one month after fruit set and second at one month after first spray reduces scab incidence effectively under south Indian conditions [37]. Chemicals and bio agents are reported to be effective against citrus scab [38].

The problem with controlling scab in lemons is the extended flowering and multiple cropping habits of these trees in warmer areas and the decision of when to apply the protective sprays. In areas where there is only one main crop a year a control strategy is simpler to implement. Obviously it is highly unlikely that the first spray would protect the fruit continuously for 6 weeks in wet conditions. The best control strategy is to get the first on weather conditions. For example, if the weather conditions are generally dry after the first spray and rainfall events are unlikely to cause a spore release then a second spray at 6 weeks may be all that is required. However if the weather is rainy and it is likely to trigger an infection then the second spray may need to be applied earlier. The relationship of increase in fruit yield was directly and linearly correlated with percentage of reduction in severity of scab and dieback diseases. Based on reduction of disease severity and increase of fruit yield, Allamanda leaf extract was noted as most effective material followed by carbendazim 50%WP**,** propiconazole**,** Cupravit 50 WP and mancozeb [39]. Trees need to be regularly pruned to keep them open and free of deadwood, which to reduce the source of inoculums, allowing for better air movement within the tree and better spray coverage inside the tree.

The effectiveness of benomyl at 0.96 g a.i./liter, fenbuconazole at 120 mg a.i./liter, and azoxystrobin at 235 mg a.i./liter was evaluated on potted seedlings of Rough lemon for scab and on grapefruit for melanose under greenhouse conditions and found that Benomyl was effective if applied up to 72 h after inoculation, and fenbuconazole and azoxystrobin were effective if applied within 16 to 48 h after inoculation. Use of post infection sprays under field conditions appears to be promising for scab control [32]. Products that induce disease resistance in plants were evaluated on potted seedlings of rough lemon for citrus scab, caused by *Elsinoe fawcettii*; grapefruit for melanose, caused by *Diaporthe citri*; and Dancy tangerine for Alternaria brown spot caused by *Alternaria alternate* pv. *citri*. Oxycom and Messenger controlled scab well in some tests. Products that induce host resistance may be useful for disease control in citrus in an integrated program with standard fungicides [33]. However, fungicides are often not adequate for disease control. Application of fungicides also raises concerns on development of resistance in the pathogens and environmental hazards [40]. Tyson and Fullerton [41] reported benomyl resistance in *Elsinoe fawcettii* in New Zealand citrus orchards for the first time. Concerted efforts are being made for the development of transgenic citrus resistant to scab pathogen, *Elsinoë fawcettii* [42]. Finally to say there is no

hard and fast rule in scab control; it is a convenience and necessity of which crop needs protecting and putting the sprays on at the right time and then fine-tuning the program according to local weather conditions [43].

Secondary Metabolites of Fungi

Several fungal secondary metabolites have been shown to be associated with fungal development [44]. Furthermore, dark brown-melanin pigments produced by many fungi by oxidative polymerization of phenolic compounds function as fungal virulence factors due to their ability to stimulate production of conidia (e.g., *Alternaria alternata*), appressoria (e.g., *Colletotrichum lagenarium*), sclerotia (e.g., *Scherotium* spp.), and sexual bodies (e.g., *Sordaria macrospora*) [45,46,47,48,49]. Melanin apparently contributes to the survival of the fungal spores by protecting against UV light damage. Cercosporin phytotoxin produced by many pathogenic *Cercospora* spp. is not required for spore development but plays a critical role during fungal pathogenesis. Phytotoxins produced by many phytopathogenic fungi often damage plant cells or influence the course of disease development and the formation of symptoms.

Elsinochromes Produced by *Elsinoë fawcettii:* Many *Elsinoë* fungal species produce light-activated, red/orange pigments, called elsinochromes. Elsinochrome pigments containing a phenolic quinine chromophore consist of at least four derivatives (A, B, C, and D). The bright red pigments, elsinochromes A, B, and C were originally isolated from cultures of a pecan pathogen, *Elsinoë randii* and their chemical structures and physical properties have been well documented [50,51]. Elsinochromes B and C can be quickly oxidized to A by chromium trioxide [51]. In contrast, elsinochrome D, likely derived from elsinochrome C by forming a methylenedioxy ring, is an orange pigment produced only by some *Elsinoë* species [52]. Although elsinochromes structurally resemble many photosensitizing perylenequinones, their toxicity has never been investigated. Photosensitizing compounds are a group of structurally diverse compounds and natural products that are able to absorb light energy and are converted to an electronically excited triplet state. The activated photosensitizers then react with oxygen molecules in two different ways to form both radical and non-radical species of activated oxygen, including superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen [53,54]. Activated oxygen species have general toxicity, as they react with biomolecules common to all cells, including lipids, proteins, and nucleic acids, and often result in cell death [55]. The toxicities of radical oxygen species have been well known. Several lines of evidence suggest that the non-radical-singlet oxygen ($1O_2$) is also highly toxic to cells [55].

Investigations on environmental factors affecting production of elsinochromes by *Elsinoë fawcettii* showed that Addition of ions such as $CaCl_2{\cdot}2H_2O$, $Ca(NO_3)_2{\cdot}4H_2O$, $CoCl_2{\cdot}6H_2O$, or LiCl decreased ESC production, whereas other ions tested markedly enhanced ESC production. Production of ESCs was also affected by the presence of multiple colonies and the distance between two colonies on the same agar plate, indicating that nutrient competition resulting in nitrogen depletion promoted ESC production. The genes viz., *RDT1*, *TSF1*, *PRF1*, *ECT1, EfPKS1,* and *EfHP1* are required for ESC biosynthesis and accumulation. All pathogenic isolates produces ESCs in cultures and/or *in planta*, further confirming the important role of ESCs in fungal virulence. Moreover, as assays on detached rough lemon and grapefruit leaves, co-inoculation of a low virulence isolate attenuated disease severity caused by the highly virulent isolate [56,57].

Citrus scab caused by *Elsinoë fawcettii* is cosmopolitan in humid citrus-growing areas. Citrus scab, caused by *Elsinoë fawcettii* (anamorph *Sphaceloma fawcettii*), is a common foliar fungal disease affecting many citrus cultivars, including grapefruit. The disease results in severely blemished fruit which reduces its marketability. Transgenic 'Duncan' grapefruit trees expressing the antimicrobial *attE*gene were produced via *Agrobacterium*-mediated transformation.

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