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Epidemiology and Control of Citrus Black Spot in South Africa

Black spot of citrus (CBS) was first noticed and described in Australia nearly 80 years ago; it has inflicted heavy losses there for many years, particularly in the coastal regions of New South Wales and Queensland. CBS appeared 30 years later in South Africa, first along the humid coastal regions of Natal, then spreading inland and later becoming a major crop destroyer in eastern and northern Transvaal, the main citrus-producing areas of the country. The disease has been known to occur in Zimbabwe for many years but did not reach epidemic proportions until 3 years ago in the northern areas.

CBS also occurs in Swaziland, Mozambique, the People's Republic of China, Indonesia, and Japan but has not yet appeared in the United States or become an important disease in the western region of any continent. Should the disease become established in the United States, the climatic conditions of Florida seem to favor an epidemic more than those of any other citrus-growing state. Climatic conditions in Brazil, a major citrus-producing country, seem particularly favorable for CBS. The chances of future occurrence of this important fungal disease in major citrus-producing countries largely depend on effective application of quarantine measures and the initial steps taken when the first outbreaks occur.

One of the interesting features of CBS is that the causal fungus may be present for many years in a particular area before symptoms appear. It may take 5–30 years from the time the first symptoms are noticed until the disease reaches epidemic proportions, depending on the presence of lemons (*Citrus limon* (L.) Burm. f.) and climatic conditions (Fig. 1). In western Transvaal, symptoms had been observed for over 30 years before control measures became necessary. Once epidemic proportions have been reached, the disease remains serious unless effective control measures are applied. CBS advances slowly but never retreats. There is not a single example in South Africa of CBS disappearing or declining after the epidemic stage has been reached.

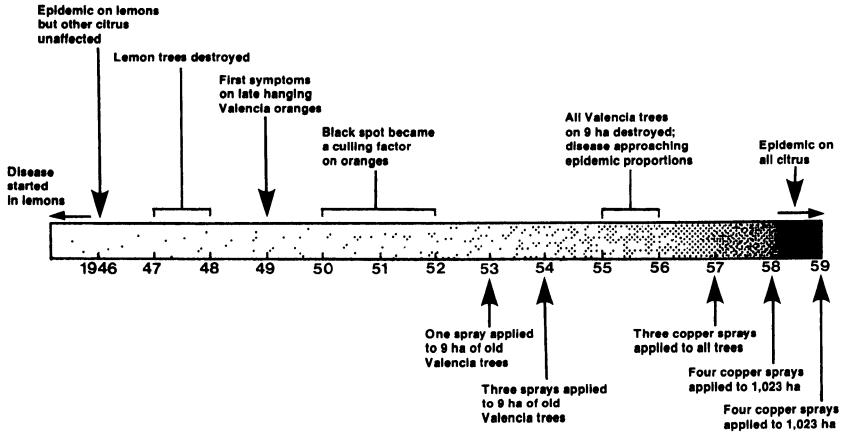

Fig. 1. Establishment of an epidemic of citrus black spot on a large citrus estate in South Africa, where conditions were ideal for the disease.

CBS is almost exclusively a fruit disease. It causes unsightly lesions on the rind that spoil the sales appeal of the fruit, but it seldom causes postharvest decay, even though the rind may become extensively necrotic. Citrus is produced in South Africa as an export commodity, and export markets demand high standards. Despite the fact that the internal quality is not affected by CBS, fruits with lesions and spots are unacceptable for export. Conversely, black spot symptoms may be taken as a sign of fruit maturity, as disease development increases with fruit maturation. The economic importance of CBS has much to do with human psychology. If spotted oranges become fashionable, CBS will lose most of its economic status as a disease.

Except for sour orange (*C. aurantium* L.) and its hybrids, all commercially grown *Citrus* spp. are susceptible. Lemons are particularly susceptible, and heavy losses may occur on Valencia and navel oranges (*C. sinensis* (L.) Osbeck) and grapefruit (*C. paradisi* Macf.)

The Pathogen

The sexual stage of the pathogen was discovered by Kiely in 1948 in New South Wales, and he named it *Guignardia citricarpa* Kiely. The imperfect stage of the causal organism was known as *Phoma citricarpa* McAlp., but the name was later changed to *Phyllostictina citricarpa* (McAlp.) Petrak.

Research results by Wager (8) and Kiely (3) brought to light that *G. citricarpa* occurs in countries or regions within a country without any evidence of disease. Furthermore, the fungus was latent in 21 plant families of cultivated and wild plants (7). More confusion was added when Kiely reported that ascocarps were never found in axenic culture, whereas Wager could produce numerous ascocarps with ease on artificial medium. *G. citricarpa* became a controversial subject, and apparently conflicting information appeared.

In 1964 McOnie (5) cleared up the mysteries by describing two morphologically identical *G. citricarpa* strains that infect citrus. One causes CBS and the other does not cause symptoms. The latter has an exceptionally wide host range, and the sexual stage develops freely in axenic culture. McOnie's work has great significance for international trade and the application of quarantine

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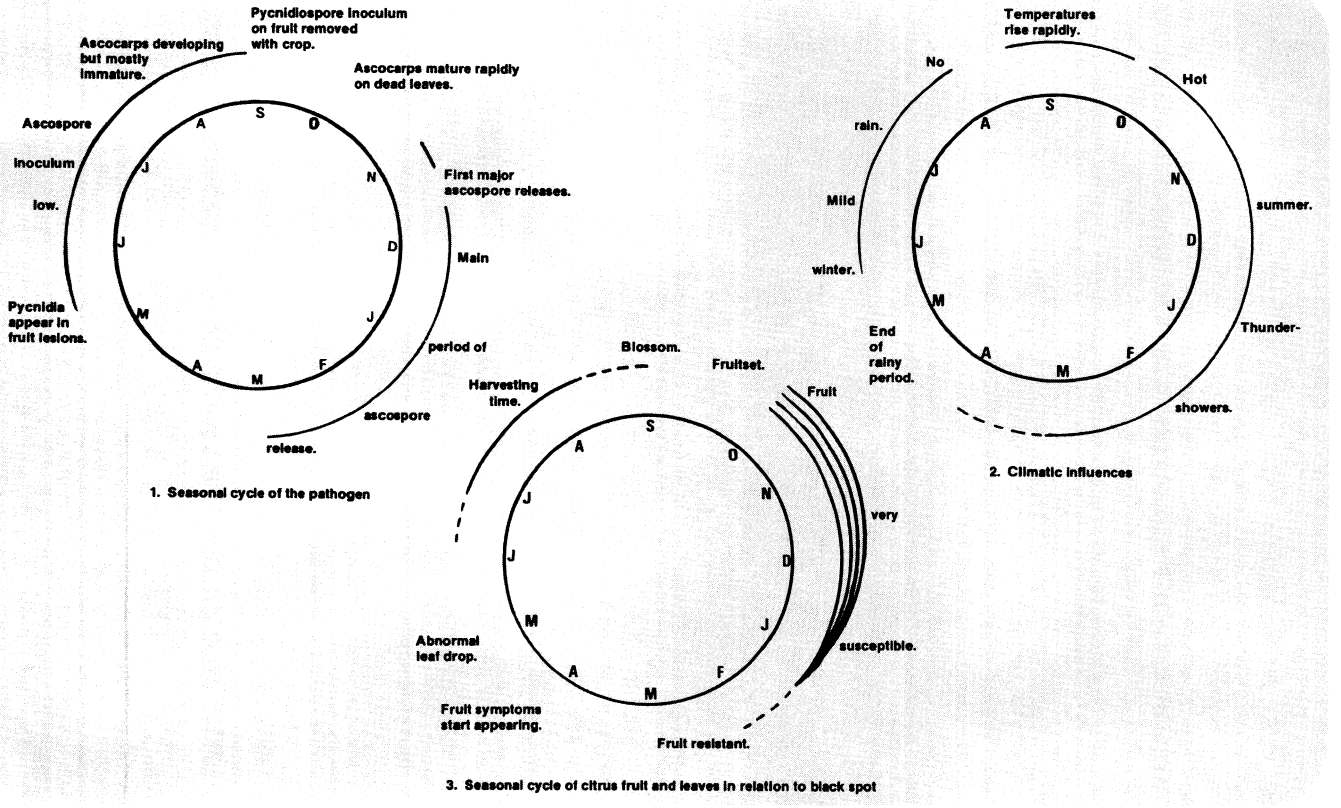

Fig. 2. The three components of a black spot epidemic: seasonal cycle of the pathogen, climatic influences, and seasonal cycle of citrus fruit and leaves in relation to black spot.

measures. Countries or regions within countries listed as "infected" could in fact be totally free from the virulent strain. The western Cape Province of South Africa is such an area; the avirulent strain is widespread there but the virulent strain is absent, so no disease occurs.

Understanding the Epidemiology

To understand the epidemiology of CBS, one must consider the availability of inoculum, the climatic conditions required for infection, and the growth cycle of a citrus tree and, particularly, fruit development in relation to disease development (Fig. 2).

Infection may come from ascospores and pycnidiospores. The relative importance of these two forms of inoculum was often misunderstood. Each has a role in establishing an epidemic, but once epidemic proportions have been reached, the importance of pycnidiospores is usually eclipsed by that of ascospores. Pycnidiospores may occur in fruit lesions (Fig. 3), on dead twigs and leaves, and, occasionally, on fruit stalks and in lesions on attached leaves. By far the most pycnidiospores are produced on dead citrus leaves on the orchard floor and, if present, on infected mature fruit.

Citrus fruits start maturing in South Africa during autumn and winter, and fruit with lesions and pycnidia are usually removed from the trees a month before the new season's fruit set. Pycnidiospores have no special mechanism of release into the atmosphere, and those occurring on dead leaves on the ground can reach the susceptible fruits only by the splashing of raindrops. Under normal orchard practices in South Africa, the odds are against pycnidiospores becoming an important source of inoculum. Pycnidiospores may be a source of inoculum where out-of-season fruit or late-hanging fruit with lesions remain on the trees after blossoming and fruit set, but such situations seldom occur in South Africa, where fruit is produced for export purposes.

Ascospores are produced on dead leaves on the orchard floor and represent the main source of inoculum once the disease has reached the epidemic stage. Whiteside (9) investigated CBS in Zimbabwe in 1965 and could find only small numbers of ascospores in infected orchards; he stated then that losses from CBS had been very slight. In 1978, however, an epidemic was well established and ascospores were abundant in the same areas.

Citrus leaves drop all year round, and ascocarps develop within 50–180 days on the leaf litter. Mature ascocarps may occur anytime during the year in a citrus orchard, but their presence and abundance depend on the frequency of moistening and drying (rain, irrigation, etc.) as well as on prevailing temperatures. Intermittent wetting and drying of leaves is believed essential to ascocarp development. There is a tendency in the Transvaal for leaves to drop more than usual during May because of red mite (*Panonychus citri* McGregor) damage. I (4) showed that leaves dropping in late autumn to early winter produce mature ascospores in November, the time when the young fruit are particularly susceptible. In New South Wales, Kiely (3) found that heavy dew is sufficient for development, maturation, and discharge of ascospores.

In the Transvaal, ascocarps develop slowly during the cool, dry winter months and may reach maturity only after the leaves have been on the ground for up to 6 months. Sometimes the leaves become decomposed before ascospores mature, and applying chemicals to accelerate decomposition may reduce inoculum. In South Africa, ascospores are discharged only during rain spells. The amount of rain has little effect on the number of spores released provided moisture is sufficient to wet the leaf litter. Continuous heavy showers seem to adversely affect spore discharge and reduce the spore load in the air. Spore discharges commence within the first hour after the leaf litter has become wet and may continue for 12 hours or longer. Kiely thought ascospores were in the atmosphere all the time, but detailed studies with spore traps showed that ascospores can be released only when the leaves harboring the ascocarps become wet (4). On rare occasions, ascospores are trapped during irrigation.

Ascospore discharges are closely influenced by the rainfall pattern. The rainy period in the Transvaal lowveld is from October to April, although occasional rains may occur outside this

period. Ascospore discharges are at a peak during November to March. In 9 out of 10 seasons no significant spore releases occur before November, but during the first 2 weeks of November a major discharge can be anticipated. I found that the first spore discharges in 1960 occurred 30 days later in the cooler citrus-growing regions of South Africa than in the Transvaal lowveld. Subsequent spray experiments confirmed that ascospore maturation is 3 weeks to a month later in these cool areas than in the Transvaal lowveld. Young fruit is highly susceptible during prolonged spells of rain the first 4 months after fruit set, then becomes resistant. Hardly any infections occur after January on in-season oranges, regardless of rain and abundance of inoculum.

The critical period for infection in the Transvaal lowveld usually starts at the beginning of November and ends in January (4). Before November, the fruit is very susceptible but the amount of inoculum is low and the rains are infrequent and of short duration; protecting fruit before November is seldom necessary. In January, the rains are frequent and inoculum is abundant, but the fruit is no longer susceptible, so the infection process fails. The danger period, therefore, stretches over 3 months. There is no easy technique or layman's aid to determine whether the fruit has become infected and to what extent. The first symptoms appear 2–5 months after the critical period has ended, which is too late for corrective action.

The Long Latent Period

An important characteristic of CBS is the long latent period after infection. The success of control with systemic fungicides hinges on exploiting the latent period. Let us consider the prelude to symptom development.

Infection takes place in the presence of moisture when the spore has germinated and produced an appressorium. A thin infection peg penetrates the cuticle and expands into a small mass of mycelium between the cuticle and the epidermis wall. This constitutes the so-called latent infection that months later produces black spot. The latent period is terminated when fruit becomes fully grown and maturity sets in. The underlying mechanism of symptom development is not understood, but several factors are influential:

1. Rising temperatures when the fruit is mature or nearly mature greatly stimulate symptom expression. In the northern Transvaal lowveld in 1959, the number of fully mature Valencia oranges with symptoms rose by nearly 2% a day from 13 August to 30 September; the climate changed during that period from mild winter to hot summer. Although not as dramatic, the picture repeats itself annually when control measures fail during or after the critical period and harvesting is not completed before the advent of summer. When the temperature rises from 20 to 27 C, fruit lesions increase significantly. The optimum growth of *G. citricarpa* on liquid basal synthetic medium is at 27 C. Postharvest development of black spot is largely influenced by the temperatures in the packinghouse and during transit to market.

2. More lesions develop on fruit exposed to high light intensities than on fruit kept in darkness. Furthermore, considerably more symptoms appear on the side of the fruit most exposed to light. I (4) found the incidence of lesions on fruit in the upper halves of old orange trees to be three times greater than that on fruit in the lower halves. Old trees usually have reduced foliage cover, and the fruit is more exposed to light. In South Africa (Southern Hemisphere), significantly more disease symptoms are found on the northern aspect of trees than on the southern aspect.

3. Drought influences symptom expression. In an elaborate field experiment, I (4) found that fruit from wilted trees that had been exposed to drought developed significantly more CBS than fruit from trees that were not wilted.

4. Symptoms may appear when the fruit is fully grown but the rind still green. The more mature a fruit becomes, with the rind changing from green to yellow or orange, the better the chances for symptoms to appear.

5. Tree condition and age influence CBS development. The disease is more severe on old trees than on healthy young trees. The fruit of vigorous young trees may not show CBS at all, even under epidemic conditions. The older the trees become, the greater the percentage of diseased fruit that can be expected.

Some Costly Mistakes

When discovered by Doidge for the first time near Pietermaritzburg in Natal in 1929, CBS caused no concern, and the climatic conditions in most of the citrus-growing areas of South Africa were thought to be unsuitable for the disease. This was a costly mistake.

Whiteside (9) fell into the same trap. He concluded that the disease would not develop to epidemic proportions in Zimbabwe. His optimism was based on the evidence that CBS had not become a problem in the inland citrus-producing areas of New South Wales and that the disease had been present for years in western Transvaal without becoming serious. He further speculated that the rain pattern of Zimbabwe was not conducive to a major CBS outbreak. Whiteside expressed these views in 1965. Thirteen years later, Zimbabwe experienced its first CBS epidemics and western Transvaal had to spray regularly to control the disease. Too much hope had been pinned on climate as a limiting factor. The buildup of inoculum to a certain threshold seems to be one of the dominating factors in establishment of an epidemic. In South Africa, numerous examples show that the first disease outbreaks always occur in lemon orchards, where inoculum builds up gradually until all adjacent citrus orchards become infected. The disease followed the same pattern in Zimbabwe, where the first disease outbreaks occurred in lemons and years later the surrounding orange groves became infected.

When CBS appears for the first time in an area, a limited number of slightly spotted fruits will be noticed in the packinghouse. At that stage the disease is not spectacular and the situation is always underestimated. These first symptoms are signals that a major disease outbreak is coming in 5, 10, 20, or 30 years. The disease has seldom failed to honor these early warnings. Farmers, however, are optimists and believe the following year will be better. In the case of CBS, the following year is worse. The epidemic does not decline but is easier to control during dry years than during wet years. Climate influences the speed of

Fig. 3. Hard spot lesion with pycnidia in the center of the necrotic tissue.

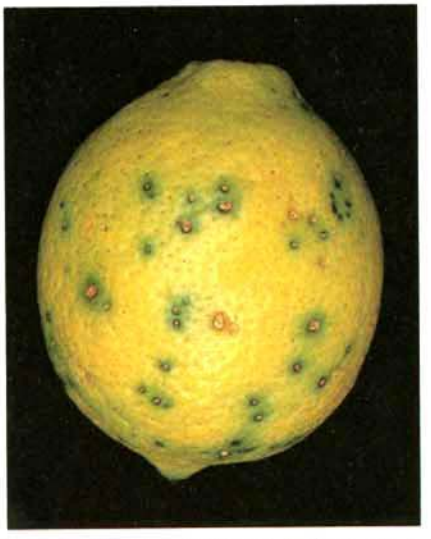

Fig. 4. Typical hard spot lesions on a lemon.

forward movement but has not stopped it yet. In areas where the winters are relatively cool, onset of the ascospores is delayed, but not enough to prevent infection.

In the various regions in South Africa where CBS occurs, the two most important factors determining an epidemic are summer rains and lemon orchards. Under these conditions, inoculum will gradually build up until the epidemic stage is reached.

Fruit Symptoms

Kiely classified fruit symptoms into three categories: hard spot or shot-hole, freckle spot, and virulent or spreading spot. A fourth category, false melanose, may be added in South Africa.

Hard spot lesions usually appear when fruit starts maturing, even before the color has changed from green to orange. On green fruit, a yellow halo surrounds each lesion. On mature fruit, a green halo surrounds a dark brown circle with a depressed light brown to gray-white center (Fig. 4). Pycnidia may be present in the center, but ascocarps never occur in fruit lesions. Although primarily a preharvest symptom, hard spot also occurs on early harvested fruit after picking. Hard spot always occurs more on the side of the fruit most exposed to sunlight.

Freckle spot usually appears after fruit color has changed from green to orange and may occur in more than one wave on the same fruit. Individual freckle spots may coalesce to form one big lesion or even a tearstain similar to melanose. The coalesced lesion may develop into a virulent spot during storage.

Virulent spot usually develops late in the season, particularly when fruit is fully mature and temperatures rise, and is also an important source of postharvest losses. The sunken necrotic lesions are brown to brick red at the periphery, irregular, and confluent (Fig. 5) and spread rapidly. Numerous pycnidia develop in the sunken lesions within a few days. The rind becomes completely necrotic, but the edible part of the fruit remains sound and decay seldom occurs. Virulent spots may appear on apparently healthy but latently infected rinds.

In South Africa, false melanose (Fig. 6) appears within months after fruit has reached the resistant stage. This symptom is not associated with *Phomopsis citri* Fawc., the melanose pathogen, but is an early season expression of infection by *G. citricarpa*. In many instances, false melanose develops into hard spot as the season progresses.

Control Measures

Quarantine. It is highly probable that human hands brought CBS to South Africa with infected plant material. Will it reach the citrus groves of California, Florida, Texas, and the "clean" countries on the South American continent? Is the answer to this danger the application of strict quarantine measures and legislation?

Modern means of communication, travel, and transport offer excellent opportunities for loopholes in a quarantine system. Any measure that will postpone the introduction of CBS is worth considering, however. When a disease outbreak occurs, every effort should be made to prevent or retard spread to other areas. When establishing new citrus farms far away from infected orchards, it may pay dividends to plant only trees from nurseries with a completely CBS-free history and to strictly prohibit introduction of plant material from sources that may carry latent infections.

The arguments against quarantine may be formidable, especially if there is commercial pressure. Probably the best argument is that the ascospores are airborne and the pathogen will reach the plant anyway. It is most unlikely, however, that ascospores will survive intercontinental spread by air currents. When one considers that inoculum buildup from the time of introduction to the onset of an epidemic may take decades, the inoculum density strongly enters the argument. The greater the initial inoculum density, the sooner the requirements for an epidemic will be fulfilled. Windborne spores from a source many miles away do not offer the same danger as planting latently infected trees, where each leaf has the potential of producing 50,000 ascospores. Fruit cannot rate high as an effective source of inoculum in international trade. Ascocarps have never been found on fruit, but pycnidiospores are produced that are not airborne.

Orchard sanitation. Normal harvesting procedures involve removing mature fruit from the orchard before the new crop sets. This takes care of one source of inoculum, namely pycnidiospores that may be washed down onto the young fruit. The ascigerous stage poses a more formidable problem. The incidence of apple scab (*Venturia inaequalis*) has been reduced considerably for decades in South Africa by the application of eradicant fungicides immediately after leaf drop, before perithecia develop. Orchard "floor" spraying with phenylmercuric chloride (PMC) was effective in preventing the ascigerous stage in the case of apple scab. I (4) carried out extensive ascospore eradication experiments on CBS using such chemicals as PMC, sodium dinitro-*o*-cresylate, lime sulfur, and nitrogenous fertilizers. All these chemicals showed promise in laboratory tests. Years later, McOnie (6) experimented with several other chemicals, but none reduced ascospore inoculum to the point where disease incidence was affected.

Chemicals. The history of chemical control of CBS up to 1971 is woven around protective fungicides. Citrus fruit hangs on the tree for about a year, which is a long time to provide protection. It was proved beyond doubt that the early part of the fruit's life on the tree is the most important for CBS infections. I (4) established, and other workers later confirmed, that a critical period of infection occurs from the beginning of November until January. The success of a protective program is based on this critical period.

Nature, however, does not operate on a calendar, and it would be naive to expect each season to be a carbon copy of the previous one. The critical period is therefore no more than a guideline. The crucial questions hinge on: 1) the commencement of the first major rains, 2) the time of the first significant discharges of ascospores, and 3) how conducive weather conditions are for infection. Preferably, this information should be known in advance. Since this is impossible, one has to rely on expectations in the light of information obtained from previous years. The experienced pathologist develops a "feeling" for predictions, but it remains a crystal-ball art. I (4) suggested that a progressive recording of ascospore maturation from spring to early summer could be used to predict the first major outbreaks, but McOnie (6) found the technique too laborious and not sensitive enough.

Hirst spore traps have been useful in interpreting research results and have been in operation at two localities in South Africa since 1959. The onset of major ascospore releases is accurately recorded and the information passed on to growers. When the grower has only protective fungicides in his store, however, knowing that an infection period has already taken place is of no value. Knowledge of the critical period served as

a fairly reliable guideline for protective action and helped with the timely application of a fungicide just before an infection period. It worked reasonably well in most cases, but losses of up to 10% were often experienced because long spells of rain made it difficult to get heavy machinery into the orchards in time. Bordeaux mixture was used almost exclusively in a three-spray program at six weekly intervals until I (4) showed that cuprous oxide and copper oxychloride provided better control.

The struggle with CBS was so intense that pathologists failed to see that while solving one problem they created another. Copper fungicides, especially when applied repeatedly, are unkind to fruit appearance. The early sprays are more inclined to accentuate minor fruit lesions than later sprays, but the rind may be damaged directly. The lesions are brown to dark brown and spoil the sales appeal of the fruit.

The dithiocarbamates were introduced in 1964, and fruit appearance improved considerably. Because of superior disease control, mancozeb replaced zineb within a year. The number of sprays per season was two or three for young orchards of navel oranges and grapefruit to four for fully grown to old orchards of Valencia oranges. The addition of spray oil to the copper fungicides, zineb, or mancozeb became an established practice.

The effect of oil. Oil formulations are well known in citriculture for their insecticidal properties, and various workers in other spheres of plant pathology have reported fungicidal properties. Oil may also adversely affect plants and reduce yields.

Wager (8) and Kiely (3) observed that the addition of spray oil to Bordeaux mixture contributed greatly toward control of CBS. I (4) experimented widely with oil as an additive to fungicides and as an orchard spray at various stages of fruit development. In all the experiments, oil tended to assist the performance of fungicides against CBS. Oil alone reduced symptom expression, depending on the time of application. Applications during March and April increase effectiveness of protective programs but retard color development of fruit and adversely affect the internal quality. I (4) showed that extremely high concentrations of oil as a postharvest treatment may control CBS but make the fruit commercially unacceptable. Oil must be considered as an additive in CBS control measures, not as a treatment on its own. Oil also plays an important role as an additive in curative control measures.

A Success Story

Seldom are years of research crowned with almost complete success. The history of black spot in South Africa is a success story.

In 1968 Kellerman used benomyl in the conventional way, namely, on a protective

Fig. 5. These late-season virulent spots on a Valencia orange will spread during storage.

Fig. 6. False melanose caused by early season infection by *Guignardia citricarpa*.

basis. The results were excellent—better than the standard mancozeb treatment and approaching complete control. If the prices had been similar, benomyl would probably have replaced mancozeb in the protective spray program; economic reasons stimulated further research to reduce dosage rates and the number of sprays. The critical period of infection was well known at this time and the long latent period was begging to be exploited. Finally a breakthrough came in 1971. It was proved beyond doubt that four or five protective sprays could be replaced by a single application of benomyl plus oil. This single spray may be applied toward the end of the critical period, and the results are nearly always better than any conventional protective program used in the past. So far, no other fungicide has quite matched the performance of benomyl. Citrus black spot is beaten. It would be foolish, however, to relax and to ignore the possibility of resistance to benomyl.

The excellent results achieved with benomyl plus oil on the fruit made us turn to the leaves, where the inoculum cycle starts. Can the latent infections in the attached leaves be eradicated so that the detached leaves fail to give rise to the ascigerous stage? If fungicidal sprays could prevent or reduce ascospore development, inoculum would be reduced and the disease cycle broken. Extensive investigations with benomyl and oil showed that neither—combined or alone—kills the fungus. They are mycelium growth inhibitors and do not prevent development of the ascigerous stage.

Traditionally, citrus trees were sprayed with high-volume applicators until about 1970, when various types of low-volume applicators were introduced. This was a tedious and expensive operation, since each tree was sprayed with up to 100 L of mixture several times a year. During the past decade, several medium- to low-volume applicators have appeared on the market. These applicators work well as long as the fungicide is evenly applied to all parts of the tree. Low-volume sprays applied by airblast equipment are giving excellent disease control.

Kellerman et al (2) tested an experimental compressed-air spray applicator with different modifications of hand nozzles and fitted booms, using benomyl plus oil as the fungicide mixture. They found this novel way of spraying not only provided satisfactory control of CBS but also saved up to 70% of spray material. This work holds considerable promise.

Aerial spraying was tried (4), but the results were variable. Kellerman and I (1) found that black spot control was poor where benomyl plus oil had been applied with a helicopter.

Resistance Not Imminent

Because of sound research and the timely arrival of an effective systemic fungicide, CBS is well under control. At the moment, benomyl plus oil rule supreme. Replacement of the dithiocarbamates has not been without repercussions, however. For example, the dithiocarbamates also controlled rust mite (*Phyllocoptruta oleivora* Ashm), so additional measures must be taken in a benomyl regimen.

There are very few permanent solutions to problems in plant pathology, and CBS will not be the exception. Resistance of *G. citricarpa* to benomyl is a possibility, but there is no indication that a replacement will be needed soon.

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