FRAMEWORK FOR MODELING THE EPIDEMIOLOGY OF SOOTY BLOTCH AND FLYSPECK DISEASES OF APPLE AND THEIR CONTROL.

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Although neither of these diseases of apple significantly affect fruit growth, they are of major economic importance. Shaffer, et al. (1983) report that over the five-year period from 1976 to 1980, damage due to these diseases in sampled commercial orchards in North Carolina averaged approximately 3.5% in Delicious and Golden Delicious, and 11 to 12 percent in the later maturing Rome Beauty and Staman varieties. Economic loss results from the unsuitability of the diseased apples for the premium priced fresh market.

Because they do not measurably affect yield, these diseases were selected as ideal systems in which to study the value of information concerning factors which affect crop quality, keeping the yield essentially constant. Yield enters only when fixed per-acre costs must be considered.

The following discussion reviews the epidemiology of the two diseases, the mechanism of action of fungicidal control with special reference to captan and zineb, and the dynamics of fungicide residue deposits and decay.

SOOTY BLOTCH

The most complete studies of sooty blotch appear to be those of Baines and Gardner (1932) and of Hickey (1960).

Sooty blotch is caused by the imperfect fungus, Gloeodes pomigena. The organism has an extremely wide host range, and can grow on twigs of a number of hosts, including apple. The implication suggested by Baines and Gardner, and reiterated by Hickey, is that spring inoculum is provided from mycelial mats overwintering on twigs within the orchard, as well as from surrounding vegetation.

In culture, Baines and Gardner found that spores are produced within cavities in the thallus and ooze to the upper surface of the thallus rather than being forcibly discharged. Hickey quotes earlier work (Anderson 1920) to the effect that initial inoculum is formed from Chlamydospores and that they are spread (Palmiter 1939) to the fruit by rain. Although the fungus is primarily superficial, it does send occasional clusters of hyphae into the waxy cuticle, occasionally penetrating the cuticle. It is conceivable that such penetration might offer some protection from fungistats.

According to Baines and Gardner, pycnidia are formed on twigs in the fall and liberate spores in the spring. They suggest that this forms the initial inoculum, and that secondary infection is caused by the spread of both spores and mycelium. It is noteworthy, however, that Baines and Gardner were not able to observe sporulating pycnidia on apple surfaces. Baines and Gardner observed spores in the field in late May and early June. Spores have been observed in North Carolina as early as mid to late May (T. Sutton, personal communication).

After infection, there is a fairly long incubation period. In the work of Baines and Gardner, the period from infection to observable symptoms varied from one to two months in 1931, but was much longer (roughly 2½ to 3 months) in 1930, possibly due to very hot dry weather. Hickey found an incubation of about 25 days in the field, but only 4 to 12 days in a moist laboratory chamber. It would seem therefore that initial infection occurs from mid-May to early June, inoculation occurs around the first of June, and the earliest time for symptom observation under field conditions should be mid-June. By this time, there may be substantial mycelial growth on the apples (though possibly not sporulation) where it is difficult for the fungistat to adhere because of the smooth waxy surface (see discussion on pesticide residue). During this time, it also seems possible that infection is developing in hidden recesses on twigs, were the rough surface provides protection from the fungistat.

For secondary infection, we may have several pathways, discounting sporulation on the surface of the apple.

1. Spread of mycelial fragments from the apple surface. As pointed out above, this is probably not important for one month or longer, depending upon temperature and moisture conditions. Since sooty blotch as well as flyspeck live primarily on the surface, they are expected to be sensitive to ambient moisture.

2. Spread of spores generated on woody tissue. If incubation on woody tissue takes the same period of time as that observed on apple surfaces, and if that incubation period (i.e., time till observable symptoms) corresponds to the time for sporulation, then secondary spores are not expected until the beginning of July. Symptoms that they would produce would not be visible until the beginning of August. It is plausible, however, that growth in crevices of woody tissue might be more closely approximated by the laboratory results of 4-12 days, so that an effectively steady stream of inoculum would reach the apples starting with early June. In such a case, conditions would be set up for a continual spread of mycelial fragments from apple surface growth starting with the beginning of July. Indeed, if incubation period on woody tissue is substantially shorter than on apple surface, a considerable pathogen population would have built up before the first symptoms were visible.

3. Spread of mycelial fragments from woody tissue colonies. If incubation period on the woody tissue is the same as that on apple surface, then this introduces no new factor into the dynamics. If it is shorter, then mycelial secondary infection is to be expected starting possibly by early June. Presumably mycelial infection would not have the same incubation period so that this would lead to observable infected fruit by early June in heavily inoculated orchards.

The diagrams of Figures 1-4 summarize the arguments from different points of view and at different levels of detail. Figure 1 summarizes the sequence of events implied by the preceding paragraph. Figure 2 condenses this information

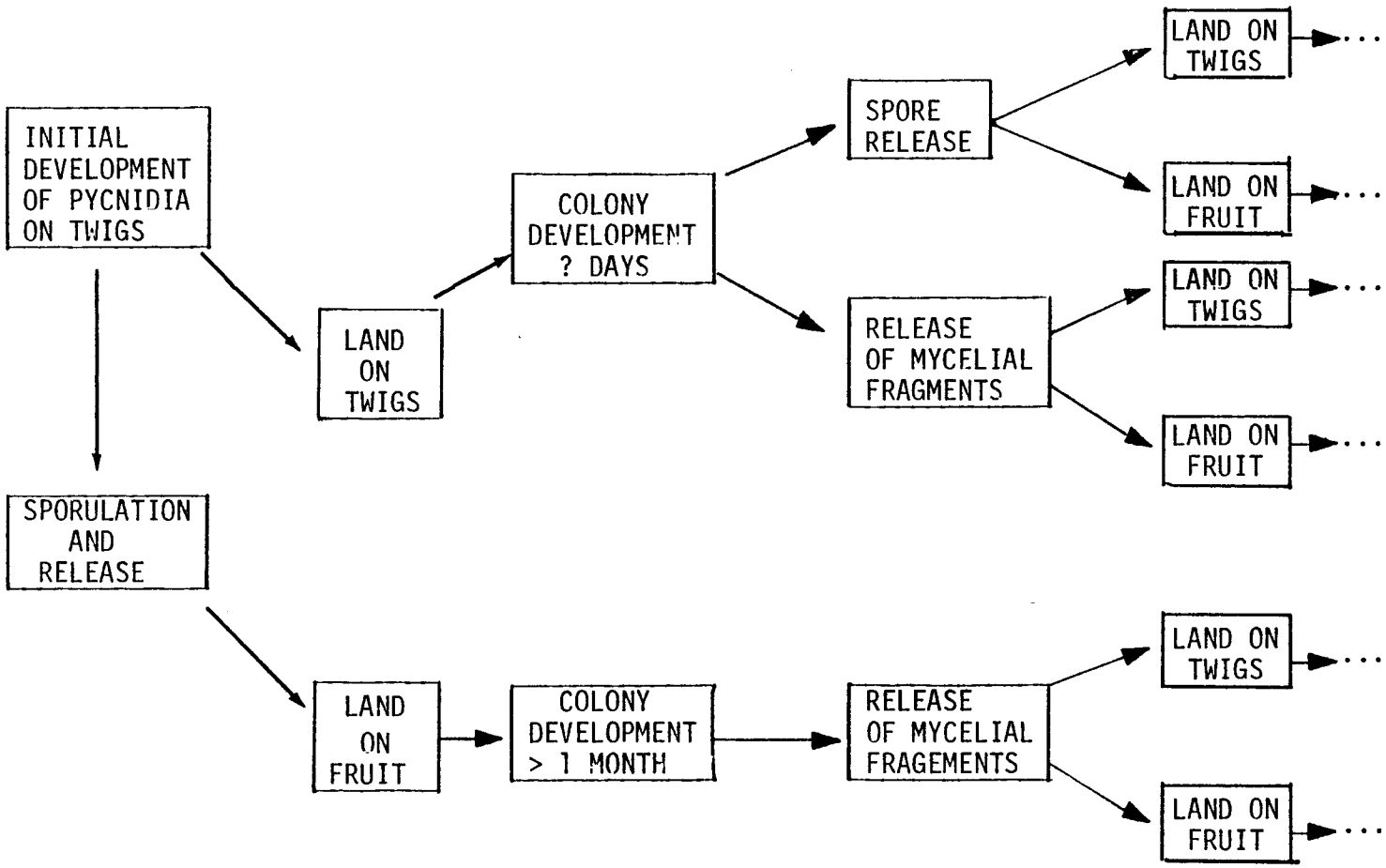


FIGURE 1. Event Tree for Sooty Blotch Infection.

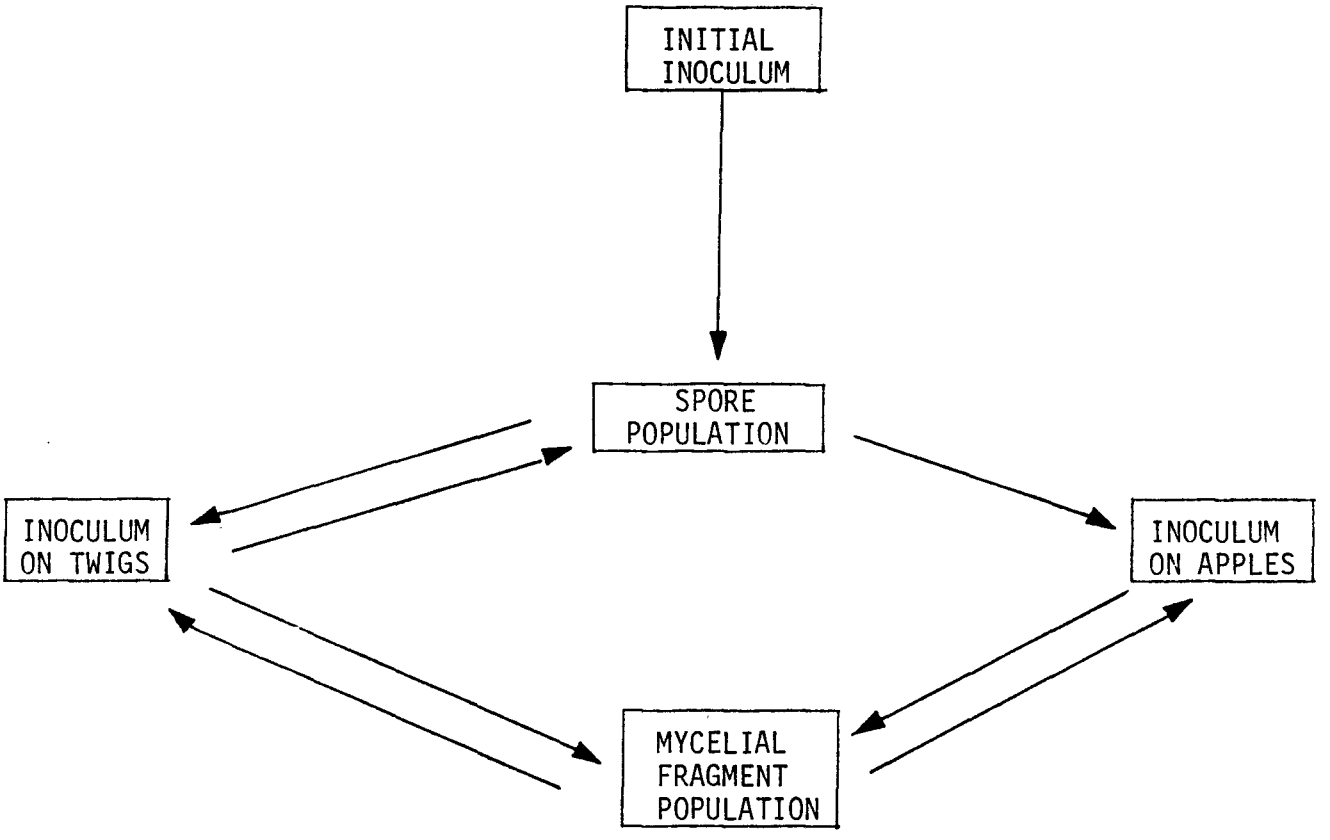


FIGURE 2. Aggregated Signal Flow Diagram for Sooty Blotch.

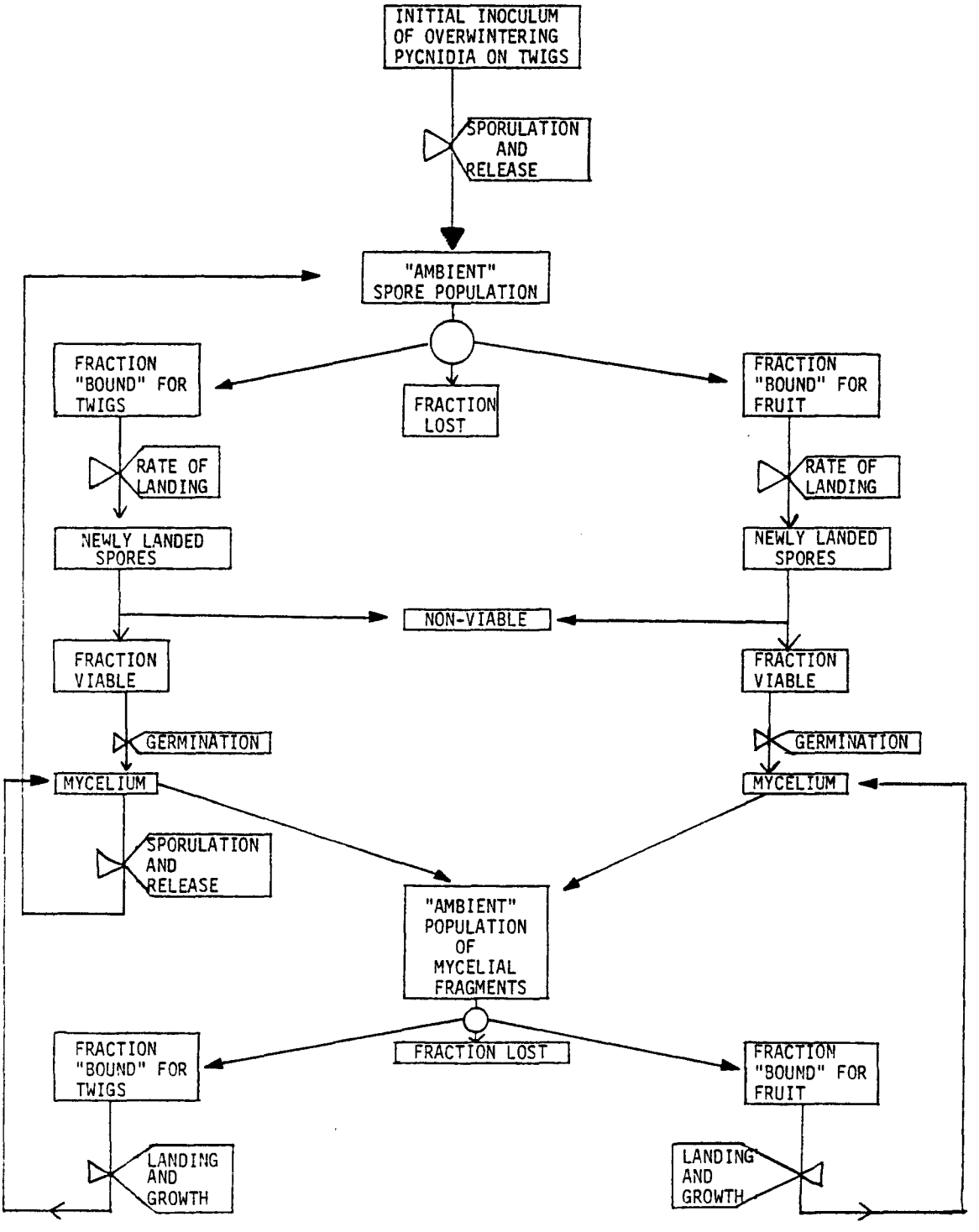


FIGURE 3. Expanded Signal-Flow Diagram for Sooty Blotch.

= Populations, = Rate Processes.

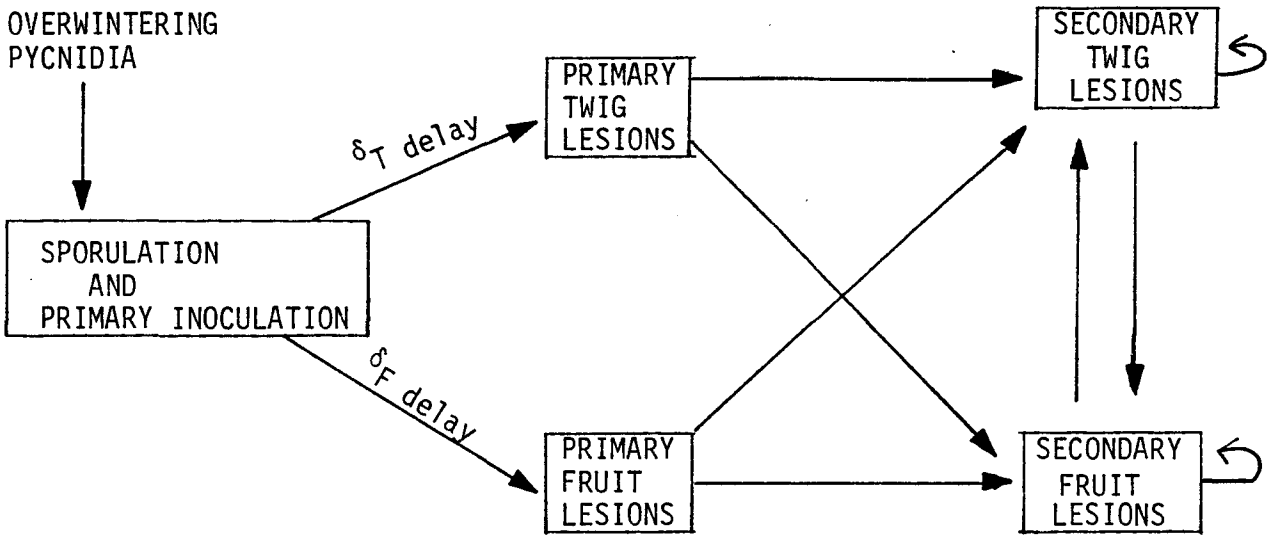


FIGURE 4. Sooty Blotch Cycle in Terms of Disease.

in terms of relationships between the various subpopulations. The dynamics of these relationships are shown in more detail in Figure 3. Finally, Figure 4 summarizes the system, not in terms of relationships between underlying subpopulations, but in terms of relationships between diseased plant parts. The dynamics associated with this last diagram may be complicated by possibly different incubation delays associated with spore and with mycelial infection. The apparent rate may therefore appear to vary even after accounting for environmental affects. However, it may not be unreasonable to assert that by the time an easily observable level of infection (say, 1% incidence) has been reached, a stationary relationship exists, so that the rate of new incidence is proportional to the amount of old incidence. It must be cautioned, however, that a model based on such an assumption and parametrized from data that meets the assumption, may not be validly extrapolated back to the nonstationary phase. Accordingly, when the logistic model is extrapolated to some initial given time, the back extrapolation may be useful for comparative and sensitivity analysis purposes, but does not have direct biological significance.

In formulating equations for the rate of infection spread, we note that this rate will be proportional to incidence only if the rate of release of mycelial fragments and the number of spores per lesion are proportional to incidence and severity.

In parametrizing the relationships suggested by any of the foregoing models, problems may exist due to the apparent genetic variability of G. pomigena. Hickey quotes Groves (1933) concerning the classification of the morphological pattern of the thallus into four separate types. In studying 46 different isolates of G. pomigena, Hickey verified the existence of the four general groups according to the morphology of the thallus. He noted that these groupings are reproducible and therefore presumably genetic in origin. However, a large number of isolates

did not fall neatly into any specific group, so that classification into distinct races did not seem appropriate. The possible difficulty is that isolates appear to differ in their quantitative nutritional responses, as well as in growth rates, and dependence of growth rate upon temperature and pH.

Environmental variables of greatest importance are likely to be temperature, rainfall, relative humidity, and wind speed. Racial differences in response of mycelial growth and sporulation to temperature and relative humidity may be important.

In the field application of laboratory data to non-linear processes, stochastic variability is always important. When the genotype is uniform, the stochastic variability arises largely in connection with the microenvironment distribution. In this case, however, it would seem that genetic variability, as reflected by morphological type, imparts an additional element of uncertainty. Dealing with this uncertainty would require (1) laboratory determination of the dependence of growth characteristics on morphological type; and (2) information on the distribution of morphological types in the field. Use of the laboratory information would be made easier if the distribution of morphological types were found to be approximately stationary.

FLYSPECK

This disease is caused by Zygophiala jamaicensis. Baker, et al. (1977) summarize the work related to this organism as it occurs on carnation and on apple. On apples, the symptoms are described as consisting of 6 to 50 shiny black round structures that appear to be superficial on the cuticle, and which develop in well-defined groups, one to two cm. in diameter. It is stated that the organism has an extremely wide host range and produces conidiophores on leaves, stem, and fruit of most hosts.

According to Durbin and Snyder (1953), the fungus overwinters on stems, leaves and discarded fruit within the orchard and possibly in the surrounding area. Inoculation with a single "ascospore culture" resulted in a cluster of ascocarps or specks. Baines (1940) observed that typical fruiting bodies also arise when apples are inoculated with mycelium. He states that attempts to induce sporulation in culture failed, although Baker, et al. state that pseudothecia sometimes form and mature in culture.

Baker, et al. (1977) cite several references which date the discharge of mature ascospores at late May to early June in France, and June in Indiana. He cites a reference by Baines and Gardner to the effect that under cool, moist conditions, it takes about three weeks for symptoms to appear. This would place the timing approximately near that of sooty blotch. Baker, et al. state that in California, production of zygophiala and mature pseudothecia take place in about one month in the field, and in about three weeks in moist chambers (on carnation). The similarity between the field and chamber results suggests that the length of the cycle on fruit, stems, and flowers might, to first approximation, be taken to be similar. Secondary infection appears to be caused primarily by conidospores (T. Sutton, personal communication). Evidence from spore trapping, as well as from direct observation of lesions on a variety of hosts, indicates that pseudothecia on primary lesions do not produce mature ascospores. Furthermore, the colonies are compact, so that spread of mycelial fragments does not seem to be an important source of infection. Figures 5 and 6 summarize the dynamics for flyspeck. The dynamics seem to be simpler than for sooty blotch, in that only conidiospore spread needs to be considered for secondary infection, and existing data do not suggest a need to distinguish between different types of surfaces. Moreover, flyspeck appears to be homothallic, so that problems related to racial variability do not arise.

However, the complication of having several processes, which may proceed

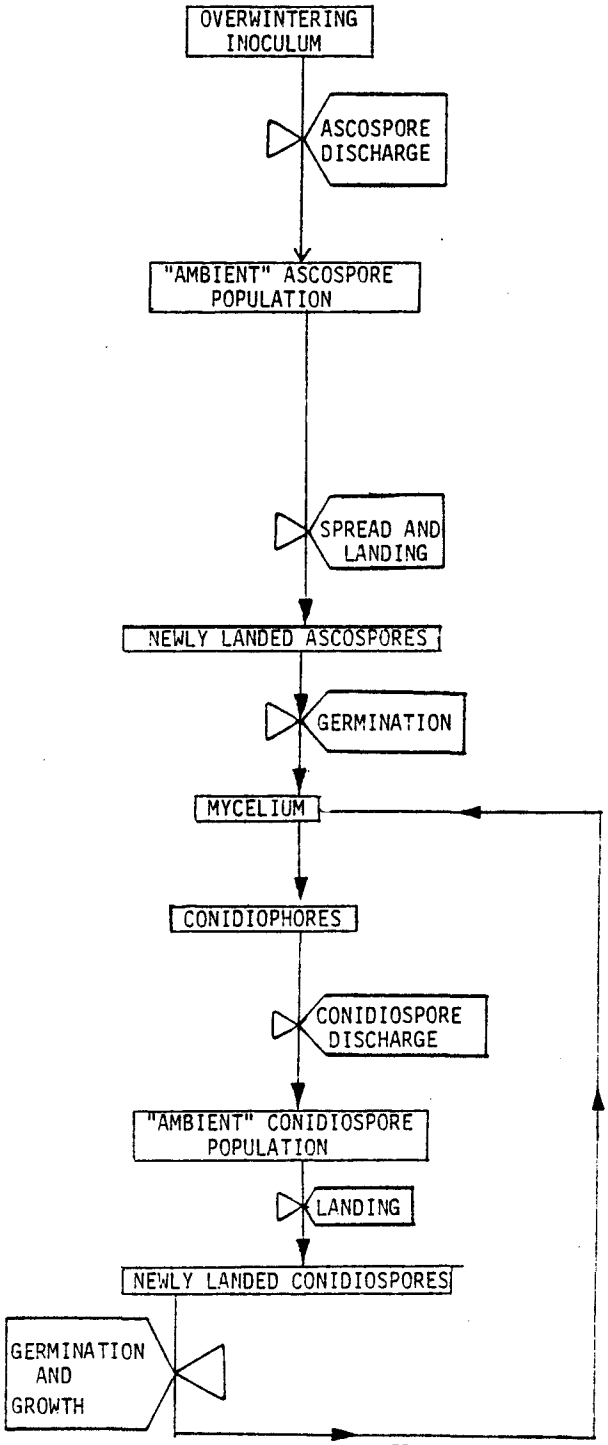


FIGURE 5. Expanded Signal-Flow Diagram for Flyspeck.

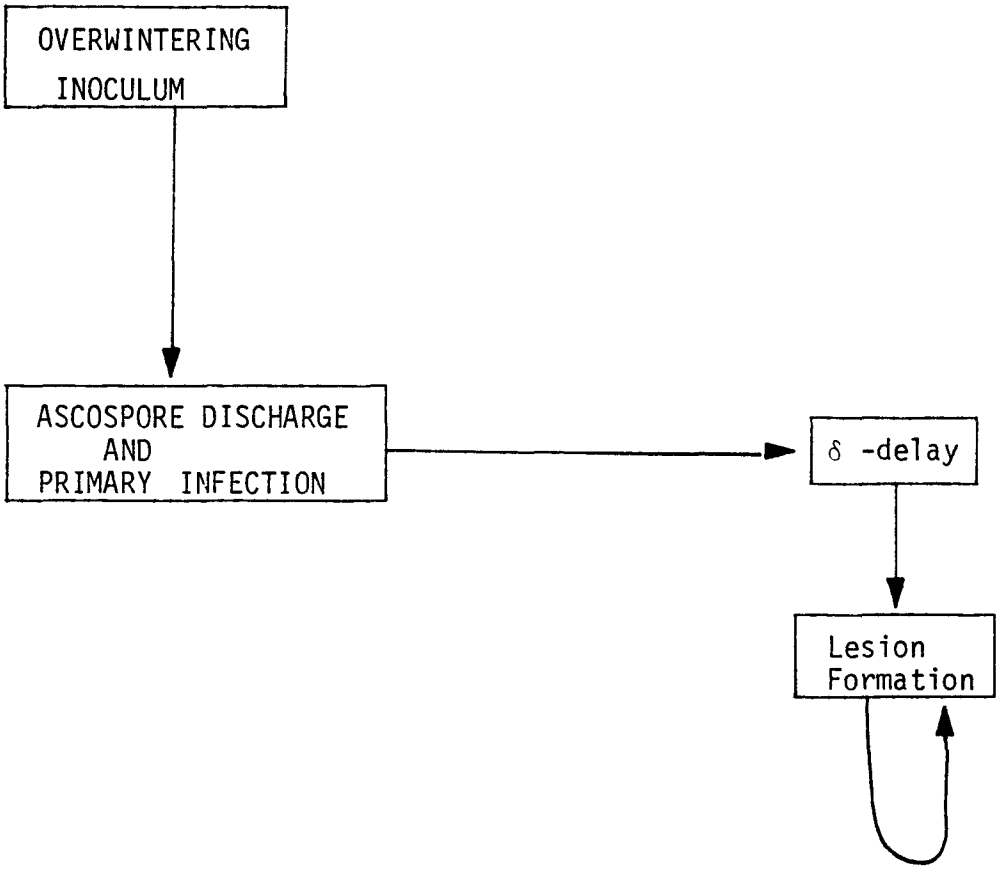
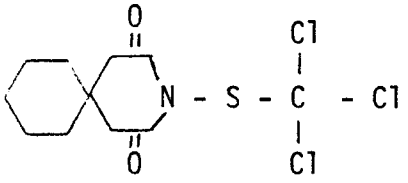


Figure 6. Flyspeck cycle in terms of disease.

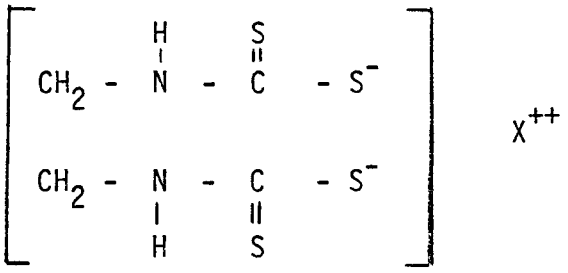
with different rates, and whose relative importance may not be constant, is a factor here as well as with sooty blotch. Therefore, the same cautions with respect to the assumption of a stationary relationship and use of the logistic equation must be observed.

ACTION OF FUNGICIDES

Figures cited by Fry (1977) indicate that in 1971, 18% of all fungicides used in the US were applied to apples. In this account, we focus principally on captan,



and salts of ethylene-bis-dithiocarbamate, namely, zineb, maneb and nabam. The general structure for the dithiocarbamates is,



where X = Zn for zineb, Mn for maneb and 2Na for nabam. The more recently developed Metiram is a mixture of polymers, with about half the zinc content.

Both of these types of fungicides are relatively unspecific and exert their effects by inactivation, or possibly inhibition, of SH-containing enzymes (Lyr, 1977). Such toxins would likely exhibit phytotoxic behavior except for physical barriers to absorption into the plant tissue. Except in sustained high doses, they appear to operate more as fungi<u>stats</u> than as fungi<u>cides</u>. Captan is detoxified within the cell by reaction with thiols; the cell, unless exhausted, may regenerate the needed enzymes. Lukens (1969) suggests that fungicidal effects result from complete exhaustion of thiols for a period of time. He also states that in most cases, captan inhibits respiration to about the same degree that it inhibits growth (see also Montie and Sisler 1962).

With reference to the epidemic cycle diagrams of Figures 3 and 5, such generalized fungistats could exert effects by:

1. Decreasing viability (e.g., probability of germination)of mature spores. Such an effect would be exerted on the surface where the spores land, and might be different for ascospores vs. conidiospores.

2. Increasing time to germination of viable spores.

3. Decreasing rate of growth and development of hyphae. Together with effect (2), this would result in an increase in observed incubation time.

4. Decreasing number of spores produced per lesion.

5. For flyspeck, changing relative amounts of conidiospores and pseudothecia produced.

6. Decreasing rate of sporulation.

7. Decreasing viability of spores produced. This would differ from effect (1) in being exerted at the cite of spore production.

Each of these effects would influence the apparent infection rate in a different way (van der Plank, 1967).

Relevant data appears to be sparse. Morehart and Crossan (1965) studied the effects of the ethylene bis-dithiocarbamates. They note that the active toxicant is actually a decomposition product, and that the pure fresh substances have little effect. Using Colletotrichum capsici (Syd.) as a test fungus, they found that exposure to the fungicide, following by washing, reduced both fraction of conidia germinated after a 24-hour test, and mycelial growth, though not by the same percentage (200 ppm produced 29% germination inhibition; 1000 ppm produced 50% inhibition of mycelial growth).

In tests run by Hickey (1960), a mixture of spores and mycelium of G. pomigena were planted in agar containing captan (150 ppm to 1200 ppm) and zineb (195 ppm to

1560 ppm). Four of the 36 plantings grew to colony at the lowest concentration of captan and one grew at all others. Growth in each case was 45% of the control. Number of growths with zineb varied from 2 to 14, and the diameter varied from 42% to 69% of control. Tests with the Zygophiala stage of flyspeck gave variable results, with the highest concentration of fungicide not necessarily giving highest inhibition. Slightly greater inhibition was observed with captan. Number of growths varied from 4 to 10 out of 24 with captan, with diameter varying from 16% to 33% of control. With zineb, number of growths varied from 6 to 12 out of 24, with diameter varying from 45% to 133%. It seems that low concentrations may have inhibited germination, but not growth.

Slide germination tests with sooty blotch gave LD-50 in three tests of 50, 4 and .45 ppm for captan; 70, 90, 3.3 ppm for zineb.

The data of Morehart and Crossan are based on exposure of the fungus to a solution of fungicide, following by washing, and then culture. The data of Hickey are based on culturing the fungus on fungicide-containing agar and on slide germination. The relation between this data and the activity of fungicide in a dried surface deposit is ambiguous. Some feeling for relative quantities involved may be obtained from the following rough calculations:

i) Hickey reports surface residue of captan of up to 10 ppm on Grimes Golden apples. Assuming this to be based on the volume of the whole apple, this would mean a surface residue of 3.33 ug/cm^2 if the apples were 2 cm in diameter, or 10 ug/cm^2 for an apple of 6 cm diameter. Based on 1200 ppm in the spray, this translates into between .0027 ml/cm^2 and .0083 ml/cm^2 of deposit, assuming apple size is between 2 and 6 cm. in diameter.

ii) Next, look at a set of data from Burchfield and Goenaga (1957), who sprayed solutions of captan onto tomato plants, and measured ability to control early blight. Concentration of the spray varied from 16 to 250

ppm. Using Michaelis-Menten inhibition kinetics, we compute a value of K_i of about 5 ppm from their data. Assuming 0.02 ml/cm^2, this amounts to 0.1 ug/cm^2 of active ingredient.

iii) Finally, if 10 ug/cm^2 is the concentration on freshly sprayed apples, 0.1 ug/cm^2 would be a relative K_i of 0.01, which is in the range that we have computed for K_i against sooty blotch, based on the data of Hickey (1960).

PESTICIDE RESIDUE

The dynamics of the pesticide residue in the system may be divided into processes related to application, redistribution, and decay.

The chemical and physical forces that influence impact and adherence of spray particles, and persistence of the deposit are reviewed in depth by Burchfield (1967). These processes are governed by interaction between the surface and the applied material, and so depend upon the electrostatic properties of the material, the surface tension of the diluent, and the detailed characteristics of surface. Both zineb and captan are classified as hydrophobic substances and are usually applied to aqueous suspensions. Burchfield makes the point that impact and adhesion are generally poorest on smooth waxy surfaces, and best on "nonwaxy rough or moderately hirsute leaves". The important implication of this statement is that residues measured on apple leaf surfaces may be higher than either those on the smooth waxy surface of the fruit, or on the very rough woody surfaces. With respect to the relation between leaves and fruit, this expectation is supported by the data of Reissig and Seem (1983).

It is evident that initial deposit will not be deterministically uniform, but will be distributed according to some random distribution. Moreover, the parameters of the distribution (and possibly the distributional family itself) will

depend upon such factors as sprayer characteristics, air currents, and position within the canopy. A detail study of distribution of pesticides deposited on Golden Delicious apple trees is reported by Travis and Sutton (in press). Mean deposition of metiram varied from 6.1 to 34 ug/cm^2, depending upon height and depth within the canopy.

Bruhn and Fry (1982a) used a gamma distribution to describe fungicide deposition on potato foliage, with the parameters depending upon stratum within the canopy. Such a descriptive framework might be useful for apple trees, with parameters depending upon height and depth.

Bruhn and Fry make the point that because of nonlinearity of the dose response relationship, the effectiveness of average residue levels will differ from the average effectiveness, so that the deterministic models may be of doubtful reliability. (see Gold, 1977, Appendix D, for a discussion on the effects of computing averages in different ways.)

After the initial deposition, the processes related to redistribution and decay begin. A rapid initial loss of pesticide is often observed, followed by logarithmic decay, which depends upon the amount of rainfall (Burchfield 1967, Reissig and Seem, 1983, Bruhn and Fry 1982b). Burchfield suggests that the high initial losses result from rapid loss of large particles, leaving the more tenacious smaller particles. In addition to causing new losses, rainfall has been identified by Bruhn and Fry (1982b) as being associated with redistribution of pesticide within the canopy.

Several factors beside physical dislodgement due to wind and rain lead to disappearances of pesticides. The first of these discussed by Burchfield is chemical decomposition. Captan is subject to substitution reactions, and especially to hydrolysis, with a half-life of 2.5 hours in neutral solution. Burchfield suggests that its longer persistence on foliage in the face of the high hydrolytic rate is attributable to its low solubility.

Sublimation, an important factor with some pesticides, appears to have some importance in the case of captan.

A factor discussed by Burchfield that is often overlooked is apparent loss due to plant growth. Burchfield cites data from Taschenberg, et al. (1963) to indicate that apparent loss of deposit on grapes due to plant growth amounted to 25% after 56 days, compared with the actual loss due to weathering of 34%. The percentages were calculated on a weight basis.

A detailed statistical model of the spatial and temporal dynamics of chlorothalonil residues on potato foliage has been reported by Bruhn and Fry (1982b). For an accurate description, they found it necessary to take into account effects of rainfall, temperature, and time since application. Redeposit on the lower strata of the canopy was associated with rainfall.

Data are reported by Reissig and Seem (1983) for decay of captan on McIntosh apple trees. While the data are quite noisy, they indicate a roughly exponential decay with number of degree days above 32°F.

DISCUSSION

Four separate processes must be described and integrated to arrive at a useful description of the dynamics of either of these diseases: 1) growth and development of the fruit, which is the entity of interest, and of other hosts for the pathogen; 2) biology and dynamics of the pathogen population and of the disease; 3) dynamics of inhibition of the fungi by chemicals, and 4) dynamics governing the concentration of chemicals within the system. It is clear that none of these processes is simple. In addition, it has been observed (Sutton, personal communication) that interaction between the two diseases is important.

Nevertheless, within the context of the framework set out in this paper, an initial description of the system has been developed, based on the following

simplifications:

1) description based on fraction of disease incidence on apples;
2) application of the stationarity assumptions discussed in the section on sooty blotch, and use of the logistic equation;
3) Michaelis-Menten inhibition kinetics;
4) exponential fungistat decay.

The model based on these simplifications has been found to fit the data of Hickey (1960) quite well. It will be reported separately together with evaluation and extension, based on work currently underway in the laboratory of Dr. Turner Sutton.

ACKNOWLEDGEMENT

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