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Parasitic and Biological Fitness of *Venturia inaequalis*: Relationship to Disease Management Strategies

Nelson (59) defines biological fitness as an organism's relative ability to persist over time. Any trait that contributes to increased reproductive capacity and/or survival is a biological fitness character. Parasitic fitness is the relative ability of a parasitic genotype or population to persist successfully over time. Both types of fitness are measured in terms of reproductive success. In Nelson's view (59), "The long process of coevolution resulted in the ultimate accumulation of many resistance and fitness genes in the host and parasite, and the result is that subsequent changes in the future course of their continuing coevolution will be largely subtle ones."

In other words, over time the relationship between a parasite and its host becomes "fine-tuned," and the relationship between *Venturia inaequalis* (Cooke) G. Wint., the fungal parasite that causes apple scab, and its host, *Malus × domestica*, appears to be finely tuned indeed. *V. inaequalis* is a successful parasite on apple because it has accumulated numerous parasitic fitness attributes (genes) that enable it to infect and reproduce, but with little apparent cost (damage) to the host. When a spore produced by *V. inaequalis* lands on a susceptible apple leaf and conditions are favorable for infection, invasion is restricted to the space between the cuticle

and the epidermis (Fig. 1), and there is almost no noticeable damage to host tissue 1 to 3 weeks later when a scab lesion appears. Apple survives because it has accumulated numerous biological fitness attributes (genes) that enable it to cope with the parasite: scab is not life threatening, and a tree can withstand numerous infections and bear fruit (produce seed).

In this article, we discuss the fitness attributes that make *V. inaequalis* a successful parasite on apple and enable apple to cope with it. *V. inaequalis* is not an obli-

gate parasite, but it is nearly so. It also has a saprophytic, sexual phase on infected fallen leaves when the host is dormant that is critical to its survival and to its success as a parasite. Fitness for nonparasitic survival is independent of a parasite's ability to induce disease (59), but with *V. inaequalis*, the biological fitness attributes of its saprophytic phase are intricately related to its parasitic fitness attributes and apple's biological fitness attributes. These fitness relationships are also discussed.

Scab as a Major Problem in Commercial Apple Production

Apple scab is now a major problem in apple-growing regions worldwide, particularly in temperate regions with cool, moist weather in spring, but how long scab has occurred on the cultivated apple is unknown. The first report of scab was in 1819 (22), but the oldest record of apple scab the authors are aware of is in a painting (c. 1600) by Michelangelo Caravaggio, "The Supper at Emmaus," which hangs at the National Gallery in London, England (Fig. 2). In "Still Life and Apple and a Pomegranate" by Gustave Courbet painted in 1871-72, which hangs in the Mesdag Museum Den Haag, each of about 16 apples has several clearly painted black spots. We can guess from this that scab was common and accepted as normal at that time.

Fig. 1. Small, undifferentiated stroma of *Venturia inaequalis* between the host cell wall and cuticular membrane. Ap, appressorium; CM, cuticular membrane; EC, epidermal cell; HCW, host cell wall; MS, mucilaginous substance; St, stroma (83). Reprinted by permission.

Fig. 2. "The Supper at Emmaus" by Michelangelo M. da Caravaggio, painted c. 1600. Enlargement of the basket of fruit shows clearly several scab lesions on the apples. Published by kind permission of the Trustees of the National Gallery, London.

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Fig. 3. The progression of orchards from a meadow orchard (upper left) of mostly crabapple seedlings grafted with sweet varieties and interspersed with pear and other fruit trees, no row structure, and trees often widely spaced (upper middle) to modern orchards with genetically uniform trees planted in rows, first of large standard trees (upper right), then of trees grafted onto semi-dwarfing (lower right and middle), and finally onto dwarfing (lower right) rootstock that resulted in much smaller trees and much greater planting densities.

Why was scab apparently not a serious problem to growers until the 1800s? One suggestion is that scab was present for centuries but remained obscure because apple trees with high resistance to scab were grown as seedlings in small plantings. In Europe during medieval times, apple plantings were in monasteries and other religious havens, and the diversity of resistance genotypes in the seedlings was most likely responsible for maintaining infection at a low frequency. In more recent times, particularly in Europe until the early 1900s, apple was also grown on farms (mainly for family use) in meadow orchards planted with trees (mostly crabapple seedlings grafted with sweet varieties) widely spaced and interspersed with pear and other fruit trees (Fig. 3). Single apple trees at the borders of fields and apple trees along roads were also common. A high number of different varieties was probably present, so we can assume that each tree was mostly surrounded by trees of a different genotype (23). The distance for ascospores (the primary inoculum) to travel from the ground to different trees, the diversity of genotypes in seedlings, and practices such as cultivation and grazing by sheep and cows that very likely removed infected leaf litter, all mitigated the severity of scab and kept the disease from rendering the fruit unusable. All that changed in the 1800s when controlled pollination followed by vegetative propagation resulted in the production of genetically uniform trees with improved characteristics (47) and increased consumer demand for fresh apples and for specific apples required changes in marketing and in apple

production. In response, farmers started to produce apples for sale at markets (before, only cider was sold, as fresh apples were for the farmer's own use): desirable cultivars were planted in the meadow orchards, and in a significant departure from custom, the trees were planted in rows (Fig. 3). These orchards were the forerunners of today's orchards and marked the beginning of modern apple production. The new practices were less effective in reducing leaf litter, and the dispersal distance for ascospores was shortened considerably. The stage was set for scab to become a major problem, and scab epidemics requiring control to save the fledgling apple industry occurred in the 1880s (60).

In the United States, apple production did not flourish until the 1800s. Before that time, orchards were comprised mostly of seedling trees, with about 10% of the trees bearing grafted scions, but by the mid-1800s, orchards of grafted clonal trees were common (8). Such orchards increased rapidly in the mid-1800s. Losses due to insects and diseases, particularly codling moth, *Cydia pomonella* (Linnaeus), and scab also increased, but the causes were poorly understood and there were few effective control practices. Consequently, by the latter part of the 1800s, orchards were being cut down (8).

Scab is unacceptable only in commercial production because the market has imposed a high standard of quality for the fruit. As Nelson (59) noted, "While natural populations of hosts learned to live with their parasites, man could not or would not." This appears to be true of apple and *V. inaequalis*, because they had apparently

Fig. 4. Negative geotropism in ascocarp formation. Top, Leaf overwintered with adaxial surface uppermost showing pseudothecium with adaxially directed ostiole; center, leaf overwintered with abaxial surface uppermost showing pseudothecia with abaxially directed ostioles; and bottom, leaf repeatedly flipped during overwintering showing biostiolate pseudothecium (27).

learned to live with one another quite well before mankind began to tinker with the natural ecosystem. In the final section, we discuss the evolution of practices and programs to control, and later manage, scab, and we conclude with speculation on future directions of management.

Saprophytism

Ascospores produced within pseudothecia (Fig. 4) in fallen infected leaves constitute the principal, and often sole, source of primary inoculum for apple scab. Pseudothecia mature only after a protracted period

of development, and ascospores do not mature until spring. What, then, are some of the salient features of the ascigerous state, and what is it about these features that might conceivably make *V. inaequalis* a better pathogen of apple?

The ascigerous state is not initiated until the infected host leaves die. Within a few days after leaf abscission, hyphae from the subcuticular stroma grow downward into the palisade and mesophyll tissues of the leaf and there form the stromatic spheres that develop, after fertilization by a compatible mating type, into immature pseudothecia.

The pseudothecium is negatively geotropic during its development (27). Irrespective of which leaf surface was infected during the parasitic phase, the ostiole will be directed toward the surface of the fallen leaf that faces away from the earth and thus toward the orchard air (Fig. 4A and B). This orientation can be observed in early spring, can even redirect growth in disturbed leaf litter (Fig. 4C), and increases the probability that ascospores will eventually be carried aloft.

Pathogen populations are comprised of two mutually exclusive mating types. Thus, there is a strong correlation between disease severity and the probability that two compatible mating types will be present within the same leaf (28). More intense disease results in a higher percentage of leaves bearing fertile pseudothecia. However, the pathogen may have a backup system that ensures that some ascocarps will form, even at the lowest levels of disease incidence. We found that even at trace levels of disease in autumn, approximately 3% of the leaves bearing single lesions produced pseudothecia (28). Exactly how this is accomplished is unclear. However, it might be accomplished by dispersal of diads, triads, or tetrads of ascospores from the same ascus. If these were dispersed and then infect together, then both mating types might be harbored within a single lesion. Another, perhaps more plausible explanation, is that bipolar heterothallic systems are not quite as cut and dried as we might like to believe, and that some isolates of *V. inaequalis* retain the capacity of selfing. This has been demonstrated in *Uncinula necator*, where certain isolates will cross in protracted associations with a second isolate that appears initially to be incompatible (31). Coincidentally, the frequency of these oddball isolates was approximately 3% in a population of *U. necator*.

Adaptation toward tree phenology (Fig. 5). Apple is a deciduous, temperate zone fruit, and there are features of the development of the ascigerous state of *V. inaequalis* that are particularly well suited to survival of apple's dormant period. The thermal optima for the phases of ascocarp initiation and development vary through time as seasonal temperatures decline after leaf fall and then rise again in spring. Pseudothecia are initiated soon after leaf abscission, most within 1 month of leaf fall (24). They increase in diameter most rapidly at temperatures near 10°C, but their numbers per unit of infected leaf are maximized at temperatures near 4°C (24). No detectable growth or differentiation occurs at or below 0°C. Most pseudothecia enter winter as spheres of stromatic tissue approximately 40 µm in diameter.

Pseudothecial development accelerates as late-winter temperatures increase, and the change in thermal optima for development reflects the rise in mean daily temperatures. The number of asci per ascocarp

Fig. 5. Adaptation of *Venturia inaequalis* development during its sexual stage while the host is dormant to host phenology in spring as evidenced by thermal optima for pseudothecia initiation, ascus density, and ascospore maturation.

Fig. 6. Changes in leaf cluster size (A); susceptibility to scab (B); and amount of airborne inoculum of *Venturia inaequalis* (C) with growth stage of McIntosh trees. The product of $A \times B \times C$ is the relative risk of infection (D). GT = green tip, CG = 12.5 cm green, TC = tight cluster, P = pink, B = bloom, and PF = petal fall (19).

is inversely proportional to temperature during ascus proliferation (late-winter) and is maximized near 4°C (29). As temperatures continue to increase, ascospores are formed by free-cell formation. Their rate of maturation is maximized near 20°C (24).

This relationship between temperature and productivity, differentiation, and maturation has several advantages for *V. inaequalis*. First, the low thermal optimum of the pathogen at leaf fall maximizes the numbers of pseudothecia formed at the cooler temperatures that prevail at this time. The very slow development at temperatures near 0°C (84) and the subsequent increase in the thermal optimum for maturation have the net effect of synchronizing the initial maturation of ascospores with the initial emergence of host tissue from flower buds of the host. The degree of synchrony is nothing short of remarkable when you consider that the overwintering structure of the pathogen is often initiated a full 5 months before bud break. Furthermore, the dates of leaf fall and bud break can vary substantially at a single site, and the duration of the period between leaf fall and bud break may be as short as 3 months or as long as 6 months. Despite this variation, the pathogen consistently produces the first mature ascospores within a few days of bud break of the host (33).

There is evidence of further adaptation toward host phenology. *V. inaequalis* consistently discharges the bulk of its ascospores at the precise host phenological stage when they are most likely to successfully infect the host (Fig. 6). The fruit buds of apple trees pass through a succession of growth stages (phenophases) during the first 8 weeks of the growing season as leaves emerge as a rosette subtending the flower cluster. Fruit buds predominate on mature trees, and their development precedes that of vegetative buds by approximately 2 weeks. Host tissue is a target for airborne ascospores of *V. inaequalis*, and the probability of a spore contacting host tissue is a function of the target size and density.

During rain, ascospores are forcibly discharged to heights of 0.1 to 13.2 mm above the surface of overwintered leaves on the orchard floor (5). Thereafter, ascospores are carried aloft by turbulence and move through tree canopies on roughly horizontal trajectories until they either settle to the ground, contact the tree, or leave the orchard (33). Host tissue can therefore be represented as a two-dimensional target, i.e., the cross-sectional or silhouette area of the susceptible leaf and floral tissue emerging from apple buds (19). For the purpose of describing temporal changes in target size, one can consider a single fruit bud or the mean development of a group of fruit buds. Initial target size is 0 in dormant buds. Target size increases slowly through green tip and 1 cm green and then increases exponentially between 1 cm green

and late pink, reaching a maximum size at petal fall (Fig. 6A).

If target size were the only factor involved, then to maximize the probability of successful establishment, ascospore discharge would be delayed until leaves are fully expanded. And yet the peak of ascospore discharge occurs earlier with respect to host phenology. An additional factor to consider is that the susceptibility of leaves that emerge from fruit buds decreases with age (Fig. 6B), i.e., the leaves acquire ontogenetic resistance (19). The probability of successful establishment is therefore maximized when the product of target size and target susceptibility is maximized. This product is maximized between the pink and bloom stages of bud development, coincident with the peak in the distribution of ascospore discharge (Fig. 6C). The pathogen effectively copes with this trade-off between a target that is expanding and, simultaneously, becoming more resistant to infection. Nearly one-half year beforehand, the pathogen begins reacting to a series of variable environmental stimuli and does so with the ultimate effect of releasing the bulk of the overwintering inoculum within a 2-week period during which reestablishment in the canopy of host trees is most likely (Fig. 6D).

Ascospore discharge attributes. The bitunicate ascus of *V. inaequalis*, a characteristic of the subclass *Loculoascomycetidae*, is composed of a relatively thick-walled and rigid exoascus and a thin-walled, extensible endoascus. When leaf tissue is wet, water moves into the ascocarp either through the ostiole or through the porous ascocarp wall and then into the asci through osmosis (5). Physiologically mature asci (32) then swell and protrude through the ostiole. If the exoascus tip ruptures, the endoascus extends further until the entire structure is approximately twice its original length and protrudes approximately 40 µm beyond the ostiole

(14,15). Following the rupture of the endoascus tip, the uppermost spore is forcibly ejected (37,82). Each successive spore within the ascus is discharged after pausing briefly at the ascus tip. The osmotic nature of the mechanism of ascospore discharge results in a slowing of the process at low temperatures (78), presumably through effects on membrane permeability and biochemical processes.

The movement of the ascus from within a closed, protective, and opaque ascocarp into the atmosphere nearly 40 µm above the leaf surface is a significant feature of the process of ascospore discharge in *V. inaequalis* and creates an opportunity for several interactions between the ascus and the external environment. The absence of light, specifically far red light from 710 to 730 nm, is known to suppress ascospore discharge in *V. inaequalis* (14,15,34). However, the suppression of ascospore discharge during darkness is not absolute. For example, senescent pseudothecia (i.e., those remaining after 90% or more of the season's total inoculum has been discharged) may freely discharge ascospores during darkness (34). Additionally, airborne ascospores have occasionally been trapped following dew at extreme northern latitudes (77). However, in the vast majority of cases, airborne ascospores are detected by volumetric spore traps, and then primarily during daylight hours (34,54) (Fig. 7).

Why should *V. inaequalis* evolve such an elaborate mechanism to restrain the discharge of ascospores during darkness? What possible selective advantage could this confer upon the pathogen? Recognizing that this is speculative, we propose that the requirement of light for ascospore discharge restricts the discharge of ascospores into dew films. Dew forms frequently during the period when ascospores are maturing (24). The typical atmospheric conditions that lead to dew formation are clear,

Fig. 7. Four-year means of the percentage of ascospores trapped per hour during 1981 to 1984 in an orchard with high ascosporeic inoculum in New Hampshire (54).

relatively cold nights during which temperatures must fall substantially below the dew point to cause a heavy dew film on leaf litter. Dew films may form abundantly on subtending leaf litter, while substantial portions of tree canopies above remain relatively dry. Dew formation is also associated with still air conditions and relatively thick boundary air layers, both of which work against escape of ascospores from the leaf litter. Additionally, the thickness of dew films may exceed the extent of ascus emergence beyond the ostiole (ca. 40 µm), resulting in discharged ascospores becoming trapped within the dew film. This entrapment is supported by two lines of evidence in previous literature: (i) with the exception of a single study from Norway (77), airborne ascospores are rarely trapped during periods of dew, and (ii) ascospores have been found abundantly in the film of dew on leaf litter without accompanying airborne catches (54,57).

Fair weather begets dew and also generally follows dew formation. What, then, would be the typical fate of an ascospore discharged during darkness? There would seem to be little selective advantage to suppressed ascospore discharge during darkness if only rain were considered. However, ascospores might face all of the above-enumerated obstacles to establishment on the host during dew: (i) relatively low temperatures during the predawn hours, (ii) entrapment in thick water films on the leaf litter, (iii) relatively poor atmospheric conditions for aerial dispersal from the leaf litter, (iv) deposition on dry leaves of the host, despite wetness of the subtending leaf litter, and (v) if the tree canopy were initially wet by dew, then rapid drying after sunrise due to fair weather. The suppression of ascospore discharge by darkness has the ultimate effect of conserving the primary inoculum for discharge during the most favorable conditions for infection. Some opportunity for discharge is lost during night rains, but

the cost of this may be outweighed by the counterbalancing (and more frequent) avoidance of discharge during dew.

Ascospore dispersal attributes. Once discharged during a suitable rain event, the ascospore's next challenge is dispersal to the host. Ascospores are produced abundantly in the leaf litter, and beneath a severely diseased tree the ascospore dose may exceed several million per square meter of ground (28). The ascospores must accomplish the task of reestablishing the parasitic relationship after a period of host dormancy. To do this, they must first either return to the canopy of their original host-tree in spring or disperse to another suitable host at some more distant location. *V. inaequalis* appears to possess traits that are primarily adapted toward short-range dispersal, with some limited longer-range dispersal.

Infected leaves drop to the ground beneath an ideal host. The tree is susceptible (otherwise there would not be infected leaves beneath it) and it is perennial. However, without capacity for longer-range dispersal, the death of the original host tree might spell the end of the pathogen population in the fallen leaves. It is important to keep in mind that *V. inaequalis* did not evolve in an orchard with multiple and contiguous hosts. In its natural environment, there would presumably be selection for effective short-range dispersal to the original host tree, but with some eventual need for dispersal of spores to neighboring trees at uncertain distances. In addition to dispersal of spores, short-range dispersal of leaves by wind may move leaves to nearby trees, and this may have played a role in dispersal of the pathogen in natural environments.

The traits that are generally associated with short-range dispersal of spores are easily enumerated for *V. inaequalis*. The ascospores are produced abundantly but are discharged during a time when mass transport is strongly downward (i.e., during

rain). They are relatively delicate structures, without the thick cell walls or heavy melanization of the conidial state to protect them from desiccation or UV radiation. Despite the apparent lack of traits that confer competence for longer-range dispersal, recently developed models indicate that some *V. inaequalis* ascospores would be transported 2 to 5 km from point and area sources of inoculum (4), and presumably at such distances desiccation and irradiation are not significant mortality factors, given that the dispersal may occur in a matter of minutes at this range, and often during a rain event. The exact number of such ascospores that survive, are deposited on host tissue, and eventually cause disease at such distances remains unknown. Presently, scab is generally managed based upon assessments of potential ascospore dose within an orchard (51).

Summary: saprophytism. *V. inaequalis* has a highly specialized saprophytic phase which allows it to survive the intercrop period of its deciduous perennial host (Table 1). Leaf death is the signal that initiates growth into the internal tissues. Thermal optima for development change during overwintering to reflect declining and then increasing temperatures. A number of attributes have evolved that increase the probability of successful reestablishment after the host resumes growth in spring. Negative geotropism orients the ostiole toward the atmosphere. Ascospores mature within days of bud break, even though the ascocarp is initiated 5 months earlier and, indeed, the bulk of the population matures when the product of leaf size and susceptibility are maximized, thereby maximizing the probability of infection. Suppression of ascospore discharge during darkness may conserve inoculum that would otherwise be wasted during dew periods. Ascospore dispersal is primarily a short-range process evolved before cultivation of apple, and it appears to be adapted toward getting the ascospores from the leaf litter back to the

Table 1. Traits of *Venturia inaequalis* and possible relationship to parasitic fitness

Trait	Possible role
Saprophytism in ascigerous state, with ascospores being parasitic	Survival of host dormancy on shed, dead leaves and later establishment on new, living leaves
Shifting thermal optima and extended period of ascocarp development	Survival of extended period of host dormancy
Low thermal optimum during early aFscocarp development	Maximizes ascocarp numbers as mean daily temperatures are declining; maximizes ascus numbers at low temperatures during late winter to early spring
Higher thermal optimum during later ascocarp maturation	Increases rate of ascospore maturation as mean daily temperatures increase
Negative geotropism in ascocarp formation	Discharge of ascospores toward atmosphere; reorientation of ascocarp in disturbed leaf litter
Suppressed ascospore discharge during darkness	Conservation of ascospore inoculum during dew periods when environmental conditions would not favor dispersal and infection
Synchrony of ascocarp maturation and host development	Ascospores discharged at host developmental stage most favorable to contact and infection

tree from which the leaves dropped. Nonetheless, their abundant production and discharge during turbulent rain events results in some longer-range dispersal.

Parasitism

The arrival of ascospores on susceptible host tissue is a signal event in the life of *V. inaequalis* and marks the immediate switch from months of saprophytism to growth as an active parasite, with all of the attendant interactions with a living, growing, and resisting host. As with the saprophytic stages, the pathogen displays a remarkable degree of adaptation toward the host, and vice versa. We now consider those features that characterize this association.

Ascospores are covered with a mucilaginous sheath and adhere readily, even to the wet but hydrophobic waxy surfaces of apple leaves and fruit. Once deposited, they rapidly attach, probably by a mucilaginous coat (Fig. 8), and germinate. The pathogen is particularly well-adapted to infect the host at relatively low temperatures. In fact, the most recent studies have illustrated that infection by both ascospores and conidia can take place in as little as 30 h at 1°C (78). All steps in pathogen development and infection are slowed by low temperatures, but unlike many biotrophs such as powdery mildew pathogens, *V. inaequalis* is a very active pathogen at low temperatures.

Germination proceeds so long as free water is present on the host surface. An appressorium develops shortly after the ascospore germinates (80), followed by the growth of a penetration peg that pierces the cuticle but not the epidermal cell wall (76). During parasitism, *V. inaequalis* exists solely as a subcuticular parasite, wholly external to the host epidermal cells (Fig. 1). How the subcuticular stroma effectively extracts what it needs from host cells in such a noninvasive fashion without benefit of specialized absorptive structures such as haustoria is not known.

Ontogenic resistance. Despite its enigmatic means of sustenance, the pathogen

can increase its biomass considerably in a period of 7 to 10 days and ramify an area up to 2 cm in diameter before conidiophores erupt through the cuticle to produce the conspicuous olivaceous lesions that typify the disease. This rapid growth, however, occurs only in leaves that have not fully expanded. A counterbalance to the susceptibility of emergent and expanding leaves is ontogenic (age-related) resistance that is expressed in fully expanded leaves (Fig. 9). Ontogenic resistance increases rapidly with leaf age and is strongly expressed in cluster leaves shortly before bloom (19) and in leaves of vegetative shoots more than three to four nodes removed from the shoot apex (19,20).

Ontogenic resistance is one of the most significant features of the natural development of apple scab in the wild. For the pathogen, highly susceptible tissue is present throughout most of the growing season, and indeed, as was previously mentioned, the flush of young cluster leaves coincides with the flush of ascospores produced in early spring. Once the tree has bloomed, the preponderance of foliage is much more resistant to infection, but so long as shoot growth continues, there will remain a small proportion of the canopy that is highly susceptible to infection. However, the bulk of the foliage will be quite resistant, and although not immune, it will persist and continue to function despite relatively heavy disease pressure.

Ontogenic resistance not only affects the absolute number of infections, but also the time that it takes for a lesion to appear and the duration of time over which additional lesions will appear. Temperature has also been shown to affect the latent period, but the effect is relatively small compared with the impact of leaf age on the latent period. For example, a temperature change from 20 to 10°C will increase the latent period

from approximately 14 days to 21 days in highly susceptible leaves. In comparison, the number of visible lesions can more than double between 21 and 80 days after infection in leaves that are older, and hence express ontogenic resistance more strongly (21).

Once conidia are produced, the disease spreads mainly within the canopy of the infected tree. The subcuticular stroma may appear as a discrete lesion, or it may be an inconspicuous network of diffuse stromatic cells, with each giving rise to widely dispersed conidiophores. In general, the older and more resistant the leaf, the more diffuse or atypical the colonization that results (10,54). Irrespective of the lesion type, conidia are released into a water film that covers the conidiophore. If this is a light film of dew, the conidia may be dispersed on the same leaf and thereby increase disease severity on that leaf. During rain, the conidia are splash-dispersed to other leaves, generally downward in the canopy from the site of their production. Rarely are conidia detected as airborne spores outside of the drip line of the tree, even when they are trapped in extremely high densities in rain splash directly beneath the tree.

Subcuticular growth of the parasite is extremely slow in mature living leaves, but when the leaves abscise in autumn, the fungus returns to the saprophytic phase and growth accelerates, no longer under the restraints of ontogenic and horizontal resistance in a living host (84).

Summary: parasitism. There is a period in early spring when ascospore infections are established in the canopy. This is followed by a protracted period of local spread in which additional tissue (on the same leaf and on additional leaves) is infected by conidia. Ascospores function to establish new infections in the canopy after

Fig. 8. Transmission electron micrograph showing reaction of the cuticle and host cell wall beneath the penetration pore of a germinated ascospore of *Venturia inaequalis* on the leaf surface and mucilage believed to anchor the ascospore to the host surface. m = mucilage (76).

Fig. 9. Diagrammatic representation of ontogenic, major gene (vertical) and minor gene (horizontal) host resistances to *Venturia inaequalis* in an apple extension shoot with respect to incubation period and expression of symptoms.

overwintering beneath the host on shed organs; yet they retain some capacity for long-distance dispersal in the event that the original host-tree dies. Conidia function to increase the amount of foliar tissue colonized by stromata and to increase the likelihood of pairing of compatible mating types on the same leaf. Rarely do trees defoliate or suffer loss of photosynthetic area that is lethal to trees. While fruit may be infected and unmarketable, they are still produced in abundance and, more importantly, contain viable seeds. In view of all the interactions and associations discussed, *V. inaequalis* and its host have coevolved to a high degree. The pathogen is committed to a life of parasitism and requires the host to survive and reproduce; yet the host, although measurably damaged, does not die from the association, and despite being parasitized continues to survive, grow, and reproduce. Given this seemingly benign association, how has this level of genetic equilibrium come about, how has mankind's manipulation of the natural system affected the relationship between host and parasite, and what has been the resultant impact on epidemic progress?

Coevolution

Nelson (59) speculated that the initial resistance of a host to a new parasite occurred by a genetic change at a single locus and that the resistance reaction was probably a hypersensitive response. The parasite population then evolved by the selection of a strain (race) that was virulent to the then-resistant host. Short-term parasitic success and host biological success were selected against in favor of long-term success: selection pressure favored host and parasite genotypes that were compatible. For example, a virulence allele that resulted in a response that killed the host would, over time, be selected against because the susceptible host population would be eliminated, or a resistance gene that conditioned a hypersensitive reaction would be ephem-

eral because it would place infection pressure on the selection of a virulence allele that allowed the parasite to become established. Thus, coevolution continued in a stepwise, gene-for-gene manner that resulted in the accumulation of many resistance genes and corresponding virulence genes. As coevolution progressed, the genetic changes conditioned more subtle response.

Ultimately, a genetic equilibrium was achieved that each could live with and, according to Nelson (59), further genetic changes incorporated into a population of the host or parasite would condition no more than slight improvements in host or parasite fitness. Was this the process by which apple and *V. inaequalis* coevolved to their level of genetic equilibrium (compatibility) at the beginning of the modern era of commercial apple production? We can't say with certainty that it was, but research has provided us with some insights that suggest it was.

Major (race-specific) genes conditioning the hypersensitive, "pit-type" reaction mentioned by Nelson (59) as a likely initial response to attack by a new parasite are present in *Malus* species and in many old domestic apple cultivars and progenies from them, but two backup resistance systems (race-nonspecific and ontogenic) combine to limit infections when major gene resistance is overcome (Fig. 9), as discussed in the following sections.

Major (race-specific) genes for resistance and corresponding virulence genes that exhibit gene-for-gene resistance have accumulated in apple and *V. inaequalis*. Much of the early work in Europe (1,2,67-72,83,84), the United States (41,44,46,61), and Canada (39) demonstrated variability of the reaction of susceptible cultivars and crabapples toward *V. inaequalis*. More detailed investigations confirmed the earlier observations and studies: particular isolates attacked the cultivar from which they originated more severely than they

attacked other cultivars (62,74,84). In the mid-1900s, genetic analysis became popular, and genes for pathogenicity (defined today as virulence/avirulence genes) were identified that had selectively overcome resistance genes in crabapple and particular selections of domestic apples. The segregation for resistance to scab in the progenies of controlled crosses with *M. atrosanguinea*, *M. floribunda*, and *M. micromalus* revealed that resistance (hypersensitive reaction) in each species was conditioned by a single gene in the heterozygous condition (reviewed in 51, pp. 81-86). Seven loci in *V. inaequalis* conditioning avirulence/virulence to several domestic cultivars were identified (13), and 19 pathogenicity genes (diallelic virulence/avirulence genes) were identified in crabapples (6,7,12,41-43,85).

Ephemeral resistance genes in domestic cultivars. Many of the species and subspecies (crabapples) that comprise the genus *Malus* (49) intercross freely (self-incompatibility is the rule) in the natural ecosystem, and some that were very likely included in the early development of the domestic apple, e.g., *M. pumila*, *M. sylvestris*, and *M. baccata* (16), also have major gene resistance to *V. inaequalis*. All domestic cultivars with lineage to these species and subspecies would be expected to have a combination of these major genes.

Knowledge acquired in the 1940s and 1950s that many older domestic cultivars such as McIntosh, Yellow Transparent, Haralson, and Red Astrachan susceptible to scab carried several alleles that conditioned avirulence/virulence to *V. inaequalis* was suggestive that all other susceptible apple cultivars with lineage to the same resistance pool also carried the ephemeral differential resistances. Nevertheless, the notion that susceptible "modern" domestic cultivars lack major resistance genes found wide consensus.

All the ephemeral resistances putatively present in *Malus* × *domestica* were forgot-

Table 2. Macroscopic symptoms^a induced by selected differential isolates of *Venturia inaequalis* on domestic apple cultivars^b

Origin of isolate	Test cultivar							
	A. Reinette	Boskoop	Glockenapfel	Golden Delicious	Jonathan	Gravenstein	R. Champagne	Spartan
Ananas Reinette	3	1	0	2	0	0	0	— ^c
Boskoop	0	2	0	0	1	0	1	0
Glockenapfel	2	1	4	3	1	0	1	—
Golden Delicious	1	0	1	4	0	0	1	—
Jonathan	0	0	0	0	3	—	0	—
Gravenstein	0	0	0	0	0	4	0	—
Maigold	2	3	0	3	3	—	—	0
Reinette de Champagne	0	1	0	1	2	0	4	—
Spartan	3	4	4	4	4	4	4	4

^a Symptoms: 0 = no visible symptoms; 1 = chlorotic and/or necrotic spots; 2 = chlorotic and/or necrotic spots with sparse sporulation; 3 = lesions with moderate sporulation; 4 = lesions with abundant sporulation.

^b The exemplary isolates were selected based on the host-of-origin and ability to differentiate as many differentials as possible. Isolate-cultivar combinations were considered compatible if they induced symptoms scored with 2, 3, and 4, and incompatible with score of 0 or 1 (adapted from 44).

^c Not tested.

ten or, at most, remained a curiosity (35) until recently when it was reported that Golden Delicious carries a differential resistance gene (36) designated Vg (9), and in another study (75), that there was differential resistance in Boskoop, Golden Delicious, and Spartan. When these findings are considered with the earlier literature, it becomes plausible that many other such ephemeral resistance alleles are present in *Malus × domestica*, and that idea has been investigated. Monosporic isolates of *V. inaequalis* were obtained from lesions caused by early-season infections in five orchards across Switzerland and tested on a range of cultivars (Golden Delicious, Ananas Reinette, Boskoop, Glockenapfel, Jonathan, Gravenstein, James Grieve, Maigold, Reinette de Champagne, Spartan, and Yellow Transparent). Differential interaction with selected isolates was shown for all cultivars (48). Avirulent and virulent isolates could be determined for each test cultivar, and the pattern corresponded to the classical vertical (differential) resistance scheme. This led the authors to conclude that functionally different resistances are present in commercial apple cultivars and that corresponding virulence/avirulences are present in the pathogen. Table 2 (selected data from 48) shows that all interactions typical for a gene-for-gene relation can be found.

The picture that emerges from this research is that functionally different resistances are present in every cultivar and that *V. inaequalis* has corresponding virulence/avirulence genes that can match these resistances: an isolate (spore) cannot successfully attack an apple cultivar unless all of the differential (ephemeral) resistances are matched by the corresponding virulence alleles. Support for this statement comes also from the appearance of races virulent to cultivars bred with major gene resistance to *V. inaequalis* (65).

Interactions between apple and *V. inaequalis* and long-term survival. Survival of apple and *V. inaequalis* over the long term has been achieved through the selection of genes conditioning interactions that allow a variable portion of the local *V. inaequalis* population to develop a stroma and produce conidia after causing only limited damage to apple leaf tissue. However, once the single tree is confronted with the corresponding *V. inaequalis* genotype, limiting secondary buildup of scab is also a requisite for long-term lifelong success of a particular apple tree in the natural system. Apple's minor (race-nonspecific) gene resistance system and its ontogenic resistance are at this stage the main determinants of long-term survival of a tree.

Minor (race-nonspecific) genes and their interaction with *V. inaequalis* genotypes (Fig. 9). Apple has a durable, race-nonspecific (horizontal) quantitative resistance system thought to be comprised of many genes that condition a basically

compatible interaction in which the parasite produces a sporulating stroma with little noticeable damage to host tissue. The genes in this system condition the subtle responses Nelson (59) predicted would occur in the long process toward achieving genetic equilibrium. The significance of this resistance system is threefold: it limits the extent the parasite can colonize and damage a susceptible host, it is effective against all races of the parasite, and it is durable. Its interactions with a pathogen genotype also determine the length of the incubation period and the amount of sporulation, two important fitness attributes that affect the rate of scab buildup. This quantitative resistance may be expressed to various degrees and is postulated to be determined by the additive effect of a variable number of quantitative trait loci.

Ontogenic resistance and susceptible tissue (Fig. 9). Ontogenic resistance reduces the number of infections, while still allowing *V. inaequalis* to be successful, by limiting the amount of susceptible tissue on each shoot almost entirely to the youngest two or three leaves, as described above. It apparently becomes inoperative in autumn, perhaps due to processes involved in leaf senescence, but this is not a direct threat to the host. In a nonbearing seedling, ontogenic resistance would slow scab-induced defoliation to nearer the time of normal leaf fall, thus lessening the weakening effect, and possibly death, an early defoliation might have on a young seedling. In a bearing tree, fruit is already produced by autumn, and the production of seed is how success (survival) in apple is measured.

The nature of ontogenic resistance is not known, but it apparently is durable because we know of no report that it was not operative in matured leaves on an actively growing shoot under natural conditions. The apparent absence of ontogenic resistance in autumn allows for the buildup of successful parasite genotypes in preparation for sexual reproduction after leaf fall. This may lessen the selective pressure for genotypes that can overcome ontogenic resistance and, if so, may account in part for the evolutionary durability of ontogenic resistance.

Strategies to Achieve Genetic Equilibrium (Compatibility)

Reproductive strategy in apple that provides the genetic diversity to cope with genetic changes in *V. inaequalis*. The production of seed with new combinations of major resistance genes is the key to the success of major gene resistance in natural *Malus* ecosystems. Each seed has a distinct combination of major resistance genes, and an ascospore can infect a seedling and develop a sporulating stroma only if it has a matching set of virulence genes. The significance of major gene resistance is that many ascospores do not have the

matching combination of virulence genes: the number of sporulating lesions on any seedling will be proportional to the number of ascospores with the correct combination of virulence genes. The seedling with major resistance genes that have the fewest matches with ascospore virulence genotypes will be the most resistant to that parasite population and the most likely to survive attack by *V. inaequalis*. Before mankind interfered with the natural ecosystem, sexual reproduction, along with horizontal and ontogenic resistance systems, was how apple kept the infection rate low enough to survive, i.e., for a seedling to grow and produce fruit.

Major resistance genes common to both parents is not desirable for apple because corresponding virulence alleles in the parasite population will become fixed, i.e., be present in all ascospores. Outbreeding (self-incompatibility) is the host strategy to diversify, as much as possible, the alleles that reduce parasite fitness. If apple were self-compatible and a cultivar were susceptible to scab, then all siblings (seedlings) of that cultivar (theoretically) would be susceptible to all ascospores produced from infections that occurred on that cultivar just as (theoretically) all conidia produced in lesions on that cultivar would infect that cultivar. Virulence alleles would become fixed in the pathogen population, and all seedlings would be susceptible. This relationship would not change until an allele appeared in the host that conditioned an incompatible response. In the natural ecosystem, the host tree has many half- and full-siblings growing nearby, and any sibling with a unique combination of resistance alleles will be best fit. In a commercial orchard, adjacent trees are likely to have an identical resistance genotype.

***V. inaequalis* reproductive strategies that provide the genetic diversity to cope with genetic changes in apple (Fig. 10).** For *V. inaequalis* to survive in the natural ecosystem, some ascospores must establish new infections when tree growth is renewed in spring. This is not a problem if the tree infected the previous growing season is still alive, because at least some ascospores will have the combination of virulence alleles needed to infect and produce a sporulating stroma. Survival is threatened if the tree dies, because apple is an obligate outbreeder and each sibling growing nearby will have a unique combination of race-specific resistance genes that an ascospore can overcome only if it has a matching set of virulence genes. Crossing experiments in the 1940s (13,40–42,44) using *V. inaequalis* strains with specific virulence to apple cultivars carrying differential resistances showed that new combinations of virulence genes do occur in ascospores and that the expression of pathogenicity (defined today as virulence) of the parasite is determined by host and pathogen genotypes. Thus, to increase the

chance that a half- or full-sibling in the vicinity produced by the dead tree, or any other apple trees nearby, will be infected, the production of ascospores is the strategy that has assured survival. Once a few ascospores have established sporulating lesions, the successful genotypes will build up through secondary cycles of asexually-produced conidia. If two different mating types infect the same leaf and sexual reproduction occurs after leaf fall, the parasite has been successful.

Cultivar Relative-Resistance Can Change

A cultivar's susceptibility/resistance to scab may vary from one region to another, or even at different sites within a region. This was first reported in 1899 by Aderhold (2), who concluded that the resistance of a particular cultivar depends on its interaction with the specific fungal population present. It has also been observed that a cultivar's resistance changed noticeably within an orchard or region. Golden Delicious trees in a secluded planting with new resistant cultivars (Freedom and Priscilla) did not develop scab for the first 5 years, even though an old, large, susceptible apple tree that became severely scabbed each year was only a few meters away, but subsequently these Golden Delicious trees were noticeably scabbed if not protected with fungicide (C. Gessler, *personal observation*). Earlier, in England, Bramley's Seedling was listed as scab-resistant, but by the early 1930s it had become highly susceptible (38). In the northeastern United States, Baldwin was considered highly resistant to scab in the late 1800s and early 1900s, and Baldwin trees grown among very susceptible McIntosh trees would have very little scab even though the McIntosh trees were severely scabbed. By

1925, however, there were instances when Baldwin apples were as badly infected as McIntosh and required the same fungicide schedule for control (17). When a well-managed Baldwin orchard in New York state yielded 98% scabbed fruit in 1910, Wallace (82) speculated that the outbreak of scab was caused by a new strain of the pathogen that had appeared by chance. Was this what happened not only with Baldwin but also with Golden Delicious and Bramley's Seedling? Research on the genetics of this pathosystem in the mid-1900s and on pathogen race composition in orchards in the late-1900s provide a plausible explanation why cultivar resistance may differ between sites or change over time at a site.

Birth of a New Pathogen Race

Six major gene resistances at different loci from six *Malus* species have been named (Vf, Va, Vb, Vbj, Vr, and Vm). Breeding programs for scab-resistant cultivars have relied mainly on these resistances, with the resistance from *M. floribunda* clone 821 (Vf) present in most released scab-resistant cultivars. Classification into physiological races was, and is, based on particular wild *Malus* sources differentiating selected pathogen isolates. Until 1993, five physiologic races had been reported and numbered (one race associated with each of the named resistances except Vf, which had not been overcome, to anyone's knowledge). In the late 1980s, scab lesions developed on seedlings of Prima carrying the Vf gene at the Institut für Gartenbauliche Pflanzenzüchtung in Ahrensburg, Germany (65). The strain causing the lesions was numbered race 6 and was distinguished from the other five physiologic races by its ability to incite sporulating lesions on hybrids carrying the Vf resistance. Knowledge gained from the

genetics research explains why Prima became susceptible to scab: until a new strain (race) appeared that had a virulence allele matching the Vf resistance allele, there were no genotypes in the pathogen population that could successfully infect Prima. What was revealed when Prima became susceptible to scab in that orchard is that (i) a major resistance gene is ephemeral, (ii) when the gene is overcome, it is no longer identifiable in that orchard even though it is present, and (iii) although it is not known how many races were in that orchard, one new race was added. After nearly a century, Wallace's speculation in 1910 that a new strain of *V. inaequalis* was responsible for the severe scab that occurred on very resistant Baldwin trees was given strong support.

Prima's becoming susceptible to scab was very likely a reenactment of an event that had occurred many times during the coevolution of apple and *V. inaequalis*. If so, considering the knowledge mentioned above, it is plausible that each cultivar has a unique combination of many ephemeral major resistance genes and that there are many races of *V. inaequalis*, as Shay et al. (73) noted nearly 40 years ago: "Few, if any, of the commercial apple varieties are resistant to a sufficient proportion of the total US or European population of biotypes of *V. inaequalis* to warrant their use as resistant parents in apple improvement," therefore "there has been no useful purpose to be served by describing and naming the hundreds of different physiologic races that can be differentiated by these hosts." It follows that the proportion of pathogen spores in an orchard that can infect a cultivar will be an important determinant of the cultivar's relative resistance to scab expressed in that orchard. This would explain why a cultivar's relative resistance to scab may differ from one orchard to another within a region or from one region to another, and recent research seems to bear this out. When 21 single conidial isolates, seven each from three cultivars (Boskoop, James Grieve, and Spartan), were pooled in equal amounts and inoculated onto the three cultivars, the only isolates recovered from each cultivar after three asexual cycles on each cultivar were those that were originally isolated (74). The virulence allele frequency toward each cultivar had changed from 33 to 100%. There was also a dramatic change of isolate frequency within each subpopulation. In all three cases, over 90% of the subpopulations were composed of two genotypes, indicating the interaction system is highly complex. In addition to the relatively simple virulence/avirulence differential-resistance relationship that exerts selection pressure, genotypes were apparently being selected that more or less fit the particular conditions, thus contributing to further diversify the pathogen population.

The research confirmed that a cultivar does not allow survival of genotypes that do not have the correct matching virulence

Fig. 10. Parasitic and saprophytic phases in the life cycle of *Venturia inaequalis* diagrammed to identify the three reproductive strategies and practices to control each reproductive strategy.

alleles, as mentioned above. It is clear from what we have presented that race composition in an orchard is an important factor determining the relative resistance of a cultivar. It has become evident from another study (48) in Switzerland that cultivar composition in an orchard provides selection pressure for pathogen genotypes. In an orchard of a single cultivar, all isolates tested were virulent to the cultivar present. Other virulence genes were present at low frequency, and some virulences were absent (or below the detection level). In an orchard with many cultivars, no virulence was fixed (present in all isolates tested), and the frequency of isolates virulent on each cultivar reflected the quantitative presence of the various cultivars. Generally, the highest frequency of any additional virulence was found in populations from orchards with a cultivar mixture; the lowest frequency of any virulence (excluding the matching virulence) was found in populations in orchards of a single cultivar. A high proportion of isolates originating from cultivar mixtures with additional virulence to Golden Delicious reflects the past and present importance of this cultivar in Switzerland and in Europe (36 and 32%, respectively) of traded apples (58). In addition, 24% of the commercial orchards are planted with cultivars having Golden Delicious as one of the parents.

The results of these studies provide insights for potential new directions in scab management. The possession of multiple virulence genes in a population gives ascospores a selective advantage in cultivar mixtures, whereas in monoculture, only one virulence pattern is needed for successful reproduction. In the natural ecosystem, the variation in resistance gene composition among apple individuals counteracts this to reduce scab incidence. If this strategy is effective in the natural system, why wouldn't it be successful in commercial apple production?

Computer simulation models with apple and *V. inaequalis* predicted a reduction in lesions of approximately 70% after six asexual generations in a mixed compared with a pure orchard (11), and preliminary results in an orchard study are in general agreement (C. Gessler, *unpublished*). Scabbed fruit on Golden Delicious-only rows was reduced from 20% (year 2000), 66% (year 1999), and 41% (year 1997) to 1, 27, and 9% in Golden Delicious, Elstar, and Pinova rows, respectively. It does not appear that the mixed-cultivar strategy alone would provide sufficient commercial control of scab, but reducing the infective inoculum by 50% could have value in a scab management program if integrated with other management strategies. For example, reducing the ascospore inoculum 50% with sanitation and 70% in a mixed-cultivar planting would, theoretically, reduce the ascospore dose by approximately 85%. This reduction in ascospore dose

might reduce scab to a level that would be manageable with less fungicide than currently recommended (see management section).

Disease Management

Sexual reproduction: the pathogen's Achilles heel. *V. inaequalis* is apparently committed to parasitism, because it is not known to live as a saprophyte in nature other than in fallen leaves that had been infected before the leaves fell. Nevertheless, the saprophytic phase is critical to the survival of *V. inaequalis*. It is closely synchronized with apple's dormant period (Fig. 5), a major reason *V. inaequalis* is a successful parasite on apple. However, apple's dormant season is also the most perilous time for *V. inaequalis*. Sexual reproduction provides the linkage from one growing season to the next, so the first few weeks after leaf fall, when the sexual stage must be initiated before the onset of freezing temperatures, is the parasite's most critical period for survival (Fig. 10). If the sexual stage doesn't occur at that time, the life cycle will end when the leaf litter is decomposed. Preventing or disrupting this vital, vulnerable stage in the pathogen's life cycle is an obvious control strategy. Why, then, have control programs relied almost entirely on fungicides to protect the trees against primary and secondary infections rather than on practices that attack this weak link in the pathogen's life cycle? The answer is found in the origin of scab control programs.

The Origin of Scab Control Programs

Apple scab became a problem that needed to be controlled in the mid-1800s when the modern era of intensive apple production was initiated. The new horticultural practices set the stage for a scab epidemic, and scab became prevalent in European orchards in the late 1800s as a result of several years of weather highly favorable for scab. By coincidence, Bordeaux mixture, the first widely used fungicide, was developed in France at that time to control downy mildew of grape. Bordeaux mixture was soon tested for scab control, and repeated applications reduced the scab problem (60).

The success with Bordeaux mixture established the strategy for scab control throughout the twentieth century. First there was the development of more efficacious, less phytotoxic inorganic fungicides, then the development of synthetic organic fungicides. An arsenal of fungicides with several new modes of action became available, and new spray schedules were developed to utilize their protectant, after-infection (eradicant), presymptom, and postsymptom activities (reviewed in 51).

The advantage of *V. inaequalis* in modern apple production. Apple culti-

vars are propagated vegetatively, and therefore the sexual process that produces seedlings with new combinations of resistance genes, some of which may have greater resistance to the local pathogen population, is circumvented. In contrast, *V. inaequalis* reproduces sexually each year, and each year it has multiple opportunities to infect and reinfect the same cultivar with new genotypes, some of which may have improved parasitic and biological fitness factors. This situation clearly favors change in the parasite, potentially at greater expense to the host, but in what ways has it affected the compatibility that had been achieved between *V. inaequalis* and apple?

There is no evidence that *V. inaequalis*, considered as a population entity, has become more aggressive. That is, scab lesions are no larger today than they were 100+ years ago, and the pathogen is still restricted to the subcuticular space until leaf fall, with little noticeable damage to underlying cells. What, then, has been the major effect of modern apple production practices on scab epidemiology? We suggest it is primarily an increase in the proportion of successful infections, i.e., an increase in infection efficiency, caused by an increase in the proportion of compatible ascospores in an orchard or region. Data from Sierotzki et al. (74) are suggestive that even between compatible genotypes, some genotypes may have a higher infective and reproductive capacity and are able to displace weaker genotypes during asexual multiplication on the same host. This cannot be proved because there are no data to compare epidemic rates in modern and earlier orchards with similar ascospore dose and infection conditions, but an analysis of horticultural practices introduced into apple production over the past century leads us to that conclusion.

Herbicide-treated strips within tree rows and repeated mowing provide an unobstructed pathway for discharged ascospores to reach the tree canopy, and semi-dwarfing and dwarfing rootstock and new pruning techniques place the tree canopy close to the inoculum source (Fig. 3). These practices allow a greater proportion of discharged ascospores to reach susceptible tissue and, consequently, increase the number of successful infections for a given ascospore dose. Considering the genetic uniformity in modern orchards planted with one or very few cultivars, if we were to design an apple orchard to optimize ascospore dispersal to a host that most of the ascospore population can infect, what more could we do? The effect of these horticultural practices on scab epidemiology was not readily recognized because new fungicides, new strategies for applying fungicide, and improvements in application technology had kept pace with the increased demands these practices unknowingly were imposing on scab control.

Although there is no direct evidence that modern orchards are under greater disease pressure (more vulnerable to scab) than orchards 100 years ago, the severe outbreak of scab that often follows a missed application or incorrect application rate of fungicide does indicate the potential for a scab epidemic that exists in orchards today. In the early 1900s, three fungicide sprays, probably not as efficient as today, provided acceptable control of scab, whereas today missing one spray at the peak ascospore season in a schedule that may include 10 to 18 sprays can result in an unacceptable level of scabbed fruit.

Pathogen Life Cycle: Clues to Management Strategies

Reproductive success is a key measure of parasitic and biological fitness, and three reproductive strategies help to ensure the reproductive success and thus survival of *V. inaequalis*. Management practices are aimed at disrupting these three strategies (Fig. 10).

Strategy 1: Establish primary infections. Beginning with renewed tree growth after winter dormancy, the first strategy helps to ensure that ascospores with recombinations of successful genotypes the previous season will be deposited on susceptible tissue when infection conditions are likely to exist. This is accomplished by having ascospores mature over several weeks (beginning around bud break), discharge during rain, and disperse (by chance) to susceptible tissue. This strategy continues until around petal fall, after the maximum amount of susceptible tissue has been produced and ascospores are nearly depleted.

Strategy 2: Build up successful genotypes. The second strategy helps to ensure that the two different biotypes required for sexual reproduction are present on the same leaf. This is accomplished by the production of conidia that are splash-dispersed from lesions to susceptible tissue, mostly within the same tree canopy. The incubation period is relatively short, usually 2 to 3 weeks, and secondary cycling can occur until leaf fall.

Strategy 3: Produce new combinations of the successful genotypes. The third strategy provides new combinations of genotypes that had successfully infected the host during the growing season, some of which may make the fungus more parasitically and/or biologically fit. This is accomplished when opposite mating types on the same leaf mate soon after leaf fall to produce immature pseudothecia.

Defensive and Offensive Strategies and Tactics

Two basic strategies have been used to disrupt or stop the three reproductive strategies: a defensive strategy to protect the tree against infection by ascospores and conidia and an offensive strategy to reduce

the ascospore dose during the parasite's saprophytic phase (Fig. 10). Defensive tactics have included fungicide treatment and apple's natural resistance. Offensive tactics have included chemical, physical, and biological means to attack the fungus in the leaf litter or to decompose or remove the leaf litter.

Defensive tactics. Protection with resistant cultivars. The most effective tactic to protect the tree has utilized apple's natural resistance. In an orchard planted with a scab-resistant cultivar, no fungicide is needed specifically to control scab because there are no races virulent on that cultivar. However, the resistances bred into scab-resistant cultivars have mainly been conditioned by a single gene effective against the local population of *V. inaequalis* that had not coevolved in the presence of this gene. New mutations or migration of compatible genotypes able to overcome this resistance followed by selective pressure for this virulence has resulted in the appearance and buildup of physiological races. To date, this has occurred with the Vf major resistance gene bred into resistant cultivars. Grower concern for the buildup of resistant populations and poor market acceptance of resistant cultivars have kept the acreage of apples planted with resistant cultivars low.

Protection with mixed-cultivars. Coevolution of apple and *V. inaequalis*, as far as we can plausibly reconstruct from the data presented above, provide some insights for potential new directions in scab management. In monoculture, only one genotype with the matching virulence pattern is needed for successful reproduction. Virulence alleles will be immediately fixed, and therefore all ascospores will be potentially able to infect. In the natural ecosystem, the variation in resistance gene composition among apple individuals counteracts this to reduce scab incidence. If this strategy is effective in the natural system, why wouldn't it be successful in commercial apple production? The preliminary results mentioned above indicate in this direction, but such trials need to be made in various locations over long periods.

Protection with fungicides: scab warning system. The main focus of scab control programs throughout the past century has been to protect the trees against primary infections through repeated application of fungicides. The typical program in 1999 centered on a warning system: a weather station to monitor and record the weather variables needed to predict Mills' scab infection periods, software to calculate Mills' infection periods, strategies to apply fungicide based on the distribution and predicted severity of infection periods, fungicide-resistance strategies, and several means to notify the grower of current infection conditions (53). Some programs also included a model to predict the daily

progress of ascospore maturation (24). The most advanced programs also included a procedure to predict an orchard's potential ascospore dose and a scab-risk action threshold based on an autumn assessment of foliar scab (50,52,53). All of the models and procedures listed above aid decision-making to select and schedule fungicides to protect the tree against infection by ascospores and conidia. Their purpose is not to replace fungicides with other practices; rather, it is to improve the efficiency in using fungicides.

The fungicide schedules developed throughout the past century were designed mainly to protect the fruit. Emphasis was on preventing infections by ascospores, and a final application was recommended around the time of terminal bud set. The intended result, i.e., a very low incidence of scabbed fruit, was achieved, but man unknowingly exacerbated the scab problem because he did not take into account the parasitic fitness significance of secondary infection cycles, which is to increase the chance that a leaf will be infected by opposite sexual biotypes. Not providing chemical protection through the remainder of the growing season allowed scab to build up in autumn, a phase of the pathogen's life cycle that was not fully appreciated until recently (51). As a result, inoculum pressure (ascospore dose) in spring was often much greater than expected. Attacking the pathogen with chemicals in autumn to reduce its overwintering stage was not overlooked, however, nor has the full potential for including eradicant practices in scab management been realized, as discussed below.

Offensive tactics. Keitt, in Wisconsin, and Palmiter, in New York, conducted eradicant studies beginning in the 1920s and continuing to the mid-1940s (reviewed in 51). Their original intent was to explore the idea that by attacking the pathogen on fallen leaves through ground applications, "...a sufficiently drastic reduction in the ascospore inoculum might ensure the success of the protectant program, even under the most favorable conditions for scab development, and that lessening the severity of the strain on protectant spraying might permit the use of milder fungicides or fewer applications" (45). Their research had immediate success. In 1937 (45), they concluded that "... (i) suitable applications of eradicant fungicides are capable of drastically reducing the ascospore inoculum and (ii) such reduction greatly retards and ameliorates the development of the disease, especially in the critical period of primary and early secondary infection," and in 1940, Palmiter (63) stated that "...such eradicant treatments can reduce a potentially heavy ascospore inoculum to such low levels that mild fungicides may be used with comparative safety."

When the eradicant studies were completed in the mid-1940s, it was clear that

an eradicator treatment enhanced the recommended sulfur program, but it was concluded that "At this stage in the development of ground treatments it is not suggested that growers omit any regular spray applications because of the ground treatment but consider it an extra spray to aid the regular wettable sulfur program clean up a bad scab situation" (64). Thus, even though the studies showed convincingly that acceptable control of fruit scab could be achieved with fewer fungicides in an orchard treated with an eradicator chemical, there was no recommendation to reduce seasonal fungicide sprays in conjunction with a ground eradicator treatment.

It has been 65 years since Keitt and his coworkers stated the basic epidemiological principles that related inoculum density to the rate of scab buildup and the time when intervention with fungicide treatment must be initiated. Yet, at the present time, there are a very limited number of management programs that base their fungicide schedule on a quantitative assessment of primary inoculum in individual orchards or integrate an eradicator treatment with a reduced seasonal fungicide program. The following may explain why. Until recently (25,26,30), (i) *all* relevant studies were conducted in high-inoculum orchards, (ii) the *majority* of these studies were concerned with the effect of eradicants on the production of ascospore inoculum, (iii) only a *few* studies had considered treatment effects on scab buildup, and (iv) *no* studies had related treatment effects on the ascospore inoculum to a reduced fungicide dose through a strategy that adjusted the standard scab fungicide schedule to accommodate the lowered ascospore dose. The recent revival of the eradicator strategy stresses the usefulness of an eradicator program (chemical, biological, and/or cultural sanitation practices) in conjunction with an *action threshold* for fungicide intervention.

Reduce leaf litter. Practices that reduce the population density of *V. inaequalis* indirectly by their action on the leaf litter may remove the leaf litter, render the leaf litter unsuitable for the saprophytic and sexual activities of *V. inaequalis* that occur in dead leaves, or prevent discharged ascospores from escaping into the orchard air. Examples include (i) physically removing fallen leaves from the orchard by hand or by machinery, (ii) raking and then plowing the leaf litter under the soil, (iii) raking and then burning the leaf litter, (iv) shredding the leaf litter with a flail mower, (v) treating the leaf litter with chemicals that hasten decomposition (by making it more vulnerable to attack or by increasing the population density of decomposers), (vi) treating the leaf litter with microorganisms that decompose the leaves or grow on the leaf surface and physically prevent discharged ascospores from further flight into the orchard air, and (vii) utilizing earthworms to bury leaves (reviewed in 51).

Attack V. inaequalis directly in leaf litter. Practices that act directly on *V. inaequalis* may restrict or prevent pseudothecial production and maturation or prevent ascospore maturation and discharge. Examples include (i) treating scabbed leaves with pesticidal chemicals, nonpesticidal chemicals, or plant extracts that suppress the development of pseudothecia and ascospores or are lethal to the fungus, and (ii) treating scabbed leaves with an antagonistic (e.g., hyperparasitic) microorganism (reviewed in 51).

Integration of Defensive and Offensive Strategies

The most advanced scab management program in 1999 integrated defensive and offensive management strategies (50,52,53, 55,56). In addition to the warning system and models mentioned above, there were action thresholds for orchards predicted to be at high risk, moderate risk, or low risk for scab determined by an autumn assess-

ment of leaf scab (Fig. 11), and several tactics were available for each level of predicted scab risk. In a high-risk orchard, the grower is alerted to prepare for a full-season fungicide schedule and to consider sanitation during the dormant season if the scab risk is very high. In a low-risk orchard, tactics can be employed that reduce the seasonal fungicide dose, reduce fungicide costs, and use fungicides more compatible with beneficials and fungicide-resistance strategies.

A sanitation action threshold identified the range of scabbed leaves assessed in autumn at which sanitation would lower the ascospore dose to that of a low-scab-risk orchard, and an orchard within that range of scabbed leaves was designated moderate scab-risk. The sanitation zone (i.e., sanitation action threshold) in Figure 11 is based on two sanitation practices, applying urea or shredding the leaf litter (Fig. 12), that lowered the ascospore inoculum by at least 50% (79). An orchard

Fig. 11. Scab-risk action threshold (left) and sanitation action threshold (right) for selecting a fungicide and sanitation program to control scab based on an autumn assessment of leaf scab. Sets of 10 trees (10 shoots per tree) are examined until the total scabbed leaves falls within the high-risk or low-risk zone. If sanitation practices are possible, sanitation applied when the assessment falls within the moderate-risk zone will lower the risk to the low-risk zone.

Fig. 12. Percent reduction in ascospores trapped above leaf litter that had been shredded or treated with urea compared with ascospores trapped above untreated leaf litter (56).

with 80 scabbed leaves in the autumn assessment, for example, would be considered to have 40 scabbed leaves if the leaf litter were shredded, and 40 scabbed leaves is within the range of a low-scab-risk orchard (Fig. 11).

The significance of the sanitation action threshold is that sanitation is coupled to tactics (e.g., urea treatment-leaf-shredding, leaf removal) that reduce the fungicide dose and lower management costs. The tactic(s) a grower selects and the timing of each fungicide application will depend on factors such as sanitation equipment available, ground-cover characteristics, cultivar susceptibility to scab, frequency and amount of rain, fungicide needs for other diseases, and the scheduling of insecticides. In addition to its use with the sanitation action threshold, sanitation enhances the effectiveness of all management practices (Fig. 13). By reducing the ascospore dose 50% or more with sanitation in a moderate- or low-scab-risk orchard, for example, (i) a mediocre fungicide might provide acceptable control and perhaps replace a more efficacious but less environmentally friendly or more expensive fungicide, (ii) it would be less likely that a more fungicide-tolerant strain of the fungus would build up, (iii) it would be less likely that a strain of the fungus capable of infecting a scab-resistant cultivar would become established, and (iv) there would be less scab in a mixed-cultivar planting.

Scab Management in the Twenty-first Century

Research over the last two decades has had two important effects on scab management programs in the northeastern United States: a shift in emphasis from a regional to an individual-orchard management program and from a predominantly defensive to a more balanced offensive-

defensive program. The shift to an individual-orchard management program is due to the development of a procedure to predict an orchard's level of scab risk (ascospore dose) and an action threshold for fungicide decision-making based on that prediction. The individual-orchard approach is significant in that it recognizes that the ascospore dose, and thus the scab risk (potential for scab buildup), can differ greatly from one orchard to another in a region. In nine commercial orchards in New Hampshire in 1983, for example, the predicted potential ascospore dose ranged from 25 to 1,505,027 ascospores per square meter of orchard floor (28). Before the development of this program, the risk of scab in all these orchards would have been assumed to be great, and consequently all would be given full-season protection with fungicide. However, modern methods would predict that five of the nine orchards were low risk.

The shift to offensive practices is due to the development of a sanitation action threshold in which sanitation practices are employed to economic advantage. Sanitation practices under investigation include the use of biological agents that attack the pathogen or decompose the leaf litter and equipment to shred or remove the leaf litter (W. E. MacHardy, *personal communication*). Planting cultivars with more durable resistance to scab, planting mixed-cultivar orchards, and increased emphasis on biologically and culturally based practices aimed at reducing initial inoculum (coupled with action thresholds and strategies to reduce the seasonal fungicide dose) are the most likely approaches for substantial further reductions in fungicide.

Biological agents may have a greater role in sanitation in the twenty-first century. A postharvest application of *Microsphaeropsis* sp., for example, reduced

the total amount of airborne ascospores trapped by 70.7 and 79.8% in 1997 and 1998, respectively, compared with untreated plots (18), and this is within the range reported for urea and leaf shredding (79). Much more needs to be learned about the leaf environment and epiphytic microbial communities, however, before significant advances can be expected in biological control of foliar pathogens. Timing the application of an antagonist is critical. If it is applied to the trees too early, some leaves may be missed or the antagonist may not survive at a suitable density or have colonized the leaf sufficiently by the time of leaf abscission; if applied after leaf fall, some leaves will be inaccessible and the biotic agent may not have time to become established and interfere with the development of *V. inaequalis* during the critical few weeks after leaf fall when mating occurs. There are also complex cultural, technological, and application problems to be solved. Andrews (3) estimated, for example, that the biomass of *Athelia bombacina* needed per hectare of apple orchard to treat leaf litter in the autumn for scab control is something on the order of 1,000 kg (wet weight). Is the market potential sufficient to justify the investment needed to produce, store, and apply the amounts of this antagonist needed to control scab?

Soil fauna, mainly earthworms, have been shown to reduce the ascospore inoculum substantially through chewing and burying leaves (66). Practices to maintain the earthworm population at or above a designated effective density must include consideration of several factors such as food availability and palatability (of apple leaves) and, most importantly, the selection of pesticides (e.g., avoid benzimidazole fungicides) and other chemicals that are not harmful to earthworms. Without appropriate chemical management, earthworms are not likely to be factored into a strategy to integrate practices for reducing fungicide dose in the management of apple scab.

Research Requirements

Nelson (59) noted that "Breeding for race-specific disease resistance and the resulting population shifts of plant pathogens has, among other things, taken the host and parasite out of the genetic equilibrium that apparently existed prior to human intervention." To date, this has happened with all major resistance genes (the ephemeral R-genes) and for Vf bred into the domestic apple. Is it possible to develop a cultivar with durable resistance to scab?

The first strategy to achieve durable resistance is to pyramid in a single cultivar several exotic resistance genes, e.g., resistance genes that are still fully effective in their region because no coevolution with the local *V. inaequalis* population has oc-

Fig. 13. Schematic representation of scab management practices.

curred (i.e., the hypersensitive response has remained effective) and strict quarantine has prevented the import of compatible genotypes. The rationale is that the pathogen would have to mutate several avirulence genes to virulence genes before it could successfully infect a cultivar with such pyramided genes.

The second strategy is to assemble several quantitative, minor-effect resistance loci. The rationale would be: they are truly horizontal resistances or, if vertical, selection pressure of each single locus was, and is, too low to select the corresponding virulence.

Combining the two strategies would probably lead to the desired level of durable resistance. To implement such strategies, however, there must be easily selectable markers of major genes and of resistance quantitative trait loci (QTLs). Some are already available, and worldwide there are several large projects (e.g., the EU-community D.A.R.E. project) that involve marker-assisted resistance breeding. Further assistance could come from biotechnology once resistance genes are identified. These genes could be cloned and replace, by positional cloning, alleles of ineffective genes, envisaging a gene therapy of cultivars defective in resistance.

There is also the need to increase the efficiency of sanitation. The most promising procedure is leaf removal. It has been demonstrated that a sweep and vacuum technique can remove over 90% of the leaf litter, thus reducing the ascospore inoculum by more than 90% (W. E. MacHardy, unpublished data), but further research is needed to determine its full potential for reducing the need for fungicide.

Concluding Remarks

V. inaequalis is not life threatening to apple, but it is a continual threat to the apple industry. The pathogen is not going to go away, nor can we expect that the pathogen and host will coevolve to a level where there is infection of fruit without production of a sporulating lesion that lowers the value of fruit. We do not envision scab being managed without fungicide in this century unless there are breakthroughs in biotechnology that result in cultivars with durable resistance. Nor do we envision new horticultural (cultural) practices that will render the host less susceptible to scab. We do expect, however, that by (i) strengthening the host's natural (horizontal) resistance, (ii) increasing host genetic diversity in orchards through cultivar selection based on our understanding of pathogen race evolution in orchards, and (iii) exploiting vulnerable links in the pathogen's life cycle through placing greater emphasis on sanitation practices that reduce the inoculum dose available to cause infection, a truly integrated program will evolve to manage scab that is acceptable to consumers and to growers and is

fully compliant with integrated crop production standards.

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