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Epidemiology and management of a periodically introduced pathogen

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Abstract

Periodically introduced plant pathogens, which are characterized by multiyear epidemics followed by multiyear absences, offer a number of unique challenges to disease management. *Peronospora tabacina*, the causal agent of tobacco blue mold, is periodically re-introduced into the northern tobacco growing areas of North America, and offers a model system to illustrate these challenges. Blue mold is very damaging, particularly to the wrapper tobacco types grown in Connecticut and Massachusetts which have little tolerance for disease, as even a limited number of leaf blemishes severely reduces their marketability. From its first introduction into Connecticut in 1937, the pathogen has exhibited a pattern of multiyear epidemics after which it disappears for a number of years. This cycle occurred three times in Connecticut between 1937 and 2001. The last two appearances of blue mold have been associated either with a change in the pathogen's tolerance of high temperatures or with resistance to a fungicide. The lessons learned from blue mold could be extended to other periodically introduced pathogens. Cooperative research, education efforts, and inter-regional contacts need to be maintained to monitor for potential changes in a pathogen's biology and epidemiology that might affect disease management. Epidemiological studies to determine the probability of different means of pathogen dispersal, forecasts of relative risk of exposure, and the development of tactics to reduce the probability of successful introduction should all help to extend pathogen-free periods and reduce crop losses due to disease.

Introduction

Many pathogens are endemic in a locale and can cause noticeable disease every year when conditions are favorable. Other pathogens appear sporadically and disappear for several years between outbreaks. These periodically introduced pathogens, which are characterized by multiyear epidemics followed by multiyear absences, offer a number of unique challenges to disease epidemiology and management. Pathogen management strategies must be developed and maintained not only when the disease is present but also when the pathogen is apparently absent. Understanding the roles of survival and long-distance dispersal in the epidemiology of the disease and pathogen are of particular importance, since understanding the most likely means of pathogen introduction and the probability of establishment and disease spread is essential to reduce the frequency of those events. Changes that occur in the biology of the pathogen over time, such as the development of increased tolerance for high temperatures or the development of resistance to fungicides, may also greatly affect the ability of disease to spread once the pathogen is re-introduced.

Full and complete information on the biology and epidemiology of periodically introduced pathogens is not easily obtained. First, it is often difficult to justify further research expenditures on a disease that has been controlled to the point that it is no longer present, especially when it has not been present for an extended time. Second, if the disease is not currently present, ongoing research efforts may be perceived to be, or even actually be, the source of the next epidemic in the crop targeted for protection. As a result, it is difficult to establish the long-term research efforts required to study disease epidemiology, to monitor changes in

biological and environmental pathogen interactions, and to develop and maintain integrated pest management strategies. Therefore, successful programs developed against periodically introduced pathogens need to incorporate collaborative research and education efforts from a number of locations.

The tobacco pathogen *Peronospora tabacina* Adam (*Peronospora hyoscyami* de Bary) is periodically re-introduced into the northern tobacco growing areas of the United States. *P. tabacina*, an Oomycete, causes a damaging leaf spot disease of cultivated tobacco (*Nicotiana tabacum* L.) known as blue mold. In addition to the often-severe impact of disease on tobacco production, blue mold is exemplar of a disease that is periodically introduced into a region.

The pathogen may have had two centers of origin, which coincide with centers of origin for the genus *Nicotiana*. The first association of blue mold with cultivated tobacco was recorded in Australia as early as 1890, (Bailey 1890, cf. Table 1), however, the disease may have occurred shortly after tobacco was first brought to Australia prior to 1840 (Angell and Hill 1932). Unlike the typical example of an exotic pathogen introduced to a host in a new area, blue mold may be an example of disease resulting from the introduction of a commercially important host to an area where the pathogen was already present on related wild plants. *Peronospora tabacina* has been recorded from several species of *Nicotiana* throughout Australia (Hill 1962; Hill and Angell 1933), and *Peronospora* spp. were also recorded from native *Nicotiana* spp. in the Southwestern United States (Farlow 1885; Harkness 1885; Clayton and Stevenson 1943; Reuveni et al. 1988) and from Argentina (Spegazzini 1891).

Blue mold was first reported on cultivated tobacco on the American continent in 1921, when it was found in Florida and Georgia, probably as a result of a human introduction of the pathogen (Smith and McKenny 1921; Schiltz 1981). Blue mold infected tobacco was brought to England in 1958 by a chemical company to evaluate fungicide trials (Klinkowski 1961), and was brought to the Netherlands in 1959 on plants used as virus host material (Klinkowski 1962). The pathogen now coexists with tobacco in the Mediterranean and Caribbean basins, and infects tobacco in temperate regions of the United States by means of long-range transport (Main and Davis 1989). Blue mold may be periodically introduced through the tobacco growing areas of the United States from these regions each year.

The blue mold pathogen(s) was named *P. hyoscyami* in Australia based on the first description of *P. hyoscyami* from the solanaceous weed *Hyoscyamus niger* L. by de Bary in Europe (Skolicky 1964), *P. sordida* Berk. on *N. bigelovii* Wats. from Nevada, and *P. nicotianae* Speg. on *N. longiflora* Cav. in Argentina (Clayton and Stevenson 1935). A review of morphology and host specificity by Adam (1933) resulted in the name *P. tabacina* Adam, commonly accepted in the literature, but not without controversy (Skalicky 1964; Clayton and Stevenson 1943; Krober and Weinmann 1964). Recently, the entire group of Oomycetes, including *P. tabacina*, has been demonstrated to be distinct from fungi, and more closely related to the brown algae (Leipe et al. 1994). In this article, the pathogen will be referred to by the commonly used name *P. tabacina*.

The northward spread of blue mold from Florida to Connecticut offers an excellent opportunity to study a periodically introduced disease. Connecticut (lat. 41°45′ N, long. 72°40′ W) is one of the more distant locations north of the typical initial sources of infection in the United States. Tobacco is grown on a relatively small acreage in the Connecticut River Valley in Connecticut and Massachusetts, isolated from the nearest large-scale tobacco production area by about 400 km. *P. tabacina* may have been introduced into the Connecticut River Valley each year during 1979–1980 and during 1996–2000.

History in Connecticut

Despite the small area of cultivation and isolation from other tobacco production areas, tobacco has a long history in Connecticut, with the earliest record of tobacco production in 1640 in Windsor. Blue mold was not reported in Connecticut until 1937 (Anderson 1937). Initially, blue mold was primarily a disease of tobacco in seedbeds, although some disease occurred in the field and in shade tents each year from 1942 to 1950, especially in cool wet years (Anderson 1952). During these years, blue mold occurred mainly early in the season in tobacco seedling production beds in most areas, and was apparently restricted from affecting plants under field conditions by high summer temperatures (Rider et al. 1961). A severe outbreak of blue mold occurred in Connecticut shade tobacco in 1951 during conducive weather conditions during July. Attempts to control the disease with the same fungicides that were effective in

seedbeds were unsuccessful, and the epidemic did not stop raging until the temperatures reached 27–32 °C for several days (Anderson 1952). Control tactics at the time consisted of sanitation, fungicide protection of seedlings in beds, and the reduction of potential overwinter survival (Anderson 1952). After 1956, there were no reports of blue mold in the Connecticut River Valley until 1979. The lack of disease prior to 1979 was no doubt due to good disease control in Connecticut and elsewhere, and the inability of the pathogen to consistently infect tobacco in the field during hot or dry conditions.

In 1979, the situation changed dramatically. An isolate of *P. tabacina* capable of causing disease during warm (hot) weather in the field resulted in a blue mold pandemic in the United States tobacco crop that caused losses of more than $250 million (Lucas 1980). The disease caused a 20% yield loss even though 60% of the crop had already been harvested by the time the pathogen was first observed (Aylor et al. 1982). In 1979, blue mold was probably introduced to Connecticut by long-distance aerial transport of sporangia from strong sources located hundreds of kilometers away (Aylor et al. 1982).

Blue mold occurred in Connecticut again in 1980, but losses were reduced compared to 1979 due to the increasing use of a highly effective systemic fungicide, metalaxyl. In 1981, blue mold was controlled so effectively by metalaxyl that few cases were reported from anywhere in the United States. Despite the near absence of blue mold in production areas, the disease was discovered in a small (15 × 15 m) area of 0.1 m tall volunteer tobacco seedlings growing among weeds and diseased tobacco trash in Windsor, Connecticut (Aylor et al. 1982). The geographic isolation of these plants combined with the comparatively small inoculum production by the few scattered locations of diseased plants elsewhere in the United States suggests that the pathogen probably overwintered in several areas, including Windsor, Connecticut (Nesmith 1982; Current Blue Mold Status Reports 8–10).

Biology of the pathogen

To put the role of re-introduction, survival, and the spread of the pathogen into context, the biology and epidemiology of blue mold bears brief review. Under suitable weather conditions, *P. tabacina* produces large numbers of sporangia that are dispersed with air currents and can very quickly cause large numbers of new lesions and secondary inoculum, resulting in the extraordinary speed of blue mold epidemics. By far the greatest source of infection occurs from sporulating lesions present within a crop canopy (Waggoner and Taylor 1958). Shade tobacco growers in the Connecticut River Valley have observed a two-week interval from first observed infection within a nonsprayed shade tobacco field to levels of infection high enough to justify crop destruction. The increase in the number of lesions with time was determined in nonsprayed tobacco in both experimental plots at the Valley Laboratory of The Connecticut Agricultural Experiment Station and in commercial seed plots in Westfield Massachusetts after the season (Figure 1). Disease becomes too severe to justify harvest when plants have more than 10 visible lesions per plant. The rapid increase in disease severity from the first detection of lesions in target plots to levels that justify destruction of the crop occurred within 14 days.

Moisture is paramount for infection and sporangiospore (sporangia) production. The primary dispersal and infection agents for the pathogen are the asexual sporangia that are produced on lesions and dispersed by wind currents. These sporangia are produced in great numbers and each has the potential to initiate a new infection on a tobacco leaf. Sporangia need a minimum of 1 h of free moisture on the leaf surface to infect, and infection increases progressively with longer periods of leaf wetness (Krober 1967). Once inside the leaf, *P. tabacina* grows vegetatively between and within leaf cells. Within four to 5 days after infection under suitable conditions of temperature and relative humidity,

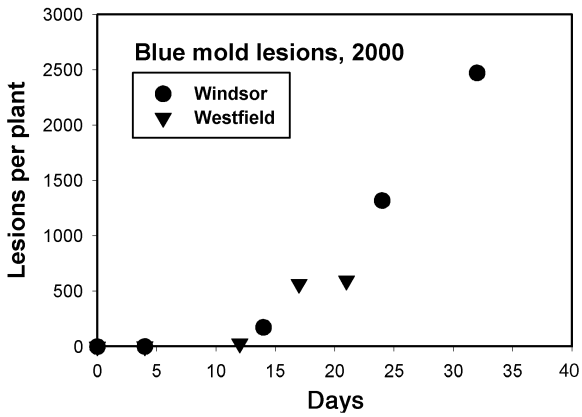

Figure 1. The increase of blue mold lesions over time in nonsprayed shade tobacco in the Connecticut River Valley in the northeastern USA.

sporangiophores grow through stomata, primarily on the lower surface under a developing yellow lesion, and a new crop of sporangia is produced (Svircev et al. 1989).

Temperature and moisture affect germination, infection, the area and rate of lesion expansion, and the timing, extent and duration of sporulation (Cruickshank 1958, 1989). Sporulation requires at least 95% relative humidity for a minimum of 3 h during a dark period of greater than 1.5 h (Cruickshank 1963), while maximum sporulation occurs at 97–100% relative humidity. Sporulation intensity is optimum at 15–23 °C, and sporulation does not occur below temperatures of 1–2 °C or above 30 °C (Cruickshank 1961). Sporulation can begin four days after infection, reach a maximum after three or four more days, and continue for up to two weeks (Cruickshank 1961).

The numbers of sporangia produced over the life of a lesion have been reported to be as high as 1 million per cm^2 lesion (Spurr and Menetrez 1990) although actual published numbers of sporangia per cm^2 over time are difficult to find. Data on sporulation intensity is often reported as relative sporulation (Cruickshank 1961), sporangium recovery from air (Aylor and Taylor 1983), or as a one-time measurement of sporulation (Reuveni et al. 1986). A number of factors in addition to environmental conditions may also affect infection and sporulation. Plant age affects both the numbers of lesions that develop and the sporulation intensity per lesion (Reuveni et al. 1986). Sporangium production decreased later in the season and as lesions aged (Rotem and Aylor 1984).

We evaluated sporangium production under field conditions in several ways. In August 1998, sporangia per unit area were compared for leaf disks of different ages as per Rotem and Aylor (1984) (Table 1). In August and September 1999, sporangia were collected from individual tobacco blue mold lesions in Windsor CT by washing them from leaves and collecting sporangia in a beaker. Sporangia were collected daily for up to 14 days until they were not produced on lesions for two consecutive days (Figure 2). Newly produced yellow lesions produced approximately 4×10^5 sporangia per lesion. Approximately half as many sporangia were collected from older lesions with necrotic flecks, and only 1×10^5 sporangia from necrotic lesions. Averages of 4×10^5 sporangia per cm^2 lesion were recovered from 10 individual lesions over a period of up to 14 days starting August 24, 1999. In repeated experiments, an average of 1.0×10^6 or 8.8×10^5 sporangia per cm^2 lesion were recovered over a period of 5 days starting August 31 or September 14, 1999. While efforts were made to collect all sporangia early in the day, these numbers are certainly an underestimate of the total produced. The large numbers of sporangia produced, and the fact that new sporangia

Table 1. Peronospora tabacina sporangia production on shade tobacco in the Connecticut River Valley in the northeastern USA, as influenced by lesion age.

Lesion stage	Sporangia ($\times 10^4$)
Yellow	39.39
Yellow/necrotic	19.38
Necrotic	10.13

The three values are each significantly different at the 0.0001 level.

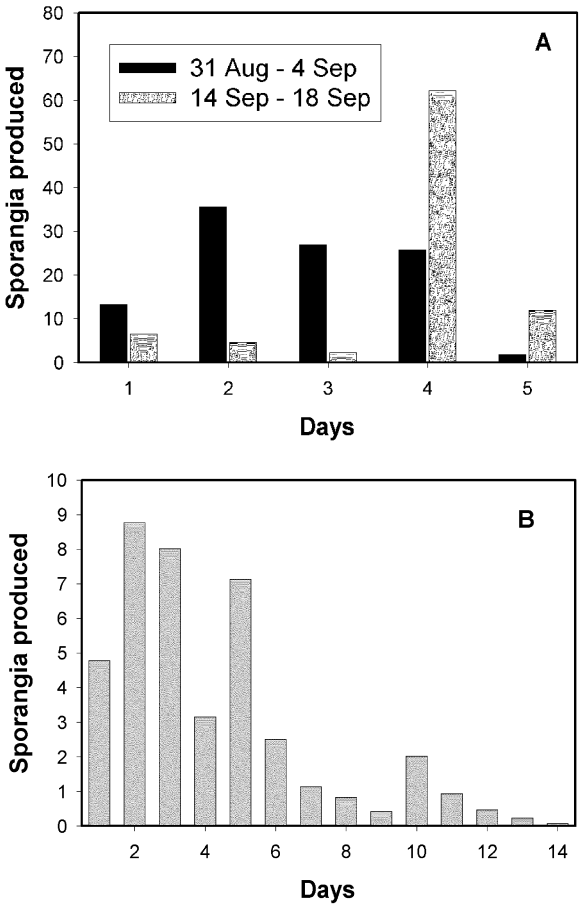

Figure 2. Peronospora tabacina sporangia ($\times 10^4$) collected per cm^2 lesion over a 5-day period (A) or over a 14-day period in 1999 (B) in the Connecticut River Valley in the northeastern USA.

are produced over time on a single lesion, help explain the explosive nature of this disease.

Release of sporangia occurs concurrently with a daily rise in air temperature and a decrease in relative humidity due to an increase in solar insolation (Cohen 1976; Hill 1961). Sporangium survival is limited by exposure to ultraviolet radiation, especially when they are airborne (Bashi and Aylor 1983; Rotem et al. 1985).

Role of the fungicide metalaxyl

Widespread use of metalaxyl essentially eliminated blue mold from the Connecticut River Valley and much of the United States until the early 1990s. The first reports of metalaxyl-resistant strains of *P. tabacina* were made in 1981 and 1982 in Nicaragua. Resistant strains were subsequently reported in Mexico in 1984 and in the United States in 1991 (Wiglesworth et al. 1988).

After 1981, blue mold did not occur in the Connecticut River Valley again until 1996, when metalaxyl-resistant isolates of *P. tabacina* were discovered in October in tobacco breeding lines left for seed production. Blue mold re-occurred in Connecticut in July 1997. Blue mold diseased transplants were brought to Pennsylvania early in the 1997 season and airborne sporangia from Pennsylvania may have initiated the Connecticut epidemic. The first confirmed reports of blue mold in the Connecticut River Valley in 1998 and 1999 were from hoop-house seedling production in Hadley, Massachusetts in June 1998, and from plant beds in northern Connecticut in June 1999. In both years, blue mold was confirmed prior to the time of most likely exposure from long-distance sporangia transport (North American Blue Mold Warning System). While there was the possibility of some early season exposures from very long-distance sources in North Carolina in 2000, blue mold was first confirmed in the Connecticut River Valley on ornamental tobacco plants in a greenhouse at the University of Massachusetts in June 2000.

The blue mold pathogen has been re-introduced periodically into Connecticut, probably via long-distance aerial transport of sporangia (discussed below), however, there is also strong circumstantial evidence for occasional survival overwinter under field or greenhouse conditions. While movement of infected transplants has not been suspected as a means of direct introduction of the pathogen to the Connecticut River Valley, it has been responsible for long-distance transport elsewhere (Nesmith 1984). Although infected transplants have not been brought to Connecticut, movement of transplants has brought the disease to locations close enough to Connecticut to be a source of sporangia to initiate an epidemic here.

Nicotiana tabacum is not the only plant capable of supporting the pathogen, nor is it the only plant capable of transporting the disease with movement of plants. Both wild tobacco species and ornamental tobacco types can support the pathogen. *Nicotiana repanda* Willd. Ex Lehm. has been associated with blue mold in the American southwest and may act as a source of annual infection (Reuveni et al. 1988). In addition, *N. alata* Link & Otto 'White perfume', *N. sanderae* (*N. alata* × *N. forgetiana*) 'Domino hybrid', and *N. sylvestris* Speg. 'Only the lonely' were experimentally infected with the blue mold pathogen under greenhouse conditions in 1997 and 1998 (LaMondia, unpublished). These species are widely grown as ornamentals in the northeastern United States and elsewhere. Young plants were more susceptible to infection and allowed greater levels of sporulation than older flowering plants. The movement of infected ornamental plants from an infested area to the Connecticut River Valley, or the local survival of the pathogen in systemically infected ornamentals under greenhouse conditions has been a major concern to tobacco growers and may have initiated the 2000 epidemic in Massachusetts.

Long-distance aerial dispersal of sporangia

The possibility that plant pathogens can be transported hundreds of kilometers through the atmosphere and initiate new disease outbreaks is very real. However, evidence for it in the case of blue mold is circumstantial. Certainly, long-distance transport of pollen, spores, and Saharan dust has been well documented. These particles can be carried high into the atmosphere and long distances by wind. Likewise, there is little doubt that sporangia can remain airborne in the atmosphere for days and be transported hundreds or thousands of kilometers during this time. Nevertheless, relatively little is known about the conditions under which plant pathogen spores can travel several hundreds, or thousands, of kilometers through the air and actually infect plants in distant fields.

Blue mold has appeared in Connecticut in only 7 out of the last 44 years (1979, 1980, and 1996–2000). Each time it caused serious economic loss. The long intervals

between outbreaks in Connecticut (1957–1979 and 1981–1996) underscore the conclusion that long-range transport of this disease to Connecticut is a relatively rare event. To better understand the probability of long-distance aerial transport, some of the biophysical and atmospheric parameters that govern long-distance transport of inoculum need to be considered. As mentioned previously, there are two main ways that the pathogen moves around. The first is by sporangia carried on the wind and the second is by the movement of infected, asymptomatic plants between fields and growing regions by people. It is possible to move the disease around unknowingly because infected plants may not show symptoms for 5–10 days. Therefore, it is possible to set transplants in the field before symptoms appear. This appears to have happened in Kentucky in 1996 and in Pennsylvania in 1997.

The probability that long-distance aerial transport of sporangia will lead to disease spread depends on the combined effect of several factors. If air movement has been in the right direction to carry sporangia on a course between a source and our crop, there are three major factors affecting the chance of successful disease transport: these are (1) sporangia production at the source, (2) dilution of the sporangia cloud, and (3) mortality of sporangia during transport. *P. tabacina* is a prolific producer of sporangia: a 500 ha area of heavily diseased tobacco can produce about 6.44×10^{13} sporangia per day. During a journey of 20–30 h (which is about the length of time it can take for air to be transported 500–700 km in the atmosphere), the concentration of sporangia in the air can be reduced by atmospheric dispersion in the absence of rain by about a factor of 10^{13}. Comparison of these numbers suggests that some sporangia will make it through, but will they be dead or alive? In full sunlight, 99% of *P. tabacina* sporangia in the air are killed in about 6 h. In full overcast (complete cloud cover) conditions, however, about 80% of the sporangia in the air survive for 6 h, or more. Clearly, sporangia have a much better chance of survival if they travel under cloud cover. Thus, depending on sky conditions during transport, there may be more (sunny) or comparable (cloudy) danger to a tobacco field from a small, potentially unnoticed, local source than from a massive source 700 km away (Aylor et al. 1982).

Northward advance of the disease front

The northward advance of the disease front can provide critical information about the potential spread of diseases such as blue mold. The northward spread of disease can be represented by trajectories in distance-time space, where the slopes of the trajectories represent the rate of progression of the disease front (Aylor 1999). The disease-front trajectories represent the dates of disease onset in growing areas starting in North Florida at about 29° N and extending to the Connecticut River Valley at about 42° N latitude. The average rate of blue mold spread from south to north for the six most recent pandemics of blue mold was 15.1, 12.8, 11.4, 18.7, 16.6, 7.8, and 7.4 km per day in 1979, 1980, 1996, 1997, 1998, 1999, and 2000, respectively. These rates are not very much different from the average rate of northward movement of the 'green wave' of planting and may sometimes be constrained by it (Aylor 1999). There are many complicating factors affecting these rates such as ground transportation of diseased transplants and changes in the pathogens response to temperature and its sensitivity to fungicides.

Thus, the spread of blue mold by long-distance aerial dispersal of sporangia in the atmosphere is unlikely, but it can happen when disease pressure is high, the source is nearby and weather conditions are conducive for infection. The fact that blue mold was absent from Connecticut for long stretches of time (between 1957 and 1979 and between 1981 and 1996) is one indication that movement of sporangia to Connecticut is an unlikely event, because there is at least some disease in the southern states almost every year. However, because of the history of follow-on or back-to-back epidemics in Connecticut, growers are probably at greater risk following a year with a lot of disease in the field. Although successful long-distance aerial transport is difficult, there was so much inoculum in the United States in 1979 and in 1996 that, in those years, it almost certainly made it to Connecticut by that route. The role of interstate transport of tobacco species must be carefully considered for its possible role in spreading this disease in the United States.

The US Department of Agriculture established a blue mold warning system in 1945 to relay information on local blue mold activity to all tobacco production areas. This service had been discontinued prior to the epiphytotic in 1979 but was re-instituted during that year (Nesmith 1984). More recently, a world-wide-web based North American blue mold forecast system was established at North Carolina State University to predict likely blue mold exposure due to local and long-distance transport (Main et al. 2000). Sporangia transport through the atmosphere is calculated using

the HY-SPLIT trajectory model (Draxler 1992), and trajectories can be obtained and calculated from the US National Oceanic and Atmospheric Administration, Air Resources Laboratory web site on the Internet.

Value of forecasts

The availability of both local and long-range forecasts of blue mold exposure and severity are most useful for farmers growing tobacco types that can tolerate significant levels of blue mold without incurring much economic loss. The long-distance forecasts may be useful in determining risk of exposure to blue mold, or in avoiding early season fungicide sprays in years when blue mold is not present in the Connecticut River Valley and was not present the previous year. Blue mold occurred early in the season in the Connecticut River Valley in 1998 and 1999, prior to predicted exposure from long-distance sources. Additionally, once the pathogen is present, the utility of local forecasts is low for a number of reasons. First, the level of disease that can be tolerated without economic loss is extremely low in cigar wrapper tobaccos where a single lesion on a leaf may result in a loss of 75% of the value of a similar noninfected leaf. Second, the small area of the Connecticut River Valley (100 km at the longest point) could allow sporangia transport and survival even under dry and sunny conditions. A 33-kph wind could move sporangia the length of the Connecticut River Valley production area with comparatively little dilution in only half the time required to kill all of the sporangia if the weather is sunny and dry. Finally, fungicide management of blue mold requires complete coverage of the leaves prior to infection. The current lack of a systemic fungicide with curative activity does not allow growers to avoid sprays on rapidly growing tobacco. The time elapsed between a forecast of exposure and recorded environmental conditions favorable for infection is not presently long enough to ensure that the disease can be controlled.

Survival of the pathogen in northern locales – overwintering

Oospores

The possibility of survival of the pathogen over the winter is controversial. The sexual phase of the life cycle may occur, although its role is poorly understood. Under certain conditions, thick walled sexual spores (oospores) can be produced. The environmental conditions necessary for oospore production and the role of vegetative mating types, if they exist, are currently unknown. In fact, the significance of oospore production on the epidemiology of blue mold is unknown. Oospore formation may range from extensive to below detectable levels, perhaps based on unknown environmental triggers, and oospore morphology may differ with different isolates of the pathogen (Spurr and Todd 1982). Wolf et al. (1936) demonstrated that oospores could survive for four years. Person et al. (1955) could not initiate oospore germination. Other workers were able to demonstrate oospore infectivity at low levels for up to 50 months in natural soils (Krober 1969; Krober and Weinmann 1964). Because of difficulties associated with the obligate nature and economic impact of the pathogen, differences in oospore production among isolates, and low infectivity of the oospores, the role of these sexual structures in blue mold epidemiology may never be fully understood. The low incidence, poor germination, and low infectivity of oospores are often cited to dismiss the importance of oospores on epidemiology (Krober 1969; Spurr and Todd 1982). However, the farther the distance of a tobacco-producing area from the source of wind-borne sporangia, the greater the economic impact of any potential infection due to oospores overwintering from a previous year's epidemic and the more difficult they become to discount. Recently, Heist et al. (2000) demonstrated that *P. tabacina* could survive as vegetative hyphae in roots of systemically infected plants, and that root transmission could occur in both wild and commercial *Nicotiana* species. This may have a significant impact on survival of *P. tabacina* overwinter in protected beds, under debris in fields, or under greenhouse conditions. It would also help to explain circumstantial evidence for overwinter survival in the absence of infective oospores or despite low oospore infectivity.

In 1997, cured shade and broadleaf leaves with blue mold were collected from 15 locations in Connecticut and Massachusetts. At least 6 leaf disks per sample location were cut from symptomatic lesions with a 1 cm diameter cork borer, and cleared in boiling 5% KOH for 20 min. Half of the leaf disks were stained with cotton blue in lacto-phenol and all disks were examined under a compound microscope. Of 100 disks examined, 14 had structures similar to the oospores described by Spurr and Todd (1982). Hyphae, haustoria, sporangia,

and sporangiophores were also present in cleared tissue. Oospore-like structures were spherical, with an echinulate wall of approximately 5 μm thickness and ranged in diameter from 25 to 35 μm. Oospore-like structures were present singly or in small clusters of up to 11 per leaf disk in the mesophyll.

To date, our efforts to determine whether the pathogen can overwinter in dried, infected tissues or seed and leaf tissue have been negative, even in leaves with oospore-like structures.

Disease management and control

Historically, blue mold has been controlled by the timely application of fungicides, and little is different today. The relatively recent development of pathogen resistance to the fungicide metalaxyl has had a large impact on the incidence and severity of disease throughout the United States. Since 1996, all isolates of the pathogen infecting tobacco in Connecticut have been uniformly resistant to metalaxyl. Because of metalaxyl-resistance and the fact that cigar wrapper tobacco must be blemish free for the crop to be marketable, both fungicide efficacy and spray techniques were investigated to achieve the superior coverage necessary for control. Coverage is especially challenging and important in shade tobacco, which is grown at high plant densities. Each plant is tied to an overhead network of wires under a cloth shade tent.

Our research has demonstrated that two new fungicides, dimethomorph and azoxystrobin, and a novel resistance-inducing systemic-acquired-resistance compound, acibenzolar-*s*-methyl, are effective against tobacco blue mold. Azoxystrobin and acibenzolar-*s*-methyl have shown potential phytotoxicity problems associated with leaf fleck damage and reduced leaf quality. Dimethomorph is currently the most effective fungicide available to growers, but as it is a local systemic, coverage remains an issue. Fungicide application within the crop canopy by means of drop nozzles or vertical boom sprayers was superior to fungicide applications over the top of the crop, especially in shade tobacco. The combination of effective fungicides with good coverage throughout the crop canopy should result in increased levels of blue mold control.

Breeding for resistance

Although resistance to *P. tabacina* is common in a number of Australian *Nicotiana* species, it is absent in American species. This might indicate that either *P. tabacina* is an introduced pathogen to North America, or that virulent strains from Australia have been introduced and established in the Americas (Rufty et al. 1990).

Fortunately, resistance to *P. tabacina* has been transferred from *N. debneyi* to *N. tabacum* in the United States (Clayton 1968) and in Australia (Lea 1963). Clayton stabilized the resistant interspecific *N. debneyi* × *N. tabacum* hybrid by crossing to a synthetic alloploid of *N. sylvestris* × *N. tomentosiformis*, prior to backcrossing to *N. tabacum*. Lea crossed *N. debneyi* × *N. tabacum* and backcrossed to *N. tabacum*. In addition, Lea crossed *N. goodspeedii* × *N. tabacum*. The resulting F1 sterile hybrids were stabilized by doubling chromosomes and backcrossing to *N. tabacum* cultivars Virginia Gold and Hicks (Wark 1970).

The genetics of blue mold resistance are difficult to interpret. Resistance appears to be conferred by a single major gene in the wild species *N. debneyi* and *N. goodspeedii*. The expression of resistance is reduced after the gene is transferred to cultivated tobacco, and segregation ratios indicate that resistance is additive and affected by modifiers in the *N. tabacum* genome (Rufty et al. 1990). Clayton (1968) recognized four distinct levels of resistance in *N. debneyi*-derived lines ranging from a high level of resistance, almost immunity, to a reduced level of resistance statistically superior to that of susceptible tobacco.

Recently, we tested seedlings of Clayton's breeding lines 292–393 and 509 obtained from the Oxford NC Seed Storage Laboratory, found them to be highly resistant to blue mold, and used them in the Connecticut resistance-breeding program. Breeding commercial tobacco cultivars for blue mold resistance is still under way. Our results indicate that the resistant line 292–393 is virtually immune to blue mold, and that line 509 exhibits a reduced level of resistance. Inbreeding quickly dilutes resistance. One blue mold-resistant F1 hybrid, CT-110, had promising leaf quality characteristics and significant levels of resistance. Although resistance is not complete in the F1 lines, CT-110 had fewer lesions, smaller lesions, and allowed less sporulation than susceptible tobacco lines. These effects of *N. debneyi* resistance in different *N. tabacum* backgrounds were also reported by Rufty and Main (1989).

Different resistance genotypes differed significantly for all components of resistance that were measured. Blue mold resistance is age-dependent, and seedlings

or young plants are more susceptible to the pathogen than older plants (Clayton 1968). Over the last few years, the greatest losses in shade and broadleaf tobacco have occurred during the middle and late part of the season. The combined effects of early season fungicide application and hybrid plant resistance would probably reduce the rate of increase of blue mold over time, and greatly reduce the losses due to blue mold.

The lessons learned from blue mold may be extended to other periodically introduced pathogens. Cooperative research, education efforts and contacts need to be maintained to monitor for potential changes in pathogen biology and epidemiology that may affect disease management. Epidemiological studies to determine the probability of different means of transport, forecasts of relative risk of exposure, and the development of tactics to reduce the probability of successful introduction all should help to extend pathogen-free periods and reduce disease losses.

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