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Management of Botrytis Leaf Blight of Onion

The Québec Experience of 20 Years of Continual Improvement

Botrytis leaf blight (BLB) of onion (*Allium cepa* L.) is caused by *Botrytis squamosa* J.C. Walker. The disease has been reported on onion crops in several of the onion production areas of the world including North and South America, Europe, Asia, and Australia (1,8,20,21,28,53,60,61), although it is not a problem in arid production regions such as the western United States. In eastern Canada, the disease is generally present every year and is especially severe on cultivars of yellow globe onion (54).

The pathogen biology and disease epidemiology have been intensively researched (1–5,12,17,18,22,23,27,41,43,46–48,50,52,55–57,64). Knowledge is available on all stages of pathogen development including dormancy, reproduction, dispersal, infection, and pathogenesis (1,2,4,5,17,23,27,55,57). Over the last few decades, in the organic soil area of Quebec, extensive research effort has been devoted to the development and evaluation of predictive models and disease management strategies. There has been an active integrated pest management (IPM) program for onions since the early 1980s, and scouting for disease has played a major role in disease management (8). In this article, we summarize the story of BLB management in eastern Canada over a period of two decades. New analyses of 20 years of data on BLB severity from the Quebec scouting program stimulated this reflection on the development and achievements of the sustained effort to manage BLB. Through this case study, we hope to deepen insight into the long-term impact of applying epidemiological tools to manage diseases.

History of Botrytis Leaf Blight of Onion

In 1925, Walker described *B. squamosa* as a new pathogen of onion and named the disease “small sclerotial neck rot” (66). In 1926, Walker reported that the pathogen was capable of causing leaf spot under prolonged periods of leaf wetness (1 week), while no lesion developed on inoculated leaves maintained wet for 48 h at temperatures of 15 to 20°C, and he concluded that *B. squamosa* was not a leaf pathogen (67). Subsequently, other researchers, including Hickman and Ashworth in 1943, Viennot-Bourgin in 1952, Page in 1953, and Hancock and Lorbeer in 1963, demonstrated that *B. squamosa* induces onion leaf blight (27,30,46,60). Hancock and Lorbeer showed that *B. squamosa* causes a disease distinct from Botrytis leaf fleck caused by *B. cinerea* Pers.:Fr. and *B. aclada* Fresen. (27). The disease caused by *B. squamosa* was named Botrytis leaf blight (BLB), but other names such as onion leaf blight or leaf blight are also used.

A considerable amount of new information was published on the biology of *B. squamosa* and epidemiology of BLB during the 1970s and early 1980s. At that time, there were three main research groups actively involved in BLB epidemiological research in North America: Cornell University (17,22,23,49–51), the University of Guelph (42,52–56), and Michigan State University (35). From the mid-1980s to the early 2000s, only a few reports on BLB were published (20). In the early 2000s, a research program aiming at improving management of BLB in Canada was initiated at Agriculture and Agri-Food Canada with the collaboration of Compagnie de recherche Phytodata Inc., the research branch of a scouting group named PRISME Consortium (9–14,58). This research program was initiated in response to: (i) the development of iprodione-resistant strains of *B. squamosa* in eastern Canada (13,58); (ii) the limited efficacy of most disease management

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schemes; (iii) increases in the number of fungicide applications used to manage the disease; (iv) the introduction of new fungicides; and (v) increased awareness of detrimental effects of fungicides in the environment. This case study draws on the long history of BLB research in the northeastern United States and the more recent work in Quebec and Ontario.

Biology of *B. squamosa*

B. squamosa belongs to the family *Sclerotiniaceae* and was first described as a parasite of the outer scales of *A. cepa* (66). The genus *Botrytis* is characterized by erect, pigmented conidiophores that branch toward the apex (29). These branches terminate in a lobe-like or globose conidiogenous cell, an ampulla, which gives rise to a cluster of holoblastic conidia (29). The sclerotia of the genus are differentiated with a heavily melanized rind surrounding a hyaline medulla. Phialidic spermatia are produced by all *Botrytis* species (31).

There are eight *Botrytis* species known to cause onion diseases (16,44,45,69). *B. aclada* (68) and *B. allii* Munn (68) cause neck rot, bulb rot, scape blight, and umbel blight. *B. tulipae* (Lib.) Lind causes neck rot and bulb rot. *B. byssoidea* J.C. Walker and *B. porri* Buchn cause neck rot. *B. cinerea* is mainly associated with brown stain and flower blight. Recently, a new species of *Botrytis* named *B. sinoallii* was associated with leaf blight of green onion, garlic, and garlic chives (69). *B. squamosa* is the only *Botrytis* species known to cause *Botrytis* leaf blight (28,29,44) on onion. *B. squamosa* can be distinguished from the other species by the large, broadly ellipsoid conidia (mostly 10–16 × 16–24 µm), which are rarely produced in culture, and by the ability to blight healthy onion leaves (27). The sclerotia are flat, scale-like, 0.5 to 4 mm in diameter, and white at first but turning black with age. Sclerotia of *B. squamosa* can be found on the outer scales of onion bulbs or on necrotic leaves or flowers (23). This phenomenon is uncommon in the semiarid onion production areas.

The teleomorph, *Botryotinia squamosa* Viennot-Bourgin, is a member of the family *Sclerotiniaceae*, in the order *Helotiales* of the *Discomycetes* (34). The stipitate and cupulate apothecia are 0.5 to 2.5 mm in diameter and can be found in the spring on sclerotia in onion debris or on cull piles (23). Wild types of *B. squamosa* are hermaphroditic, self-sterile, and cross-fertile. Asci are 162.5–200 × 13.8–12.5 µm, and each ascus contains eight ascospores of 15.0–17.5 × 10.0–12.5 µm (43). Infections by ascospores result in lesions with water-soaked margins similar to those produced by conidia (19,23).

Symptoms of *Botrytis* Leaf Blight

The disease develops in two stages: a leaf spotting phase followed by a leaf blighting phase. The disease appears first as leaf lesions 24 to 48 h after inoculation. Tissue maceration then occurs as a result of pectolytic enzymes produced over several days. The lesions on leaves are whitish, 1 to 5 mm in length, and generally

surrounded by a greenish-white halo that first appears water-soaked (Fig. 1, left). As the lesion ages, the center becomes sunken, straw-colored, and sometimes develops a slit oriented lengthwise along the lesion. Older onion leaves are more susceptible than younger leaves (3). Partial or complete leaf blighting generally occurs within 5 to 12 days after initial lesion development under optimum conditions for BLB development (Fig. 1, right). Onion fields with severe infections often take on a yellowish cast as a result of coalescing lesions on the leaves, leaf tip dieback, and leaf blighting (Fig. 2). Necrotic tissue plays an important role in the epidemiology of BLB, as it is the site for secondary conidial production by *B. squamosa* under moist conditions (Fig. 3) (27).

Yield Losses Caused by BLB

There is little information on the relationship between BLB development and yield losses. Most yield losses occur when the bulb formation commences and bulb filling occurs, as this is the time when onions are most sensitive to BLB infection. In fungicide trials, Shoemaker and Lorbeer (49) reported yield losses caused by *B. squamosa* ranging from 7 to 30% in nonsprayed plots. Similarly, yield losses of 26% were reported in the Netherlands (20). Early management actions that prevent BLB from reaching the exponential phase of an epidemic at the time of bulb formation may be more efficient in managing the disease than action taken later in the season.

Fig. 2. Onion field severely infected by *Botrytis squamosa*.

Fig. 1. Discrete lesion (left) and upper leaf blighting (right) caused by *Botrytis squamosa* on onion plants.

Fig. 3. Sporulation of *Botrytis squamosa* on dead onion leaves.

Fig. 4. Life cycle of *Botrytis squamosa* on onion crops.

Disease management strategies for BLB of onion in Canada have been developed using an adaptive approach in which increased effort is expended on control during the period of bulb filling, and less effort after this critical period (8,9,40,46,54). Typically, fungicide sprays are not applied after the end of August, when the crop is becoming senescent and bulbs are left in the field to dry prior to harvest and storage by mid-September.

Epidemiology of *Botrytis* Leaf Blight

As with most polycyclic diseases, epidemics of BLB involve a sequence of events starting with the production and dispersal of initial inoculum, followed by primary infection, repeated cycles of production and dispersal of secondary inoculum, and completed by the production of survival structures of *B. squamosa* (Fig. 4) (53).

B. squamosa overwinters as sclerotia formed on infected onion leaves and necks of onion bulbs that remain in the field as crop debris or in cull piles. Ascospores produced within apothecia, which develop from the sclerotia (Fig. 5, top), can infect onion leaves but are not considered a significant source of primary inoculum (22,23). In northern onion production areas, such as eastern Canada, New York, and Michigan, the main source of initial inoculum is conidia produced on sclerotia that overwinter in soil, on onion debris, or in onion cull piles (Fig. 5, bottom) (22,23). In areas of year-round onion cropping, onion leaves can also be infected by conidia produced on infected leaves of neighboring fields.

Sclerotia of *B. squamosa* have the ability to produce conidia repeatedly, which results in production of initial inoculum over a prolonged period in spring and early summer (22,23). Air temperature generally does not limit inoculum production, as conidia are produced on sclerotia at temperatures ranging from 3 to 27°C (optimum of 9°C) (22,23). The conidia, when deposited on onion leaves, produce the “simple interest” phase of the disease (59) (leaf spotting phase), with lesion number increasing slowly (53). As leaf blighting and leaf tip dieback progress, conidia are produced on the necrotic tissues (53,55). This occurs primarily at night, provided

Fig. 5. Production of an apothecium containing ascospores (top) and conidia from sclerotia of *Botrytis squamosa* (bottom).

the leaves are wet for at least 12 h and the mean temperature during the leaf wetness period is between 8 and 22°C (4). If the leaf wetness period is between 5.5 and 12.0 h, conidiation will occur only if the preceding day was humid (RH > 70% for at least 6 h) and the necrotic tissues are moist.

The concentration of airborne conidia of *B. squamosa* follows a diurnal pattern with maximum numbers of conidia trapped during the day with increasing temperatures and decreasing relative humidity. Lorbeer (37) reported that 80% of the airborne conidia

were caught with a Hirst spore trap between 800 and 1600, while Sutton et al. (55) reported that peaks in the number of airborne conidia trapped occurred between 900 and 1200. During the subsequent phase of BLB epidemics, the concentration of airborne conidia increases logarithmically with accumulation of dead leaf tissue in the crop except when the soil temperature is $>30^{\circ}\text{C}$ (52,53).

Infection of onion leaves by conidia is mainly influenced by temperature and duration of leaf wetness. Despite the absence of a complete model describing the combined effect of a wide range of temperatures and leaf wetness durations on lesion development, several studies have addressed the effects of temperature and/or leaf wetness on infection of onion leaves by *B. squamosa* (41,48,50,56,57). The optimum temperature for BLB development was reported to be 18°C (41,57) and 20°C (50,56). The maximum temperature for lesion development was reported to be 24°C (41,50,57). At temperatures between 9 and 25°C , lesion number increases with increasing temperature and leaf wetness duration; however, conflicting reports have been published on the optimum leaf wetness duration. Minimum leaf wetness requirements of 5, 6, 9, and 12 h at 18 to 20°C have been reported, while maximum lesion development was reported to occur at leaf wetness durations of 12, 18, or 48 h (41,50,56,57). Using dry spore inoculation techniques, Alderman and Lacy (2) reported that dry spores applied onto dry onion leaves survived up to 2 days in the absence of leaf wetness, and kept the potential to induce lesions when the leaves were subsequently wet. The authors also reported that lesion production was optimal at 20°C , lower at 15°C , and greatly reduced at 25°C ; and that lesion production was initiated after 6 h of leaf wetness, with lesion number increasing sigmoidally through 32 h of leaf wetness. When the leaf wetness period was interrupted, the number of lesions was reduced proportionally to the length of the dry period, and as few as 0.3 to 1.7 h of dryness was sufficient to significantly reduce the number of lesions produced (5).

Following the leaf spotting phase of the disease, *B. squamosa* further colonizes infected leaves as they blight and die back (2,27,52). Sporulation occurs almost exclusively on dead leaves, mainly the dead leaf tips (Fig. 3), and rarely on living leaf tissue (27). Even though dead leaf tissue must be available for sporulation to occur, under field conditions, the relationship between sporulation and environmental conditions has not been defined clearly. In controlled studies, sporulation on dead leaves increased with increasing temperature, leaf wetness duration, and leaf age, and was inhibited at temperatures $>30^{\circ}\text{C}$ (3,53).

Sclerotia are formed on infected onion leaves and on the necks of onion bulbs before or after harvest, and on seed stalks (23), and buried in the soil with these disintegrated and decayed tissues. Sclerotia can survive up to 21 months when buried 15 cm below the surface of organic soils (23). In the northeastern United States and eastern Canada, formation of sclerotia on dead infected leaves is more prolific during wet periods in late August and early September (23).

Chemical and Biological Control of BLB

Commercially acceptable control of BLB was achieved with dithiocarbamate fungicides in New York State until the early 1970s, when failure to control BLB with this group of fungicides was reported (38). As a result, chlorothalonil became the standard fungicide for BLB management (38). The situation was different in eastern Canada. Monitoring for BLB was introduced to the Holland Marsh region of Ontario, Canada, in the early 1980s (40). The first fungicide spray was recommended when a threshold of one lesion per leaf was reached (40). Growers alternated applications of mancozeb with applications of chlorothalonil or difolatan, but included benomyl in the spray regime when disease pressure was high (40). Difolatan and benomyl are no longer registered in the United States and Canada (7).

Currently, in eastern Canada, growers typically start fungicide programs with a preventive fungicide, such as a dithiocarbamate, applied at 7- to 10-day intervals, followed by fungicides such as

chlorothalonil or iprodione, often mixed with a dithiocarbamate, depending on disease pressure (9). In this region, mixtures of fungicides are used to provide control of both BLB and downy mildew (*Peronospora destructor*). The fungicides Pristine, a mixture of pyraclostrobin and boscalid (BASF Canada Inc., Mississauga, Ontario), and Switch, a mixture of cyprodinil and fludioxonil (Syngenta protection des cultures Canada Inc., Guelph, Ontario), are incorporated into the spray program when disease pressure is perceived to be high.

In the organic soils of southwestern Montreal, the first application of dithiocarbamate fungicides is recommended when the number of lesions on the oldest and youngest leaves reach a critical disease level of five and three lesions, respectively (9). If disease continues to increase, growers are advised to apply fungicides from other chemical groups. A common fungicide program for BLB in eastern Canada involves 6 to 14 sprays per season, of which 3 to 4 sprays are with iprodione alone or in a fungicide mixture (13,58).

Pathogen insensitivity to fungicides can be a serious problem, and good disease forecasting and management decisions can slow or prevent the development of fungicide insensitivity. The sensitivity of 35 field isolates of *B. squamosa* from the organic soils of Southwest Montreal to mancozeb (Dithane DG), iprodione (Rovral), vinclozolin (Ronilan DF), and chlorothalonil (Bravo 500) was tested under laboratory conditions (58). All isolates were sensitive to mancozeb (FRAC group M) and chlorothalonil (FRAC group M), but four isolates were insensitive to two dicarboximide fungicides, iprodione and vinclozolin (FRAC group 2) (6). Cross-resistance to these two fungicides was observed. In the onion production area of Quebec, the percentage of isolates resistant to iprodione varied from 8 to 21% (13).

Over the last decade, new fungicides belonging to chemical groups different from those that were available were registered for management of BLB, including boscalid (FRAC group 7, Lance or Endura, BASF Canada Inc.; Endura S.p.A., Bologna, Italia), boscalid + pyraclostrobin (FRAC groups 7 and 11, Pristine, BASF Canada Inc.), and cyprodinil and fludioxonil (FRAC groups 9 and 12, Switch, Syngenta protection des cultures Canada Inc.) (6). The availability of these fungicides allows for a better rotation of fungicides with different modes of action (fungicide groups) and, hence, better management of fungicide insensitivity in *B. squamosa* populations than previously possible. The fungicide industry continues to test and release new fungicides that may be useful for BLB management. These include fluopyram (Luna, Bayer CropScience, Research Triangle Park, NC) and metconazole (Valent Canada, Inc., Guelph, Ontario) (26,36). Effective fungicides are needed to obtain maximum benefit from disease forecasting. For BLB, true systemic fungicides could be very useful because they become rainfast quickly and are not washed from leaves during the rain events that can contribute to BLB development. The redistribution of systemic fungicides within a leaf increases the effectiveness of the fungicide, which is especially important for onion leaves, which are cylindrical and covered with a wax layer that can repel fungicide (24).

Effective biological controls could provide additional disease management options for BLB. Several potential strategies for biological control of BLB were investigated from 1974 to 2006 (10). Conidia of *B. squamosa* require an exogenous source of nutrients for germination (17), hence competition for nutrients was studied as a potential biological control strategy. First, in 1974, Fokkema and Lorbeer investigated the antagonistic potential of saprophytes in the onion phyllosphere and concluded that, despite the large number of bacteria, yeast, and filamentous fungi inhabiting the onion leaf surface, most were poor antagonists of *B. squamosa* (25). Similarly, in 1977, Clark and Lorbeer studied the role of phyllosphere bacteria in the infection process of *B. squamosa* and concluded that, even though some bacteria were able to inhibit conidial germination in vitro, only a few significantly reduced lesion development on onion leaves (18).

Because *B. squamosa* sporulates on dead onion leaves, it was hypothesized that using saprophytes to suppress *B. squamosa*

sporulation on dead leaves may slow disease progress (11,32). In 1992, Köhl et al. studied the effects of removing necrotic tissues on sporulation of *B. squamosa* (32). Removal of 30 to 50% of the necrotic tissues resulted in 34% reduction in airborne conidial concentration and delayed epidemics by 7 to 10 days. Later, Köhl et al. reported that weekly applications of *Gliocladium roseum* did not result in colonization of necrotic tissues and had no effect on BLB development (33).

The biological control product Serenade (*Bacillus subtilis* QST 713 strain, AgraQuest, Davis, CA) is registered for management of BLB on onions in the United States and Canada (7). Serenade enhances host defense and hence provides some disease control. AgraQuest recommends the use of this product in combination with other fungicides. In one trial, application of Serenade resulted in a significant increase in plant health, indicated by the number of green leaves per plant near the end of the season, even though BLB pressure was very low and no differences in number of lesions per leaf were detected (42).

Recently, the biological control agent *Microsphaeropsis ochracea* (Carisse & Bernier) was evaluated for its ability to control sclerotia-borne inoculum, colonize onion leaves, and reduce production of conidia (11). Under laboratory conditions, the number of conidia produced per sclerotium treated with *M. ochracea* was reduced by 75.5% compared with nontreated sclerotia. Under field conditions, *M. ochracea* colonized only necrotic leaves and reduced production of conidia by *B. squamosa* on these leaves by an average of 82% compared with nontreated leaves. However, when the experiment was repeated in commercial onion fields, the effects of *M. ochracea* on reducing initial inoculum and sporulation on dead leaves was insignificant, mainly due to incoming inoculum from adjacent fields. It was thus concluded that *M. ochracea* may be successfully used only if there is a regional consensus to use this biological control agent to manage sclerotial populations.

Predictors for Botrytis Leaf Blight Developed in New York, Michigan, and Ontario

In eastern Canada, recommendations to manage BLB include a crop rotation of 2 to 3 years with nonsusceptible crops, removal of volunteer plants and onion cull piles, use of less susceptible cultivars (if available), and application of fungicides to reduce disease progress (40). In eastern Canada and the northeastern United States, most onions are produced on organic soils at highly specialized farms where crop rotation sometimes is considered impractical or not cost-effective. Hence, there may be no crop rotation, or growers may practice only short-term rotations with other high-value crops such as carrots, lettuce, or celery. Onion production is concentrated in a few production areas with many adjacent onion fields or a high density of fields located within a few square kilometers. This allows conidia to move readily between fields even where crop rotation is common. Because there is little resistance to BLB in commercial cultivars, most management programs do not consider host resistance.

The conventional fungicide spray program for onion leaf blight management in eastern Canada comprises application of fungicides at 7- to 10-day intervals from the three- to four-leaf growth stage until shortly after the onion lodge (40). The frequency and timing of fungicide sprays for BLB control may be crucial in some years and less important in others (51). In years with low rainfall, BLB severity may be so low that there is no need to apply fungicides for disease control. However, in most years, leaves need to be protected from disease to avoid yield losses and to ensure that plants have sufficient green leaves to absorb the sprout inhibitor, maleic hydrazide, which is used on onion crops for which the bulbs are destined for long-term cold storage. Also, since there is often a premium price for large onions, maintenance of a maximum green leaf area can have large economic consequences. Disease may reduce overall yields but also reduce the value of the crop by reducing the proportion of “jumbo” onions. Some growers and crop advisors state that early application of fungicides is important to avoid infection of the first leaves by spores from exogenous sources, and because the growing season is occasionally so rainy that opportunities to spray are limited or do not occur at optimal times. Onion fields tend to be too small, and too close to other crops, to allow aerial applications of fungicides when the fields are too wet for ground sprayers.

Notwithstanding these constraints, determining the risks of BLB and optimum timing of fungicide applications can improve crop protection in most situations while avoiding unnecessary sprays. Two strategies have been developed to improve timing of fungicide applications. The first strategy consists of timing the initiation of a fixed-interval fungicide spray program based on monitored or predicted disease levels (39,53). The second strategy involves determining the optimum interval between fungicide sprays based on predicted disease risks or observed disease severity. In practice, both strategies are used in conjunction.

In organic-soil areas of New York, Michigan, and Ontario, BLB monitoring networks were implemented in the late 1970s and early to mid-1980s (8,51). To improve BLB fungicide spray timing, critical disease level (CDL) and BLB forecasters were developed during that period. Forecasters developed in New York, Michigan, and Ontario for BLB management are presented in Table 1. Initially, Shoemaker and Lorbeer proposed the use of a CDL of one lesion per 10 leaves to prompt a calendar-based fungicide spray schedule (51). This threshold was modified over the years to a more conservative threshold of the first lesions detected in a field (54), then to one lesion per leaf (39), a threshold that was also adopted in Ontario by 1980 (40). In Ontario, the spray threshold is currently one lesion per leaf based on examination of the three oldest green leaves (80% green tissue) on 50 to 100 plants randomly selected per field (15). In the organic soil area of southwestern Montreal, the CDL was adapted for the oldest and youngest leaves. Initially, the CDL was set at one, then at five and three lesions on the oldest and youngest leaves, respectively, for application of protective fungicides, and at 10 and five lesions for curative fungicides, respectively (10). Sequential sampling plans have been developed to increase the efficiency of sampling (8,62).

Table 1. Characteristics of forecasters developed in Ontario, Michigan, and New York to time fungicide sprays for management of Botrytis leaf blight caused by *Botrytis squamosa*

Forecaster ^a	Input	Output	Use for timing	Threshold
BOTCAST	Temperature (°C) Leaf wetness (h)	Cumulative disease severity values (CDSV) since 50% onion emergence	First spray	Warning CDSV = 21-30 Spray CDSV = 31-40
PREDICTOR	Temperature (°C) Vapor pressure deficit (mb)	Sporulation index (SI) on a 0-100 scale	Spray interval	SI = 50 SI = 70 SI = 80
BLIGHT-ALERT	Temperature (°C) Relative humidity (%) Probability of rain (%)	Inoculum production index (IPI) on a scale of 0-25 Infection probability (IP)	Spray interval	IPI > 7 IP > 30%

^a Forecasters BOTCAST, PREDICTOR, and BLIGHT-ALERT were described by Sutton et al. 1986 (53), Lacy and Pontius 1983 (35), and Vincelli and Lorbeer 1988 (65), respectively.

Aerobiology of BLB and Monitoring of Airborne Inoculum

Airborne conidia of *B. squamosa* drive epidemics of BLB (35,55). Depending on weather conditions, the numbers of conidia produced above the onion canopy vary greatly daily and annually (35,55). Three leaf blight prediction systems have been developed that are based on an estimation of sporulation potential from weather information (35,53,55,62). Sutton et al. (55) reported a significant correlation between the concentration of airborne conidium and severity of BLB. Vincelli and Lorbeer (63) reported that large increases in BLB severity were associated with days in which >10 conidia m⁻³ of air were detected. Carisse et al. (9) assessed the usefulness of measuring airborne inoculum as an aid for improving BLB management. The results of their study suggested that applying a fungicide when airborne inoculum concentrations reach 10 to 15 conidia per m⁻³ can reduce the amount of fungicide applied by 56 to 75% compared with a conventional spray program. Monitoring airborne inoculum helped to determine the need for fungicide applications and the scouting efforts required to efficiently monitor BLB on individual farms. However, the adoption of such a spray decision strategy by growers depends, in part, on the cost of sampling, including the number of spore samplers required for adequate estimation of airborne inoculum concentrations. If conidia of *B. squamosa* are uniformly distributed above the onion canopy, the use of one or a few spore samplers per field may provide an adequate estimate of airborne inoculum concentrations. Otherwise, monitoring airborne inoculum in individual fields is not practical.

Recent studies showed that during nonmanaged (no fungicide applied to control BLB) epidemics of BLB, lesion density and airborne inoculum concentrations have random spatial patterns during the lag phase of epidemics. When disease progressed rapidly, spatial aggregation increased, and when the rate of disease increase plateaued, spatial patterns became random again. Conversely, managed epidemics (fungicides were applied to manage BLB), typically with low levels of disease, were associated with random patterns of both lesion density and concentration of airborne conidia (12). These results indicated that adequate monitoring of airborne inoculum for management of BLB can be achieved using only one or a few samplers per field.

Comparison of Predictors for BLB Management

The major predictors of BLB risk (Table 1) have been mostly evaluated in the areas where they were developed, mainly based on the number of fungicide applications required to control the disease with and without the predictors. Regardless of the predictor used, there have only been a few attempts to determine the best action thresholds. De Visser tested the cumulative disease severity values (CDSV) from the forecaster BOTCAST combined with the sporulation index (SI) from the forecaster PREDICTOR at various CDSV and SI values (20). A spray advisory system with the first fungicide spray applied when CDSV = 40 and subsequent sprays at an action threshold of SI = 70 reduced the number of fungicide sprays by 54% compared to a weekly spray schedule, without yield losses or significant increase in disease severity (20).

In 1988, Vincelli and Lorbeer (63) examined spore trap and weather data collected over 11 years (1969, 1970, 1972, 1976 to 1981, 1985, and 1986) and evaluated the reliability of the BOTCAST DINOv (53) and SI values (35) to predict the importance of *B. squamosa* spore episodes, grouped as insignificant, minor, or major, corresponding to <1, 1 to 9, and ≥10 conidia/m³ air, respectively. The DINOv component of BOTCAST correctly predicted minor spore episodes (82% of correct predictions); however, it failed to correctly predict major spore episodes in 43.5% of the cases (Table 2). SI values at a threshold of 50 correctly predicted the importance of minor and major spore episodes in approximately 50% of the cases.

The requirement for several years of field data has probably limited the evaluation of forecasters or risk indicators. Recently, the

predictive accuracy of seven monitoring- or weather-based BLB predictors was assessed using Receiver Operating Characteristic (ROC) curves (10). ROC curve analysis was used to quantify the effectiveness of the predictors developed in New York, Michigan, and Ontario (Table 1) to correctly distinguish situations in which BLB was above or below a damage threshold defined as the acceptable amount of damage on the crop. A perfect predictor that predicts risk and the absence of risk for 100% of the situations will have an area under the ROC curve (AUC) equal to 1, and less accurate predictors produce AUC < 1.

Among the seven predictors evaluated (10), three were monitoring-based: (i) the daily concentration of airborne conidia per m⁻³ (ACC); (ii) the average number of lesions on the oldest leaves (OLD); (iii) the average number of lesions on the youngest leaves (YNG); and four were weather-based: (iv) the daily sporulation index (SI); (v) the daily inoculum potential index (IPI); (vi) the infection probability (IP); and (vii) accumulated disease severity index (DSV) since the last fungicide spray. The areas under the ROC curve (AUCs) were significantly higher for the three monitoring-based predictors, ranging from 0.80 to 0.94, than for the weather-based predictors, which ranged from 0.55 to 0.74 when AUCs for IP were excluded. However, there were no significant differences among predictors within each type of predictor with the exception of IP, which was not significantly different from the no-information line (AUC = 0.5). As the damage threshold was increased from one to 10 lesions per leaf, the differences between AUCs among predictors diminished (10).

Based on ROC analysis, the action thresholds for airborne inoculum (ACC) were determined to be 8.06 and 13.76 conidia per m⁻³ of air for damage thresholds of one or five lesions per leaf, respectively (10). The analysis also suggested that optimum CDL 3 to 5 lesions per old leaf and 0.16 to 0.5 lesions per youngest leaf (10). These values are higher than the thresholds used in Ontario and New York and lower than thresholds currently used in Quebec (10). Among all the weather-based predictors, SI proposed by Lacy and Pontius (35) had the highest AUC and was best at an action threshold of SI = 80 (10).

From a disease management perspective, a disease predictor should be selected based on the proportion of adequate decisions made when using the predictor. An adequate decision can be defined as the proportion of true positives, when the disease was above the damage threshold and a fungicide spray was advised,

Table 2. Evaluation of *Botrytis squamosa* sporulation risk indicators for reliability at predicting the importance of *B. squamosa* airborne spore release episodes, based on 7 years of spore trap and weather data collected over 11 years (1969, 1970, 1972, 1976 to 1981, 1985, and 1986) (63)

Sporulation forecast	Importance of spore episodes ^a		
	Insignificant	Minor	Major
BOTCAST^b			
Predicted (DINOv = 1)	38.7% (12)	81.8% (9)	56.5% (13)
Not predicted (DINOv = 0)	61.3% (19)	18.2% (2)	43.5% (10)
Sporulation index^c			
Predicted (SI ≥ 50)	27.9% (43)	51.2% (43)	58.7% (71)
Not predicted (SI < 50)	72.1% (111)	48.8% (41)	41.3% (50)

^a Insignificant, minor, and major spore episodes are days when the mean *B. squamosa* airborne conidial density for a 24-h period following the forecast was 1.0 spore/m³, >1.0 to 9.9 spore/m³, and ≥10.0 spore/m³, respectively. Number of episodes is in parentheses.

^b DINOv is daily inoculum value from the forecast system BOTCAST (53).

^c SI is sporulation index proposed by Lacy and Pontius (35).

and by the proportion of true negatives, when the disease was below the damage threshold and a fungicide spray was not advised. From a grower's perspective, a disease predictor should also be selected based on the risk associated with the use of the predictor. This risk can be defined as the proportion of false negatives, when the disease was above the damage threshold but a fungicide spray was not advised. Finally, from the perspective of reducing fungicide applications, the disease predictor should minimize the proportion of false positives, situations where a fungicide spray was advised but not needed because the disease was below the damage threshold.

When considering data for the entire season, at a damage threshold of 5 lesions/leaf, the proportions of adequate decisions were higher for the monitoring-based predictors than for the weather-based ones (Fig. 6). The monitoring-based predictors and the weather-based predictors SI and DSV were the most efficient at providing adequate decisions. Considering that disease and pathogen monitoring is generally more expensive than weather monitoring, it was proposed to combine monitoring- and weather-based predictors (SI and DSV). A method to reduce the cost of using biological monitoring-based predictors is to use these early in the season to determine the first action thresholds. Once the first fungicide is applied, the interval between sprays could be determined using weather-based predictors combined with less intensive monitoring-based predictors at a higher action threshold. A modification

of this approach is used in the Bradford region of Ontario. When the DSV is very low (<10), pest management scouts spend very little time looking for BLB lesions, since lesions are more difficult to find. When the DSV gets close to 15, scouts routinely check 100 plants/field for lesions twice a week and continue to do so until the threshold of one lesion/leaf is reached in individual fields. Results of the ROC analysis suggest that this approach is sound but that the spray threshold could be increased to 3, and probably 5 lesions per old leaf. Once the fungicide spray program begins, scouts no longer count lesions but monitor the general level of disease control in the field.

An effective disease forecaster also depends on the effectiveness of the delivery system. The BLB forecasting program used in Quebec is part of a larger Integrated Pest Management (IPM) program that includes monitoring and forecasting other onion diseases and insect pests, and provides information on risks, spray timing, and resistance management. There is a high level of confidence in the personnel, a good delivery system, training for IPM scouts, and an effective communications network. All of these contribute to the use of disease forecasts by growers.

Long-term Evidence of Improved Management of BLB

Annual BLB epidemics are a component in the long-term dynamics of the disease. Historically, these annual epidemics have been the sole focus of attention in the development of disease management tactics, but one can ask whether the continuing effort to reduce the annual impact of BLB has had any impact on long-term disease pressure. The capacity to answer this question depends on the availability of suitable data. Fortunately, there are crop scouting data for more than 20 years of BLB epidemics in Quebec Province.

Figure 7A shows the average number of BLB lesions per leaf in each of 19 years from 1987 to 2005. These data are based on field scouting in individual fields over the main part of the growing season from June to early August. Each point is the average over all fields and sampling visits and summarizes between 900 and 3,500 individual observations depending on the overall size of the scouting plan in each year. A simple linear regression of the average lesion count per leaf per year against time reveals that average lesion numbers per leaf declined by 0.17 per year over the 19 years of data. There was evidence that the parameters of the fitted regression model differed from zero: the estimated intercept was 3.91 lesions per leaf (s.d. = 0.693, $P < 0.001$); the estimated slope was -0.17 lesions/year (s.d. = 0.066, $P = 0.021$); $R^2_{\text{adj}} = 0.23$, ($F(1,17 \text{ df}) = 6.5$, $F_{\text{pr}} = 0.021$).

The negative linear trend in the disease intensity data shows the average year-on-year reduction in BLB in southwest Quebec during the period of IPM implementation. However, the trend accounts for only 23% of the variance in the observed data; the year-to-year fluctuations around the trend account for the remaining 77%. Since growers are concerned with both the level and predictability of disease, any reduction in the year-to-year variability of BLB epidemics is also a welcome outcome for disease management. There is some evidence that the dynamics of BLB in southwest Quebec have indeed become more stable over time. To illustrate this, we divided the observed disease time series into three consecutive sections of 7, 6, and 6 years in length running from 1987 to 1993, 1994 to 1999, and 2000 to 2005, respectively. Within each of these sections, we calculated the absolute difference in average lesion counts between pairs of consecutive years to measure year-to-year variation in disease. An effective long-term disease management program should have a low level of disease and small year-to-year differences. In the first section of 7 years, the average difference between years was 1.2 lesions per leaf and the largest absolute difference was 3.4 lesions. For the middle section, the corresponding values were 3.6 and 5.6 lesions per leaf, respectively, and in the final section 0.4 and 1.1. The pattern of BLB epidemics over the 20 years of IPM implementation can be summarized as a transition from an early period with relatively high levels of disease and moderate year-to-year variations in disease levels, through a period of gradually decreasing disease but in-

Fig. 6. Proportion of adequate (dark bars) and inadequate (dashed bars) decisions for seven predictors of Botrytis leaf blight (BLB) risk evaluated for a damage threshold of 5 lesions/leaf for **A**, entire season and **B**, after the first fungicide application (10). Proportion of adequate decisions was calculated as the sum of proportion of true positives (BLB above the damage threshold and a fungicide spray was advised) and proportion of true negatives (BLB below damage threshold and a fungicide spray was not advised). Proportion of inadequate decisions was calculated as the sum of proportion of false negatives (BLB above damage threshold and a fungicide spray was not advised) and the proportion of false positives (BLB below damage threshold and a fungicide spray was advised).

creased year-to-year variation, to the current period in which both average disease levels and year-to-year variation in disease are lower.

As indicated in the title of the article, development of the disease management strategy in southwest Quebec has been a process of continual change and elaboration. One of the components of this process has been a move from what might be a traditional “watch and respond” tactic, in which scouting was used to determine if disease levels had reached damage thresholds, to an “early warning” or “preemptive” approach in which disease control has increasingly focused on reducing primary infections. The change in emphasis can be seen in the data on crop scouting.

The time series for the proportion of the sampling effort (i.e., number of scouting visits made in a year) over the whole scouting program in June, July, and August each year is shown in Figure 7B. These series were obtained by counting the total number of scouting events made each season over all farms and then calculating the monthly totals as proportions of the grand total within each year. Examining these sampling effort data, it can be seen that about 25 to 30% of sampling was carried out in June each year from 1987 until 1998. After 1998, the sampling effort allocated to June increased to between 40 and 50% with a corresponding decrease in the amount of sampling carried out later in the season. This change in the pattern of sampling effort corresponds with the introduction of spore-sampling technology into the disease management system and an increasing awareness of the benefits of getting good, early disease control to prevent, as far as possible, initial inoculum from establishing the foliar disease epidemic. Long-term data such as these are not available from the Bradford area. However, lesion counts from fields that were scouted in 1981 and 1982 are available for comparison with lesion counts from 2006 and 2007. In 1981, a dry year, the average number of lesions per fourth oldest leaf per plant was 3.5 on 13 August (40). This is much higher than the average number of lesions per leaf on the oldest three leaves in 2008, which never reached the spray threshold of 1.

As noted above, the pathogen is carried over from season to season as soilborne sclerotia which germinate in the following spring and early summer to produce the primary inoculum. Since the amount of primary inoculum is thus a function of the size of the sclerotium population, there is clearly a link between the final intensity of the foliar epidemic in one year (which determines the amount of colonized tissue available for sclerotium production) and the amount of initial inoculum available at the start of the next year. Even though growers follow crop rotation, the close proximity of many fields allows for an increase in the pool of airborne primary inoculum in the area. The direct link between sclerotia production in the fall and inoculum in the spring suggests that disease levels in individual fields could be reduced by reducing the sclerotium population. However, since the airborne conidia of *B. squamosa* are highly mobile, attempts to reduce the soilborne phase of the pathogen by individual growers in an intensively farmed area are unlikely to succeed. Cooperative, long-term efforts by all, or a large proportion of growers may prove successful, particularly where the within-season efforts by growers are combined with research to target control efforts more effectively. We are currently investigating this strategy using a combination of statistical time series methods and life cycle modeling to examine the sensitivity of the long-term data to growers' actions and life cycle stages of the pathogen.

Potential of Newly Developed DNA-Based Forecasts for BLB

Knowledge of the aerobiology of BLB and evaluation of disease risk indicators promoted the implementation of a network of spore sampling sites in the southwest of Montreal. After only a few years, it became evident that it is not possible to quantify spores of *B. squamosa* in a timely and reliable fashion by the traditional method of spore counts using a microscope. Hence, Carisse et al. (14) developed and validated a TaqMan qPCR assay to quantify *B.*

squamosa spores from the collecting surface (rod) of rotating-arm samplers. When developing the qPCR assay, the authors considered the following constraints: spores are collected on a silicone-greased surface; the limit of detection should be below the action threshold; and the spore quantification procedure must be fast (14). Using the TaqMan qPCR assay, it was possible to extract DNA from the silicone greased rods and detect as few as 2 spores/rod. No signals were detected from rods free of *B. squamosa* spores, and a linear relationship was obtained between the known numbers of conidia deposited on silicone greased rods and the numbers of spores determined with the qPCR assay. No signals were obtained with the qPCR assay when tested with DNA from seven species of the family *Sclerotiniaceae* (*B. aclada*, *B. porri*, *B. cinerea*, *B. elliptica*, *B. ficariarum*, *B. ranunculi*, *Sclerotium cepivorum*, and *Sclerotinia sclerotiorum*) and 36 airborne fungal contaminants isolated from air samples; however, a weak signal was obtained with DNA from *B. elliptica*, a lily pathogen unlikely to be present in a large onion field. For 25 samples, the whole procedure from spore extraction from field samples to estimation of the number of conidia/rod took approximately 2 to 3 h (14).

ROC analysis showed that the qPCR assay was reliable at identifying the risks of BLB. However, the best action thresholds were lower when conidial concentration was estimated using the qPCR assay than when using microscope spore counts, probably because spores of similar appearance to those of *B. squamosa* conidia may be counted when using the microscope method. The proportion of adequate decisions was higher when airborne inoculum was estimated with the qPCR assay than with the microscope (14).

Fig. 7. A, Time series for *Botrytis* leaf blight lesion counts on plants sampled on each of two farms in Quebec Province over 19 years. Each value is an average of the number of lesions per leaf on one upper and one lower leaf per plant for 15 to 20 plants per field for approximately 10 weekly samplings each year. B, time series for proportions of total sampling effort expended in June, July, or August for *Botrytis* leaf blight over 19 years. Vertical dotted line is a reference point showing the year 1999 in both sets of series. Data were collected in the organic soil area of southwest Montreal.

It is thus possible to implement a network of spore traps combined with qPCR assays to assess risks of BLB in individual fields. More research is needed to determine if air sampling should be implemented at the regional, farm, and/or field level. Once the air sampling network is fully implemented and DNA from different fields and sampling dates is available, it will be possible to enhance the value of air-sampled DNA by providing information on the presence of fungicide resistance genes in *B. squamosa* samples. Starting in 2009, the scouting group PRISME (<http://www.prisme.ca>) is responsible for running a network of spore samplers, at both the farm and the regional levels. The samplers at each onion farm are located in fields sown with the onion cultivars most susceptible to BLB and with the highest risk of disease development based on microclimate, preceding crop, and history of BLB. Because the first conidia of *B. squamosa* are produced on sclerotia that overwintered in fields that had onions the previous year, the regional samplers are installed in fields where onions were grown the previous year and where a high level of BLB was observed.

Conclusion

Despite intensive research and disease management efforts, BLB is still present in most onion fields in the northeastern United States and eastern Canada. However, over the last few years, the disease appears to have reached equilibrium at relatively low disease levels (Fig. 7A) in southern Quebec. It is difficult, however, to determine if this equilibrium is due to sustained efforts in disease management, especially scouting, or to greater efficacy of the new *Botrytis* fungicides available, or both. The onion production area in southern Quebec has proved to be an excellent large-scale test site for disease forecasting and disease management, in part because of the long-term data available.

In the eastern Canadian provinces of Quebec and Ontario, for the past 25 years, most onion growers have used critical disease levels and disease forecasts to time fungicide sprays, with some gaps in IPM delivery in Ontario. Thus, growers tolerate a certain amount of BLB, hopefully below the damage threshold, because of an awareness of action thresholds and disease risk, and because none of the registered fungicides provides complete BLB control. This might explain why the disease is still present and continuously requires monitoring and management practices to reduce losses. Regardless of the onion production areas, forecasters for BLB are widely adopted by growers, implying that these tools are perceived by growers as useful. The question remains as to what would happen if disease management efforts were relaxed. Reaching a low and sustainable level of disease in such an intensive production system could certainly be considered a success.

Recent studies have highlighted the gaps in our knowledge on BLB, which include management of sclerotial populations, detection and management of fungicide-insensitive isolates, and improved assessment of the influence of weather on pathogenesis (10,13,14). These studies suggest that BLB management strategies may be improved by collective efforts to reduce populations of sclerotia, using more conservative critical disease levels for fungicide sprays, and not delaying the first fungicide application too long. Stability in the system may be achieved by concentrating scouting efforts in June (Fig. 7B) and initiating fungicide sprays at or before disease onset in order to maintain a low level of disease during onion bulb formation. The availability of very effective fungicides improves the effectiveness of disease forecasting and grower confidence. The successes achieved to date in managing BLB argue for maintaining scouting programs, promoting the development and implementation of disease risk indicators, and maintaining a good communication network among scientists, horticultural specialists, and growers.

Accepting that it is not possible to disentangle the contributions to improved disease control made by new fungicides and by implementation of an IPM approach, this evaluation of the monitoring and forecasting of BLB suggests that successful implementation of these management tools has short-term benefits of protecting onions with a minimum number of fungicide sprays, and long-term benefits of reducing initial inoculum and overall disease pressure.

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Mr. Tremblay has worked for Agriculture and AgriFood Canada as a research professional in Dr. Carisse's laboratory since 2000. He graduated in biology in 1999 at Sherbrooke University, and since then he continues to refine his training in the field of molecular biology. His role in the molecular plant pathology and epidemiology team is to develop and adapt molecular techniques for detecting and quantifying plant pathogens. Recently, he participated in the development of rapid fungal DNA extraction techniques and TaqMan quantitative PCR assays to quantify inoculum from the collecting surface of spore samplers. Those new techniques are useful for rapid quantification of airborne inoculum from a vast network of spore traps.

Dr. McDonald is a professor in the Department of Plant Agriculture, University of Guelph. She obtained a B.Sc. in plant protection in 1978, and an M.Sc. (1981) and Ph.D. (1994) in plant pathology from the University of Guelph. Dr. McDonald was the provincial Pest Management Specialist for muck vegetables and a research scientist with the Ontario Ministry of Agriculture and Food before joining the university in 1997. Currently, she teaches a graduate course in plant disease epidemiology and is the Research Program Director for the Plant Production Systems Program at the University of Guelph.

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M. Brodeur is the leader of the 'Compagnie de recherches Phytodata', the research branch of a grower's association, PRISME (Productions en Régie Intéressée du Sud de Montréal enr), well known in Quebec for its 27 years of involvement in Integrated Pest Management (IPM). Phytodata is owned by 35 vegetable growers, all members of the scouting and crop management consulting service PRISME, and by one agronomist, M. Luc Brodeur. Over the years, Phytodata has developed and/or validated several IPM services now offered to growers through PRISME, among which are farming activities and pesticide applications tracking, weather forecasts, pest warnings, irrigation warnings, scouting, development and evaluation of risk indicators, and integrated crop management and traceability software (Datatrace).

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