EPIDEMIOLOGY OF APPLE LEAF SPOT*

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ABSTRACT

Apple leaf spot (ALS) caused by *Colletotrichum* spp. is a major disease of apple (*Malus domestica*) in Southern Brazil. The epidemiology of this disease was studied in experiments carried out in the counties of Passo Fundo and Vacaria, State of Rio Grande do Sul, from February 1998 to October 2000. The disease was found in all the six apple orchards sampled in the growing seasons of 1997/98 and 1998/99. The fungus isolates associated with ALS fit the characteristics of *C. gloeosporioides* (75%), *C. acutatum* (8%), and *Colletotrichum* sp. (17%). The pathogen overwintered in dormant buds and twigs but not in dropped leaves or fruit mummies. Two sprays of copper oxychloride (at 0.3%) reduced the fungus initial inoculum by 65-84.6% in buds and 85.6-93.7% in twigs, but had no effect on the early season progress of the disease. Disease severity increased proportionally to elevation of temperature from 14 to 26-28 °C. At 34 °C, however, infection was completely inhibited. The duration of leaf wetness required for infection ranged from two hours at 30 °C to 32 h at 16 °C. The relationship of temperature (T) and leaf wetness (W) to disease severity (Y) was represented by the model equation $Y = 0.00145[((T\text{-}13)^{1.78})((34.01\text{-}T)^{1.09})]$ * $25/[1+14\exp(-0.137W)]$, $R^2 = 0.73$ and $P < 0.0001$. Currently, this information is being used to manage the disease and to validate a forecast system for ALS.

Additional key words: *Colletotrichum*, disease forecast, disease management.

RESUMO

Epidemiologia da mancha foliar da macieira

A mancha foliar da macieira (*Malus domestica*), causada por *Colletotrichum* spp., é uma das principais doenças da macieira no Sul do Brasil. Estudos sobre sua epidemiologia foram conduzidos em Passo Fundo e Vacaria, RS, no período de fevereiro/1998 a outubro/2000. A doença foi encontrada nos seis pomares amostrados em 1998 e 1999. Isolados do fungo obtidos a partir de lesões em folhas e frutos corresponderam às espécies *C. gloeosporioides* (75%), *C. acutatum* (8%) e *Colletotrichum* sp. (17%). O patógeno sobreviveu em ramos e gemas dormentes, mas não em folhas caídas ao solo e frutos mumificados. O tratamento de inverno com duas aplicações de oxycloreto de cobre (0,3%) reduziu o inóculo do patógeno em gemas (65 a 84,6%) e ramos (85,6 a 93,7%), mas não diminuiu o progresso inicial da doença. A severidade da mancha foliar da macieira aumentou à medida que a temperatura ambiente foi elevada de 14 para 26-28 °C. A 34 °C, entretanto, a infecção foi completamente inibida. A duração do molhamento foliar requerido para infecção variou de duas horas a 30 °C para 32 h a 16 °C. A relação da temperatura (T) e do molhamento foliar (W) com a severidade de doença (Y) foi representada pela equação $Y = 0{,}00145[((T\text{-}13)^{1,78})((34{,}01\text{-}T)^{1,09})]$ * $25/[1+14\exp(-0{,}137W)]$, $R^2 = 0{,}73$ e $P < 0{,}0001$. Atualmente, estas informações estão sendo utilizadas para manejo da mancha foliar da macieira e para validação de um sistema para sua previsão.

INTRODUCTION

Apple leaf spot (ALS), also named *Glomerella* or *Colletotrichum* leaf spot, is a major disease of apple (*Mallus domestica* Borkh.) in Brazil. The importance of this disease results from its wide distribution (Leite *et al*., 1988; Bonetti *et al*., 1999), the high amount of damage (50 to 75%) (Cerezine *et al*., 1992; Muller *et al*., 1993), and the numerous fungicidal sprays (10 to 12/season) needed for its control (Cerezine *et al*., 1992).

The ALS is caused by species of the genus *Colletotrichum*, especially *C. gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph *Glomerella cingulata* (Stonem.) (Spauld. & Schrenk) (Leite *et al*., 1988; Bonetti & Katsurayama, 1999; Sanhueza, 1999). Great variation in both pathogenicity and cultural/morphological features of *Colletotrichum* isolates causing ALS have been reported (Bonetti & Katsurayama, 1999), so that some questions regarding the etiology of the disease still remain unclear.

The control of ALS is difficult because of rapid disease development. Its incubation period can be as short as two days

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(Leite *et al*., 1988). Additionally, most of the economic and efficacious fungicides used for the control of ALS are only protectants. Therefore, it is risky to wait for the first disease symptoms to initiate spray applications. On the other hand, preventive use of fungicides may represent increased and unnecessary costs. Usually, growers apply fungicides to plants every 5-10 days, or at each 25 mm-rain event, from blooming to harvest (Bonetti & Katsurayama, 1999).

One strategy for optimizing disease management is to predict its occurrence. This can be done by monitoring the weather variables needed for infection. Although some data about weather effects on ALS are available (Bonetti & Katsurayama, 1999), more information is needed to develop a forecast system for such disease.

This research was oriented to study the weather variables that lead to plant infection and to the need for disease control. Because little is known about the epidemiology of ALS, additional work was performed to study pathogen survival and dissemination, as well as the effect of winter applications of fungicides on the fungus initial inoculum.

MATERIAL AND METHODS

Frequency of disease and characteristics of fungus isolates

In February 1998, six apple orchards located in Lagoa Vermelha, Passo Fundo, Sertão, Soledade, and Vacaria counties had their leaves and fruits examined for symptoms of ALS. After isolation and purification in PDA medium, 12 monosporic isolates were obtained from these leaves and fruits. In March 1999, one hundred isolations were carried out from symptomatic leaves collected in Passo Fundo and Vacaria. The isolates were characterized according to their colony color, shape and size of conidia, growth rate at different temperatures (26, 28, 32, and 35 °C), and sensibility to the fungicide benomyl (0, 2, and 5 ppm). Five replicates (Petry plates) were used for all *in vitro* tests. The pathogenicity of the isolates was tested on leaves of Gala plants (two plants per isolate) sprayed with 10^5 conidia/ml and incubated in the growth chamber at 26 °C, 12-h photoperiod, for seven days.

Survival and dissemination of the pathogen

Leaves on the soil surface (15 g), fruit mummies (10 g), dormant buds (10 g), and twigs (50 g) were collected from non-sprayed Gala plants in Passo Fundo (June 8, June 30, and July 27, 1998; September 6 and October 10, 1999) and Vacaria (September 10, 1999). At each collect, the material was chopped, suspended in distilled water plus Tween 20, shaken for 30 min., diluted to 10^{-1}, 10^{-2}, and 10^{-3}, and transferred (0.1 ml) to Petry plates with PDA medium plus cloranfenicol (20 drops per liter). Plates were incubated for 48 h at 25±1 °C, 12-h photoperiod, and examined for colonies of *Colletotrichum* spp, which were counted, transferred to new plates, and later used to inoculate fruits and leaves of the Gala cultivar. To study the dissemination of the pathogen, spore traps bearing sticky glass slides were placed at 0.6 m (1998 and 1999) and 1 m (1999) above the soil surface, under apple trees and near to boxes containing diseased apple leaves. Twice a week or every day following rainfalls, the sticky slides were replaced by new ones and examined for the presence of both conidia of *Colletotrichum* and ascospores of *Glomerella*.

Winter application of fungicides

In 1999 and 2000, dormant plants of the Gala cultivar were sprayed twice (June and August) with either calcium polysulfate (4 °Bé) or copper oxychloride (0.3%). Each treatment was applied to four replicates of three plants, at a volume of 1000 l/ha, in the counties of Passo Fundo (1999 and 2000) and Vacaria (1999). Either two (1999) or three (2000) times after fungicidal applications, twigs and dormant buds from both sprayed and non-sprayed plants were collected and examined for the presence of the pathogen as described in the previous paragraph. In 2000, sprayed and non-sprayed plants were also examined for initial disease incidence through four weekly samplings (7, 13, 20, and 26 Dec 2000) of 400 leaves per treatment (four plants × 100 leaves).

Temperature and leaf wetness requirements

Seventy seven combinations of temperature (14, 16, 18, 20, 22, 24, 26, 28, 30, 32, and 34 °C) with leaf wetness duration (2, 4, 6, 8, 16, 24, and 32 h) were tested to determine their effect on infection of apple leaves by *C. gloeosporioides* in the growth chamber. A mix of three isolates (10^5 conidia/ml) was sprayed on 30 cm-high plants, being one plant (minimum of 25 leaves) for each tested combination of temperature and leaf wetness duration. Upon completing each period of leaf wetness, the plants were transferred to a growth chamber adjusted to 26 °C and a 12-h photoperiod, incubated for additional six days, and then assessed for disease severity by means of an electronic leaf area meter (LI-COR, Inc., Lincoln, NE).

Disease progress curves were established by organizing the disease data in ascendant values of temperature and leaf wetness duration. A logistic model $Y = B_1/(1+B_2\exp(-B_3W))$, where Y is the average severity, B_1 the asymptotic stabilization of the curve, and B_2 and B_3 are, respectively, parameters related to initial inoculum and to rate of infection, while W represents the wetness duration in hours, was fitted to the data obtained by non-linear regression techniques, at the average temperature of 26 °C.

A general beta function, $Y = B_1((T-B_2)^{B3})((B_4-T)^{B5})$, in which B_2 represents the minimum and B_4 the maximum temperature for disease development, while B_1, B_3, and B_5 are parameters for the equation (Hau & Kranz, 1990), was used to examine the relationship between disease severity and temperature when leaf wetness duration was longer than 24 h. The analysis was performed using the SAS statistical package, version 6.12 (SAS, 1995).

RESULTS

Frequency of disease and characteristics of fungus isolates

ALS was detected in all orchards sampled in 1998 and 1999. In 2/3 of the orchards the disease was either unknown

by the growers or mistaken as a physiological plant disorder. Eleven of 12 isolates obtained in 1998 presented gray colonies, oblong conidia (0.49-0.54 × 1.35-1.47 µm), and they induced disease symptoms on apple leaves. One non-pathogenic isolate formed white colonies with creamy, salmon masses of fusiform conidia. Eight isolates (LF, LM, RM, GSTM, UPFF, UPFM, ZF, and STM) did not grow in PDA medium with 2 or 5 ppm of benomyl (Figure 1-A), three (GSTF, STF, and RF) grew slowly, and one (EF) was not affected by such concentrations of fungicide. Most isolates grew well (colony diameter ≅ 80 mm) at 26 and 28 °C but they were greatly inhibited (≤ 11 mm) at 35 °C (Figure 1-B). The EF isolate differed from the others by being able to grow at 32 °C (66 mm) and 35 °C (26 mm). In 1999, all isolates presented gray cultures and formed stromata, perithecia, and ascospores. Because these isolates formed few conidia they were not tested for pathogenicity.

Survival and dissemination of the pathogen

The amount and the characteristics of the overwintering inoculum of *Colletotrichum* spp. varied according to the plant part, year, date, and orchard sampled. The mean number of colony forming units (CFU) in PDA (Table 1) decreased from fruit mummies (60,000) to leaves (21,667), dormant buds (2,073), and twigs (340). Usually, the CFUs were more numerous in early rather than late collects, and more in 1998 than in 1999. While isolates from buds and twigs caused ALS on leaves, those from fruit mummies and leaves induced bitter rot in Gala fruits. No conidia nor ascospores were captured by the spore traps used in 1998 and 1999.

Winter application of fungicides

Copper oxychloride was more efficacious in reducing fungus inoculum than calcium polysulfate (Table 2). The mean number of CFUs varied from 4013 (copper) to 6969 (calcium polysulfate) and 13243 (check) in buds and from 210 (copper) to 226 (calcium polysulfate) and 3197 (check) in twigs. The winter treatment had lower efficacy on buds, where the amount of inoculum was higher. The initial incidence of the apple leaf spot (Figure 2) was not affected by applications of either calcium polysulfate or copper oxychloride.

Temperature and leaf wetness requirements

The various combinations of temperature and leaf wetness duration resulted in disease severity between zero and 28.4% (Figure 3). The minimum, optimum, and maximum temperatures for infection were 14, 24, and 32 °C, respectively. The relationship of temperature (T) to disease severity (Y) was represented by the model equation 1.

$$Y = 0.00145[((T\text{-}13)^{1.78})((34.01\text{-}T)^{1.09})] \quad (1)$$

The duration of leaf wetness required for infection ranged from two hours at 30 °C to 24 h at 14 °C. The logistic equation 2 represented the effect of leaf wetness (W) on disease severity (Y).

$$Y = 25/[1+14\ \exp(-0.137W)] \quad (2)$$

Multiplication of equations 1 and 2 resulted in the joint model 3 ($R^2 = 0.73$, $P < 0.0001$), which produced the response surface (Figure 4), combining the effects of temperature and leaf wetness duration (Hau & Kranz, 1990).

$$Y = 0.00145[((T\text{-}13)^{1.78})((34.01\text{-}T)^{1.09})] * 25/[1+14\ \exp(-0.137W)] \quad (3)$$

DISCUSSION

Characterization of *Colletotrichum* species involves morphological and cultural features such as colony color, shape and size of conidia, optimum growing temperatures, and sensibility to benomyl (Berstein *et al*., 1995; Shi *et al*., 1996; Freeman *et al*., 1998). According to these features, 75% of the *Colletotrichum* isolates obtained in 1998 fit the characteristics of *C. gloeosporioides*, 8% of *C. acutatum* J. H. Simmonds, and 17% of *Colletotrichum* spp (Crusius, 2000). Therefore, *C. gloeosporioides* is the main fungal species causing apple leaf

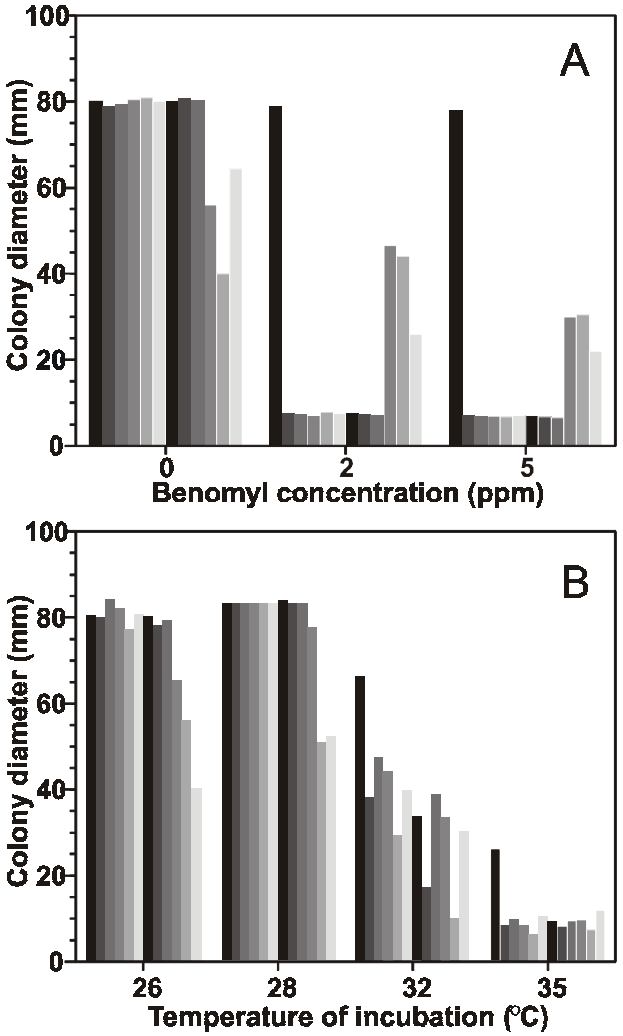

FIG. 1 - Colony diameter (mm) of *Colletotrichum* isolates after seven days of incubation at different levels of benomyl (A) and temperatures (B). For each concentration of fungicide or temperature of incubation, the bars are sequenced according to the EF, GSTM, LF, LM, RM, STM, UPFF, UPFM, ZF, GSTF, RF, and STF isolates.

TABLE 1 - Number of colony forming units (CFU) of *Colletotrichum* spp. from fruit mummies, leaves on the soil surface, buds, and twigs from dormant apple (*Malus domestica*) trees in Passo Fundo and Vacaria counties, RS

Location/Date	Colony forming units (n°/g)			
	Fruits	Leaves	Buds	Twigs
Passo Fundo				
8 Jun 98	60,000 (10,000)[z]	40,000 (5,000)	2,500 (350)	1,333 (185)
30 Jun 98	-	20,000 (2,500)	6,250 (1500)	0
27 Jul 98	-	5,000 (1,000)	250 (29)	0
6 Sep 99	-	-	1,146 (105)	396 (46)
10 Oct 99	-	-	938 (84)	20 (6)
Vacaria				
10 Sep 99	-	-	1,354 (193)	292 (38)
Mean	60,000	21,667	2,073	340

[z] The value between parentheses indicates the standard deviation of the mean for five replicates.

TABLE 2 - Colony forming units (CFU) and control of *Colletotrichum* spp. in dormant buds and twigs from apple (*Malus domestica*) trees sprayed twice with calcium polysulfate (CP) or copper oxychloride (CO) in winter, in Passo Fundo and Vacaria counties, RS

Year, location, and plant part	Colony forming units (n°/g)			Control (%)	
	Check	CP	CO	CP	CO
1999 - Passo Fundo[w]					
Buds	1,042 (173)[z]	2,656 (234)	365 (59)	-	65.0
Twigs	208 (33)	42 (17)	21 (6)	79.8	89.9
1999 - Vacaria[x]					
Buds	1,354 (143)	1,458 (159)	208 (36)	-	84.6
Twigs	292 (45)	104 (22)	42 (15)	64.4	85.6
2000 - Passo Fundo[y]					
Buds	37,333 (5,602)	16,792 (2,284)	11,467 (1,410)	55.0	69.3
Twigs	9,092 (1,146)	533 (61)	567 (69)	94.1	93.7
Mean					
Buds	13,243	6,969	4,013	-35.8	72.9
Twigs	3,197	226	210	79.4	89.7

[w] Mean of two assessments at 19 and 53 days after treatment; four replicates/treatment.
[x] Mean of two assessments at seven and 30 days after treatment; four replicates/treatment.
[y] Mean of three assessments at eight, 14, and 28 days after treatment; four replicates/treatment.
[z] The value between parentheses indicates the standard deviation of the mean.

spot in Rio Grande do Sul. In a similar survey done by Bonetti & Katsurayama (1999) in the State of Santa Catarina, the frequencies of *C. gloeosporioides*, *C. acutatum*, and *Colletotrichum* spp. were 43.6, 50, and 6.4%, respectively. Recently, Carvalho *et al*. (2000) reported that among 38 isolates of *Colletotrichum* spp. obtained from different apple growing regions in Southern Brazil, only those of *C. gloeosporioides* were pathogenic to leaves of the apple cultivar Gala and caused typical symptoms of ALS. It was not possible to determine the frequency of fungus species in 1999, since the drier summer season may have restricted the occurrence of the anamorph *Colletotrichum* and induced more formation of the teleomorph *Glomerella*.

The survival of *C. gloeosporioides* depends on the dormant apple tree. Only isolates obtained from buds and twigs caused apple leaf spot on inoculated plants. In contrast, *Colletotrichum* isolates recovered from leaves on the soil surface

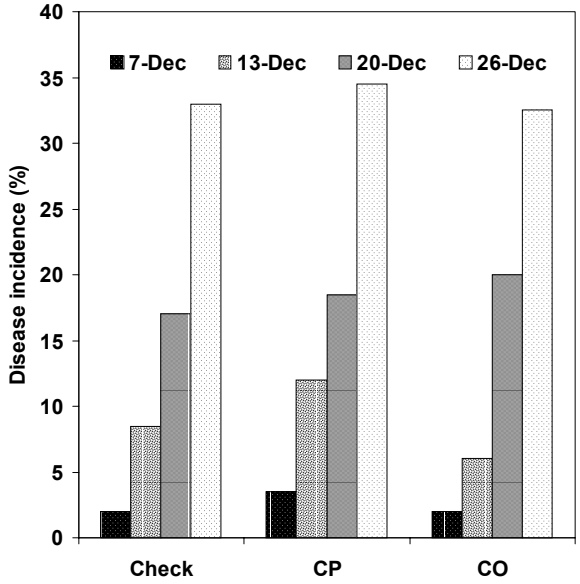

FIG. 2 - Percent incidence of apple leaf spot on apple (*Malus domestica*) trees sprayed twice with calcium polysulfate (CP), copper oxychloride (CO), or water (check) in winter (19 Jul and 17 Aug 2000).

and fruit mummies induced symptoms of bitter rot in fruits (Crusius, 2000). These findings are in agreement with previous reports by Jones & Sutton (1996) and Sanhueza (1999) who have already indicated the dormant plant as the main source of inoculum for apple bitter rot and apple leaf spot, respectively. The absence of pathogen's propagules on spore traps placed near to boxes with diseased apple leaves on the soil surface may have resulted from failure of the spore trapping method (i.e. the spores may have germinated during the time elapsed between collects) or it may be additional evidence that leaves on the ground are not a source of inoculum to the disease. Therefore, management of dropped leaves in order to control ALS may be useless.

Winter application of fungicides did not eradicate the primary inoculum of the fungus. Instead, higher numbers of colony forming units were found in some dormant buds treated with calcium polysulfate (Crusius, 2000). This fungicide may have eliminated antagonist epiphytes, thus allowing a greater recovering of *Colletotrichum* from treated buds compared to the check treatment. The same fungicide was more efficacious on twigs and reduced fungus inoculum by 64.4-94.1%. Although better results were obtained with sprays of copper oxychloride, reduction of the initial inoculum for apple leaf spot requires improved spray schemes, perhaps earlier and more frequent sprays of fungicide to avoid the inoculum establishment into the dormant buds. Also, the lack of difference in initial disease incidence between sprayed and non-sprayed plants indicates that even minimum amounts of inoculum can lead to epidemics of apple leaf spot.

Apple leaf spot occurred in a wide range of temperatures

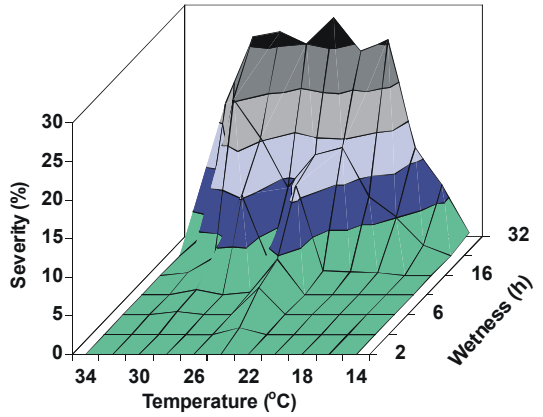

FIG. 3 - Observed severity (%) for apple leaf spot on apple (*Malus domestica*) as affected by temperature (°C) and leaf wetness duration (h).

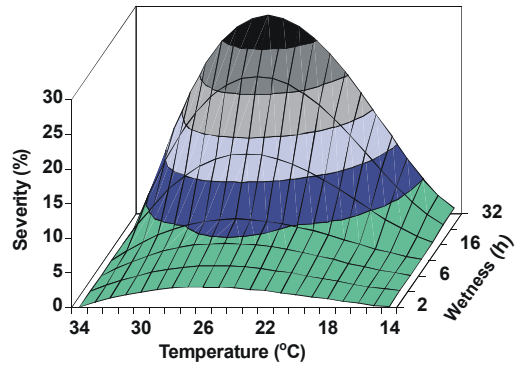

FIG. 4 - Percent severity of apple leaf spot on apple (*Malus domestica*) estimated by non-linear regression of temperature (T) and leaf wetness duration (W) to disease (Y); $Y = 0.00145[((T-13)^{1.78})((34.01-T)^{1.09})]* 25 / [1 + 14*\exp(-0.137W)]$, $R^2 = 0.73$, $P < 0.0001$.

(14 to 32 °C), which is somewhat similar to that (12 to 28 °C) obtained by Katsurayama & Bonetti (1999). At temperatures between 24 and 30 °C, only 2 to 4 h of leaf wetness are required for infection. In contrast, 6 to 16 h are needed when the temperatures are in the range of 16 to 22 °C (Crusius, 2000). Since the latter temperatures are common in both spring and summer, leaf wetness duration seems to be the main factor limiting the development of apple leaf spot. Additionally, the release of conidia and ascospores is rain dependent (Sutton & Shane, 1983; Jones & Sutton, 1996). Therefore, moisture (rain and leaf wetness) is essential for fungus dissemination and plant infection.

A visual comparison of the observed and simulated surface responses of disease severity as a function of temperature and leaf wetness duration indicates that the model closely represents the real system. This information on temperature and leaf wetness requirements is being used to develop a forecast system for apple leaf spot, which will also include a model for rain splash release of conidia, so that the fungus dissemination and the plant infection can be accurately predicted. After its validation in different apple orchards and growing seasons, this forecast system will contribute to improve the control of apple leaf spot and to reduce fungicide usage.

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