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Forecasting the Pepper Gray Mold Rot to Predict the Initial Infection by *Botrytis cinerea* in Greenhouse Conditions

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We determined threshold environmental factors to initiate infection of pepper plants by *Botrytis cinerea*, a fungal pathogen of pepper gray mold, in two greenhouse conditions. A new efficient spore-trapping method was developed to estimate population density of air-borne conidia in the greenhouses, and spore release was measured using a Kerssies' selective medium. At a given day, spores were released greater during daytime (mostly from 7:30 am to 10:30 am and at 4:30 pm) than nighttime. Diurnal and nocturnal temperatures in the greenhouse-1 were about 25°C and 17°C, and relative humidity was 100% for prolonged 24 h due to rain on December 17, 1997. Population density of air-borne conidia was 3.0×10^3 conidia/ 0.5 m^3 after two days, and the initial infection occurred in ten days. During the same period of time in the greenhouse-2, diurnal temperature was about 25°C and nocturnal temperature was below 15°C, and population density of air-borne conidia was 10^4 conidia/ 0.5 m^3. Under these conditions, the initial infection started in three days. This indicates that the early infection occurs under which diurnal temperature is approximately 25°C, nocturnal temperature is maintained below 15°C, and population density of air-borne conidia is 10^4 conidia/ 0.5 m^3 at saturated relative humidity condition.

Keywords **:** *Botrytis cinerea*, Botrytis infection index, pepper gray mold.

Botrytis cinerea is known to be a facultatively parasitic pathogen whose inocula are enhanced from large debris-borne saprophytic bases. It produces conidia at temperatures above 12°C (best at about 15°C) in subsaturated atmospheres, releases them by a hygroscopic mechanism in conditions of rapidly changing humidity, and generally infects plants from conidia in a film of water. Prolonging the persistence of dew, rain, and irrigation water have been recognized as predisposition as etiolated and soft tissues. However, this fungus often infects plants directly from a saprophytically-based-inoculum such as a fallen petal adhering to a leaf or fruit surface. It can also establish quiescent infections, which in tomato stems can last for up to 12 weeks before becoming aggressive. This behavior has profound implications in the design of prophylatic, disease-escape, and therapeutic control measure (Jarvis, 1980; Jarvis, 1989).

However, no one has yet pointed out the low temperature stress to petals as the most critical predisposing factors for primary infection to occur in greenhouses. Therefore, we determined the optimum threshold environmental conditions for predisposition of hosts that result in initial epidemics, established the "Botrytis infection index" based on etiology of pepper gray mold rot, and constructed disease progress patterns.

Materials and Methods

Evaluation of disease development in greenhouses. To evaluate disease development, percent infection of petals, and numbers of infection on new shoots, leaves, green fruits and shoot branches per 20 plants were obtained. From the detailed and repeated observation on etiology of Botrytis gray mold rot, we developed standard evaluation system referred to as the "Botrytis infection index." Temperature and relative humidity in greenhouses were recorded hourly by data logger (Optic StowAway™ Temp USPH5373346, StowAway™ Relative Humidity Logger SRHA08, ONSET Computer Co., MA, USA). Probes were positioned among the canopies at a height of 1.5 m in greenhouses from December 8 to 27, 1997 to determine the environmental parameters.

Monitoring air-borne conidia. To facilitate trapping air-borne conidia, we developed a new spore trapping device equipped with millipore filter (Whatman International Ltd, Maidstone, England) on electric suction pump (12-V DC, 2Å; Daelim Electronic Co., Korea). It was used to monitor air-borne conidia at the rate of 0.25 m^3 air per minute. To observe the periodic release of spores, the spore sampling was done at 7:30 am, 10:30 am, 2:30 pm and 4:30 pm. Spores trapped in millipore filters were suspended in 1 ml sterile distilled water followed by enumerating with haemocytometer under light microscope.

Monitoring spore release. Kerssies' selective medium (Kerssies, 1990) consists of the following components (g/L distilled water): $NaNO_3$, 1.0; K_2PO_4, 1.2; $MgSO_47H_2O$, 0.2; KCl, 0.15; glucose, 20 agar, 25. The medium was sterilized for 20 min at 121°C. After

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cooling to 65°C, the following ingredients were added (g/L distilled water): Tetrachlor (PCNB, 75% WP), 15×10^{-3}; Maneb (manganese ethylene bisdithiocarbamate), 1.0×10^{-2}; chloramphenicol (antibiotic), 5×10^{-2}; $CuSO_4$, 2.2; Rubigan (fenarmol), 0.1 ml/L; tannic acid, 5.0. The pH of themedium was adjusted to 4.5 with 5.0N NaOH and was used to estimate conidia release. The medium was exposed to air above the pepper plant canopies for day (7:30 am-4:30 pm) and night (4:30 pm-7:30 am), immediately before and after initial stage of infection and at epidemic stage, followed by incubation at 20°C for 20 days. The number of colony on plates were counted.

Results

Because of rain on December 17, 1997, diurnal and nocturnal temperature and relative humidity in two greenhouses were about 25°C, 17°C, and 100%, respectively (data not shown). In greenhouse-2, nocturnal temperature maintained below 15°C for several days since December 17 and relative humidity remained higher than 90% from December 9 to 27. Under two different environmental conditions, the number of air-borne conidia as a primary inoculum source for pepper plants was estimated using the spore sampler that can suck air at 0.25 m^3 volume per min. Numbers of air-borne conidia were increased to about 10^4 conidia/0.5 m^3 in the greenhouse-2 on December 17 and 19, but population density of conidia in the greenhouse-1 was negligible in the same period and increased to 3.0×10^3 conidia/0.5m^3 on December 19 (Fig. 2A). The spore sampler was able to detect air-borne conidia in a relatively short time and improved detection limit by 100 folds compared to the conventional method of using Kerssies' selective medium (Table 1). This new equipment allowed only 1 hr to detect and enumerate conidia (Table 1).

When spore release was measured at height of 1 m from the ground, spores were mostly released from 7:30 am to 10:30 am and at 4:30 pm, but not at 2:30 pm and were released 1,000 fold more during daytime than nighttime (Fig. 1). Spores were released more in the greenhouse-2 than in the greenhouse-1 after December 17, which is coincided with disease development (Fig. 2). In greenhouse-2, initial infection of gray mold started as 0.33 according to

Table 1. Comparative spore montoring efficiency of spore sampling and Kerssies' selective media (KSM)

	Time to conidia trapping	Time to detect and enumerate conidia	Detection limit	Remarks
Spore sampler	2 min	1 hr	$\geq10^4$	New method
KSM	9 hr	20 days	$\geq10^6$	Conventional method

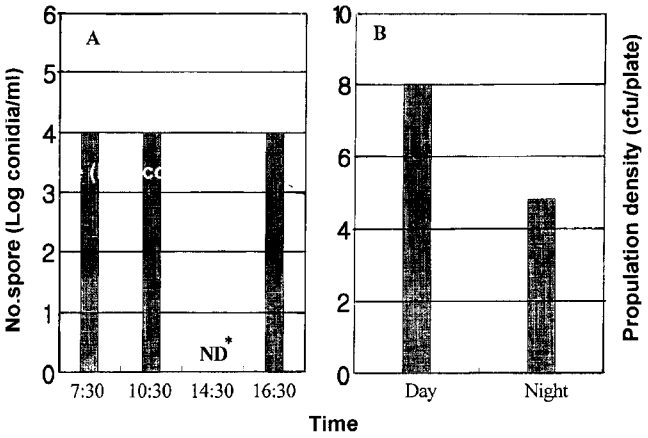

Fig. 1. Counts of air-borne spores released at indicated time by vacuum pump-facilitated trapping (A) and by exposing Kerssies' selective media (B) to air in greenhouse. Selective media were exposed to trap air-borne conidia for 9 h from 7:30 am to 4:30 pm (day) and 5 h from 4:30 pm to 7:30 am (night), followed by incubation at 20°C for 20 days. *; means not detected.

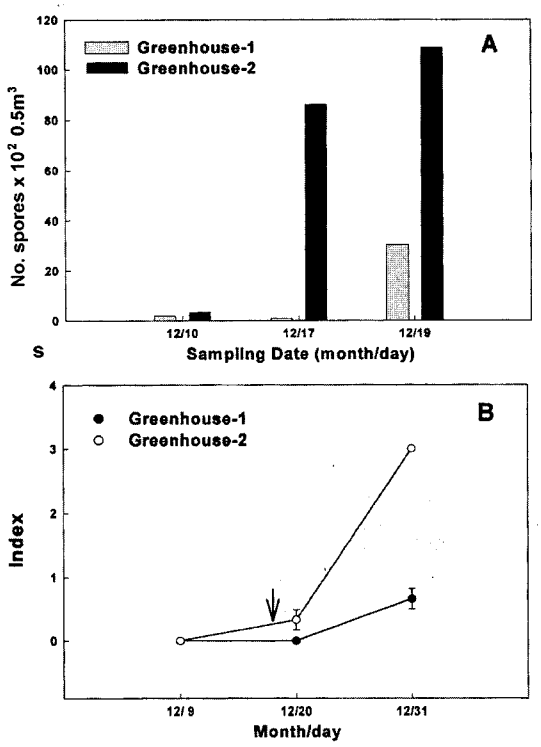

Fig. 2. Population density of air-borne conidia as a primary inoculum (A) and disease progress of gray mold in terms of *Botrytis* infection index (B) under two different experimental conditions. Arrow indicated the date from Dec. 17 to Dec. 19 when air-borne conidia was trapped in the greenhouse-2 opposed to the greenhouse-1. Temperatures were low and relative humidity was extremely high in both greenhouses at corresponding period, followed by temperature conditions dropped to below 15°C in the greenhouse-2 as opposed to greenhouse-1. Vertical bars indicate standard errors of the mean.

Table 2. *Botrytis* infection index[1]

	Petal	New shoot	Leaf	Green fruit	Shoot branch
Index	(%)	(No.)	(No.)	(No.)	(No.)
0	0-20				
1	21-30	0-5	0-5		
2	31-	6-10	6-10		
3	-30	11-20	11-20	0-2	0-2
4		20-	20-30	3-5	3-5
5				6-	6-

[1]Data, collected in all field experiments, included percent infection of petals and numbers of infection on new shoot, leaves, green fruits and shoot branches per 20 plants.

Botrytis infection index 3 days after rain, and disease incidence was rapidly increased because of low nocturnal temperature below 15°C for several days. However, only early symptom of gray mold rot was apparent on December 31 in the greenhouse-1 (Fig. 2B).

Discussion

We examined the threshold environmental conditions for the initiation of infection of gray mold in pepper greenhouse and developed a new efficient spore-trapping method for air-borne conidia.Spore release wasmostly observed from 7:30 am to 10:30 am and at 4:30 pm. In case of using a selective medium, spores were released greater during the day than during the night. Periodic release pattern was determined by spore trapping and KSM method. Conidia have been reported to be released during the morning hours when relative humidity is dropping, transported by air currents, and deposited on the onion leaf in the absence of free water (Lacy and Pontius, 1981; Lacy and Pontius, 1983). Conidium release is effected by a hygroscopic mechanism. Jarvis (1980) found that a basic circadian pattern of two periods of prolific conidium dispersal in the air, corresponded to two periods of rapidly changing relative humidity. The first occurrs from mid-morning until noon, typically a time of dew evaporation with the relative humidity falling from about 85% to 65%; the second does with dew fall or with the relative humidity rising rapidly again through the same range (Jarvis, 1980). Spore release episodes of downy mildew pathogens have been associated with decreasing humidity, rising temperature, and the evaporation of leaf wetness (Scherm and van Bruggen, 1995). Our results indicate that most of the inocula were detected during early morning or late afternoon when moisture and temperature are fluctuating. We determined conidia density as high as 10^4 conidia/0.5 m^3/2 min in the air to cause the initial infection of *B. cinerea* on greenhouse-grown pepper plants at spore release period. The spore sampler developed in this study is more efficient and requires less time to enumerate the air borne inocula, about 2 hr than KSM which took over 20 days of incubation for brown colonies to develop on the media. The recovery rate of KSM was relatively low: 510 colonies for 10^6 conidia. This information, together with that of environmental conditions, would provide us with optimal timing for control practices.

Defining specific environmental factors most critical to disease development could serve as the template for the development of a spray advisory or disease warning system (Gross et al., 1998). Dew deposition in the greenhouse is common on cool nights followed by warm and humid days, and the more so at high humidities with a large day-night temperature difference (Jarvis, 1980). Spores of *B. cinerea* require a film of water for at least 7 hr to germinate (Shitenberg and Elad, 1997). Disease tends to be more serious in certain areas of the greenhouse where adverse conditions such as poor drainage and roof drips occur (Jarvis, 1989). Therefore, we selected several places where disease occurs annually in the greenhouse.

Because the plants are protected from direct exposure to the rain in the greenhouse, the source of leaf wetness is not rain droplets, but is the condensation of dew instead. During rain events, growers avoid opening the vents, the RH within the greenhouse increases, and the temperature differences between air and leaves result in the formation of a film of water on plant organs (Shitenberg and Elad, 1997). Moreover, condensation of water on the inner side of the polyethylene covers drops on the plants, adding to foliage wetness.

We developed standard evaluation system referred to as the "Botrytis infection index" by which precise field data representing disease severity was obtained from the detailed and repeated observation on the etiology of Botrytis gray mold rot. Consequently, initial infection of the greenhouse-2 based on Botrytis infection index started faster than that of greenhouse-1. These critical factors were determined to be the threshold environmental conditions leading to predisposition of hosts that resulted in initiation of infection. Shoemaker and Lorbeer (1977) suggested that relative humidity close to 100% for 24 hr was necessary for the onion leaf spotting phase. Epidemics in strawberry are expected when the relative humidity remains higher than 90% and the temperature not less than 14°C (Jarvis, 1980). Bulit et al. (1970) qualified the valid infection period criteria by a count of airborne conidia or *B. cinerea*; a low concentration, of the order of 40/m^3 was not sufficient to initiate an epidemic despite suitable temperature and relative humidity conditions.

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