

An Infection Model of Apple White Rot Based on Conidial Germination and Appressorium Formation of *Botryosphaeria dothidea*

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Regression models for determining infection periods of apple white rot were developed based on conidial germination and appressorium formation of *Botryosphaeria dothidea*. A total of 120 apple fruits were inoculated with the fungal conidial suspension and subjected to 6 temperatures and 10 wetness periods. Conidia germinated and produced appressoria, exhibiting swollen tips of germ tubes on the fruit surface. Conidial germination (G) increased with temperature (T) and wetness period (W), and was described as $G = -89.273 + 7.649T + 7.056W - 0.109T^2 - 0.085W^2 - 0.066TW$ ($R^2=0.75$). Less than 2 hr of wetness period were enough for conidia to germinate at 25 to 30°C. Effects of temperature and wetness period on appressorium formation (A) could be explained as $A = -1.540 - 2.375W + 0.045W^2 + 0.213TW$ ($R^2=0.77$). The relationship between conidial germination and appressorium formation (A_g) was described as $A_g = 0.381 - 0.227G + 0.005G^2$ ($R^2=0.67$), suggesting that conidial germination may have to reach approximately 43.7% to initiate appressorium formation. Using the regression equation for conidial germination and the criterion of 43.7% conidial germination, an infection model was developed to determine infection periods based on temperature and wetness period. The infection model with the criterion of 43.7% conidial germination was apparently more conservative than the appressorium formation model in determining possibility of apple infection. The infection model seemed sensitive to variable weather conditions, suggesting possible use of the model for timing fungicide sprays to control white rot of apples in practice.

Keywords : apple, appressorium, *Botryosphaeria dothidea*, germination, white rot

Botryosphaeria dothidea Ces. & De Not. (synonymous with *B. ribis* Grossenb. & Dug.) is found in most tropical and warm temperate regions as a pathogen of many plants (McGlohon, 1982; Smith, 1934). The fungus belongs to

Ascomycota, and its anamorph is *Fusicoccum aesculi* Corda (Punithalingam and Holliday, 1973). It causes white rot, one of the most destructive apple (*Malus domestica* Borkh.) diseases in the world (Sutton, 1990). In Korea, the disease has been a serious problem since the introduction of Fuji, a highly susceptible cultivar to the disease, from Japan in early 1970's (Kim and Kim, 1989; Lee et al., 1993). The disease results in soft and light-colored rot on fruit, and extensive cankers on limbs, eventually resulting in tree death. The main inoculum of *B. dothidea* is conidia, which are present in orchards throughout the growing season of apple fruit (Kim et al., 1995; Sutton and Arauz, 1981). The fungus forms appressoria, that are directly involved in host penetration and adherence to host surfaces, on the fruit surface or enters fruit through natural openings such as lenticels and surface cracks (Kim et al., 1999). Apple fruit can be infected by conidia within 7 weeks after petal fall, and symptoms usually become noticeable 6 to 8 weeks before harvest (Parker and Sutton, 1993b). Ultrastructural aspects of the latent infections and fungal survival were reported (Kim et al., 2001, 2004).

The control of apple white rot relies mainly on chemical practices by periodic fungicide sprays (Sutton, 1990). Season-long benomyl spray at 2-week-intervals was known to be an effective measure for reducing the disease development (Brown and Britton, 1986; Sutton, 1990). Kohn and Hendrix (1983) suggested initiating preventive fungicide sprays when sugar content level reached 10.5% of apple fruits. However, improper timing of fungicide sprays can result in a considerable yield loss in spite of a full-season protection program. Timing of fungicide sprays can be improved through a better understanding of factors that influence infection processes of the pathogen. Both temperature and wetness period apparently play critical roles in the growth of fungal pathogens and development of various diseases (Arauz and Sutton, 1989; Broome et al., 1995; Jones, 1986; Michailides and Morgan, 1992; Yun and Park, 1990). In order to effectively manage the disease by proper timing of fungicide applications, it is crucial that the effects of temperature and wetness period on the fungal growth are quantitatively described. Then, such a predictive model can

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be used as part of a disease forecasting model (Arauz et al., 1990).

Several infection models have been developed to predict the development of apple white rot. A model made by Kohn and Hendrix (1983) was to predict fruit rot incidence using sugar content of apple fruits throughout the growing season. However, sugar content level fluctuates throughout the growing season, so that it was not assumed to be a reliable indicator of fruit susceptibility (Parker and Sutton, 1993b). Two disease models developed by Parker and Sutton (1993a) were derived from the effect of temperature and wetness period on symptom development on apple fruit pieces. In their study, one model was modified from the other by eliminating additional lesion quantity due to latent infections that occurred prior to fruit sampling in an orchard, which often cause overestimation of infections in artificial inoculation experiments. In order to make a model unaffected by latent infections, parameters involved in the fungal pre-penetration behavior can be employed for model development. Either conidial germination or appressorium formation might be suitable for this purpose, as they are crucial steps on host surface for disease development. Little information is available on the interaction of temperature and wetness period with regard to conidial germination and appressorium formation of *B. dothidea*. The objectives of this study were i) to determine effects of temperature and wetness period on conidial germination and appressorium formation of *B. dothidea* and ii) to develop an infection model for determining infection periods of apple white rot based on hourly temperature and wetness data. Sensitivity of the infection model to weather conditions was compared with a disease model previously developed by Parker and Sutton (1993a) using four sets of weather data obtained from different orchards.

Materials and Methods

Inoculum preparation. An isolate (BD-3) of *B. dothidea* was obtained from a naturally infected apple fruit exhibiting typical white rot symptoms. The isolate was grown on potato sucrose agar (PSA) slants at 25°C for 2 weeks. The culture surface on the PSA slants was flooded with 10 ml of sterilized distilled water and scraped to make a mycelial suspension. The suspension was spread evenly with a sterile bent-glass rod over the surface of freshly prepared acidified (0.2% lactic acid) PSA plates amended with streptomycin sulfate at 100 µg/ml to inhibit bacterial contamination. The fungus was then incubated at 27°C under continuous fluorescent light with a 12-hr photoperiod for 3 weeks. Pycnidia of the fungus were collected by flooding the plates with sterilized distilled water and scraping the surface of the culture plates. Conidia were

dislodged by disrupting pycnidia in sterilized distilled water with a mortar and pestle. The suspension was filtered through 2 layers of cheesecloth to remove mycelial fragments. Inoculum concentration of the conidial suspension was adjusted to 1.0×10^5 conidia/ml using a hemacytometer.

Inoculation of apple fruit. A total of 120 asymptomatic apple (cultivar Fuji) fruits at full maturity were carefully harvested to avoid bruising in an apple orchard at Anseong, Korea. The fruits were washed with mild detergent to remove dirt, insect honeydew, and pesticide residues. They were then surface-sterilized with 0.1% sodium hypochlorite solution for 2 min, rinsed with tap water, and allowed to air dry. The fruits were inoculated to runoff with the conidial suspension using a hand-held sprayer. Immediately after inoculation, the fruits were placed in a plastic tray (32 × 43 × 8 cm) lined with moist paper towels, and put into a polyethylene bag for maintaining wetness throughout treatment periods. They were placed in each of 6 incubators at 10, 15, 20, 25, 30, and 35°C. After the assigned 10 wetness periods of 2, 4, 8, 12, 16, 20, 24, 32, 40, and 48 hr at each temperature, 2 fruits were removed for each combination of temperature and wetness period treatments to examine conidial germination and appressorium formation on the fruit surface. The wetness period in this study was defined by the length of time that the inoculum droplet remained as free water on the fruit surface. The wetness treatments were conducted under continuous complete darkness at each temperature.

Specimen preparation for light microscopy. Ten squares of epidermal peels (each 1 × 1 cm with approximately 1 mm thickness of underlying tissue) were excised from the inoculated fruits using a sterile razor blade. The epidermal peels were dipped in EtOH-chloroform (75:25, v/v) mixture amended with trichloroacetic acid at 0.15% (w/v) to prevent further conidial germination and decolorize specimens (Wolf and Fric, 1981). The peels were then immersed in 10 ml of distilled water amended with 2 drops (approximately 50 µl) of 0.1% lactophenol-cotton blue, stained for 10 min, and examined using a light microscope (BH; Olympus Optical, Tokyo, Japan). A conidium was considered germinated if the germ tube was at least one-half the length of the conidium. Percent conidial germination was determined by examining 10 conidia selected randomly on each epidermal peel, a total of 200 conidia from 20 epidermal peels of 2 fruits for a combination of temperature and wetness period treatment. Percent appressorium formation was the number of appressorium-formed conidia out of the germinated conidia.

Model development and sensitivity test. Two multiple

regression models describing effects of temperature and wetness period on conidial germination and appressorium formation of *B. dothidea* on apple fruits were developed. The quantitative relationship between conidial germination and appressorium formation was also determined. The stepwise procedure was used to select the best set of independent variables using SAS PROC GLM (SAS Institute, Cary, NC). Criteria for selecting the best statistical model included the significance of the *F* value and coefficient of determination (R^2).

Regression models were tested for their sensitivity in response to four sets of weather data collected from apple orchards in 1993, 1994, 1996, and 2003. For comparison, a disease model which was previously developed by Parker and Sutton (1993a) based on the results of laboratorial experiments determining effects of temperature and wetness period on symptom appearance on apple fruit pieces was also evaluated for its sensitivity to the same weather data sets. The Parker and Sutton's disease model is expressed as follows:

$$D = -0.1546 + 0.0123T + 0.0329W - 0.00169W^2 + 0.0000225W^3 - 0.00153TW + 0.000111TW^2 - 0.00000151TW^3$$

where *D* is the percentages of diseased fruit pieces. *T* and *W* are hourly temperature (°C) and wetness period (hr), respectively.

Each weather data set for the model sensitivity tests consisted of hourly temperature and wetness period from June 1 to September 30. The 4-year microclimatic data were collected using automated weather stations installed in apple orchards at 4 different locations in Korea; i) Chonan in 1993, ii) Suwon in 1994, iii) Pyongtaek in 1996, and iv) Naju in 2003. The automated weather stations consisted of a datalogger (CR10; Campbell Scientific, USA), temperature (Model 107; Campbell Scientific, USA) and leaf wetness sensors (Model 237; Campbell Scientific, USA). In order to avoid overestimation of the infection risk, the simulation was performed to end infection periods by interrupted wetness period based on the results of *B. obtusa*, a closely related species of *B. dothidea*, causing apple black rot (Arauz and Sutton, 1990). The model sensitivity was evaluated in terms of the amount of variations in duration of daily infection periods (hr) over the test periods. As for the Parker and Sutton's model, the amount of variations in daily percentages of diseased pieces over the test periods was used as the indicator of model sensitivity.

Results

Conidial germination. Conidia of *B. dothidea* were fusoid and straight in shape. Conidia germinated and produced

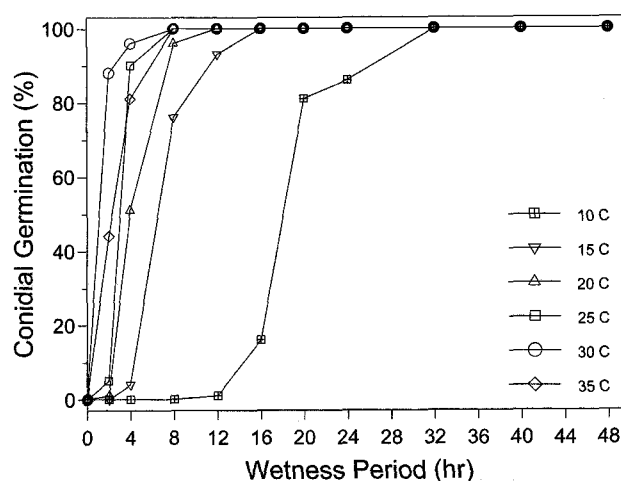


Fig. 1. Effects of temperature and wetness period on conidial germination of *B. dothidea* on apple fruits.

germ tubes from either one or both ends of conidia. There was active conidial germination even in short wetness periods on the fruit surface (Fig. 1). Approximately 31, 88, and 44% of conidia germinated after 2 hr at 25, 30, and 35°C, respectively. After 8 hr, all the inoculated conidia germinated at 25, 30, and 35°C. The optimum temperature for conidial germination of *B. dothidea* ranged from 25 to 30°C. Lower temperatures required longer wetness periods for conidial germination. Only 17% of conidia germinated at 10°C even after 16 hr of wetness period.

Appressorium formation. Conidial germ tubes grew on the fruit surface and formed appressoria, exhibiting swollen tips of germ tubes. Percent appressorium formation increased with temperature up to 30°C as in conidial germination (Fig. 2). Appressorium formation required longer than 2 hr

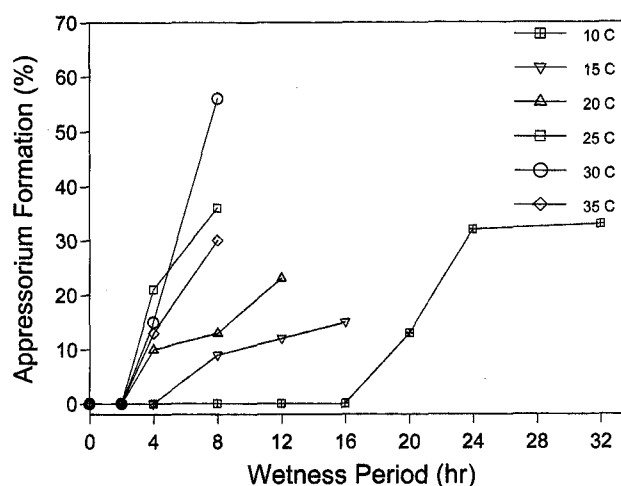


Fig. 2. Effects of temperature and wetness period on appressorium formation of *B. dothidea* on apple fruits.

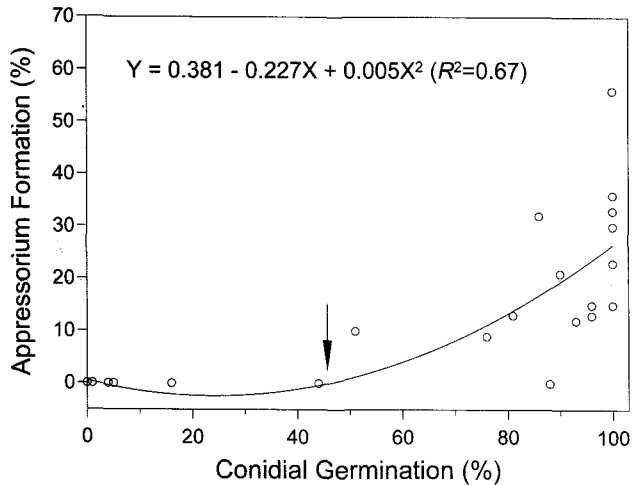


Fig. 3. Relationship between conidial germination and appressorium formation of *B. dothidea*. Appressorium formation was initiated when conidial germination reached approximately 43.7% (an arrow) at a given temperature and wetness period.

of wetness period even at 25°C. After 8 hr, 36, 56, and 30% of germinated conidia produced appressoria at 25, 30, and 35°C, respectively. Longer than 16 hr of continuous wetness were required to produce appressoria at 10°C. The optimum temperature for the fungal appressorium formation ranged from 25 to 30°C. As appressoria usually formed at the tips of germ tubes grown long from a conidium, some appressoria could not be determined from which conidia they had originated. Consequently, percent appressorium formation could not be determined after 8 hr of wetness at 25, 30, and 35°C, 12 hr at 20°C, and 16 hr at 15°C.

Relationship between conidial germination and appressorium formation. Appressorium formation increased as conidial germination increased under continuous wetness periods (Fig. 3). The relationship between conidial germination (G) and appressorium formation (A_g) could be described by the following regression model:

$$A_g = 0.381 - 0.227G + 0.005G^2 \quad (1)$$

The coefficient of determination of Eq. (1) was 0.67, indicating that 67% of variations in percentage of appressorium formation could be accounted for by conidial germinations. All estimates of parameters were statistically significant ($P=0.01$). Until conidial germination reached approximately 43.7% at a given temperature and wetness period, appressorium formation was hardly observed. However thereafter, gradual increase of appressorium formation was evident on germinated conidia.

Model development and sensitivity test. The combined

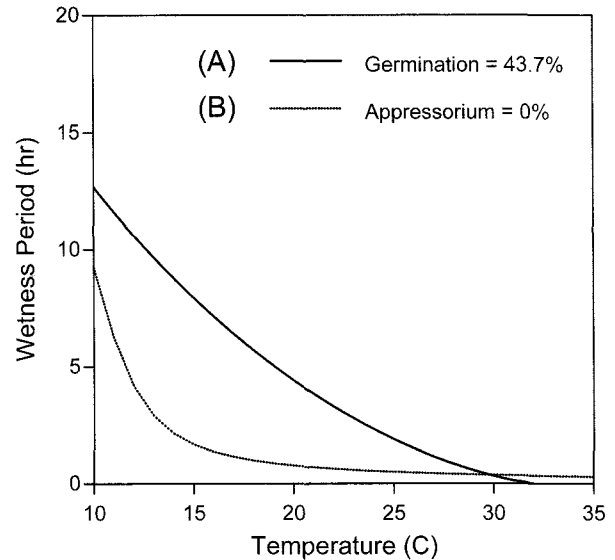


Fig. 4. Critical temperature and wetness period conditions required for (A) 43.7% conidial germination and (B) appressorium formation of *B. dothidea*.

effects of temperature (T) and wetness period (W) on percent conidial germination (G) and appressorium formation (A) could be described by the following regression models:

$$G = -89.273 + 7.649T + 7.056W - 0.109T^2 - 0.085W^2 - 0.066TW \quad (2)$$

$$A = -1.540 - 2.375W + 0.045W^2 + 0.213TW \quad (3)$$

The coefficients of determination of Eqs. (2) and (3) were 0.75 and 0.77, respectively, indicating that 75% and 77% of variations in percentages of conidial germination and appressorium formation, respectively, could be accounted for by temperature and wetness period. All estimates of parameters were statistically significant ($P=0.01$).

From Eqs. (2) and (3), wetness periods required for 43.7% conidia to germinate and appressoria to form at certain temperatures were calculated by substituting G in Eq. (2) and A in Eq. (3) with 43.7 and 0, respectively (Fig. 4). According to the relationship between conidial germination and appressorium formation in Fig. 3, appressorium formation was hardly initiated on the surface of apple fruits until conidial germination reached approximately 43.7% at a given temperature. Therefore, both graphs in Fig. 4 determine the temperature and wetness period conditions under which *B. dothidea* could form appressoria to infect apple fruits. Eq. (2) with the criterion of 43.7% conidial germination was apparently more conservative than Eq. (3) in determining possibility of apple infection. In this study, we decided to use Eq. (2) with the criterion of 43.7% conidial germination as the model to determine infection

Table 1. Comparison of sensitivity of two infection models of apple white rot using four sets of weather data obtained from different orchards

Location	Year	Variance of daily model output ^a	
		Infection model ^b	Parker and Sutton's disease model ^c
Chonan	1993	86.83	49.94
Suwon	1994	38.44	34.74
Pyongtaek	1996	90.69	68.71
Naju	2003	119.46	80.98

^a Variances of daily output values generated by the models using daily temperature and wetness period data from June 1 to September 30.

^b The infection model developed in this study is based on the effects of temperature and wetness period on conidial germination with 43.7% conidial germination as the appressorium formation. Data are variances of daily infection periods (hr) determined by the model.

^c A disease model previously developed by Parker and Sutton (1993a). Data are variances of daily percent disease fruit pieces determined by the model.

periods of the fungus. For clarity in the text, this model is called 'the infection model' hereafter.

Using four weather data sets collected from apple orchards, the daily infection periods in hours and the percentage of daily diseased fruit pieces were calculated from the infection model and the Parker and Sutton's disease model, respectively. Since two models generate outputs in different units, their sensitivity in response to variable weather conditions was compared in terms of variances of daily outputs from the models (Table 1). The variance of daily infection periods determined by the infection model developed in this study was always greater than that of daily diseased fruit pieces determined by the disease model developed by Parker and Sutton (1993a), indicating that the infection model responded more sensitively to weather conditions than the Parker and Sutton's disease model.

Discussion

This study demonstrated that temperature and wetness period directly affected conidial germination and appressorium formation of *B. dothidea*. Both conidial germination and appressorium formation increased at high temperatures (25 to 30°C) within less than two hours of wetting on the surface of apple fruits. Furthermore, *B. dothidea* produces conidia over the 6 to 30°C range with a maximum sporulation at 18 to 24°C (Copes and Hendrix, 2004) and the conidia are primarily discharged by rainwater in summer months (Kim et al., 1995; Sutton and Arauz, 1981). These previous observations and the results from this study suggest that either fungal penetration into host tissues or entrance through natural openings on apple fruits can occur

easily during the apple growing seasons in Korea. The responses of conidial germination to temperature in this study are consistent with those obtained by Sutton and Arauz (1991) who found the optimum temperature for conidial germination ranged from 26.7 to 29.5°C.

Regression models for conidial germination and appressorium formation responding to temperature and wetness period were developed to provide information on possible infection by *B. dothidea* for scheduling fungicide sprays. The infection model developed in this study was derived from the effects of temperature and wetness period on conidial germination on apple fruits, and the quantitative relationship between conidial germination and appressorium formation. Consequently the daily infection period determined by the infection model was the time period in hours being continuously wet after 43.7% of conidia on the surface of apple fruit germinated in a day.

In comparison with the Parker and Sutton's disease model, the infection model in this study appeared to be relatively sensitive to weather conditions in apple orchards. Although the infection model has not yet been tested for its validity in predicting fungal infections in apple orchards, the relatively high sensitivity of the model in response to variable weather conditions would probably suggest its effectiveness or better performance than the Parker and Sutton's disease model in identifying favorable weather conditions for fungal infection. The small variance of daily infection periods at Suwon in 1994 was attributed to the relatively unfavorable weather conditions due to less frequent rainfall events over the period as compared with other years (data not shown).

The infection model in this study was developed based on the experimental results obtained under continuous wetting conditions. However, interruptions of wetness periods often occurs in cases of nocturnal wetting and diurnal drying cycles, and intermittent rain showers. Therefore, fungal spores and germ tubes should be exposed to alternating wet and dry periods before host infection. It remains to be revealed whether interrupted wetness periods are detrimental to the growth of *B. dothidea* or not. In order to be widely practiced in apple disease management, the infection model should be evaluated for its effectiveness in scheduling fungicide sprays in apple orchards. Comparison of season-long disease progress under predicted and conventional fungicide spray programs would provide information on the improvement of current management strategy of the disease.

Acknowledgments

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