SHORT COMMUNICATION

EFFECT OF LEAF WETNESS DURATION, TEMPERATURE AND INOCULUM CONCENTRATION ON INFECTION OF EVERGREEN AZALEA BY *COLLETOTRICHUM ACUTATUM*, THE CAUSAL AGENT OF ANTHRACNOSE

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SUMMARY

Colletotrichum acutatum is the causal agent of anthracnose of azalea (*Rhododendron azalea*). This disease, first observed in Italy in 2002 on cv. Palestrina in nurseries located in the Verbano-Cusio-Ossola province of Piedmont (northern Italy), causes serious losses. Although most azalea cultivars grown are resistant to the disease, some of the most popular are very susceptible. In order to understand the effect of environmental parameters on anthracnose, the effect of inoculum density, leaf wetness duration and temperature on the development of the disease was studied. Conidial concentration of 10^5 and 10^6 produced the most severe symptoms. The disease was severe at 15°C and 20°C, and severity decreased at temperatures outside this range. At 20°C the pathogen required at least 24 h of leaf wetness to develop significant symptoms, whereas at 15°C it needed extended periods; with 48 h of leaf wetness the pathogen produced 85 to 100% infection of leaf surface. No symptoms developed at 5°C or 30°C. Knowledge of the factors favourable to disease development could help improve management tactics, based on the control of environmental factors.

Key words: Rhododendron, disease epidemiology, disease management.

Rhododendron species are popular ornamentals that include both rhododendrons and azaleas. In Italy, they are widely grown in Piedmont, in the Lake Maggiore area. Azalea (*Rhododendron azalea*) is the most popular species grown, accounting for 50% of the total production (Rabbogliatti, 2004).

Azaleas are susceptible to several foliar diseases (Benson and Williams-Woodward, 2001), including anthracnose, resulting in leaf spots and defoliation, with severe financial losses (Coyer and Roane, 1986). *Colletotrichum acutatum,* isolated in 2002 from plants of cv Palestrina, was identified as the causal agent of anthracnose on azalea in Italy. The disease was observed in several nurseries in the Lake Maggiore area (Garibaldi *et al.*, 2004).

Anthracnose on azalea was reported for the first time in 1895 in Florida on swamp azalea (*Rhododendron viscosum*) and the causal agent was identified as *C. azaleae* (Ellis and Everhart, 1895). Since 1954, anthracnose on azalea has been observed and described in Louisiana, where the causal agent was identified as the conidial stage of *Glomerella cingulata*, the teleomorph of *C. gloeosporioides*. *C. azaleae* is now considered synonymous with *C. gloeosporioides* (Farr *et al.*, 1989; Von Arx, 1957). Severe outbreaks of anthracnose have been reported on *Rhododendron* species in Sweden and Latvia and the causal agent of the disease was identified as *C. acutatum*, although in some cases *C. dematium* was isolated from infected plants. Both species reproduced symptoms of the disease in pathogenicity tests (Vinnere *et al.*, 2002).

After the first appearance of anthracnose in 2002, severe outbreaks were observed on several cultivars of azalea grown in nurseries in the area where the disease was first observed, causing severe economic losses. Although many of the azalea cultivars grown in Italy are resistant or partially resistant to anthracnose, some of the most popular are susceptible to the disease (Bertetti *et al.*, 2007). *C. acutatum* can reproduce on many different types of tissues on various hosts and can exist as necrotroph, biotroph, endophyte or epiphyte, depending on plant species and tissues involved (Peres *et al.*, 2005). In ornamental production areas, it might affect several hosts. However, few investigations of cross pathogenicity and host range of *C. acutatum* isolates in nature have been conducted (Peres *et al.*, 2005). Temperature and leaf wetness duration affect infection and development of anthracnose diseases of other crops, such as cucumber (Thompson and Jenkins, 1985), bean (Tu, 1992), lentil (Chongo and Bernier, 2000) and strawberry (Wilson *et al.*, 1990; Howard *et al.*, 1992). There is no information available on the effect of such parameters on anthracnose of azalea. Studies on the effects of environmental factors on anthracnose of azalea could provide a better understanding of the disease and lead to improved disease management.

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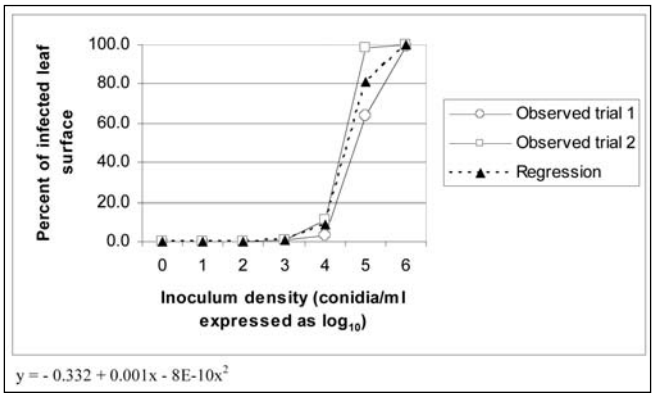

Fig. 1. Effect of inoculum density on severity of anthracnose caused by *Colletotrichum acutatum* on *Rhododendron azalea* cv. Palestrina (trials 1 and 2). Data from these experiments showed similar variances with the Levene test, so conidial concentration and disease severity can be related using a single equation obtained by regression analysis and shown at the base of the graph.

The present study was undertaken to understand the effect of inoculum density, leaf wetness duration, and temperature on the development of anthracnose on azalea under controlled conditions.

From April 2006 to May 2009, six trials were carried out in our laboratory under the conditions reported in Fig. 1-3. The effect of inoculum density, leaf wetness and temperature on disease severity was evaluated in two tests each. Evergreen azaleas (*Azalea japonica* cv. Palestrina), which are very susceptible to anthracnose (Bertetti *et al.*, 2007), were supplied by Tecnoverde S.r.l. (Verbania – Fondotoce, Italy) and used throughout our study. Three weeks before the tests, three-month-old rooted cuttings grown in 10 cm diameter plastic pots were pruned, fertilized and raised in a glasshouse at 18-23°C so that most of inoculated leaves were 21-day-old. The container substrate used was peat moss: perlite (9:1 vol/vol). During the trials, plants were maintained in a growth chamber and arranged randomly in complete block design with three single-plant replications. Each trial was conducted twice.

C. acutatum inoculum consisted of a conidial suspension of an isolate of the pathogen obtained from infected azalea cv. Palestrina, prepared in distilled water, just before inoculation, from 20-d-old cultures grown on potato dextrose agar (PDA) maintained at 20±1°C. Five ml of the spore suspension were applied using a manual sprayer to inoculate each plant thoroughly, covering all the leaves. Control plants were sprayed with distilled water. All plants were maintained under moist conditions

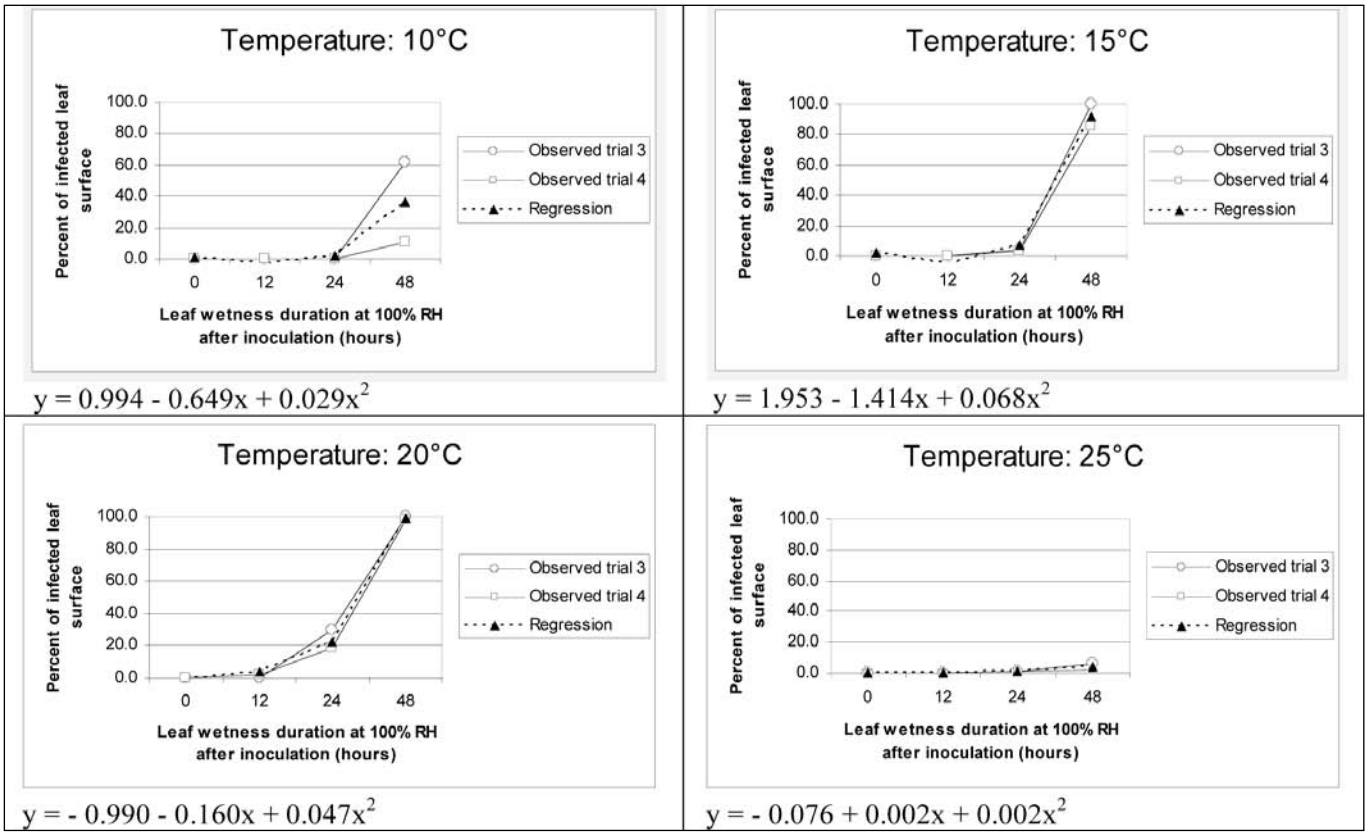

Fig. 2. Effect of leaf wetness duration on severity of anthracnose caused by *Colletotrichum acutatum* on *Rhododendron azalea* cv. Palestrina (trials 3 and 4). For each temperature tested, data obtained showed similar variances with the Levene test. Thus leaf wetness duration and severity can be related by using a single equation obtained by regression analysis as shown at the base of each graph. There were no symptoms on inoculated plants maintained at 5 and 30°C.

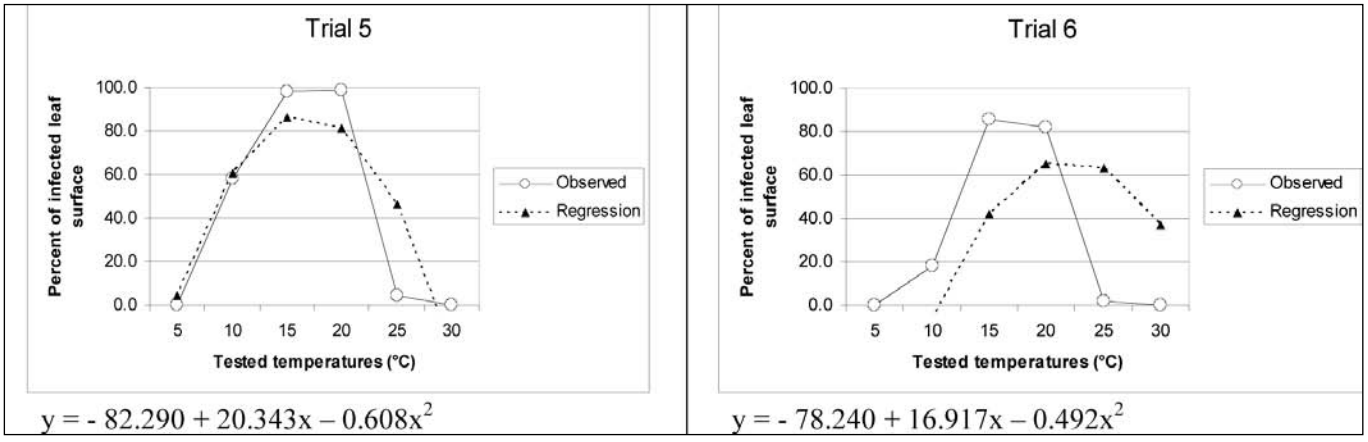

Fig. 3. Effect of different temperatures on severity of anthracnose caused by *Colletotrichum acutatum* on *Rhododendron azalea* cv. Palestrina (trials 5 and 6). Temperatures and severity were related by the regression analysis equations obtained and shown at the base of the graphs.

after inoculation, as indicated below. Relative humidity and air temperature were measured by a data logger (Fourier Systems Inc., USA) placed among the plants.

The effect of inoculum density was investigated by inoculating plants with suspensions containing 10^1, 10^2, 10^3, 10^4, 10^5 and 10^6 conidia/ml. Inoculated plants were placed in growth chamber at 20°C with a 12 h/d fluorescent light regime (Fig. 1). Immediately after inoculation, plants were covered with a moist plastic bag to maintain a saturated atmosphere for 48 h.

The effect of leaf wetness duration was investigated by inoculating plants with a suspension of 10^6 conidia/ml. Inoculated plants were incubated with continuous 100% RH obtained by covering the plants with moist plastic bags for 0, 12, 24 and 48 h in growth chambers at 5, 10, 15, 20, 25 and 30°C, with a 12 h/d fluorescent light regime (Fig. 2).

The effect of different temperatures was investigated by inoculating plants with a suspension of 10^6 conidia/ml. Inoculated plants were maintained with continuous 100% RH for 48 h in growth chambers at 5, 10, 15, 20, 25 and 30°C (Fig. 3).

Disease development was evaluated daily starting at the first appearance of symptoms. The percent of infected leaf surface was evaluated on 40 leaves arbitrarily selected per plant, considering only leaves that were about 25-30-day-old. The data were presented graphically and subjected to regression analysis (SPSS for Windows, 17.0 version, SPSS Inc., USA). Equations were developed to relate inoculum density, leaf wetness and temperature to disease severity (Fig. 1, 2 and 3). Data obtained from trials 1 and 2 showed similar variances with the Levene test. Data are presented in one graph with a single equation (Fig. 1). The same method was adopted for trials 3 and 4 (Fig. 2).

This study confirmed that the azalea cv. Palestrina was highly susceptible to *C. acutatum*. The inoculation method used produced consistent symptoms, which started to develop 2 days after the inoculation at 15 and 20°C, 3 days and 5 days after inoculation at 25°C and 10°C, respectively. Non-linear regression analysis of the data showed that the best fit was obtained with a 2nd order polynomial curve.

Regarding the effect of inoculum density, a conidial concentration of at least 10^4 conidia/ml was needed to cause infection. Concentrations of 10^5 and 10^6 conidia/ml caused the most severe infection (Fig. 1).

At 20°C, the pathogen needed at least 24 h of leaf wetness to cause significant infection, whereas at 10, 15 and 25°C, it needed extended periods (Fig. 2). With 48 h of leaf wetness at 15 and 20°C, the most severe disease was observed, with 85 to 100% of the leaf surface infected (Fig. 2).

Temperatures of 15 and 20°C provided the most favourable conditions for infection. At 10°C, disease severity was intermediate. At 25°C, the disease developed poorly (Fig. 3). No symptoms were observed on inoculated plants maintained at 5°C or 30°C.

Thus the development of anthracnose on azalea can be influenced by inoculum concentration, duration of wetness period, and temperature. Previous studies indicated that host genotype affected susceptibility (Bertetti *et al.*, 2007). Inoculated azalea plants given 12 h of wetness showed no or very limited symptoms, even at the most favourable temperatures.

Anthracnose was most severe at temperatures of 15°C and 20°C, and severity decreased significantly at 10 and 25°C. These results are in line with the observations of the local extension workers, who reported anthracnose occurrence during the spring and autumn.

In recent years, anthracnose on azalea in Italy has caused severe financial losses to the industry in the Lake Maggiore area, where production is concentrated. Although the majority of azalea cultivars are at least par-

tially resistant to *C. acutatum*, those such as 'Palestrina', which are very susceptible are still widely grown. Our results provide some practical guidelines for growers. Best cultural practice consists in keeping wetness duration lower than 12 h when temperatures exceed 15°C. The information obtained in this study should help to develop a more rational control of anthracnose when conditions favour disease development and may help to limit the use of chemical treatments.

In northern Italian conditions, particularly with younger, more susceptible plants, fungicides should be applied during spring and autumn, when rain is frequent and temperatures are favourable. At least partially resistant cultivars should also be selected.

Results of this study, along with work on the same pathogen in other crops, such as strawberry (King *et al*., 1997; Madden *et al*., 1992) may explain the increasing importance of *C. acutatum* in northern areas, due its ability to grow at lower temperatures and its short latent period.

ACKNOWLEDGEMENTS

We thank Mattia Sanna for statistical advice. Research supported by a grant from Regione Piemonte. We also thank the anonymous referees for their valuable suggestions.

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Received January 21, 2009
Accepted July 30, 2009