Effect of Temperature and Wetness on Infection of Black Raspberry by Aeciospores of *Arthuriomyces peckianus*

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ABSTRACT

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The optimum temperature range and leaf wetness duration for germination and infection of black raspberry by aeciospores of *Arthuriomyces peckianus* were determined experimentally. The germination of aeciospores on water agar increased with temperature from 12 to 28°C. Maximum germination of aeciospores occurred in free water and declined as relative humidity was reduced below 100%. The effect of temperature on the ability of aeciospores to infect leaf disks of black raspberry was described by the model $y = -171.688 + 21.609T - 0.544T^2$, where y = percent successful infection of aeciospores and T = temperature (C). The predicted optimum temperature for infection was 19.9°C. The optimum duration of leaf wetness for infection was 10 h at 22°C, but infection could occur in as little as 6 h at 22°C.

Orange rust of black raspberry (*Rubus occidentalis* L.), caused by the demicyclic rust *Arthuriomyces peckianus* (E. Howe) Cummins & Y. Hiratsuka (4), commonly occurs in the eastern United States (2,5,8). The disease is named for the large orange aecia present on infected plants in the spring. Infections by aecia are local (14), resulting in small, inconspicuous clusters of teliospores on the underside of the leaf. Teliospores are produced in the fall, but the time of year when basidiospores infect the plant has not been determined. Basidiospore infections are systemic (6), and cause the most damage, resulting in plants that do not produce fruit (7). Since the time of year when basidiospore infection occurs is unknown, aeciospore infections in the spring are the primary candidates for control. Control of aeciospore infections should decrease production of teliospores and reduce the number of systemic infections. Pady (14) described infection by aeciospores, and although the environmental conditions necessary for infections were not determined, he reported that aeciospores germinated from 6 to 30°C.

Currently, no fungicides are registered for use in the United States for the control of orange rust. However, Kleiner (9) reported that protectant and eradicant fungicides were effective against aeciospore infections. In order to effectively time applications of these fungicides, knowledge of the infection requirements of aeciospores is necessary. Therefore, the objective of this study was to determine the optimum conditions for germination of aeciospores and infection of black raspberry by aeciospores of *A. peckianus*.

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MATERIALS AND METHODS

Plant material. All infection experiments used leaflets of the black raspberry cultivar Bristol (Nourse Farms, South Deerfield, Mass.), taken from plants produced in a greenhouse. Leaf material was collected from mature leaves one to three nodes from the bottom of a cane. Dodge (6) reported that only older, fully expanded leaves were susceptible to infection by aeciospores of *A. peckianus*.

Inoculum. Aeciospores were collected exclusively from naturally infected *R. occidentalis* plants to avoid collecting *Gymnoconia nitens* (Schwein.) F. Kern & H. W. Thurston, a microcyclic rust related to *A. peckianus*. The host ranges of both rusts are similar, but *R. occidentalis* is host only to *A. peckianus* (5). Plants located at Walnut Springs park in State College, Pa., served as a source for aeciospores. Aeciospores were collected from 27 May to 7 July 1991, using a cyclone spore collector (16). Aeciospores not used immediately were stored at 4°C in a cold room. Stored aeciospores were re-hydrated before use at approximately 22°C for 24 h in a 100 × 15 mm petri dish that contained 10 ml of sterile distilled water.

The effect of temperature on germination. Freshly collected aeciospores were dusted onto the surface of 1.5% water agar. Dishes were flooded with water and incubated at constant temperatures ranging from 12 to 28°C at 2°C increments. The percentage of aeciospores germinating in each dish was determined by observing 100 aeciospores per dish in random fields of a microscope (×200) after the dishes incubated 24 h in the dark. No additional germination was observed after 24 h in preliminary experiments. A germ tube more than half the length of the aeciospore was considered germinated (1). If more than 100 aeciospores were present in a field, all aeciospores were scored to ensure against bias. Treatments were arranged in a completely randomized design with four separate petri dishes representing experimental units. The experiment was repeated once using stored aeciospores.

The effect of relative humidity on germination. Relative humidity (RH) chambers were prepared by amending NaCl with 2% water agar in concentrations of 0, 0.6, 1.1, 2.2, and 3.3 *m*, which established humidity levels of 100, 98, 96, 92, and 88%, respectively, at 22°C (1,10). The dishes equilibrated for 15 h before use in an experiment. Four coverslips dusted with freshly collected aeciospores were placed in each humidity chamber and incubated for 24 h in the dark. Germination at the different levels of RH was compared with that of aeciospores dusted onto the surface of 1.5% water agar and flooded with water for the same incubation period. The germination percentage of each treatment was scored as described above. The treatments were arranged in a completely random design with four humidity chambers as experimental units. Each coverslip inside the humidity chamber was considered a subsample. The experiment was repeated once.

The effect of temperature on infection. Raspberry leaf tissue was excised with a 12-mm-diameter cork borer from a fully expanded leaf taken one to three nodes from the bottom of a cane. Five disks were placed onto water-saturated filter paper in an inverted petri dish, and inoculated by dusting freshly collected aeciospores onto the surface of the leaf disk with a camel-hair brush. A 40-µL drop of water was placed in the center of each disk, and the petri dish was sealed with Parafilm (American National Can, Greenwich, Conn.). The inoculated disks were incubated at constant temperatures ranging from 12 to 28°C in 2°C increments. After 30 h in the dark, leaf disks were cleared and stained as described by Bruzzese and Hasan (3) for examination with a light microscope.

The number of successful infections was determined by observing at least 50 aeciospores using random fields of a microscope (×200) where the drop of water was

placed. If more than 50 aeciospores were in the field of view, all aeciospores were counted to ensure against bias. Infections were considered successful when mycelium was present in the epidermal cell below an appressorium. Treatments were arranged in a randomized complete block design with two replications within the blocks. The replications consisted of dishes containing five leaf disks. Each leaf disk was one subsample. The blocks consisted of dishes containing disks taken from different aged leaves since the area of a single leaf was insufficient for the necessary number of subsamples. The experiment was repeated once using stored aeciospores.

The optimum wetting period for infection. Four leaf disks were inoculated as described above and placed in petri dishes. Leaf disks were subjected to 6, 8, 10, 12, and 30 h wetting durations in the dark at 22°C. After the wetting duration for each treatment, the drop of water was broken on the disks, and disks were allowed to air dry for 15 min. The dish was re-sealed and allowed to incubate for a total of 30 h at 22°C to allow sufficient observable mycelium to develop in epidermal cells. After incubation the leaf tissue was cleared and stained (3). The percentage of infection was determined as in previous germination experiments. The amount of infection in the 30-h treatment was compared with that in the shorter treatment periods because the maximum number of infections had occurred by that time. Only aeciospores previously stored at 4°C were used in the experiment since fresh aeciospores were no longer available. Treatments were arranged in a completely random design with four replications. Each petri dish was an experimental unit, and the leaf disks were subsamples. The experiment was performed twice.

Analysis of variance and regression analysis was done using SuperANOVA statistical software (Abacus Concepts, Inc., Berkeley, Calif.). Mean separation was done using Tukey's honestly significant difference test (15). Data from germination experiments were subjected to an arcsine square root transformation in order to stabilize the variance.

RESULTS

The effect of temperature on germination. Aeciospore germination increased linearly with temperature (Fig. 1). Germ tubes were more than 10 times the length of the aeciospore. Aeciospores that germinated on water agar did not produce appressoria. The germination increase in response to temperature was greater with freshly collected aeciospores than with stored ones. The R^2_{adj} for the regression of germination against temperature for fresh and stored aeciospores was 0.446 (P = 0.0001) and 0.213 (P = 0.005), respectively.

The effect of relative humidity on germination. Germination of aeciospores of *A. peckianus* was significantly less at ≤98% RH (Table 1). Germ tubes observed in free water were more than 10 times the length of the aeciospore, while germ tubes in the RH treatments were less than five times the length of the aeciospore. At 92 and 88% RH, some of the aeciospores appeared to be desiccated.

The effect of temperature on infection. The most common method of infection observed was the formation of an appressorium adjacent to the aeciospore. The germ tube was short and often unobservable. Long germ tubes were uncommon but could be found in all samples. At 28°C most of the germ tubes and appressoria were long and distorted, similar in appearance to germ tubes of aeciospores sown on water agar.

Infection at 28°C was rare with either fresh aeciospores or aeciospores stored at 4°C. Because variance for both experiments was found to be similar using Bartlett's test at χ^2 of 0.95, data were combined prior to regression analysis.

The relationship between temperature and the amount of successful infection was described by the equation $y = -171.688 + 21.609T - 0.544T^2$ (R^2_{adj} = 0.73, P = 0.001) where y = percent successful infection of aeciospores and T = temperature (C) (Fig. 2). The standard errors for the parameters are as follows: B_0 = 10.034, B_1 = 1.050, and B_2 = 0.026. The predicted optimum temperature for aeciospore infection was 19.9°C.

The optimum wetting period duration for infection. Successful infections occurred with as little as 6 h of wetness; however, the greatest number of infections occurred with 10 or more hours of wetting (Fig. 3).

In both leaf disk infection experiments, an unidentified fungus contaminated some samples by attaching to the aeciospores and inhibiting germination. These samples were subsequently removed from the experiments. The contaminant was found to be present with aeciospores collected from wild *R. occidentalis* plants.

DISCUSSION

Free water was more important than temperature for successful infection by *A. peckianus*, because aeciospores are not limited to a narrow range of temperatures for germination and infection. Dew of more than 6 h in duration may be sufficient to result in infection. Aecia occur in late spring when dews and rainy weather produce wetness periods adequate for infection to occur.

Because wetting period duration is the limiting factor in aeciospore infections, control measures such as pruning and trellising may be beneficial. A trellising system that spreads the canes may increase air circulation and promote more rapid drying, shortening the wetting period and the potential for infection. This control measure may prove most effective when dew or light rains trigger an infection period.

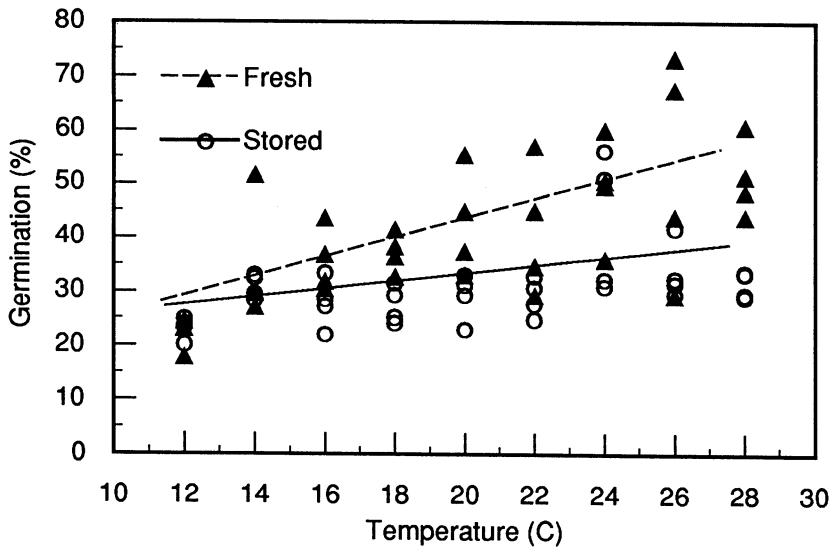

Fig. 1. The effect of temperature on the percentage of germination of both stored and freshly collected aeciospores of *Arthuriomyces peckianus*.

Table 1. The effect of relative humidity on the percentage of germination of aeciospores of *Arthuriomyces peckianus* after 24 h

Treatment	Percent germination
Free water	35.2 a[z]
100% relative humidity	11.8 b
98% relative humidity	0.5 c
96% relative humidity	1.0 c
92% relative humidity	0.0 c
88% relative humidity	0.3 c

[z] Means followed by the same letter are not significantly different according to Tukey's honestly significant difference test (P = 0.05). Mean separation was performed on transformed data.

The wetting period experiments used aeciospores stored at 4°C, which exhibited lower germination than freshly collected aeciospores. If the experiment were to be repeated with fresh aeciospores, the optimum wetting period may be less than 10 h. Germination and infection by aeciospores stored at 4°C may have been reduced due to dehydration. Stored aeciospores functioned better when hydrated for 24 h before use.

Low temperature does not appear to have a detrimental effect on *A. peckianus*, as the fungus germinated readily at 12°C. Fewer infections were observed at 12°C; however, more might have been observed if the wetting period had been longer than 30 h. Many appressoria had emerged at 12°C, but had not penetrated the epidermal cells.

The optimum temperatures (16 to 23°C) for infection of raspberry leaf disks are temperatures that are common in central Pennsylvania during May and June when *A. peckianus* releases aeciospores (Table 2). We hypothesize that the sequence of events for aeciospore infection begins during a wetting period with germination of aeciospores and formation of appressoria at a lower night temperature and penetration of epidermal cells as the temperature rises. By the time the temperature rises above 26°C, colonization by the fungus may have begun.

The percentage of germination of aeciospores in all experiments was lower than previously reported (9). The maximum percentage of germination observed in this study was 73.3%. The quality of the inoculum collected may have been poor, due to high temperatures during production of aecia of *A. peckianus* in May and June 1991 (Table 2). We observed that aecia commonly aborted in wild black raspberries. Often, aeciospores could be collected only in the morning. This was not experienced while collecting aeciospores for preliminary experiments in 1990, when temperatures were not unusually high. In 1990, aeciospore collection was possible in both the morning and evening. The collection technique also may have contributed to the poor germination. The vacuuming technique may have collected both mature and immature aeciospores, reducing the number of viable aeciospores from the collected total.

Table 2. The daily average temperatures (C) for State College, Pa., for May and June, 1989 to 1991 (11,12,13)

Date	Average minimum	Average maximum	Average for the month
May 1989	7.6	18.9	13.3
May 1990	8.2	18.4	13.3
May 1991	12.1	24.8	18.5
June 1989	13.6	25.3	19.4
June 1990	13.8	25.4	19.6
June 1991	14.5	27.2	20.8

Control of both aeciospore and basidiospore infections is necessary to control orange rust in the field. Correct decisions for control of aeciospore infections may now be feasible. Use of environmental monitoring could identify infection periods based on data presented in this study, allowing proper timing of eradicant fungicide applications. Control of aeciospore infections would then be possible following registration of eradicant fungicides identified by Kleiner (9). Infections by aeciospores are local, occur during raspberry flowering, and are unlikely to have any effect on fruit production the year of infection. Basidiospore infections are systemic, resulting in the commonly observed symptoms of orange rust. The time of year when basidiospores infect the host has not been identified. Limiting aeciospore infections may reduce the number of telia produced, and provide easier control of basidiospore infections. Further research on the time of basidiospore infections and the knowledge of environmental conditions favoring infection by basidiospores is needed to complete the knowledge of infection periods for *A. peckianus*. Knowledge of basidiospore infection periods combined with the knowledge of aeciospore infection periods may enable control of orange rust in the field.

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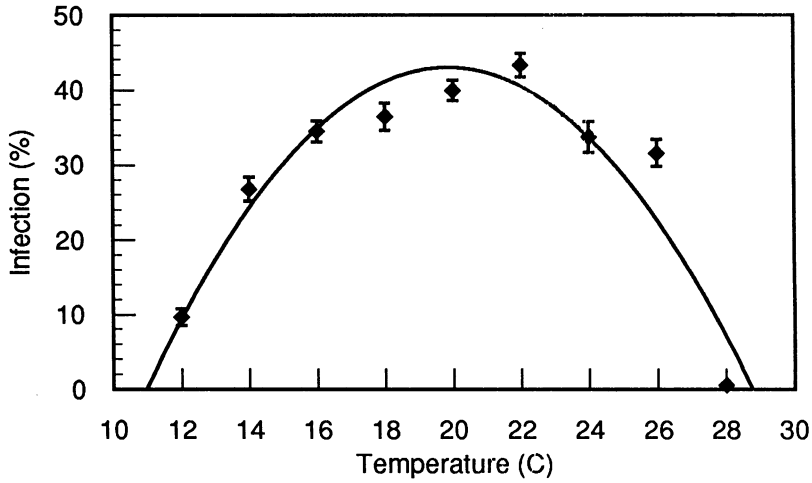

Fig. 2. The effect of temperature on the infection of black raspberry leaf disks by aeciospores of *Arthuriomyces peckianus*. The error bars represent the standard error of the means. The curve is described by the model $y = -171.688 + 21.609T - 0.544T^2$, where y = percent successful infection of aeciospores, and T = temperature.

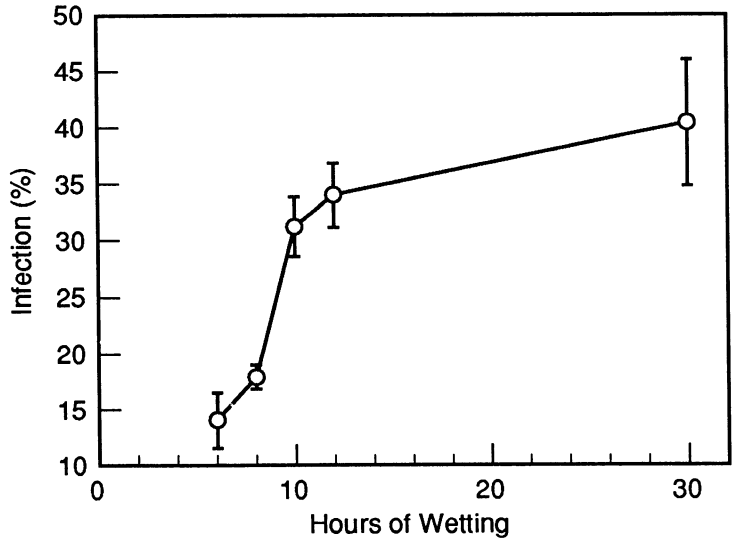

Fig. 3. The effect of wetting duration at 22°C on the percentage of infection of aeciospores of *Arthuriomyces peckianus*. Error bars represent 95% confidence intervals.

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