Effects of Temperature, Light, and Relative Humidity on Powdery Mildew of Begonia

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ABSTRACT

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Under controlled temperature, relative humidity, and light conditions, conidia of *Oidium begoniae* (the causal agent of powdery mildew of begonia) germinated on glass slides and excised leaves at temperatures ranging 4–32 C, with the most rapid germination at 23–25 C. Haustoria did not form above 30 C. Temperatures of 28 C or above caused reduction or cessation of hyphal growth, inhibition of sporulation, and eventual eradication of the pathogen. Temperatures at 20–21 C were optimal for colony development as measured after 7 days of incubation. Decreasing relative humidity caused only slight decreases in conidial germination and the development of mildew colonies. Water killed most submerged conidia within 10–30 min. Floating conidia germinated well with little appressorial formation. Sporulation occurred in response to diurnal cycles of light and dark. Changing the onset of the light period changed the time of release of conidia.

Powdery mildew, which is caused by *Oidium begoniae* Puttemans, is a devastating disease of elatior begonias. Where protectant fungicide programs were not followed in Ohio and surrounding states, epidemics have ruined many crops. Eradication with registered fungicides is difficult (5). The appearance of pathogen races capable of infecting previously immune cultivars, such as *Begonia* × *hiemalis* 'Aphrodite,' have caused further problems (3,6,8,9).

In greenhouses, it may be possible to control the disease by manipulation of the environment. Longree (2), however, concluded that rose powdery mildew could not be economically controlled by manipulating temperature or relative humidity in the greenhouse. Temperatures above 27 C were inhibitory to the fungus, but were also detrimental to the host. However, the feasibility of eradicating the powdery mildews pathogens with short periods of high temperatures in greenhouses remains to be investigated.

In this study, the response of *O. begoniae* to temperature, light, and relative humidity was investigated to determine whether powdery mildew of begonias can be controlled by manipulating of the greenhouse environment.

MATERIALS AND METHODS

Effects of temperature and relative humidity on germination, appressorium formation, and shriveling of conidia were studied in growth chambers. Conidia were incubated on glass slides or mature excised leaves of *Begonia* × *hiemalis* 'Schwabenland Red' (the cultivar used throughout this study) in double petri plates suspended on screening over trays containing saturated salt solutions (NaCl [85–95% RH], $MgCl_2 \cdot 6H_2O$ [50–60% RH], anhydrous $CaSO_4$ [15–30% RH], or water [100% RH]) and enclosed in plastic bags (Tables 1 and 2). Double petri plates were two stacked plates with a hole between them through which the petiole of a leaf placed in the upper plate was immersed in water in the lower plate (Fig. 1). The upper lids of plates were removed for the relative humidity experiments. The relative humidity was allowed to equilibrate inside the bags for 24 hr before the leaves or slides were placed in them. Cool-white fluorescent light (intensity

TABLE 1. Effects of temperature and relative humidity on germination, formation of appressoria, and on shriveling of *Oidium begoniae* conidia after 24 hr on glass slides

Temp (C)	RH[x] (%)	Conidia (%) observed[y]: Ungerminated	Without appressoria	Shriveled
15	100	56 bc[yz]	71 b[z]	19 a[z]
15	57	61 cd	85 cd	56 cd
21	100	48 b	72 b	35 b
21	88	33 a	54 a	43 bc
21	57	47 b	73 b	59 d
21	23	76 de	···	90 f
29	100	66 cde	96 e	30 ab
29	88	76 e	81 bc	50 cd
29	57	75 e	93 de	71 e

[x] Relative humidity regulated by saturated salt solutions in trays in plastic bags. Glass slides were supported on screens above the salt solutions.
[y] Spores from leaves infected for 7 days were dusted on each glass slide and 500 spores per slide were observed on each of 16 slides per treatment.
[z] Numbers in the same column followed by the same letter do not differ significantly, $P = 0.05$, according to Duncan's new multiple range test.

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range 2,500–3,000 lux) was provided from 0700 to 1900 hours. For determinations of the effects of the environment on spore germination and appressorium formation, spores harvested from leaves that had been infected for 7 days were dusted on glass slides. Five hundred spores per slide or leaf were observed on each of 16 slides per treatment.

In a further study, germination and appressorium formation on excised leaves were determined by dusting 100–200 spores from leaves infected for 7 days onto each excised leaf and observing them. Hyphal growth, extent of sporulation, and colony formation at various temperatures and relative humidities were also observed on excised leaves (Figs. 2–5). Hyphal growth was determined by measuring the longest hyphae in 10 randomly selected colonies per leaf (four leaves per treatment) by using a dissecting microscope equipped with an ocular micrometer. Sporulation was determined by counting the number of spores in 10 colonies on each of six leaves per treatment. Colony number (18 leaves per treatment) was determined by observing with the naked eye.

TABLE 2. Effects of temperature and relative humidity on germination, formation of appressoria, and on shriveling of conidia of *Oidium begoniae* after 24 hr and on number of visible colonies after 7 days on excised leaves

Temp (C)	RH[x] (%)	Conidia (%) observed[y]: Ungerminated	Without appressoria	Shriveled	Visible colonies[z]
15	100	82 b	89 b	56 a	62 cd
15	88	86 b	92 b	52 a	56 de
15	57	84 b	90 b	59 a	51 de
15	23	...	...	...	38 e
21	100	56 a	78 a	69 a	100 a
21	88	64 a	78 a	67 a	101 a
21	57	62 a	74 a	86 b	90 ab
21	23	...	...	...	80 bc
29	100	77 b	92 b	60 a	0 f
29	88	81 b	91 b	63 a	0 f
29	57	83 b	89 b	91 b	0 f
29	23	...	...	...	0 f

[x]Relative humidity regulated by saturated salt solutions contained in trays in plastic bags. Leaves were in double petri dishes supported on screens above the salt solutions.
[y]Based on counts of 100–200 conidia on each of eight leaves per treatment. Numbers in the same column followed by the same letter do not differ significantly, $P = 0.05$, according to Duncan's new multiple range test.
[z]Germinated conidia forming visible colonies on the leaves at 21 C and 100% RH represent the 100% level.

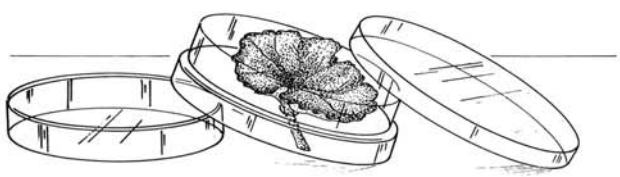

Fig. 1. Infected, excised begonia leaf in a double petri plate. The left portion contains the water reservoir, in which the end of the petiole was immersed.

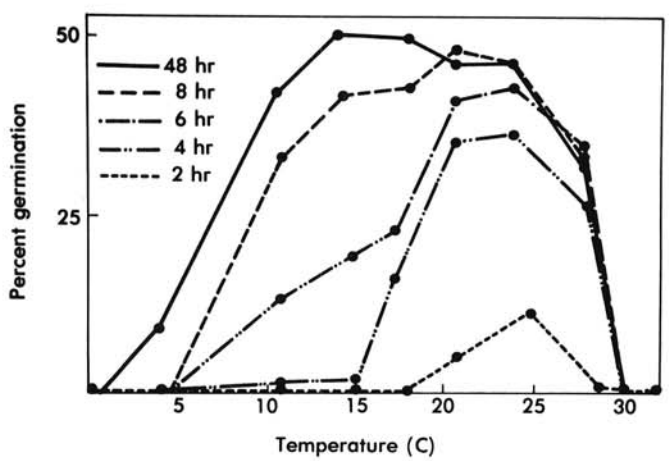

Fig. 2. Effect of temperature on germination of *Oidium begoniae* conidia on glass slides. Time of reading given in hours. The curves represent readings made at varying hours after slides were dusted with conidia.

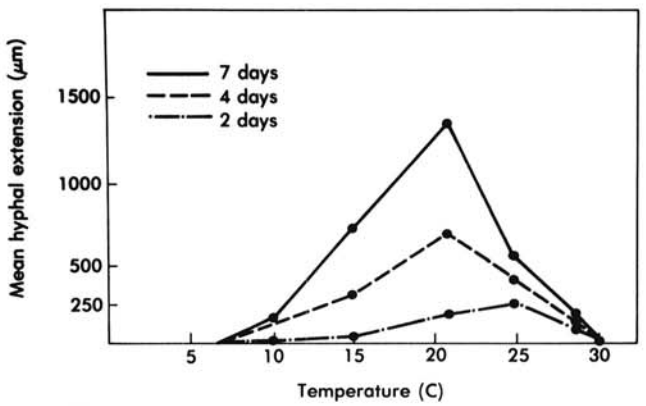

Fig. 3. Effect of temperature on mean extension of hyphae of *Oidium begoniae*. The curves represent readings made 2, 4, and 7 days after excised begonia leaves were dusted with conidia.

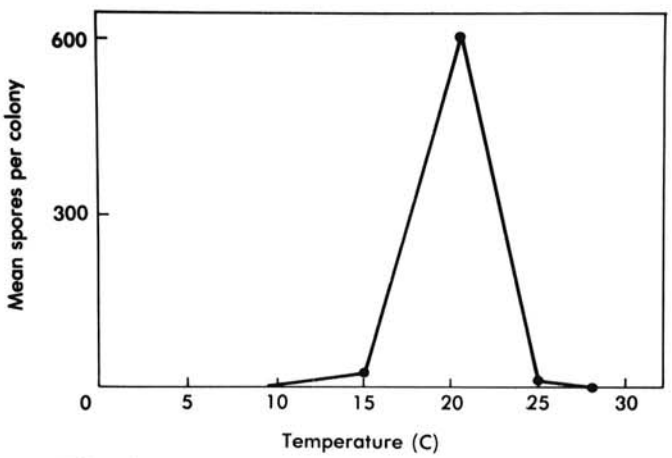

Fig. 4. Effect of temperature on sporulation of *Oidium begoniae* on excised begonia leaves 9 days after inoculation.

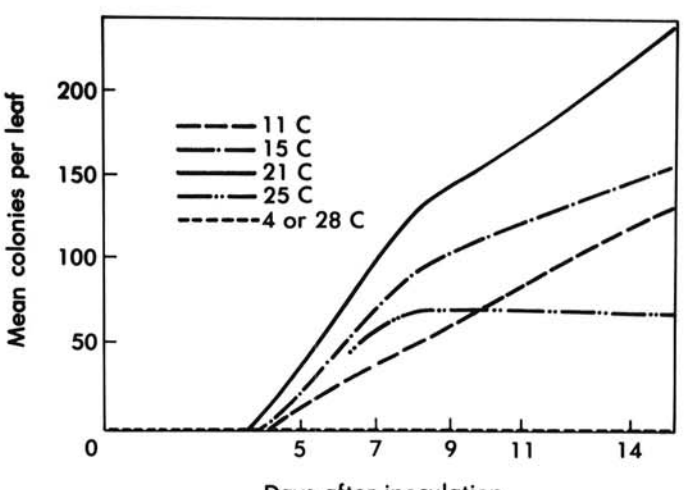

Fig. 5. Effect of temperature on number of *Oidium begoniae* colonies per begonia leaf visible to the eye over time.

Effects of temperatures of 28, 32, or 40 C on conidia, haustoria, and hyphae of *O. begoniae* were studied on mature excised begonia leaves in double petri plates. Leaves inoculated by dusting with conidia from 7-day-old infections were incubated at 21 C for 2 days prior to the heat treatment to observe effects on hyphal growth or for 7 days prior to the heat treatment for conidial responses. After temperature treatment and observation, the plates were returned to 21 C and the leaves were observed for 14 to 28 days for signs of fungal recovery. Conidia attached to heat-treated conidiophores were observed for shriveling and germinability by appressing leaves to glass slides (five leaves per treatment; one slide per leaf), incubating the slides at 21 C and 100% RH for 6 hr, and observing 400 spores per slide. Shriveling and encapsulation of haustoria were observed in epidermal peels (four peels per treatment; 50 haustoria per peel) made from heat-treated leaves. Haustoria were made more visible when the peels were autoclaved in a solution of 0.05% Trypan blue in a lactic acid:glycerine:water (2:2:1, v/v) at 103 kPa for 1 min to aid penetration of the stain. Hyphal growth responses to eradicative temperatures were determined by observing 20 randomly selected colonies per treatment under a dissecting microscope. The heat treatment experiments on conidia and haustoria were repeated using whole plants instead of excised leaves. Length of heat treatment and recovery periods were lengthened to 1 and 28 days, respectively, but experiments were otherwise the same as with excised leaves.

TABLE 3. Effect of changing onset of light on percent of mature conidiophores and conidia of *Oidium begoniae* at the indicated stage of development

Observation time	Developmental stage[y]	Conidiophore/conidia maturation (%)[z] after lights were turned on at: 0100 hours	0500 hours	0800 hours
0800 hours	2+1	9	3	5
	3+1	31	97	88
	3+1d	60	0	6
1100 hours	2+1	47	6	9
	3+1	53	49	83
	3+1d	0	45	8
1600 hours	2+1	···	55	10
	3+1	···	37	13
	3+1d	···	8	77

[y] Number of cells in conidiophore plus conidium. d = detached conidium. See Fig. 1 for further illustration of development.
[z] Percentage derived from observing the development of 40–65 conidia per photoperiod variation.

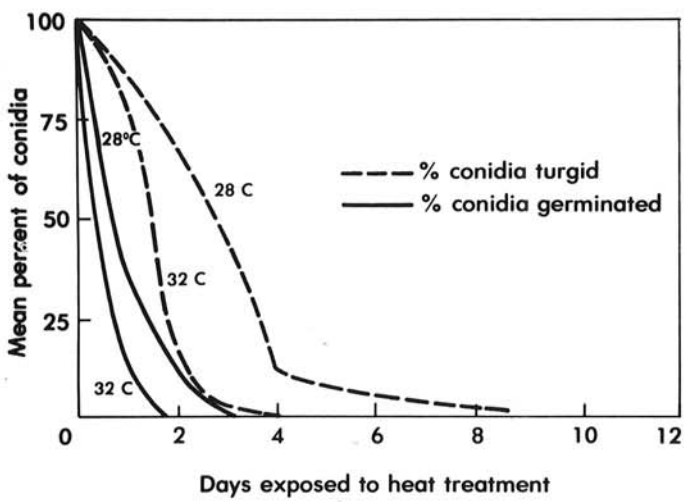

Fig. 6. Effect of eradicative heat treatments of 28 or 32 C on conidia of *Oidium begoniae* in 7-day-old colonies on excised begonia leaves exposed to such heat for varying lengths of time (days).

Diurnal release of conidia was investigated in two 4-day periods in March 1978, using a Burkard spore trap set in the greenhouse amid 50 heavily diseased plants. Formation of conidia in response to onset of the light period was observed by placing leaves in double petri plates on a microscope stage so that conidiophores could be observed for several successive days. Microscopes were placed in a growth chamber at 21 C in which light was 2,500–3,000 lux for 12 hr. The timing of onset of the light period was periodically changed and the effect of this change on conidium development noted (Table 3); 40 to 65 conidiophores were observed per treatment.

Light and temperature interactions were investigated by exposing conidia on leaves infected for 7 days to 3,000 lux of light for 5 hr, inoculating excised leaves by dusting them with the conidia, placing the leaves in an infection promoting 21 C or inhibitory 29 C incubator for varying lengths of time up to 24 hr. The next 6 days after temperature treatment, the leaves were incubated in double petri plates at 21 C with 12 hr of light (2,500–3,000 lux) per day. Seven days after inoculation the number of visible colonies per leaf was determined. Fifteen leaves were used per treatment.

The effects of free water on conidia were tested by washing conidia with distilled water from leaves that had been infected for 7 days and shaking the wash water well so that the conidia were submerged. This suspension was periodically sprayed with a DeVilbiss atomizer onto uninfected begonia leaves. Also, conidia were floated on drops of distilled water and placed on dry Parafilm M (American Can Company, Greenwich, CT 06830) and the percentage of conidia dessicated, germinated, and with appressoria was determined after 6 hr at 21 C and 100% RH.

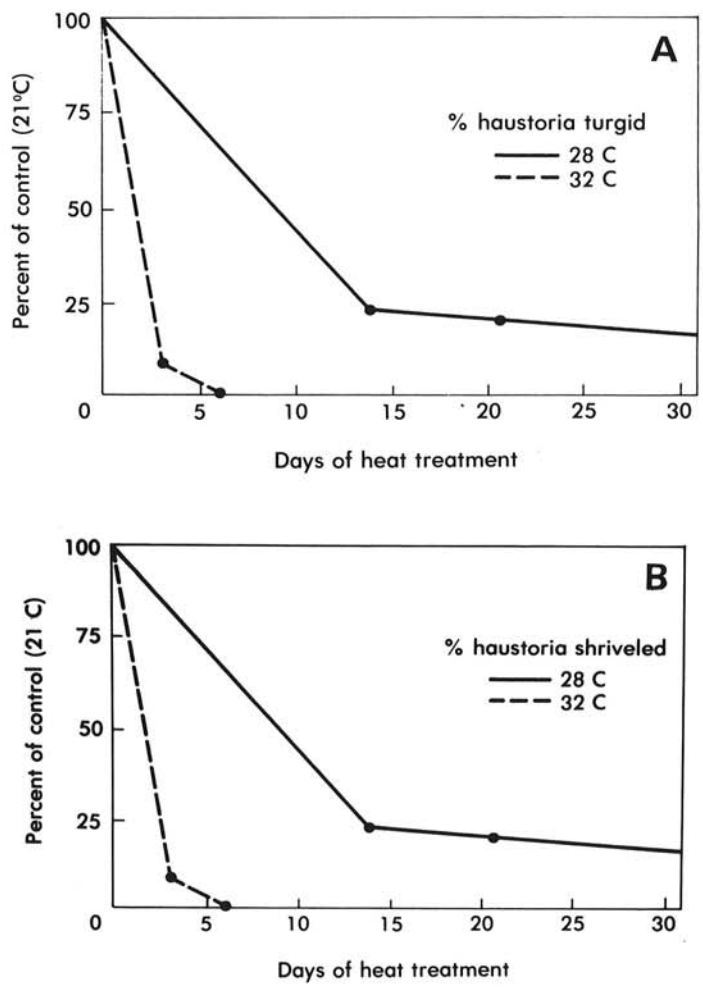

Fig. 7. Effect of eradicative heat treatments (28 or 32 C) on **A**, turgid and **B**, shriveled haustoria of *Oidium begoniae* in 7-day-old colonies on excised begonia leaves exposed to such heat for varying lengths of time (days).

RESULTS

Figures 2–5 summarize temperature responses of *O. begoniae* on excised leaves. The minimum, optimum, and maximum temperatures for germination of conidia of *O. begoniae* after 48 hr were less than 4 C, 11–25 C, and 30 C, respectively (Fig. 2). Appressorial formation had the same cardinal temperatures as germination. The 25 C treatment resulted in the highest percent of germination after 6 hr and the longest hyphae after 48 hr, but thereafter hyphal length was greatest at 21 C (Fig. 3). Sporulation after 9 days was much greater at 21 C than at 15 or 24 C (Fig. 4). The minimum, optimum, and maximum temperatures for formation of colonies visible to the naked eye were 11, 21, and 25 C, respectively (Fig. 5). After 9 and 11 days of incubation at 28 C, mean hyphal lengths were 200 and 191 μm, respectively, suggesting that hyphae stopped growing some time prior to the mean 7-day reading of 208 μm shown in Fig. 3. Cessation of growth at 28 C was accompanied by shriveling and encapsulation of haustoria, which were often found in browned cells.

Colonies were considered eradicated by 28, 32, or 40 C when exposed conidia were shriveled and would not germinate, haustoria were shriveled, or hyphal growth was stopped and could not be induced to resume at 21 C. On mature, excised leaves it took 14, 3, and 2 days at 28, 32, and 40 C, respectively, to totally eradicate *O. begoniae* (Figs. 6–8). Heat eradication of the disease on whole plants required more time (Figs. 9 and 10). Colonies tended to survive longer on the younger leaves. Many of these leaves had just emerged from the buds at the onset of the heat treatments. At 28 C, a period of dormancy or extremely slow growth of hyphae was induced (Fig. 8, days 4–14). When excised leaves were treated for 12 days or less, hyphal growth resumed at a high rate after the leaves were returned to 21 C.

The optimum relative humidity for germination, appressorial formation and visible colony development was 80–100%, but the effects of humidity were less striking than temperature (Tables 1 and 2). Low relative humidity caused some shriveling of the conidia on glass slides, but had little effect on conidia on detached leaves.

Light influenced the production and release of conidia in a diurnal fashion. Conidia caught in the Burkard spore trap reached a strong peak between 1100 and 1300 hours. Thirty-three percent were caught between 1100 and 1200 hours and 63% were caught between 1200 and 1300 hours. Production of conidia began ~5 hr after onset of the photoperiod by division of the terminal cell of the conidiophore (Fig. 11, 2 + 1 to 3 + 1 stage). This newly formed cell began swelling 24 hr later, after the previously formed conidium had matured (Fig. 11, stage 3 + 1d). Delimitation of the fully formed conidium occurred about 48 hr after cell division took place on the conidiophore. When the onset of illumination was changed to 0100 hours, a corresponding change in the time of conidial formation took place (Table 3).

We hypothesized that exposure of host plants to 29 C only at that time of day when release of conidia, penetration, and haustorial formation took place (8–16 hr after onset of photoperiod) would be necessary to prevent infection. Exposure of plants to 29 C 8–16 hr after inoculation did greatly inhibit colony formation, but did not

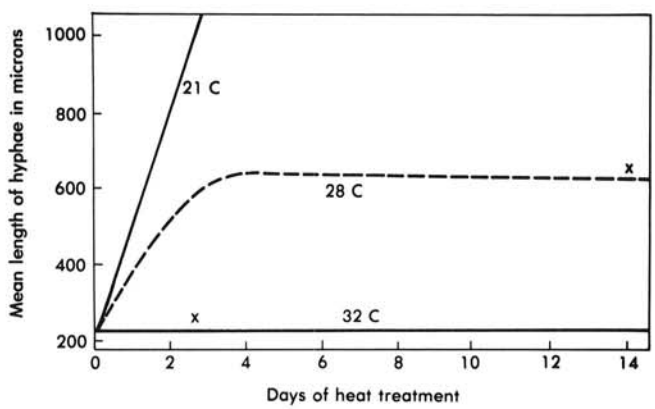

Fig. 8. Growth of mycelia of 2-day-old *Oidium begoniae* colonies on excised begonia leaves when exposed to eradicative heat. The x on 32 and 28 C curves designates the point at which growth will not resume when excised leaves are returned to 21 C.

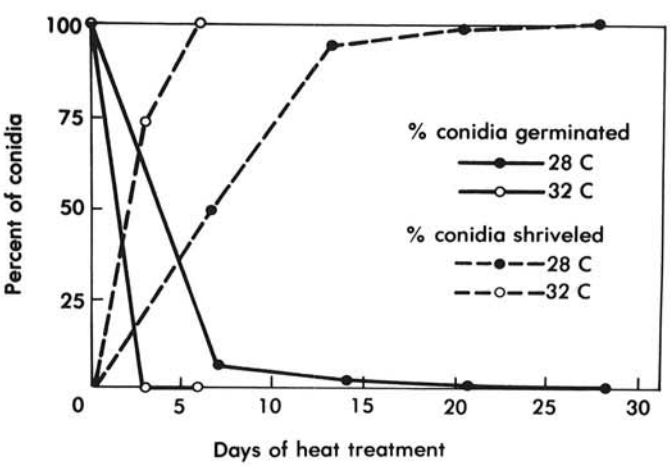

Fig. 9. Effect of eradicative heat treatment on *Oidium begoniae* conidia on 7-day-old colonies on whole begonia plants exposed to 28 or 32 C for varying lengths of time (days).

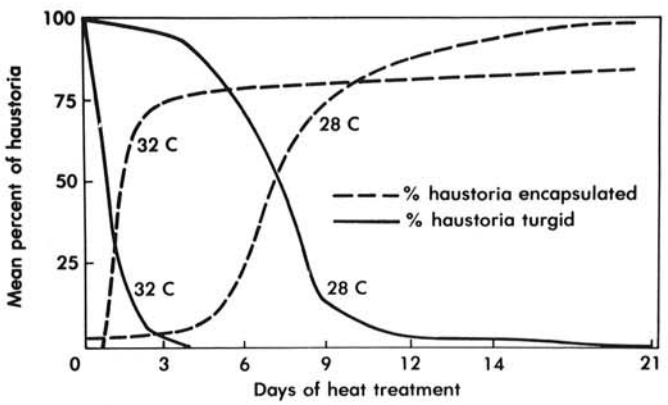

Fig. 10. Effect of eradicative heat treatments on haustoria of *Oidium begoniae* in 7-day-old colonies on whole begonia plants over time exposed to 28 or 32 C for varying lengths of time (days).

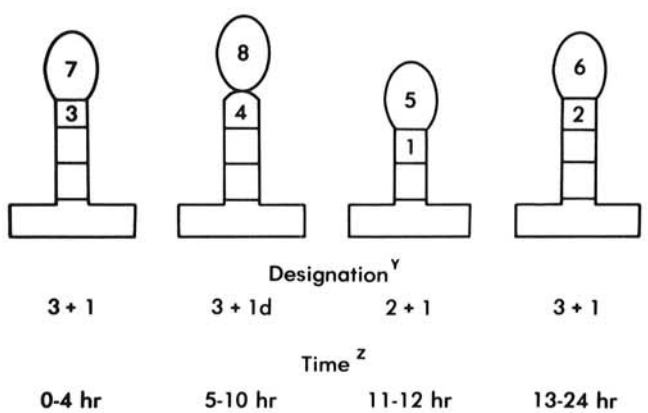

Fig. 11. Ontogeny of conidiophore and conidia production by *Oidium begoniae* in relation to the onset of photoperiod. Numbers on cells and conidia refer to order in which conidial formation takes place. The first event is division of the terminal cell (1) to form a conidial initial (2). This cell, traced by following numbers 3–8, becomes a fully developed conidium in 48 hours. y, Number of cells in conidiophore + conidium. z, Hours after onset of photoperiod, designated for first 24 hr only.

TABLE 4. Effects of exposure to 29 C for various periods before or after inoculation on the number of visible *Oidium begoniae* colonies on excised *Begonia* × *hiemalis* leaves after 7 days at 21 C

Exposure to 29 C relative to inoculation time[y]		
From	To	Colonies per leaf
No (control)	Immed.	172 d[z]
0 hr	7 days	0 a
−24 hr	0 hr	138 cd
0 hr	4 hr	126 c
4 hr	8 hr	131 cd
8 hr	16 hr	25 b
16 hr	24 hr	98 c
0 hr	24 hr	21 b

[y]After exposure, excised leaves in double petri dishes were returned to 21 C and incubated for 7 days at 100% RH over trays of water in platic bags.
[z]Numbers followed by same letter do not differ significantly, $P = 0.05$, according to Duncan's new multiple range test. There were 15 leaves per treatment.

result in the complete control of the disease (Table 4).

Immersion of conidia in water caused a progressive loss in their ability from 13 visible colonies per leaf after immersion for 1–10 min, down to four visible colonies per leaf after immersion for 30 min, and to no visible colonies per leaf after 30–60 min of immersion. However, conidia floating on water exhibited germination increase 6 hr after inoculation and decreased conidial shriveling compared to conidia on Parafilm M. Germ tubes grew away from the face of water droplets and were longer than germ tubes of conidia on Parafilm M.

DISCUSSION

The cardinal temperatures for *O. begoniae* are near the means for previously studied powdery mildews (7,10). Since *B.* × *hiemalis* (Rieger elatior begonias) is a greenhouse-grown crop, control of temperature is a possible means of controlling the disease. Exposure to 32 C for 6 days was an effective eradicative treatment, but testing in a commercial greenhouse is necessary to evaluate its practicality. Elatior begonias must be heavily shaded at high temperatures to avoid sunscald. High temperatures also promote vegetative growth, which may inhibit flowering and, if used for long periods, may produce leaves less fit for propagation (1). High temperature eradicative treatments may be best applied, therefore, when vegetative growth is desired. Such a time might be after cuttings are taken from stock plants, prior to or during the rooting and plantlet differentiation period. The small unit size of the plants at this time would make treatment in a confined area more feasible as well.

Erysiphe polygoni DC, *Microsphaera* spp., and *Uncinula* spp. are less affected by relative humidity than other powdery mildews (11). It is likely that *O. begoniae* is a member of the genus *Microsphaera* (6). Therefore, venting and drying greenhouse air, a control measure for powdery mildew of rose, may have little effect on *O. begoniae*. Diurnal periodicity is also a commonly observed trait of this group of powdery mildews (4). Control of the disease by beginning the photoperiod earlier in the morning so that vulnerable stages of the fungus are exposed to naturally occurring midday heat may be feasible when risk of mildew epidemics is high. The longer days also would inhibit flowering and so should only be used for disease control during periods when vegetative growth is desired. This treatment was not totally effective and therefore could not be recommended in place of protective fungicides.

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