Influence of Night Temperature on Disease Development in Fusarium Wilt of Chrysanthemum

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ABSTRACT

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Constant high temperatures are known to favor development of Fusarium wilt of florists' chrysanthemum caused by *Fusarium oxysporum* f. sp. *chrysanthemi*. However, temperatures in greenhouses and the field rarely remain high for long periods of time. Rooted cuttings of *Chrysanthemum morifolium* 'Royal Trophy,' 'Mandalay,' and 'Torch' were grown for 2 wk and root-inoculated with *F. o.* f. sp. *chrysanthemi*. Plants were exposed to regimes with a day temperature of 35 C and night temperatures of 13, 18, 24, 29, or 35 C. Symptoms were rated daily on a scale of 0 (no symptoms) to 5 (dead) for 28 days after inoculation. Average total ratings (ATR) were obtained by summing the daily ratings and dividing by the number of inoculated plants. For cultivars Royal Trophy and Mandalay, ATR were greatest at a night temperature of 29 C. For Torch, ATR did not vary significantly with night temperature. Symptoms in Torch were not apparent until night temperatures were 24 C or greater, whereas other cultivars exhibited symptoms at all temperatures. Symptomless plants of all cultivars were colonized frequently. Effects of diurnal temperatures on mycelial growth and sporulation of the pathogen on agar media were not correlated with disease development.

In a previous study, a constant temperature of 35 C was most favorable for development of Fusarium wilt of chrysanthemum caused by *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *chrysanthemi* Litt., Armst. & Armst. (6). However, temperatures in greenhouses and fields rarely remain this high for long periods of time and generally, a diurnal fluctuation in temperature occurs. Littrell (8) maintained temperatures at 28–30 C during the day and 23 C during the night while studying the effects of nitrogen nutrition on disease development. Woltz and Engelhard (9) examined the effects of nitrogen source and lime on disease development and grew plants at 33 ± 2 C during the day and 25 ± 2 C at night. In another study, they raised the day temperature to 38 ± 2 C (4). Because diurnal temperature conditions are more representative of field and greenhouse conditions, the objective of this investigation was to determine the effect of diurnal temperatures on the pathogen and on infection and disease development. We know of no reports on the influence of varied night temperature on Fusarium wilt of chrysanthemum.

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MATERIALS AND METHODS

Procedures used were identical to those described previously with a few exceptions (6). Culture-indexed rooted cuttings were planted in an autoclaved 1:1 peat-perlite medium containing Osmocote slow-release fertilizer and were grown under greenhouse conditions for 2 wk before placement in controlled temperatures. The cultivars Royal Trophy, Mandalay, and Torch were used in all experiments, and experiments were performed three times. Isolate 0-807 of *F. o.* f. sp. *chrysanthemi* was used twice and isolate 0-693 was used once. These isolates were obtained as lyophilized cultures from the Fusarium Research Center of The Pennsylvania State University and were grown on potato-dextrose agar (PDA) slants under fluorescent lights at a temperature of 22 C for 2 wk. Suspensions of microconidia and macroconidia concentrations were adjusted to 60,000 spores/ml, as measured with a hemacytometer. Plant roots were wounded by cutting two trenches 2 cm from the stem on opposite sides of the plant to a depth of 5 cm and a 100-ml spore suspension was added for inoculation. The temperature regimes in the controlled environment chambers were diurnal with a photoperiod of 14 hr. Day temperatures were 35 C and night temperatures were 13, 18, 24, 29, and 35 ± 1 C. Air temperatures were monitored continuously with Rustrak model 88 chart recorders. Soil and stem temperatures were monitored in one trial with a Honeywell multipoint strip chart recorder.

Plants were rated daily using the six point rating system described previously (6), in which 0 = no symptoms; 1 = chlorosis and/or wilting of one or two leaves with possible curvature of leaves in some cultivars; 2 = necrosis following chlorosis and wilt; 3 = necrosis, chlorosis, wilt, and curvature of more than two leaves; 4 = as above and stunted; and 5 = dead plants. Rating summations were made for each cultivar at each temperature and divided by number of inoculated plants to give an average total rating (ATR). Average total ratings were analyzed by regression. In two of the trials, all asymptomatic plants were cultured after 4 wk. Stems were cut at the soil line and leaves were removed. An outline of the plant was made on lined paper. Stems were then disinfested in 5.25% sodium hypochlorite for 5 min. These were dried on paper toweling and sections were removed at 2.5-cm intervals and placed on carnation leaf agar (CLA) (5). Plates were examined after 3–5 days for fungus growth. Sections of the stem in which the pathogen was present were noted on the outline, thus giving a diagram of the location of the pathogen in the plant.

Isolates 0-693, 0-734, and 0-807 of *F. o.* f. sp. *chrysanthemi* were used to study the effects of temperature on the pathogen. Initial inoculum was grown from lyophilized cultures of each of the three isolates placed on PDA and CLA and allowed to grow for 1 wk at room temperature. Plugs of hyphae were removed from the advancing margins. Those from PDA were placed on PDA and those from CLA were placed on CLA. Ten plates of each isolate on the selected medium were placed in each chamber. Linear growth of the colonies was measured on both PDA and CLA after 10 days. Factorial analysis and *F* tests were performed on data to determine significance among treatments.

To study the effect of temperature on pathogen sporulation, five plates were chosen from each treatment and were flooded with 10 ml of sterile, distilled water. Mycelia were rubbed lightly with a

rubber policeman to loosen conidia. The resulting suspension was poured through two layers of cheesecloth to remove most of the bits of hyphae. The concentration of combined microconidia and macroconidia was determined by making two counts with a hemacytometer. The two counts were averaged to give one value for each plate. Factorial analysis and *F* tests were performed on data to determine significance among treatments.

RESULTS

The results of three trials on the effect of night temperature regimes on symptom development are presented in Table 1. Average total ratings for Royal Trophy were consistently higher than those for the other cultivars, Torch always had the lowest ratings, and Mandalay was intermediate. Regression analysis confirmed these observations. The line that best described the response for Torch did not differ significantly from one with a slope of zero indicating that there was no variation in the ratings at the various temperatures. The lines describing the responses of Royal Trophy and Mandalay were significantly different from zero. Average total ratings decreased when the night temperature went from 13 to 18 C, then increased with night temperatures to 29 C, and leveled off or decreased at 35 C. This was most noticeable in the cultivar Mandalay (Fig. 1).

Average total ratings for Royal Trophy were greatest when it was inoculated with isolate 0-693. At most temperatures, symptoms were initiated earlier by this isolate than they were by isolate 0-807. However, regression analysis also showed that there was no significant difference between the isolates nor was there an interaction between isolate and cultivar.

The rate at which symptoms developed and the extent to which they progressed varied with temperature. The number of days before symptom initiation decreased as the night temperature increased, with a few exceptions. Generally, the number of days that a plant was assigned a rating of three or four increased with increasing night temperature. Plants were assigned a rating of five earlier and for longer periods of time when the night temperature was 29 or 35 C.

Symptoms in Royal Trophy included chlorosis as the most prominent early symptom at night temperatures of 13 and 18 C followed by leaf wilting, stunting, and death of plants 26 days after inoculation. At night temperatures of 24 C and above, the first noticeable symptom was wilting, apparent at the tips of one or two leaves at or above the middle of the stem. Plants commonly advanced from a rating of one to a rating of three when more leaves wilted without the development of necrosis in previously affected leaves. This condition occurred most frequently when the night temperature was 29 C. Under these conditions, plants of Royal Trophy were dead approximately 21 days after inoculation.

Symptom progression in Mandalay was similar at all temperatures and consisted of chlorosis and curvature of upper leaves, wilting, necrosis, stunting, and occasionally death of plants. However, at night temperatures of 24 C and below, necrosis did not always develop. Inoculated plants were killed only when night temperatures were 29 or 35 C.

Inoculated plants of the cultivar Torch exhibited symptoms only when night temperature was 24, 29, or 35 C. At 24 C, symptoms consisted of chlorosis and curvature of leaves at the tip of one branch followed by leaf wilting. Symptoms were similar at the higher night temperatures except that wilting occurred earlier and was followed by necrosis of affected leaves. One plant from the 29 C regime received a rating of four when it appeared stunted and chlorotic compared with control plants. This was the highest rating that a plant of the cultivar Torch ever received.

At the end of two trials, symptomless plants were cultured to determine whether infection had occurred and the extent to which colonization had progressed. As the night temperature increased, more plants were infected and developed symptoms (Table 1). Plants of Royal Trophy and Mandalay were infected at all temperature regimes. When the night temperature was 24 C or above, plants that were infected usually developed symptoms. The best combination of temperatures for infection and symptoms in these cultivars appeared to be 35 C day/29 C night and 35 C day/35 C night.

When the night temperature was 13 or 18 C, some plants of Torch became infected and colonized but developed no symptoms. All six treated plants that were incubated at 35 C day/18 C night and inoculated with isolate 0-807 were infected. When inoculated with isolate 0-693, none of the plants were infected. The best temperature combination for infection and symptom expression in Torch was 35 C day/29 C night.

Table 1. Effects of diurnal temperatures on symptom expression in chrysanthemum cultivars Royal Trophy, Mandalay, and Torch inoculated with *Fusarium oxysporum* f. sp. *chrysanthemi*[a]

Effects	Night temperature (C)				
	13	18	24	29	35
Royal Trophy					
ATR[b]	30.3	29.6	42.6	64.4	65.3
Days to symptoms[c]	17.7	18.5	14.6	12.1	11.5
Total with symptoms[d]	17	18	18	18	18
Total cultured[e]	1	...	...	...	...
Total infected[f]	1	...	...	...	...
Mandalay					
ATR[b]	13.4	7.3	14.4	47.4	34.7
Days to symptoms[c]	20.8	22.8	18.9	14.8	15.3
Total with symptoms[d]	13	10	12	18	15
Total cultured[e]	3	6	5	...	1
Total infected[f]	2	1	1	...	1
Torch					
ATR[b]	0	0	0.9	3.6	2.3
Days to symptoms[c]	...	...	26.3	24.2	22.3
Total with symptoms[d]	0	0	4	8	5
Total cultured[e]	12	12	8	5	9
Total infected[f]	4	6	4	1	4

[a] Plants were grown under 14-hr day/10-hr night regimes. Day temperature was 35 C.
[b] ATR is average total ratings of symptoms. Values are means of three trials.
[c] Values are means of three trials.
[d] Total is number of plants from three trials that exhibited symptoms. Total number of inoculated plants at each regime was 18.
[e] Values are the sum of two trials. All symptomless plants were cultured.
[f] Values are the sum of two trials. Of plants that were cultured, these were found to be infected.

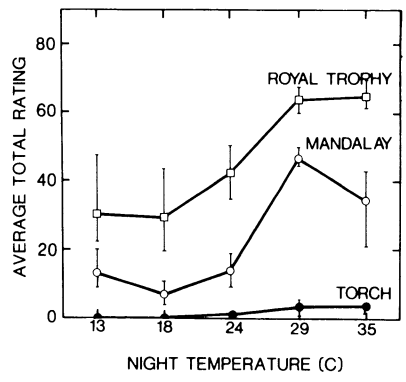

Fig. 1. Means of average total ratings of symptoms for the chrysanthemum cultivars Royal Trophy, Mandalay, and Torch inoculated with *Fusarium oxysporum* f. sp. *chrysanthemi* and grown under diurnal temperature conditions where day (14 hr) temperature was 35 C. Bars indicate ranges of rating.

The extent to which asymptomatic plants were colonized was determined for the cultivars Mandalay and Torch inoculated with isolate 0-807 (Fig. 2). There were three symptomless, infected plants of Mandalay and these occurred at night temperatures of 13, 24, and 35 C. Although plant growth was best at the lower night temperatures, colonization by the pathogen was greatest at the median night temperature. The extent of colonization of Torch also was greatest at a night temperature of 24 C. Three symptomless, infected plants were found at this temperature. At night temperatures above and below 24 C, colonization decreased, except for one plant of Torch that was symptomless at 29 C, but was completely colonized.

All three isolates of *F. o.* f. sp. *chrysanthemi* grew at all temperature regimes on PDA and CLA (Fig. 3). Linear colony growth was best on both media when the night temperature was 24 C. Linear growth was better on CLA

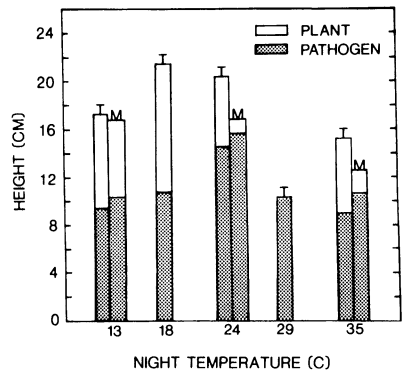

Fig. 2. Average height of asymptomatic plants and extent of colonization of chrysanthemum cultivars Torch (T) and Mandalay (M) 28 days after inoculation with isolate of *Fusarium oxysporum* f. sp. *chrysanthemi*. No asymptomatic, infected plants of Mandalay were found at 18 and 29 C. Plants were grown under 14-hr day/10-hr night regimes. Day temperature was 35 C.

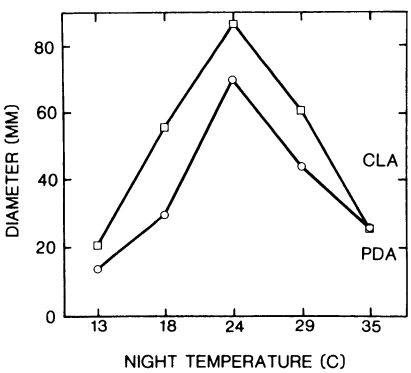

Fig. 3. Average colony diameters of three isolates of *Fusarium oxysporum* f. sp. *chrysanthemi* grown on carnation leaf agar (CLA) and potato-dextrose agar (PDA) after incubation at five diurnal (14-hr day/10-hr night) temperature regimes for 6 days. Day temperature was 35 C.

than on PDA at all temperatures with the exception of 0-807, which grew less on CLA when the night temperature was 35 C. Factorial analysis and *F* tests showed that there were significant ($P = 0.01$) effects due to blocks, temperature, and medium as well as a significant interaction between temperature and medium. There was no difference in linear growth among the three isolates.

All isolates produced significantly more spores on PDA than on CLA. On CLA, sporulation was best when the night temperatures were 13 or 35 C. Night temperatures of 24 and 29 C resulted in the lowest spore production. All isolates reacted similarly to temperature, except at 35 C. At this temperature, 0-807 produced approximately four times more spores than the other isolates. On PDA, all three isolates produced 18–112 times the number of spores that they produced on CLA. Isolate 0-693 sporulated best at a constant temperature of 35 C and 0-734 produced the most spores at night temperature of 13 and 18 C. Isolate 0-807 produced significantly more spores than the other isolates at all temperature regimes and a constant temperature of 35 C appeared to be best for sporulation of this isolate. Factorial analysis and *F* tests showed that there were significant ($P = 0.01$) effects due to isolate, temperature, and medium as well as interactions between isolate, temperature, and medium. Interactions between isolate and temperature, isolate and medium, and temperature and medium also occurred (Table 2). There was also a significant interaction between all three factors.

DISCUSSION

The highest average total symptom ratings for Royal Trophy, Mandalay, and Torch at diurnal temperatures are similar to those obtained in the study of the effect of constant temperature on symptom expression (6). To compare these investigations, diurnal temperature regimes were converted to an average daily temperature. The conversion was made by multiplying the day temperature by 14 (the number of day hours), the night temperature by 10 (the number of night hours), and dividing the sum by 24. Average daily temperatures for the five regimes were 26, 28, 30, 33, and 35 C. In most cases, ATR from diurnal regimes were considerably higher than those from comparable constant temperatures. Differences were least when constant 29 C and 35 C day/24 C night were compared for Royal Trophy and when constant 27 C and 35 C day/18 C night were compared for Mandalay. As Torch did not respond significantly to temperature, there was no pattern to the differences between constant and diurnal temperatures.

In at least one other case, diurnal changes in temperature affected disease severity. Clayton (1) studied the effects of soil and air temperatures on the development of tomato wilt caused by *F. o.* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. He found that the most favorable conditions for disease were a soil temperature of 27 C and an air temperature of 28 C with short periods where the temperature rose to 33 or 34 C. For Fusarium wilt of chrysanthemum, 29 C with increases to 35 C were best for symptom development, although we do not know how long these increases in temperature must last to affect disease development significantly.

Symptom progression varied somewhat with increasing night temperature. When night temperature was 13, 18, or 24 C the predominant initial symptom was chlorosis of upper leaves in Royal Trophy and Mandalay. This was similar to symptoms described by Engelhard and Woltz (3). At higher night temperatures of 29 and 35 C, wilting of leaves was the first symptom noted. Plants of Royal Trophy tended to advance from the initial wilt stage to a severe wilt stage without becoming necrotic under these conditions. In Mandalay, the necrotic stage became more apparent at high temperatures. At night temperatures of 13 and 18 C, this symptom never developed. Thus, it is difficult to develop

Table 2. Factorial analysis and *F* tests on sporulation of three isolates of *Fusarium oxysporum* f. sp. *chrysanthemi* on potato-dextrose agar or carnation leaf agar at five diurnal (14-hr day/10-hr night) temperature regimes

Source	df	Sum of squares	Mean square	*F* value
Blocks	4	402153.97	100538.49	1.46
Isolates	2	28720995.00	14360498.00	209.21**[a]
Temperature	4	5448345.30	1362086.30	19.84**
Medium	1	58746111.00	58746111.00	855.86**
Isolate × temperature	8	6831699.60	853962.45	12.44**
Isolate × medium	2	27041308.00	13520654.00	196.98**
Temperature × medium	4	4354844.50	1088711.10	15.86**
Isolate × temperature × medium	8	5852214.60	731526.83	10.66**
Error	116	7962236.00	68639.97	···
Total	149	145359910.07		

[a]** = Significant at the $P = 0.01$ level.

a rating system that adequately describes symptom progression in all cultivars at all temperature conditions.

The recommended temperature conditions for growing chrysanthemums are 15.6 C nights with 21 C day temperatures (7). Although these specific conditions were not used, extrapolations can be made with the data that was obtained. Average total ratings for these conditions might range from 0 for a resistant cultivar to approximately 46.5 for a susceptible one. Plants that did not exhibit symptoms might still be infected based on the results from this study.

The symptoms observed in the cultivar Torch were not as severe as those reported previously (3). No symptoms developed at night temperatures of 13 or 18 C and most inoculated plants received ratings of one, two, or three at higher night temperatures. Only one plant received a rating of four, and this occurred at a night temperature of 29 C. Although it was shown statistically that symptom development in Torch did not respond to temperature, a temperature trend was found that was consistent with that shown for Royal Trophy and Mandalay (Table 1). Torch was essentially resistant to *F. o.* f. sp. *chrysanthemi*. However, resistance in Torch was one of symptom expression and not of infection and colonization. Plants frequently became infected and colonized under conditions that were not favorable for symptom development (Table 1). Night temperatures of 13 and 18 C allowed the pathogen to colonize plants extensively, although there was no external evidence of disease. At higher night temperatures, plants tended to develop symptoms. Those plants that remained symptomless were colonized as extensively as plants of susceptible cultivars. This phenomenon may be due to the effects of temperature on growth and/or sporulation of the pathogen in the plant. Extent of colonization (Fig. 2) and linear growth on artificial media (Fig. 3) were similar in that both were greatest when the night temperature was 24 C, and decreased gradually when the night temperature was above or below this point. Stem temperatures were found to be 1 or 2 C below air temperatures so the pathogen was exposed to similar temperature conditions and responded similarly in vivo and in vitro. However, this effect alone did not explain the enhanced expression of symptoms at the 35 C day/29 C night regime.

Sporulation of the pathogen also did not provide an adequate explanation of the results. Generally, sporulation in vitro was best at the extreme temperatures of 13 and 35 C. Isolation studies showed that infected plants were colonized continuously, whereas discontinuous colonization might be expected if conidia were important for movement in the vascular system. Inoculation with isolate 0-807 would be expected to result in rapid symptom development and higher symptom ratings because this isolate sporulated more profusely than the other isolates. Statistical analysis of ratings showed that there was no significant difference between the isolates. Histological studies have shown that conidia rarely occur in advance of hyphae in the xylem of the cultivar Yellow Delaware (2). Sporulation of the pathogen in the vascular system appeared to have no effect on disease development.

The effects of diurnal temperature on Fusarium wilt of chrysanthemum appeared to be due to more than a simple, direct effect on growth and sporulation of the pathogen. Tolerance in Torch and Mandalay was expressed as a decrease in symptom expression. However, plants that were tested in these experiments proved to be colonized to the same extent as more susceptible cultivars. Thus, resistance or tolerance in Torch appears to be a physiological response, but influenced less by temperature in Torch than in Mandalay. This hypothesis is supported by reports of previous investigations (6). Furthermore, plants became infected and colonized by the pathogen under conditions that were unfavorable for symptom development. Therefore, culture-indexing used by commercial propagators is a useful and important procedure for the production of chrysanthemums that are free of vascular pathogens. Temperature effects on the pathogen and the plant, and the interactions between the two, needs more detailed study.

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