Symptom Enhancement of Fusarium Wilt of Chrysanthemum by High Temperatures

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ABSTRACT

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Chrysanthemum morifolium cvs. Royal Trophy, Mandalay, Glowing Mandalay, and Torch were inoculated with conidia of *Fusarium oxysporum* f. sp. *chrysanthemi* and grown under a 14-hr photoperiod at constant temperatures of 24, 27, 29, 32, or 35 C. Symptoms were rated on alternate days on a scale of 0 (no symptoms) to 5 (dead) for 28 days. Ratings were totaled for each cultivar at each temperature and divided by the number of inoculated plants to give an average total rating (ATR). The ATRs for Royal Trophy and Glowing Mandalay increased with temperature and were greatest at 35 C. The ATR for Torch was greatest at 29 C although there was no significant response to temperature. Symptomless plants of all cultivars were colonized by the pathogen. In vitro growth of the pathogen was not correlated with ATR and did not explain the observed responses.

Fusarium wilt of chrysanthemum is caused by *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *tracheiphilum* race 1 (E. F. Sm.) Snyd. & Hans. (1) and *F. oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *chrysanthemi* Litt., Armst. & Armst. (2). A review of the disease was published recently (5).

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During the past 15 yr, investigations of the influences of various factors on the development of Fusarium wilt of chrysanthemum have included the effects of nitrogen source (12), nitrogen and lime (20), and nitrogen, lime, and fungicides (6,8,9). However, most of these investigations were performed under greenhouse conditions with day temperatures as high as 38 C and night temperatures as low as 23 C. The effects of temperature alone on disease development have not been determined.

Symptom development in vascular wilts caused by *Fusarium* is enhanced by high temperatures. The optimum temperature for testing susceptibility of carnation to *F. oxysporum* f. sp. *dianthi* (Prill. & Del.) Snyd. & Hans. was 26 C although air temperature could be lower if the soil temperature remained at 26 C (10). Wilt of coriander was more severe at 28 than at 20 C (16). Our preliminary studies showed that symptoms developed infrequently in chrysanthemum inoculated with *F. o.* f. sp. *chrysanthemi* when the air temperature was 24 C. Hence, temperatures of 24 C and higher were used to determine the effects of constant temperatures on symptom development.

MATERIALS AND METHODS

Culture-indexed, rooted cuttings of *Chrysanthemum morifolium* (Ramat.) Hemsel (California-Florida Plant Co., Fremont, CA) were planted in an autoclaved 1:1 peat-perlite medium containing Osmocote slow-release fertilizer in 13-cm plastic pots. Cultivars used were Royal Trophy, Torch, and Mandalay or Glowing Mandalay. The only difference between cultivars Mandalay and Glowing Mandalay is the color of flowers produced; there is no other known morphological difference. Moreover, preliminary experiments revealed a similar pattern of symptom expression in the two Mandalay cultivars. Plants were grown in greenhouses for 2 wk before inoculation and incubation at the various temperature regimes.

Plants were inoculated with a conidial suspension of isolate 0-734 or 0-807 of *F. o.* f. sp. *chrysanthemi*. A third isolate, 0-693, was included in the in vitro growth studies. These were obtained as lyophilized cultures from the Fusarium Research Center, The Pennsylvania State University, and are deposited there

as reference cultures. Cultures were grown on potato-dextrose agar (PDA, Difco) slants under fluorescent lights at a temperature of 22 C for 2 wk.

Suspensions of microconidia and macroconidia were prepared by flooding PDA slants with 10 ml of sterile, distilled water and gently rubbing the mycelium with a rubber spatula to loosen conidia. The resulting suspension was poured through two layers of cheesecloth to remove hyphal pieces, and conidia were counted with a hemacytometer (Improved Neubauer, Levy Ultraplane, AO Instrument Co., Buffalo, NY); the final concentration of conidia was adjusted to 60,000/ml.

Plant roots were wounded by cutting two trenches about 2 cm from the stem on opposite sides of the plant to a depth of 5 cm with a spatula and adding 100 ml of the conidial suspension. Control plant roots were wounded, and 100 ml of sterile, distilled water was added.

Two days before inoculation, plants were placed in M-4 reach-in controlled-environment chambers (Environmental Growth Chamber Corp., Chagrin Falls, OH). Relative humidity was maintained at 50% or less, and the photoperiod was 14 hr light/10 hr dark in all chambers. Light measurements were made before the first trial, and intensities were adjusted to $1.89 \times 10^4 \pm 0.23 \times 10^4$ lux. All plants were watered daily with a Chapin watering system (Chapin Watermatics, Watertown, NY) connected to a timer. Chamber temperatures were constant at 24, 27, 29, 32, or 35 ± 1 C.

Plants were rated for symptoms on alternate days for 4 wk. The rating system was a modification of that described by Engelhard and Woltz (7) 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 the number of inoculated plants to give an average total rating (ATR).

Eight plants of each cultivar were placed in each chamber, and six were inoculated and rated for symptom development while two served as uninoculated controls. The experiment was performed twice with isolate 0-807, and one trial included the cultivar Glowing Mandalay substituted for Mandalay. The temperature experiment was performed once with isolate 0-734, using cultivars Royal Trophy, Mandalay, and Torch. Regression analysis was used to determine differences among ATRs of each treatment. Soil and stem temperatures were monitored in one trial with a multipoint strip-chart recorder (Honeywell Industrial Division, Fort Washington, PA).

In two of the trials, some asymptomatic plants were tested to determine whether the pathogen was present after 4 wk. Stems were cut at the soil line and leaves removed. A rough outline of the plant was made on lined paper. Stems were then disinfested in 0.525% 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) (14). Plates were examined after 3–5 days for fungal growth. Sections of the stem in which the pathogen was present were noted on the outline, thus giving a diagram of the distribution of the pathogen in the plant.

Effects of temperatures on linear growth of the pathogen were also studied. Pieces of lyophilized cultures were placed on PDA and allowed to grow for 1 wk at 22 C. Plugs of hyphae 5 mm in diameter were removed from the advancing margin and placed on PDA in 9-cm plastic petri dishes. Ten dishes of each isolate were sealed with Parafilm, placed in plastic bags, and the plastic bags placed in the controlled-environment chambers. Linear growth, quantified by diameter of mycelial growth, was measured over a 6-day period. Diameters of growth on 10 dishes per isolate per temperature were averaged, and an analysis of variance and *F*-tests were performed to determine differences.

RESULTS

The sequence of symptoms that occurred in a particular cultivar was essentially the same regardless of temperature, but the rate at which symptoms occurred, the extent to which they developed, and the number of days to the onset of symptoms varied with temperature (Table 1). There was no difference in symptom response of the cultivars Mandalay and Glowing Mandalay.

In Royal Trophy, symptoms were first observed as wilting and/or chlorosis in one or two leaves. This was the extent of symptom development at 24 C. At 27 C, wilted leaves became necrotic and new leaves were curved. At 29 C, symptoms consisted of more extensive leaf wilting confined to one side of affected plants. Wilting of entire plants occurred at 32 C, and some plants became severely wilted without showing necrosis. Symptoms developed most rapidly and were most severe at 35 C and consisted of severe wilting followed by death of most plants 21 days after inoculation. Dark, sunken streaks developed along one side of the stem in which orange sporodochia were produced. Plants not killed were stunted compared with uninoculated control plants.

Initial symptoms in Mandalay and Glowing Mandalay were chlorosis and/or slight curvature of the upper leaves. At temperatures of 29 C and higher, these symptoms were accompanied by a slight wilting of the leaves. Stunting was observed at 32 and 35 C, and a few of the plants incubated at these temperatures were dead when the experiment was terminated.

Only 13 of 90 inoculated plants of Torch developed symptoms. At 27 C, one plant developed symptoms consisting of slight chlorosis of upper leaves. Chlorosis and curvature were apparent in leaves of plants at 29 C, and occasionally, these leaves developed irregular margins resulting in a serrated appearance followed by necrosis of affected leaves. At 32 C, initial chlorosis and curvature were accompanied by wilting of leaves. Symptom progression at 35 C was similar to that at lower temperatures except that plants were also stunted when the experiment was terminated.

ATRs of all trials showed that symptom development in cultivars varied

Table 1. Effects of constant temperatures on symptom expression by the chrysanthemum cultivars Royal Trophy, Mandalay, Glowing Mandalay, and Torch inoculated with *Fusarium oxysporum* f. sp. *chrysanthemi*

	Temperature (C)				
	24	27	29	32	35
Royal Trophy					
ATR[a]	0.8	6.3	15.3	23.8	34.0
Days to symptoms[b]	24.0	21.6	16.7	12.2	12.0
Total with symptoms[c]	5	14	17	16	18
Mandalay					
ATR[a]	0	0.7	4.1	14.1	17.3
Days to symptoms[b]		24.0	20.9	15.9	16.0
Total with symptoms[c]	0	4	8	12	15
Torch					
ATR[a]	0	0.1	1.4	0.4	0.9
Days to symptoms[b]		28.0	24.4	14.0	14.5
Total with symptoms[c]	0	1	4	2	6

[a] ATR = average total ratings of symptoms. Values are means of three trials.
[b] Values are means of three trials.
[c] Number of plants from three trials exhibiting symptoms. Total number of inoculated plants at each temperature was 18.

with temperature as measured by the ATRs of symptoms (Fig. 1). The vertical lines in Figure 1 indicate the range of the ATRs used to calculate each data point. For samples of the size used in our experiments, the range is essentially the same as the variance.

Regression analysis of ATRs of all trials showed that three straight lines with significantly different slopes best described the results. The line for Torch was not significantly different from one with a slope of zero, although there appeared to be a greater response at temperatures of 29 C and higher. The line that described the response pattern for Royal Trophy had the greatest slope, whereas that for the Mandalay cultivars was intermediate. Symptom development in three cultivars was directly proportional to temperature. There was no significant difference between the two isolates of *F. o.* f. sp. *chrysanthemi.*

Isolations from asymptomatic plants showed they were colonized by the pathogen. Only two of the 12 plants at 24 C from which isolations were made were infected, and these were Royal Trophy and Mandalay plants. At 27 C, of seven plants tested, five were colonized including one Royal Trophy, two Mandalay, and two Torch. Plants incubated at 29 C that were tested included only Mandalay and Torch because all plants of Royal Trophy showed symptoms. Three of the six symptomless Mandalay were tested, and all were colonized. Two of the three plants of Torch that were tested were also colonized. At 32 C, there were 14 symptomless plants of which five were tested and one Royal Trophy and one Torch plant were colonized. Three of eight symptomless plants from 35 C were cultured, and only one plant of the cultivar Torch was colonized. There was no pattern of colonization at the various temperatures, nor was there a difference between isolates.

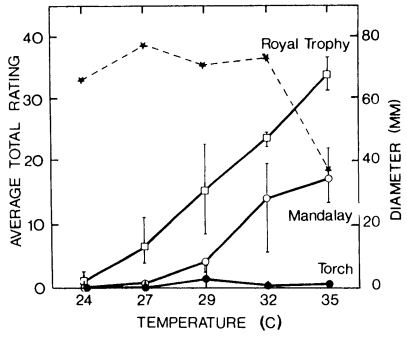

Fig. 1. Means of the average total ratings of symptoms for the chrysanthemum cultivars Royal Trophy, Mandalay, and Torch. Bars indicate ranges of ratings from three trials. Dashed line shows average colony diameters after 6 days for the three isolates of *Fusarium oxysporum* f. sp. *chrysanthemi* grown on potato-dextrose agar at five constant temperatures under a 14-hr light/10-hr dark photoperiod.

Linear growth of the three isolates on PDA was greatest at 27 C for 0-807, 27–32 C for 0-734, and 27 and 32 C for 0-693 although there was no statistically significant difference among isolates. However, the effects of temperature on all isolates were significant over the temperature range tested ($P = 0.05$). Linear growth in diameter of mycelia increased slightly from 24 to 37 C, whereas at 27, 29, and 32 C, diameters were about equal, and growth of all isolates was poor at 35 C (Fig. 1).

DISCUSSION

Symptom progression in susceptible plants was somewhat different from that described in the literature. Engelhard and Woltz (7) found that unilateral chlorosis of one or more leaves occurred initially followed by slight to pronounced curvature of leaves. Their rating system was based on this progression. In these experiments, Royal Trophy showed wilting as an early symptom, and a revised rating system was used. It became obvious that symptom development depended on the cultivar and the incubation temperatures used.

In cultivars Royal Trophy and Mandalay infected with *F. o.* f. sp. *chrysanthemi*, symptom development showed a linear response to temperature. The ATR of Royal Trophy was higher than those of the other two cultivars at all temperatures tested. Mandalay was moderately susceptible and did not develop pronounced symptoms until the temperature was higher than 27 C. Torch appeared to be tolerant because symptoms did not develop until temperatures were 29 C or more and were never severe. These results differed somewhat from those of Engelhard and Woltz (7), who considered Torch to be susceptible to wild isolates of *Fusarium* sp. but somewhat tolerant to *F. o.* f. sp. *chrysanthemi.* Engelhard and Woltz did not identify the species of wild isolates of *Fusarium.*

The rating that a plant received was not correlated with colonization of the plant by the pathogen, because some symptomless plants were colonized at lower temperatures. As the temperature increased, fewer symptomless, infected plants were found. Not enough plants were cultured to determine if this phenomenon was significant. This is important for flower producers, because recommended temperatures for chrysanthemum production are 16 C at night and fluctuating temperatures during the day (11). Cuttings from plants that are infected and show no symptoms become symptomatic when planted and grown at higher temperatures. Chrysanthemums are vegetatively propagated, and these cuttings may be an important means of pathogen transmission. The only method for control is to establish production plants from culture-indexed mother plants.

Fusarium wilt of chrysanthemum was unusual in its response to temperature when compared with other diseases caused by *F. oxysporum.* The optimum temperature for development of tomato wilt, caused by *F. o.* f. sp. *lycopersici* Sacc., was 28 C (4). Above 34 C or below 20 C, symptoms were not expressed. When soil temperatures were 35 C, plants did not become infected. Soil temperatures around 27 C were most favorable for rapid symptom development in Fusarium wilt of watermelons caused by *F. o.* f. sp. *niveum* (E. F. Sm.) Snyd. & Hans. (19), and no infection occurred above 33 C. The optimum temperature for wilt of muskmelons caused by *F. o.* f. sp. *melonis* (Leach & Currence) Snyd. & Hans. was 22 C (13), and symptom expression decreased rapidly from 22 to 34 C. In the studies mentioned above, the optima for symptom expression ranged from 22 to 31 C, and at temperatures higher than 31 C, there was a rapid decline in symptom expression. For the chrysanthemum cultivars Royal Trophy and Mandalay, the optimum temperature for symptom development had not been reached at 35 C. This explains why Fusarium wilt of chrysanthemum is more noticeable in greenhouses and production areas during the summer, when day temperatures are likely to be high.

Although the optimum temperature for in vitro growth of the chrysanthemum wilt pathogen was similar to other formae speciales of *F. oxysporum*, the finding that the optimum for symptom expression was higher than that for in vitro growth was not typical. In many studies, it has been shown that these two temperatures are identical or nearly so (4,18,19). Some studies have shown that the optima for symptom development were lower than those for in vitro growth by as much as 8 C (13,15). The optimum temperatures for in vitro growth of three isolates of the chrysanthemum wilt pathogen ranged from 27 to 32 C. These were lower than the optimum for symptom development. Stem temperatures were 1–2 C lower than the air temperature. This might explain why the optimum for symptom expression in cultivars Mandalay and Royal Trophy was 35 C whereas the optimum for fungus growth was 32 C. However, the response of Fusarium wilt of chrysanthemum to temperature may involve more than the direct effect of temperature on development of the pathogen.

The cultivar Mandalay was considered tolerant to Fusarium wilt under greenhouse conditions and was used to study the mechanism of tolerance (17). Plants were maintained at ambient air temperature with a constant soil temperature of 27 C. Anatomical responses in the suscept to *F. o.* f. sp. *chrysanthemi* were

limited and were not considered to account for tolerance in this cultivar. Tolerance appeared to have a physiological basis.

In this study, the tolerance mechanism appeared to break down as the temperature increased. Mandalay showed severe symptoms when the temperature was 29 C or higher. The ATRs were 0, 0.7, and 1.3 at 27 C and increased to 4.5, 3.3, and 4.5 at 29 C in the three trials carried out at these temperatures. It was also noted that control plants did not differ morphologically at these temperatures, although plants at 32 and 35 C were slightly stunted. Stems were not sectioned, so it is not known whether there were anatomical changes caused by temperature. However, temperature does affect physiological processes in the plant, and a physiologically based tolerance might be expected to vary with temperature as was observed.

The mechanism responsible for the apparent resistance in the cultivar Torch did not respond significantly to temperature. If chrysanthemum plants localized the pathogen by anatomical changes, as in tomato (3), then plants of the resistant cultivar would be less extensively colonized by the pathogen. However, plants tested in these experiments proved to be colonized to about the same extent as more susceptible cultivars. Resistance or tolerance in Torch appeared to be a physiological response although one that was less influenced by temperature than that which occurred in Mandalay.

It should be emphasized that tolerance in the cultivars Torch and Mandalay was expressed as a decrease in symptom expression. Plants became infected and colonized by the pathogen even under conditions unfavorable for symptom development. Therefore, culture indexing continues to be a useful and important procedure for the production of chrysanthemums that are free of vascular pathogens.

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