Effect of Temperature on Development of Kabatiella caulivora in Trifolium subterraneum

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ABSTRACT

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Effects of temperature on development of clover scorch (northern anthracnose), which is incited by *Kabatiella caulivora,* were examined in *Trifolium subterraneum* 'Yarloop'. Relatively high day/night temperatures (30/25 C or 33/28 C) for 1-7 days before inoculation accelerated leaf development and predisposed plants to infection. For establishment of infection during days 1 to 4 after inoculation (under low light and high humidity), temperatures of 16, 20, and 24 C were more favorable than 28 C. For development of the disease during days 4 to 11 after inoculation (under natural light and uncontrolled humidity), the optimum temperature range was 8-20 C. For plants kept at 20, 24, and 28 C during days 4 to 11, a reduction in light intensity to one-tenth of natural light did not significantly affect development of the disease.

Clover scorch (northern anthracnose), which is incited by *Kabatiella caulivora,* is distributed widely in red clover grown in the moist, temperate regions of the world (1, 2). In recent years, the disease has caused considerable damage in subterranean clover in southern parts of Australia. This has prompted studies of physiological factors (such as plant age) and environmental factors (such as humidity and light) that affect development of the disease in that host (3, 4, 5). In general, conditions which were unfavorable for plant growth, favored the development of the disease. The aim of the present paper was to examine the effects of temperature on predisposition of plants to infection, on the establishment of the disease under low light and high humidity during the first 4 days after inoculation, and on the subsequent development of the disease under natural light and uncontrolled humidity.

MATERIALS AND METHODS

Procedure for plant growth, infection, and disease assessment.—Plants of *Trifolium subterraneum* L. 'Yarloop' were grown as described previously (3) in a naturally-lit area of the Canberra phytotron which was maintained, except when stated otherwise, at 21 C during an 8-hour day and at 16 C during the night. Four weeks after sowing, plants were inoculated with a suspension of spores of isolate WA_1 of *Kabatiella caulivora* (Kirchn.) Karak by means of an atomizer (3). For establishment of infections, during days 1 to 4 after inoculation (early post-inoculation, EPI) plants were maintained at specified temperatures in Plexiglas humidity boxes in artificially-lit cabinets in which they received an 8-hour photoperiod of fluorescent light [753 lux (= 70 ft-c = 1.7 W m^{-2})] (3). For development of the disease, during days 4 to 11 after inoculation (late postinoculation, LPI) plants were placed in a C-type cabinet (7) maintained at specified temperatures and with natural light supplied during an 8-hour photoperiod. On day 11, attached leaves were rated as diseased, dead or normal (folded and expanded leaves). Leaves were rated as diseased when one or more lesions were identified on the petioles or petiolules, or occasionally on the leaflets (3). The total number of leaves per plant was the sum of the attached leaves.

In each experiment, there were six pots per treatment and five or six plants per pot (i.e., 30 or 36 plants per treatment). Usually, experiments were repeated three or four times on different occasions.

Effects of preinoculation temperature.—In the first experiment, plants were exposed to four different temperature conditions during 1 week before inoculation. Test plants were grown for 3 weeks after sowing at a day/night temperature of 21/16 C and then were transferred for 1 week before inoculation to temperatures of 30/25, 27/22, or 24/19 C. Control plants were grown continuously at 21/16 C. After inoculation, all test and control plants were kept at 20 C. In the second experiment, plants were grown at 21/16 C, as above, and then were transferred to 33/28 and 30/25 C for 7, 5, 3, or 1 day before inoculation. Control plants were grown continuously at 21/16 C. After inoculation, all plants were kept at 20 C.

Effects of postinoculation temperature.—The first experiment was done in six parts over a period of 2 months (February and March 1975). During EPI half of the test plants were kept at 16 C and half at 20 C; during LPI, six pots of plants from both 16 and 20 C were kept at each of 16, 20, 24, and 28 C (parts one and two), at 12, 16,

20, 24, and 28 C (part three) and at 8, 12, 16, and 20 C (parts four to six). In the second experiment, during EPI, half the test plants were kept at 24 C and half at 28 C; during LPI, six pots of plants from both 24 and 28 C were kept at 20, 24, and 28 C.

Effects of postinoculation temperature and light.—During EPI, plants were kept at 20 C; during LPI they were kept at 20, 24, or 28 C. At each of these LPI temperatures, one treatment received natural light and another one-tenth natural light by shading with Sarlon shade cloth (Sarlon Industries, Waterloo, New South Wales).

Analyses.—Data were analyzed by analyses of variance. Original data were used, except for numbers and percentages of diseased leaves in the experiments on the effects of postinoculation temperature on disease development. In the first of these, the susceptibility of six successive lots of test plants (parts one to six), increased during the experimental period, presumably due to a reduction in light intensity and/or photoperiod associated with seasonal change. For example, the mean percentages of diseased leaves for EPI 16 and 20 C (the two treatments common to all parts), were 22.2, 21.0, 27.8, 30.5, 27.5, and 31.2% for parts 1, 2, 3, 4, 5, and 6, respectively. Therefore, it was necessary to adjust the data to a common level of susceptibility, so that valid comparisons could be made between the LPI temperatures. Before the adjustments were made, the data for numbers of diseased leaves per plant were given a $\log_{10}$ transformation (because the means and variances were correlated), and the data for percentages of diseased leaves were given an arcsine transformation (because some percentages were very low). Part four was chosen arbitrarily as the standard to which all other parts were adjusted as follows: (i) the mean of the data for 16 C and 20 C (these being the temperatures common to all six parts) was calculated for each part; (ii) the differences between each of these means and that of part four were calculated; (iii) for each part (except part four), the differences calculated in (ii) were added to each data item to obtain the adjusted transformed data, which were used in the analyses. In the second experiment, the percentage data were given an arcsine transformation.

RESULTS

Effects of preinoculation temperature.—A greater percentage of leaves developed lesions in plants exposed to 30/25 C during 1 week before inoculation, than in plants exposed to lower temperatures during the same period (Table 1); i.e., a high day/night temperature predisposed plants to infection.

To determine if plants exposed to high temperatures for less than 1 week before inoculation would become more susceptible, disease development was examined in plants kept at 33/28 or 30/25 C for 7, 5, 3, or 1 day before inoculation. Data from the two high-temperature treatments were similar and were combined for analysis and presentation (Table 2). For plants kept at high temperatures for 1 to 7 days before inoculation, the percentage of diseased leaves was greater than that for control plants. Among the high-temperature treatments, the differences between days 7 and 5 and between days 7 and 3 were significant.

Because previous data (3) showed that symptoms developed only on petioles and petiolules of leaves with leaflets expanded at the time of inoculation (expanded leaves), an increase in susceptibility of plants following exposure to high temperatures before inoculation could be related to a temperature-induced increase in numbers of expanded leaves. Data in Tables 1 and 2 show that exposure of plants to high temperatures for short periods of time increased the numbers of expanded leaves per plant. Differences reached significance in Table 2 but not in Table 1. At the time of harvest, 11 days after inoculation, there were no significant differences between treatments, in respect to the total numbers of leaves per plant (mean total number of leaves per plant at harvest was 10.5 and 14.6 in Tables 1 and 2, respectively).

Effects of postinoculation temperature.—The effects of postinoculation temperature on disease development were examined during EPI and LPI (Table 3). There were no differences in the percentages of diseased leaves, between plants which received 16 and 20 C during EPI, except for plants that were kept at 8 C during LPI. Differences in percentages of diseased leaves between

TABLE 1. Effect of preinoculation temperature on development of *Kabatiella caulivora* in *Trifolium subterraneum* and on expansion of leaves[x]

Day/night temperature (C)	Expanded leaves per plant at inoculation[y] (mean no.)	Diseased leaves (%)[y,z]
21/16	5.7 a	23.5 ab
24/19	6.6 a	25.5 b
27/22	6.7 a	21.6 a
30/25	6.6 a	31.4 c

[x]Plants grown at 21/16 C for 3 weeks after sowing and transferred to higher temperatures for 1 week before inoculation. After inoculation, plants received 20 C continuously. Only petioles and petiolules of leaves with leaflets expanded at inoculation became infected (3).

[y]Data followed by the same letter in each column are not significantly different ($P = 0.05$).

[z]Ratio of number of diseased leaves over total number of leaves at harvest multiplied by 100.

TABLE 2. Effect of high temperatures, during 1 to 7 days before inoculation, on susceptibility of *Trifolium subterraneum* to *Kabatiella caulivora* and on expansion of leaves[x]

Length of treatment (days)	Expanded leaves per plant at inoculation[y] (mean no.)	Diseased leaves (%)[y,z]
0	3.9 a	16.9 a
1	4.2 a	23.2 bc
3	4.6 b	21.1 b
5	4.8 b	22.2 b
7	4.9 b	25.5 c

[x]Plants grown at 21/16 C and transferred to 33/28 and 30/25 C for 1 to 7 days before inoculation at 4 weeks after sowing. Data for the two high-temperature conditions were combined for analysis. Only petioles and petiolules of leaves with leaflets expanded at inoculation became infected (3).

[y]Data followed by the same letter in each column are not significantly different ($P = 0.05$).

[z]Ratio of number of diseased leaves over total number of leaves at harvest multiplied by 100.

plants which received 24 and 28 C during EPI were significant at LPI temperatures of 20, 24, and 28 C.

In general, temperatures of 16, 20, and 24 C during EPI, were more favorable for disease development than 28 C.

For plants kept at 20 C during EPI, the highest percentage of diseased leaves developed during LPI at 8 C and decreased with increase in temperature (Table 3). In contrast, for plants kept at 16 C during EPI, the highest percentage of diseased leaves developed during LPI at 12 to 20 C. This difference between the data for plants kept at 16 and 20 C during EPI was largely a consequence of the method of assessment. Use of percentages (ratio of numbers of diseased leaves over total numbers of leaves at harvest) can be misleading when variations in numbers of diseased leaves are small relative to variations in total numbers of leaves. In this experiment, numbers of diseased leaves per plant kept at 16 and 20 C during EPI changed very little during LPI temperatures of 8 to 20 C, although changes for plants kept at 16 C were greater than those of plants kept at 20 C (data in brackets in Table 3). With the same temperature treatments, changes in the total number of leaves per plant were pronounced (Table 3). Since the pathogen did not spread from the inoculated leaves to leaves which developed during the course of the experiment (3), it was valid to assess the effects of temperature on disease development in terms of numbers of diseased leaves per plant. Using this method, the optimum temperature range for disease development in plants kept at 16 and 20 C during EPI was 12 to 20 C and 8 to 20 C, respectively (data in brackets in Table 3). These results are considered to be more valid than those obtained with percentages, since the patterns of disease development in response to temperature for plants exposed to 16 and 20 C during EPI are more similar; moreover, they are in accord with observed symptoms (the size and darkness of lesions on the petiole), which were most severe at LPI temperatures of 12, 16, and 20 C and least severe at 28 C.

The data show that once the pathogen had become established, the disease developed favorably over a wide range of low temperatures (8 to 20 C).

Postinoculation temperature and light.—The effect of light intensity on development of the disease was examined during LPI at 20, 24, and 28 C. In agreement with previous data (Table 3), the percentage of diseased leaves decreased with increase in LPI temperature. However, the percentages of diseased leaves for plants grown in natural light and shade (brackets) did not differ significantly: LPI 20 C-25.41% (23.93%); LPI 24 C-13.24% (19.75%); and LPI 28 C-5.70% (8.25%).

DISCUSSION

Predisposition (10) may play a significant role in the epidemiology of clover scorch in subterranean clover. Susceptibility was affected by the age of plants and by wounding (3), and also by high temperatures (33/28 or 30/25 C) to which plants were exposed 1 to 7 days before inoculation. Since expanding petioles are highly susceptible (3) and high temperatures accelerate expansion of leaves (Tables 1 and 2), the exposure of plants to high temperatures for short periods could increase susceptibility by promoting cell expansion; e.g., the pathogen may penetrate expanding cell walls more readily than mature cell walls. Alternatively, treatment with high temperature could increase susceptibility by affecting biochemical constituents of the cells.

High temperature (28 C) during EPI and LPI was inhibitory. This could be due to a direct effect of high temperature on the pathogen (9); alternatively, since mature leaves are less susceptible than newly expanded leaves (3) and since leaves are reduced in size at high temperatures (8), the inhibitory effect could be due to the development of resistance to growth of the pathogen, in leaves which cease expansion or mature relatively early.

The optimum temperature for the development of the disease ranged from 8 or 12 C to 20 C. These temperatures

TABLE 3. Effect of postinoculation temperature on development of *Kabatiella caulivora* in *Trifolium subterraneum* and on growth of the host plant[s]

LPI[t]	Mean total leaves per plant at harvest EPI[u]	Diseased leaves (%)[v] EPI[u]					
	16 and 20[w,x]	16[x]		20[x]		24[y]	28[y]
8	16.1 a	25.2 bc	(3.9 b)[z]	40.1 e	(6.3 c)[z]		
12	18.7 b	33.6 d	(6.2 c)	36.9 de	(6.7 c)		
16	22.0 c	33.5 d	(7.4 c)	30.8 cd	(6.7 c)		
20	25.5 d	29.6 cd	(7.5 c)	28.1 c	(7.3 c)	23.0 c	5.2 c
24	27.5 e	18.1 b	(4.7 b)	16.0 b	(4.1 b)	16.3 b	2.7 b
28	27.8 e	7.1 a	(1.7 a)	5.2 a	(1.3 a)	7.6 a	0.4 a

[s]Data obtained 11 days after inoculation. Means followed by the same letter in each column are not significantly different ($P = 0.05$).

[t]LPI = late postinoculation temperature (C); i.e., during days 4 to 11 after inoculation when plants were exposed to natural light and uncontrolled humidity.

[u]EPI = early postinoculation temperature (C); i.e., during days 1 to 4 after inoculation when plants were exposed to low light and high humidity.

[v]Ratio of number of diseased leaves over total number of leaves at harvest multiplied by 100.

[w]Mean data for EPI 16 C and EPI 20 C.

[x]Means of original data from the first experiment, adjusted as described in the text.

[y]Means of original data from the second experiment.

[z]Mean number of diseased leaves per plant.

extend from well below the optimum to near the optimum for plant growth measured in terms of leaf number (Table 3) or dry weight production; viz., a mean daily temperature of 18.6 C (6). The optimum temperature range for development of the disease in subterranean clover is lower than that reported for red clover; viz., 20 to 24 C (1, 2). However, the experimental data available for red clover at temperatures below 20 C are few.

Over a range of postinoculation temperatures from 20 to 28 C, a reduction in light intensity to one-tenth that of natural light produced no significant effect on the development of the disease. This result is in accord with previous data (4) which showed that at 20 C a reduction in light intensity to less than 5,380 lux (500 ft-c) was required before an increase in the percentage of diseased leaves occurred. It is evident that light intensity is unlikely to influence development of the disease, except in dense stands.

The present data provide further support for previous evidence that *K. caulivora* is a weak pathogen that can cause severe symptoms in plants grown under suboptimal conditions (3, 4).

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