Effects of Water Stress on Thyronectria Canker of Honeylocusts

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ABSTRACT


Drought-stressed 1- to 3-yr-old honeylocust trees (Gleditsia triacanthos) developed significantly (P = 0.05) smaller Thyronectria cankers (Thyronectria austro-americana) than well-watered seedlings, as measured by bark canker size. Analysis of variance of vertical canker size and percent girdling indicated significant interactions of linear trends across time with isolate and stress factors. Xylem (staining and colonization) canker size was not affected by the stress treatment. Pressure bomb assessment of the trees' water potentials was well correlated with both bark and xylem studies. Two isolates obtained in 1981 (429) and 1982 (433) from canker margins on honeylocusts in eastern Nebraska were used in the Nebraska studies; these isolates are referred to in the text as isolates 1 and 2, respectively. Before inoculation, the fungi were grown on potato-dextrose agar for 10–14 days at 26–28 C.

Inoculation. The bark surface of trees was disinfested with 70% ethanol, and a cork borer wound of 4 or 5 mm was made by removing the bark to the xylem on each tree 5 or 10 cm above the soil line. In one experiment in Colorado, a second, higher wound 20 cm above the soil was used. A 5-mm agar plug containing mycelium and conidia was placed in the wound. A Parafilm wrap on the wound was applied for 2 wk. Nine trees per isolate-stress combination were used in the Nebraska experiment, and 8 to 15 trees per treatment were used in each of three experiments in Colorado.

Water-potential measurement—Nebraska. All trees designated for water-stress treatment were water stressed by withholding water starting 4 wk before inoculation. Nonstressed trees were watered every 3–4 days during this time. At 2 wk before inoculation (May 2, 1985), two predawn water-potential measurements were made on each tree with a pressure bomb using two leaflets per tree. Weights of each pot were recorded at this time. Mean water potentials 2 wk before inoculation for nonstressed and stressed trees were −0.19 ± 0.07 (n = 18) (mean ± standard deviation) and −0.82 ± 0.43 MPa (n = 18), respectively. The stressed trees were watered and then allowed to dry twice at 7-day intervals before inoculation. Stress levels were maintained by adding water to maintain pot weights. Two predawn water-potential measurements on each tree also were taken 11 days after inoculation and at approximately 3-day intervals thereafter until the test was terminated 46–47 days after inoculation. Immediately after each water-potential measurement, weights of all pots with trees were reestablished by watering to weights obtained 2 wk before inoculation.

Water-potential measurement—Colorado. Water potentials were measured with a pressure bomb at 4:00 A.M. twice per week for one leaflet per tree. The nonstressed trees were watered weekly, and the stressed trees were watered with 500 ml/pot when the water potential approached −3.0 MPa. There were three to four drying cycles for stressed trees in an experiment. Stressed treatments were imposed for 2 wk before inoculation.

Data collection—Nebraska. The size of cankers was determined when water potential was measured, by measuring discoloration of the bark surface above and below the inoculation wound (excluding the size of the wound). The percentage of a stem's cir-
cumference girdled was determined using the method of Kessler (17). At the end of the experiment, bark was removed from cankers, and the length of the elliptical, discolored, and colonized xylem area was measured and recorded as xylem canker size. Isolations were made at margins of discolored xylem above and below the inoculation wound on each tree. The length of narrow red streaks in the xylem also was measured.

**Data collection—Colorado.** Bark canker sizes were recorded 32–38 days after inoculation by measuring the horizontal and vertical diameters of discolored bark. At the end of the experiment, the bark was stripped and the xylem canker size was determined. In the three experiments in Colorado, the total length of narrow red streaks in the xylem that originated at the cankered and discolored area was measured. Isolations were made from margins of the discolored xylem on 50% of the cankers.

**Data analysis—Nebraska.** Vertical canker size and percent girdle expansion over time were analyzed by a repeated measures analysis of variance with two factors: isolate and stress treatments. Polynomial regressions were used to characterize the time trends displayed in Figures 1 and 2. Because the isolates’ rates of vertical growth and percent girdling differed depending on whether their hosts were stressed, the initial analysis was followed with a one-factor analysis of variance of isolate-stress combinations for each date. Pair-wise differences in total vertical canker size among isolate-stress combinations were assessed using Tukey’s HSD procedure (α = 0.05), with results presented in Table 1. No interaction between isolate and stress treatments was found for percent stem girdle, but pressure bomb data were found to be a significant covariate. Results, both unadjusted and adjusted by analysis of covariance, are presented in Table 2. Differences among isolate-stress main effects were tested using one-factor analysis of variance. All computations were produced using the Manova procedure in the SPSS-X User’s Guide (28).

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**Fig. 1.** Predicted values (unadjusted for pressure bomb values) of average total vertical canker size on nonstressed and stressed trees of *Gleditsia triacanthos* inoculated with isolate 1 (429) or 2 (433) of *Thyrocentria austro-americana* 11 to 47 days after inoculation (Nebraska test). Predicted values are from cubic least-squares regression of canker size on date.

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**Fig. 2.** Predicted values (unadjusted for pressure bomb values) of average percent of stem circumference girdled on cankered nonstressed and stressed trees of *Gleditsia triacanthos* inoculated with isolate 1 (429) or 2 (433) of *Thyrocentria austro-americana* 11 to 47 days after inoculation (Nebraska test). Predicted values are from cubic least-squares regression of percent girdle on date.

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**Data analysis—Colorado.** Horizontal and vertical diameter measurements were added together to calculate canker size. Canker size was compared between stress treatments using analysis of variance with the log10-transformed pressure bomb readings for each tree used as a covariate (Table 3). Log transformations were used because the data were skewed. All computations were produced using the Manova procedure in the SPSS-X User’s Guide (28).

**Influence of osmotic stress on fungal growth.** Colorado isolate (83–28) of *T. austro-americana* was used in this study. The fungus was grown for 2 wk before use in osmotic tests on a basic medium consisting of agar (20 g/L), glucose (20 g/L), asparagine (2 g/L), KH2PO4 (1 g/L), MgSO4 (0.5 g/L), Fe (0.2 mg/L), biotin (5 μg/L), thiamine (100 μg/L), and a combination of Zn (0.2 mg/L) and Mn (0.1 mg/L). To produce various osmotic levels, the basic medium, minus agar, was amended with 0, 158, 230, 301, and 373 g/L of polyethylene glycol 3350 (PEG) and autoclaved. A similar set of media without glucose was prepared to assess the nutritional effects of PEG.

The osmotic potentials of the media were assessed using a Wescor C-51 psychrometer (Wescor, Inc., Logan, UT) calibrated against known NaCl solutions. The osmotic potentials of the five treatments before inoculation were −0.36, −1.17, −1.82, −3.15, and −4.77 MPa in media with glucose and −0.78, −1.45, −2.43, and −4.64 MPa in media without glucose.

Three 250-ml flasks containing 125 ml of media per treatment were inoculated with 8-mm-diameter disks of the fungus with minimal agar retained on the disks. Two flasks per treatment were left uninoculated as controls. Flasks were placed on a rotary shaker and incubated for 8 days at 22–24°C. The flask contents were vacuum filtered through Whatman No. 4 filter paper that had been dried previously at 100°C for 4 days and weighed. The residue on the filter paper was rinsed twice with hot water to remove excess PEG. Filter papers with fungal residue were dried for 4 days at 100°C, and dry weights were measured to the nearest 0.0001 g. The experiment was repeated three times.

The increase in dry weight of the filter papers minus the dry weights of the two papers from uninoculated flasks was analyzed. The uninoculated controls were used to correct for PEG residue on the filter papers. Because there were unequal variances among treatments, log10 transformations of the corrected weights plus one were performed before analysis. An analysis of variance using the Manova program (28) was used to analyze fungal growth at each osmotic concentration. An analysis of linear, quadratic, and lack of fit relations of the treatment with glucose was performed using a Manova program with polynomial contrasts.

**Table 1.** Average vertical canker sizes on young trees of *Gleditsia triacanthos* inoculated with isolates of *Thyrocentria austro-americana* and subjected to water stress for 47 days — Nebraska test.

<table>
<thead>
<tr>
<th>Treatment interactions</th>
<th>Bark canker size (mm); days after inoculation*</th>
<th>Xylem canker size (mm)</th>
<th>Mean water potential* (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No stress — isolate 1</td>
<td>25.2 b 45.0 b 50.2 a 53.7 a 54.4 a 0.61</td>
<td>25.2 b 19.8 ab 20.2 a 19.8 ab 19.8 ab 20.2 a</td>
<td>25.2 b 19.8 ab 20.2 a 19.8 ab 20.2 a</td>
</tr>
<tr>
<td>No stress — isolate 2</td>
<td>19.8 ab 43.9 b 53.1 a 58.0 a 59.4 a 0.55</td>
<td>19.8 ab 43.9 b 53.1 a 58.0 a 59.4 a 0.55</td>
<td>19.8 ab 43.9 b 53.1 a 58.0 a 59.4 a 0.55</td>
</tr>
<tr>
<td>Stress — isolate 1</td>
<td>20.2 a 44.9 a 56.4 a 56.4 a 56.4 a 1.65</td>
<td>20.2 a 44.9 a 56.4 a 56.4 a 56.4 a 1.65</td>
<td>20.2 a 44.9 a 56.4 a 56.4 a 56.4 a 1.65</td>
</tr>
<tr>
<td>Stress — isolate 2</td>
<td>13.7 a 36.4 a 43.7 a 56.1 a 2.24</td>
<td>13.7 a 36.4 a 43.7 a 56.1 a 2.24</td>
<td>13.7 a 36.4 a 43.7 a 56.1 a 2.24</td>
</tr>
</tbody>
</table>

*Data from every third reading are given. Means in a column followed by similar letters are not significantly (P = 0.05) different as tested by Tukey’s HSD procedure. Each mean represents nine trees. Mean length of cankers excludes the inoculation wound area.

*Xylem canker size is the vertical size of discolored and colonized xylem tissue measured 47 days after inoculation.

*Mean water potential is the average of pressure bomb readings 11–47 days after inoculation.
RESULTS

Nebraska. Water-stressed 1-yr-old trees had significantly smaller bark cankers than trees watered regularly (Tables 1 and 2). Analysis of variance of total vertical canker size indicated significant interaction of linear trends across time (date) with isolate and stress (Fig. 1). These results indicate that the changes in canker size over time for each of the four isolate-stress combinations were different and should be considered individually. Because significant quadratic and cubic polynomial contrasts occurred, differences in canker size should be examined over time. Significant linear and quadratic stress by date interactions for both vertical canker size and percent girdling also were found (Fig. 2). Thus, the effects of stress apparently changed over time. Significant differences in canker sizes between stressed and nonstressed trees ended 30 days after inoculation. Cankers expanded more above the wound than below for all readings. The total vertical size was chosen, however, to represent vertical canker expansion because it correlated well with the relation of the below-wound and above-wound canker expansion.

Stems of several nonstressed trees were girdled completely by the end of the experiment. The average stem circumference girdled was 89%. None of the stressed trees was completely girdled by the cankers. In Nebraska, stems above the inoculation site on four of nine nonstressed trees inoculated with isolate 1 died within 22–32 days after inoculation because of complete girdling of the bark and cambium. Similarly, stems above inoculation sites on three of nine nonstressed trees inoculated with isolate 2 died within 20–35 days because of complete girdling.

Isolates did not induce significantly different sized cankers as measured by vertical canker size or percent girdling. Isolate differences seen after 22 days could be accounted for by pressure bomb data used as covariates (Table 2). Significant isolate-stress interactions for vertical canker size indicated that one should study both isolate and stress factors in assessing canker size (Table 3).

Pressure bomb readings were significantly higher on stressed trees than on nonstressed trees (Table 1). Pressure bomb values were well correlated with canker size and explained the stress effect on canker size (vertical) and percent girdling 17–32 days after inoculation. After 32 days, pressure bomb readings were not correlated with canker size.

Significantly more stromatic conidiomata formed on nonstressed than on stressed trees. For example, 41 days after inoculation, the number of fruiting stroma per tree averaged 25.3 on nonstressed trees and 5.7 on stressed trees. The first stroma appeared 14 days after inoculation, but the largest increase appeared 30–40 days after inoculation. Only a slight (nonsignificant) effect of isolates on the number of stroma was found, with isolate 1 having more fructifying bodies than isolate 2. All sampled cankers yielded the pathogen. Narrow red streaks (2–6 mm) developed in the sapwood immediately adjacent to the wound in five of 18 control honeysuckles in the Nebraska test and were assumed to be a host reaction to wounding. T. austro-americana was not isolated from these streaks. Narrow red streaks developed extensively in the xylem of nonstressed (11–14 cm) and stressed (19–21 cm) inoculated trees, but there was no significant relationship between streak length and stress for both isolates.

Xylem canker size did not vary significantly among the isolate and stress treatments (Table 1).

Colorado. Significantly larger bark cankers were found on nonstressed 2- to 3-yr-old honeysuckles in the Colorado tests (Table 3). The xylem canker measurements were not significantly different among stress treatments and thus matched the Nebraska data. No significant relationship between the size of the red, discolored streaks and stress was found although some streaks were 10 cm in length.

The average pressure bomb readings were significantly different between stressed and nonstressed trees. As with the Nebraska data, no correlation between pressure bomb values and canker size occurred after 32 days. All cankers sampled, including the discolored xylem tissue and narrow red streaks, yielded the pathogen.

In vitro osmotic stress. An increase in PEG significantly reduced the growth of the pathogen in the glucose-based liquid culture. A significant linear relationship (P < 0.01) between fungal growth and PEG concentration was found using the log₁₀ of the average dry weights of the fungal growth in cultures with glucose and PEG minus the dry weights of the uninoculated controls (Fig. 3). Higher order polynomial effects were not significant. The equation relating the log of dry weight to negative MPa of solution was as follows:

\[ y = -0.14627 \text{ (MPa)} + 3.57 \]

Growth of the fungus was not stimulated by osmotic stress because growth was reduced between −0.4 MPa (normal medium conditions) and the addition of PEG at the lowest concentration, which produced an osmotic stress of about −1.2 MPa.

The PEG acted as a minor carbon source for the fungus. Analysis of variance of the dry weights with and without sugar indicated that, at each osmotic concentration, growth was sig-

|TABLE 2. Average percent stem circumference girdled on young trees of Gleditsia triacanthos inoculated with two isolates of Thyronectria austro-americana and subjected to water stress for 47 days — Nebraska test |
|---------------------------------|-------------|-------------|-------------|
|Treatment main effects          | Days after inoculation  |
|                                | 11 | 22 | 32 | 47 |
|No stress                       | 45.3 b | 80.0 b | 89.6 b | 89.1 b |
|Stress                          | 40.8 a | 58.1 a | 65.1 a | 69.0 a |
|Isolate 1                       | 46.4 a | 75.5 b | 82.6 b | 83.0 b |
|Isolate 2                       | 39.7 a | 62.7 a | 72.1 a | 75.1 a |
|Isolate 1 (adjusted)b \( \ldots \) | 72.8 a | 80.7 a | 81.6 a |
|Isolate 2 (adjusted)b \( \ldots \) | 65.3 a | 74.4 a | 76.5 a |

\( a \) Data from each third reading are given. Mean effect means followed by similar letters are not significantly (\( P = 0.05 \)) different as tested by analysis of variance without a pressure bomb value as a covariate. Each mean represents cankers on 18 trees, nine trees per stress per isolate combination.

\( b \) Isolate means are adjusted by the coefficient derived by using pressure bomb values as covariates.

\( c \) No pressure bomb readings were available to use as covariates on this date.

|TABLE 3. Average canker sizes on 2- and 3-yr-old trees of Gleditsia triacanthos inoculated with Thyronectria austro-americana and subjected to water stress for 32–38 days — Colorado test |
|---------------------------------|-------------|-------------|
|Experiment treatment \( a \) | Mean canker size \( b \) | Mean water potential \( c \) |
|                                | Bark (mm) | Xylem (mm) | (MPa) |
|1 Nonstressed                   | 28.5 a    | 41.9 a    | 0.97 |
|Stressed                        | 24.9 a    | 47.5 a    | 2.35 |
|2 Nonstressed                   | 21.9 b    | 73.2 a    | 0.96 |
|Stressed                        | 17.6 a    | 68.7 a    | 2.99 |
|3 Nonstressed                   | 32.2 b    | 49.7 a    | 0.86 |
|Stressed                        | 28.0 a    | 52.6 a    | 2.38 |
|Total nonstressed               | 28.7 b    | 52.2 a    |      |
|Total stressed                  | 25.6 a    | 54.4 a    |      |

\( a \) Stressed trees were drought stressed by withholding water. Nonstressed trees were watered regularly. Stressed trees were stressed 2-4 wk before inoculation and maintained in this condition by adding only enough water to prevent wilting for 32–38 days.

\( b \) Mean canker size at 32–38 days after inoculation — average of combined vertical and horizontal diameters. Means in a column within an experiment followed by similar letters are not significantly different as tested by analysis of variance. \( N = 8, 10, \) and 30 for the three experiments.

\( c \) Average pressure bomb values for the entire experiment.
DISCUSSION

The results of experiments with 1- to 3-yr-old honeylocusts indicate that drought-stressed trees had smaller bark cankers than nonstressed trees. The pathogen's reduced growth in culture under increased osmotic stress suggests that the reduced growth of the fungus may partly explain the smaller cankers on stressed trees. In contrast, *Cytospora kunzei* Sacc., a pathogen of drought-stressed spruce, produced maximum growth in culture between -1.5 and -2.0 MPa (22). Additionally, hyphae of *Botryosphaeria dothidea* (Moug.;Fr.) Ces. & de Not. in nonstressed trees were thin, contorted, and restricted, whereas growth was good in stressed trees (20).

Honeylocusts traditionally are thought to be somewhat drought tolerant and well suited for urban environments. Honeylocusts have small leaves, small stomatal dimensions, and low stomatal density, characteristics that may provide tolerance to drought (21). But honeylocusts also have poor stomatal control over water loss, which does not provide tolerance to drought stress (21). Our results, however, suggest that honeylocusts are tolerant of short-term (up to 30 days) drought as measured by canker expansion.

There was no definite relationship of xylem canker size to stress even though the fungus colonized a slightly larger area of xylem compared with bark canker size. Thus, separate interactions seem to occur between the pathogen and bark (periderm and phloem) and xylem tissues. Other trees and pathogens similarly showed larger colonized areas of xylem than of bark with drought, defoliation, and freezing stresses (24).

The colonization of xylem tissue may not be important, especially in relationship to canker expansion and drought-stressed trees. Bark colonization is restricted by host defenses, and the cambium is not killed above a majority of the area of colonized xylem. Thus, the cambium appears to be able to bury the fungus in the old xylem and thus contain the pathogen. The large colonized area of xylem and long periods of discolored xylem tissue do suggest, however, that the pathogen may move relatively easily through the vascular system. The pathogen is reported as a vascular wilt of Japanese honeylocust (*G. japonica* Miq.); therefore movement in the xylem is not surprising (26).

Pressure bomb readings gave a good measure of stress and correlated well with bark canker size until 30 days after inoculation. The lack of correlation after 30 days was shown in both the Colorado and Nebraska experiments. Thus, it is important to measure canker size before 30 days to show any relationship between drought stress and canker size. These results suggest that drought stress does not have a constant effect on host and pathogen interactions. The host may become more tolerant of drought and/or more resistant to pathogen colonization after 30 days of stress.

The aggressiveness of *T. astrop-americana* may be affected by low osmotic potential conditions. Possibly bark defenses or active biochemical defenses (20) are not affected adversely by drought stress in honeylocusts. Previous work on other woody plants, however, indicates slowed periderm production in drought-stressed plants (23); stressed poplars showed decreased lignification, reaction zone size, and organization of cells around wounds (7).

The production of fruiting stroma was correlated with canker size, and more fruiting bodies formed on nonstressed trees. Thus, overall it would appear that drought stress does not favor canker expansion and fungus sporulation. However, the results of these greenhouse studies may not be directly applicable to field conditions where trees are exposed to fluctuating drought and excess water. Field studies are needed to simulate drought cycles in a natural system where drought and overwatering may cause root damage and possibly a different set of stresses than were tested in this study.

LITERATURE CITED