TOTAL LEAF NITROGEN CORRELATED WITH WALNUT ANTHRACNOSE RESISTANCE

by Dan Neely

Abstract. Greenhouse studies of *Juglans nigra* confirmed the findings of field trials that fertilization with nitrogen reduces the severity of anthracnose caused by *Gnomonia leptostyla*. Total nitrogen content of leaves on seedlings of *Juglans* treated with a complete Hoagland's solution, with Hoagland's minus nitrogen, or with Hoagland's plus nitrogen ranged from 2.14 to 4.13%. Numbers of anthracnose lesions decreased with increased leaf nitrogen content (R=0.719).

Additional key words: epidemiology, cultural control, host nutrition/pathogen interaction.

Because nitrogen is essential for plant growth but of limited availability in soil, its effect on host nutrition and the severity of disease has been extensively studied. Generally, juvenile plant tissues are more vulnerable to disease than mature tissues because natural defense mechanisms are poorly developed in immature plants(6). This general observation, however, proved not to apply to anthracnose incited by *Gnomonia leptostyla* on black walnut, *Juglans nigra* (11). Nitrogen fertilizer enhanced the resistance of black walnut to anthracnose and delayed premature defoliation (11). Rate of application rather than form of nitrogen was correlated with disease resistance.

Juvenile foliage of black walnut has been observed to be less severely infected by anthracnose than more mature foliage. Researchers have postulated that the application of nitrogen fertilizer prolongs juvenility in black walnut (10). A thorough study of leaf age and susceptibility, however, revealed that this apparent resistance was due primarily to leaf expansion during the period between inoculation and the development of symptoms (approximately 14 days) (3). Trichome density on juvenile leaves also hampers placement of conidia on leaves (3). Lesion size and acervulus development are reduced on juvenile leaves (3). The concentration of juglone in walnut leaves decreases with leaf age, and this compound has been associated with disease resistance to walnut anthracnose (4) and pecan scab (7).

The purpose of this study was to correlate the effects of nitrogen fertilization and walnut anthracnose resistance by establishing the nitrogen content in leaves of plants subjected to five fertilization regimes and by observing the development of lesions following artificial inoculation under controlled conditions.

Materials and Methods

The seedlings used in this study were progeny from one tree and originated from nuts stored at 4 C over the winter and placed in sand on a mist bench for germination and shoot emergence as needed. When 3 to 6 cm tall, each seedling was placed in a greenhouse in a 3-L can containing vermiculite and washed sand (3:1 v/v). Seedlings were watered but not fertilized. After 2 wk, when the seedlings were 15 to 20 cm tall, the nut was removed from the seedling and the plant was repotted in the vermiculite and sand mixture. In preliminary tests, I found that the retention of nuts greatly modified the effects of nutrient treatments. Nutrients were supplied daily throughout the remainder of the test.

Modified Hoagland’s solutions were supplied to five groups of seedlings; these were Hoagland’s complete, Hoagland’s minus nitrogen, Hoagland’s minus phosphorus, Hoagland’s minus potassium, and Hoagland’s plus additional nitrogen (9). The plus-nitrogen treatment was a Hoagland’s complete plus 10 ml of 1 M NH₄NO₃ per liter. Each plant was irrigated daily with 100 ml of solution. Six tests were done from April through October in 1982. Within each test, there were 11 to 14 seedlings per treatment. Test 3 was twice as large as the others and divided into 3a and 3b. All treatments were not included in each test.

Leaf samples for chemical analysis were obtained immediately prior to inoculation. The second leaflet from the point of attachment of the rachis on the second youngest of the four leaves to be inoculated (leaf three) was removed from each plant. In each of tests two through six, leaflets
from one treatment were combined, oven-dried, ground, and total nitrogen content determined by the Kjeldahl method (13).

*Gnomonia leptostyla*, isolated from black walnut the previous summer, was grown in petri dishes on oatmeal agar for 2-3 wk at 21°C under a 12-hr photoperiod. Conidia were washed from the agar surface and adjusted to 1 \( \times 10^6 \) conidia/ml. Four mature (greater than 25 cm long) leaves on each seedling were sprayed with a conidial suspension and covered with plastic bags for 48 hr. After the bags were removed, trees were maintained in a greenhouse where the temperatures ranged from 20-24°C. The number of lesions on 4 sq cm of the leaf surface of each leaflet on each of four leaves on each seedling was recorded after approximately 2 wk. The leaves varied in number of leaflets.

**Results**

The number of anthracnose lesions increased as the nitrogen content of black walnut leaves decreased. The data from leaf three are correlated in Figure 1. The total nitrogen content in the 25 leaf samples ranged from 2.14 to 4.13%. Leaves of seedlings receiving no nitrogen averaged 2.66% nitrogen; those of plants receiving nitrogen averaged well above 3%. Leaves of seedlings receiving the complete Hoagland's averaged 3.59% nitrogen; those receiving Hoagland’s minus phosphorus, 3.51%; those receiving Hoagland’s minus potassium, 3.69%; and those receiving Hoagland’s plus nitrogen, 3.90%. The number of lesions on leaf three per 4 sq cm from trees in the five treatments ranged from 0.4 to 113.0 (Table 1). The number of lesions on leaf three were also representative of lesion counts on the older leaves one and two (Table 2). The low infection obtained in Tests 1 and 3 could not be explained. With the exception of Test 3, trees receiving Hoagland’s minus nitrogen had substantially and significantly more

![Table 1. Average number of lesions on leaflets of leaf three in 4 sq cm of surface area in six greenhouse tests. The walnut trees were growing in vermiculite and sand, and nutrients were supplied through modified Hoagland’s nutrient mixtures.](image)

![Fig. 1. Effect of nitrogen concentration in walnut leaves on the severity of walnut anthracnose; lesion number from five tests with counts 10 to 14 days after inoculation. Trees fertilized with modified Hoagland’s nutrient solutions.](image)
lesions than did trees receiving the complete nutrient formula. Trees receiving Hoagland’s nitrogen but minus phosphorus had more lesions than those receiving Hoagland’s minus potassium. The addition of nitrogen to a complete Hoagland’s resulted in an even greater, although not a significantly greater, reduction in lesions.

The distribution of lesions on the leaflets from the base to the tip from plants receiving a complete Hoagland’s and those receiving the Hoagland’s minus nitrogen are given in Table 2. There were fewer lesions on leaf four (5 to 7 days younger than leaf three) than on leaves one, two, or three.

Discussion

The greatest benefits from using fertilizers to control diseases have been observed with moderately susceptible or partially resistant plants. Diseases on immune plants or on highly susceptible plants are not affected by the application of nitrogen (8). Black walnut infected by G. leptostyla falls into the moderately susceptible category. Primary infection by ascospores occurs in May, and secondary cycles of reinfection by conidia do not cause serious defoliation until late July and August (2). Even in epidemic years, anthracnose has been only weakly correlated with tree growth (5). Variability in susceptibility is great between individuals in families, and within-family selection for anthracnose resistance has been recommended in black walnut breeding programs (5).

Table 2. Distribution of lesions on the leaflets of four black walnut leaves in six greenhouse inoculation tests.

<table>
<thead>
<tr>
<th>Leaflet position</th>
<th>Base</th>
<th>Tip</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaf</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Complete Hoagland's</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (oldest)</td>
<td>5.5</td>
<td>7.8</td>
</tr>
<tr>
<td>2</td>
<td>7.0</td>
<td>8.3</td>
</tr>
<tr>
<td>3</td>
<td>6.6</td>
<td>7.1</td>
</tr>
<tr>
<td>4</td>
<td>5.1</td>
<td>4.3</td>
</tr>
<tr>
<td><strong>Minus nitrogen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (oldest)</td>
<td>29.5</td>
<td>36.7</td>
</tr>
<tr>
<td>2</td>
<td>37.2</td>
<td>35.2</td>
</tr>
<tr>
<td>3</td>
<td>34.8</td>
<td>36.7</td>
</tr>
<tr>
<td>4</td>
<td>22.7</td>
<td>23.3</td>
</tr>
</tbody>
</table>

Disease control is achieved through practices that follow the epidemiological principles emphasized by Berger (1). The progress of the epidemic is slowed by host resistance through reduction of spore deposition, reduction in total number of infections, reduction in lesion expansion, reduction in sporulation, lengthening of the latent period, and a shortening of the infectious period. The application of nitrogen fertilizers to black walnut is beneficial in each of these ways.

The nature of the interaction of nitrogen with other nutrients in walnut anthracnose disease resistance remains undetermined. In field trials the addition of phosphorus and potassium to nitrogen in fertilizers was not beneficial and often was detrimental in controlling anthracnose (11). In this study, the presence of potassium did not increase the uptake of nitrogen, a finding that fails to support Huber and Watson (8). Trees receiving phosphorus but not potassium had a slightly higher leaf nitrogen content and essentially the same disease resistance as trees receiving nitrogen, phosphorus, and potassium.

Increasing the nitrogen content in walnut does not greatly alter ontogenetic maturity of leaflets. Individual leaves on trees receiving increased nitrogen are slightly larger but cease growing in approximately the same period of time as those receiving less nitrogen (3). Nitrogen fertilization, however, can greatly extend the period of indeterminate growth on black walnut, and more leaves are produced. Trees in nursery-type situations may show a 200 to 300% increase in annual height growth (12). The management practice of applying nitrogen to stimulate tree growth has coincidentally reduced the probability of a disease epidemic in black walnut.

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Literature Cited
Abstract


A sidewalk block heaved upon a tree's roots is not the tree's fault, and in many cases, blaming the tree for sidewalk problems is like blaming the bearer of bad news. A raised or cracked block may be an indicator of other contributing factors--heavy clay soils, severe freeze/thaw cycles, poor construction, even improper watering practices. These "non-tree" factors can predispose blocks of concrete to root intrusion. Depending upon locale and climate, only 10-30 percent of all sidewalk failures in most cities are related to trees and tree roots. A common solution to tree-related sidewalk problems is to cut back the roots of the tree. This is usually a temporary remedy that involves removing the unacceptable concrete blocks and cutting the problem root(s) back to a point where the two-inch-thick sidewalk form can be set. It is generally safe to remove any root with a diameter of less than 4 1/2 inches. A way to literally circumvent the drawbacks that go along with extensive root cutting is to use cutouts and bypass walks. This method is inexpensive, and it solves the problem for a longer period of time than cutting roots does. Another solution is to vary construction standards for sidewalks. In certain cases it may be advantageous to pour the concrete to a depth slightly less than conventional standards. It is recommended that a base of cinders be laid under the concrete. Roots tend to follow the path of least resistance, so the cinders will encourage roots to branch out in that area rather than toward the surface. Whichever method or combination of methods you and your professionals decide is best to solve your particular tree/sidewalk conflict, give your tree the benefit of the doubt and do what you can to protect it during sidewalk repair and construction.