Infection Site, Infection Period, and Latent Period of Canker
Caused by Anisogramma anomala in European Filbert

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ABSTRACT


Ascospores suspended in sterile distilled water were hypodermically infiltrated into buds infested with Phytophthora avellaneae and into noninfested buds resulted in 46.5 and 29.0 percent infection, respectively. Infection in buds not infested with P. avellaneae was attributed to mechanical injury during inoculation. There was a general increase in percent infection as inoculation dates approached the normal infection period from February through late May which was determined from disease incidence in trap plants. Infection was correlated with the duration of rainfall during the same season. Ascospores were recovered on slides exposed to 10 mature cankers continuously from each of 10 different exposure periods from 26 November to 3 April. The data indicate that the period of spore release is at least 6 mo in duration, extends well beyond the spore-trapping dates, and thus lasts much longer than the natural infection period. Heaviest discharge was recorded during periods of constant wetting of the stroma. Following infection there was normally a 12-16 mo latent period for symptom expression in the orchard. Under greenhouse conditions symptoms developed within 6 mo of inoculation. Phomopsis was isolated frequently from both cankered and healthy tissue, so it is unlikely that it is involved in the Anisogramma anomala canker syndrome.

Additional key words: infection court, inoculation techniques, Corylus avellana, eriophyid mites.

Anisogramma anomala (Peck) E. Müller, an indigenous pathogen of the American hazelnut, Corylus americana Marsh, also causes severe stem canker ing of several cultivars of commercial European filbert, Corylus avellana L. (1,12). Ellis and Everhart (6) suggested that Anisogramma was more damaging to the introduced C. avellana than to the native filbert. According to Bass (1) the failure of continued attempts to cultivate the European filbert in the eastern United States was largely due to this disease.

In 1973, the disease was discovered in the Pacific Northwest, which had previously been kept free of the disease by isolation and quarantine (5). A 1979 survey reported 49 infected orchards (3,8).

Perennial cankers over 200 cm in length, which often girdle entire main scaffold limbs, have been found in severely diseased trees. Active cankers contain between five and 200 stromata each containing 40 to 60 perithecium. Once such cankers spread into the main trunk the tree is usually girdled completely within a few years (Gottwald and Cameron, unpublished). Cameron and Gottwald (4) reported a 10- to 14-mo latent period between inoculation and symptom expression. They suggested that 2 yr may be required for ascospore production (7). There is apparently no comidal state (7).

Variatel susceptibility to Anisogramma canker is highly correlated with susceptibility to bud initiation and galling by the eriophyid mite Phytophthora avellaneae Nal. Barcelona, the main commercial cultivar, is only moderately susceptible to both Anisogramma and eriophyid mites (2), but the commercial pollenizer cultivars Daviana and DuChilly are more susceptible to mite infestation and bud galling and are severely affected by the disease (3,10).

Objectives of our research were to study several aspects of the disease cycle that had not been previously investigated. These were the duration of the natural infection period, the length of the latent period, and the duration of ascospore discharge. This study also included comparison of inoculation methods to test the susceptibility of various filbert tissues to ascospore inoculum and tests of pathogenicity of Phomopsis sp. isolates.

MATERIALS AND METHODS

Preliminary inoculations. Two-year-old filbert Corylus avellana "Daviana" seedlings were inoculated in the greenhouse by four different methods with a sterile water suspension of ascospores of A. anomala from perithecia taken from diseased filbert branches in Clark County, Washington. Spores were harvested aseptically by cutting off the top of the stroma with a hot scalpel and collecting the perithecial ooze on a sterile transfer needle. The inoculum was then suspended in sterile distilled water. This spore slurry was agitated to wash the spores and then centrifuged. This process was repeated five times until the pellet began to resuspend without agitation, signifying that the ascospores were free of the slime in which they were collected. Microscopic examination of the slurry revealed that this method caused hydration of the spores. All trees were inoculated with this slurry during February 1977. Five inoculations were made in each of five trees to test each of the four following methods: (i) hydropemic inoculation into artificial stem wounds, (ii) hydropemic inoculation of leaf petioles, (iii) hydropemic infiltration of mite-infested, galled buds and, (iv) spray inoculation of the entire tree with an atomizer. After inoculation, plants were put outside in isolation. Symptoms were evaluated 6, 18, and 24 mo after inoculation.

On 15 January 1977, twelve 10-year-old orchard-grown cultivar Daviana C. avellana trees in Clark County, Washington, were inoculated by three methods with a suspension of ascospores of A. anomala prepared as above. Five inoculations were made per limb and five limbs were inoculated per tree. Four trees were used per method giving a total of 100 inoculations for each of the following methods: (i) hydropemic inoculation of artificial stem wounds, (ii) hydropemic infiltration of male catkins and, (iii) hydropemic infiltration of healthy or mite-infested buds. Symptoms were evaluated 6, 18, and 29 mo after inoculation.
Inoculation of mite-infested versus healthy buds. Mite-infested and healthy buds of 2-yr-old potted cultivar Daviana C. avellana seedlings in the greenhouse were hydropodically infiltrated with a sterile suspension of ascospores of A. anomala prepared as above. Seedlings were inoculated 12 December 1977, 10 January 1978, and 10 February 1978 during what was presumed to be the infection period. Trees selected for inoculation had the highest number of mite-infested buds. All of the mite-infested buds on 10 trees were inoculated on each inoculation date. Only two trees were inoculated in all of their healthy buds because there were so many more healthy buds than mite-infested buds. Inoculated seedlings were kept moist under a mist sprayer for 7 days, and then placed in isolation outside. Symptoms were evaluated 6 mo and 18 mo after the final inoculation date.

Twelve, 10-yr-old C. avellana cultivar Daviana trees in the orchard were pruned of all existing A. anomala infections. Buds of four trees were inoculated by the hydropodemic infiltration technique with a concentrated suspension, 30–40 × 10^{12} ascospores per cubic centimeter of A. anomala on 3 December 1977, 8 January 1978, and 4 February 1978. On two of the four trees 100 mite-infested buds were inoculated and on the other two, 100 healthy buds were inoculated. Symptoms were evaluated at 4 mo and 14 mo after the final inoculation date.

**Determination of the infection period and latent period.** Healthy 2-yr-old "trap seedlings," cultivar Daviana, which are susceptible to Anisogramma canker, were exposed to rain splash inoculum to determine periods of natural infection by placing them beneath the canopy of a severely cankered filbert tree for 1-mo intervals from 20 October 1976 to 13 October 1977. Twenty-four replications of five trees per replication were each exposed for 1-mo with each replication overlapping the previous and succeeding replications by 2 wk. Ten control trap seedlings, which had been severely wounded by stem breakage and bark laceration, were exposed for the entire period, 15 January 1977 to 20 October 1977. Following exposure, trees were placed in isolation at the Southwest Washington Experiment Station in Vancouver, Washington, and evaluated for symptoms in July 1977, July 1978, and June 1979. Data were correlated with rainfall, temperature, and humidity recorded in the diseased orchard in Clark County, Washington, where the trees had been exposed.

**Ascospore discharge.** Ten cankers were studied from 26 November 1978 to 4 April 1979 for ascospore release. Ten microscope slides, coated with Vaseline petroleum jelly, were each positioned 1–2 mm above several sporae-laden stromata of each canker. Each stroma contained 40–60 perithecia. Relative humidity and temperature were measured with a sheltered 7-day recording hygrothermograph (Honeywell Corp., Portland, OR 97214). Periods and amounts of rainfall were recorded with a 7-day recording rain gauge (Weather-Measure Corp., Sacramento, CA 95841). Both instruments were placed ~0.5 m above the ground in the orchard under study. Trap slides were replaced 10 times during the course of the study over the same area of the canker. Exposure periods of 5 to 20 days were used due to the low incidence of discharge and the prolonged period of possible spore discharge under study. Slides were stained and made permanent simultaneously by applying a thin layer of 1% polyvinyl alcohol into which acid fuchsin was incorporated. Spore discharge was rated on a modification of the spore discharge index (SDI) scale used by Johnson and Kuntz (9) in which 0 = no spores, 1 = a few scattered spores usually in groups of eight, and 2 = numerous spores found clumped together. Spore counts were made under phase contrast optics at ×400 with a Zeiss compound microscope. Spore identification was made possible by the morphologically distinct aborted cell at the anterior end of the ascospores of A. anomala which is visible with phase-contrast optics at ×1,000.

**Isolation and test of pathogenicity of Phomopsis sp.** To test the possibility that Phomopsis sp. may be the imperfect state of Anisogramma anomala (W. Brant, personal communication), 250 isolations were made in June 1977 from several diseased twigs in each of two orchards blighted with Anisogramma canker. As a control, 157 and 265 isolations also were made from healthy wood taken from diseased and disease-free orchards, respectively. The disease-free orchard is 145 km (90 miles) south of the southern border of the diseased area. Wood pieces approximately 3.5 × 1.0–2.0 mm in size were cut from diseased or healthy wood and surface sterilized for 5 min in 0.05% sodium hypochlorite. These wood pieces were then stabbed into potato dextrose agar (PDA) in petri plates with one half of the piece above and one half below the agar surface. From these plates 100 Phomopsis isolates obtained only from diseased wood were axenically cultured on PDA slants. During March 1978 all isolates were transferred to PDA agar petri plates to increase inoculum. Each isolate was then inoculated into five healthy 10-yr-old cultivar Daviana filbert trees at the time A. anomala was determined to be spreading in the phloem. The bark was removed with a 3-mm diameter cork borer, and a 3-mm diameter by 2-mm thick disk of agar from a Phomopsis culture was inserted into the resulting hole and sealed with black plastic electrician's tape. Evaluations of these inoculations were made in June 1979.

**RESULTS**

**Preliminary inoculations.** Two infections on seedlings were discovered 6 mo following inoculation. Both infections resulted from hydropodemic injection of ascospores into Eriophyid mite-galled buds. No infections resulted from hydropodemic inoculation of leaf petiole or stem wounds or from atomizer inoculations of the entire seedling. No subsequent infections were discovered upon reexamination of seedlings 18 and 22 mo after inoculation.

No symptoms were detected on 10-yr-old orchard trees 6 mo after inoculation. Three infections, all from hydropodemic infiltration of ascospores into buds, were discovered 18 mo after inoculation. No infections resulted from hydropodemic inoculation of either catkin or stem wounds. No further infections were discovered during the subsequent 24 mo.

**Inoculation of mite-infested versus healthy buds.** Infections resulted from hydropodemic infiltration of both healthy and mite-infested buds on seedlings in the greenhouse on all three inoculation dates. There was a general trend toward a higher

<table>
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<th>Inoculation date</th>
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<th>Infections (no.)</th>
<th>Infection (%)</th>
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<tr>
<td>12 December 1977</td>
<td>galled 68</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>healthy 93</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>10 January 1978</td>
<td>galled 68</td>
<td>3</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>healthy 84</td>
<td>4</td>
<td>6.9</td>
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<tr>
<td>10 February 1978</td>
<td>galled 33</td>
<td>4</td>
<td>12.1</td>
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<tr>
<td></td>
<td>healthy 93</td>
<td>8</td>
<td>8.6</td>
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Buds galled by mite infestation and healthy buds were inoculated by hydropodemic infiltration with an ascospore suspension.

<table>
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<tr>
<th>Inoculation date</th>
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<th>Infections (no.)</th>
<th>Infection (%)</th>
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<tbody>
<tr>
<td>3 December 1977</td>
<td>galled 200</td>
<td>43</td>
<td>21.5</td>
</tr>
<tr>
<td></td>
<td>healthy 58</td>
<td>58</td>
<td>29.0</td>
</tr>
<tr>
<td>8 January 1978</td>
<td>galled 200</td>
<td>40</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>healthy 29</td>
<td>29</td>
<td>14.5</td>
</tr>
<tr>
<td>4 February 1978</td>
<td>galled 200</td>
<td>93</td>
<td>46.5</td>
</tr>
<tr>
<td></td>
<td>healthy 33</td>
<td>33</td>
<td>16.5</td>
</tr>
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Buds galled by mite infestation and healthy buds were inoculated by hydropodemic infiltration with an ascospore suspension.
percentage of infection during the second and third inoculation periods (Table 1).

No symptoms had developed on inoculated trees in the orchard by the 4-mo evaluation. Symptoms were first detected 14 mo after inoculation. Percent infection resulting from ascospore infiltration of mite-galled buds was greatest in trees inoculated in February, while percent infection resulting from inoculation of healthy buds was greatest in trees inoculated in December. Maximum percent infection was 46.5% in trees with mite-galled buds inoculated on 3 February 1978 (Table 2).

Determination of the infection period and latent period. "Trap plants" exposed for approximately 1-mo during overlapping intervals from 20 October 1976 to 30 October 1977 were partially evaluated for symptom expression during July 1977 and fully evaluated July 1978 and again June 1979. Six trap trees, exposed for different 1-mo periods during the interval 12 February 1977 to 21 May 1977, and six of the 10 control trees exposed continuously from 15 January 1977 to 20 October 1977 developed Anisogramma canker (Table 3). In no case did trees develop symptoms during the growing season immediately following exposure. Symptoms did not develop until July 1978, 10–14 mo following exposure. No further infections were discovered during the June 1979 evaluations. Infected trees were classified into two distinct infection periods (Fig. 1). The infection period roughly correlated with rainfall recorded during that year and with moderate temperatures above freezing. No diseased trees were found among those set out during the second year of the study from 3 November 1977 to 3 March 1978. These trees were evaluated July 1978 and again June 1979.

Ascospore discharge. Spore discharge occurred during all 10 periods that spore trapping was attempted (Fig. 2). In nearly all cases, spores were recovered in small or large masses with definite rounded edges indicative of droplet dry-down following water splash dispersal. Only one octet of ascospores, suggesting forcible discharge, and a few cases of individual or scattered spores were found.

Isolation and test of pathogenicity of Phomopsis sp. Although Phomopsis sp. was isolated more frequently from diseased wood (76.4%), than from healthy samples taken from diseased orchards (21.9%), a substantial percentage (44.5%) of Phomopsis also was recovered from 265 nondiseased C. avellana cultivar Daviana samples collected in a nondiseased orchard approximately 145 km (90 mi) south of all known infected trees. No infections resulted from the 500 inoculations of 100 Phomopsis isolates.

**DISCUSSION**

Either mite-infested or healthy buds can serve as the infection courts for germinating ascospores of *A. anomala*. Under artificial conditions such as in a greenhouse, *A. anomala* may be capable of

<table>
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<th>Exposure period</th>
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<th>Infections per tree (no.)</th>
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<tr>
<td>12 February–22 March</td>
<td>CD-48</td>
<td>2</td>
</tr>
<tr>
<td>22 March–23 April</td>
<td>CD-62</td>
<td>1</td>
</tr>
<tr>
<td>6 April–7 May</td>
<td>CD-65</td>
<td>2</td>
</tr>
<tr>
<td>23 April–21 May</td>
<td>CD-67</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>CD-74</td>
<td>2</td>
</tr>
<tr>
<td>15 January–20 October</td>
<td>CD-1</td>
<td>1</td>
</tr>
<tr>
<td>20 July–29 August</td>
<td>CD-2</td>
<td>1</td>
</tr>
<tr>
<td>29 August–29 September</td>
<td>CD-3</td>
<td>1</td>
</tr>
<tr>
<td>29 September–29 October</td>
<td>CD-5</td>
<td>1</td>
</tr>
<tr>
<td>29 October–29 November</td>
<td>CD-6</td>
<td>1</td>
</tr>
<tr>
<td>29 November–29 December</td>
<td>CD-7</td>
<td>1</td>
</tr>
</tbody>
</table>

*Overlapping exposure periods and exposure periods between those listed resulted in no detectable disease. All dates were in 1977.

The number of diseased trees listed for each exposure period represents the number in a five-tree replicate group that became infected. The number of diseased trees listed for the control exposure period represents the number of a 10-tree replicate group that became infected.

**Fig. 1.** Correlation of meteorological data from a European filbert orchard in Clark County, Washington, with the infection period for *Anisogramma anomala* canker as determined by exposure of "trap seedlings" under infected trees. Seedlings were exposed for 1 mo each, and a total of 11 infections on six seedlings developed on seedlings exposed during the indicated infection periods.

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completing its life cycle within a single year; however, this has never been observed in the orchard. All evidence points to a 10- to 16-mo latent period under normal orchard conditions.

The beginning of the natural infection period determined with trap plants in this study correlates well with the appearance of new, expanding mite-galled buds (10). Although artificial inoculation will effectively initiate new infection earlier, the bud scales of mite-infested buds are too tightly appressed, for effective penetration by ascospores prior to the natural infection period. A great deal of entanglement, wounding, and exposure of vascular tissue in mite-infested Corylus buds results from the feeding of P. avellanae (11). Hypodermic infiltration of ascospores into healthy buds also mechanically damages young tissues. This may account for the successful inoculation of uninfested buds.

The natural infection period occurred during the beginning of sap flow, but prior to resumed growth. Inoculated healthy buds were susceptible to infection during the dormant period prior to resumed growth but became less susceptible as natural callusing resumed. Mite-infected wounds in mite-infested buds tend to resist callusing and may remain receptive longer.

The percent infection following inoculations of greenhouse-grown trees was lower than the percentage of field infection. Many of the inoculated branches of potted trees died back due to winter injury before symptoms could develop, giving a lower percent infection than would have occurred under more favorable conditions.

A 12- to 16-mo latent period appears to be the normal course of events during the disease cycle of Anisogramma canker. This could account for the gap between the two distinct infection periods detected during this study. Anisogramma canker has been found to be sporadic in its build up and spread (8). Several years often elapse between outbreaks and periods of rapid buildup of this disease.

Results of several different independent experiments indicate that the 1976–1977 season was much more conducive to infection than was the 1977–1978 season. This also could account for the relatively low number of natural infections in trap plants. In addition, some infected buds may have been accidentally dislodged during handling of the plants.

Examination of the cream-colored discharge produced by individual stromata reveals a heavy concentration of both free ascospores and a few intact deliquescent asci suspended in a viscous ooze (7). This viscous mass is easily dispersed in water and the individual asci can be seen to burst. Free moisture in the form of rain washes the ooze down branches and causes it to drip. Dispersal patterns within individual infected filbert trees suggests that inoculum is dispersed by water or rain splash. The massed or streaked pattern of ascospores trapped on slides also suggests splash dispersal. Forcible discharge, although detected once, appears to be of much less significance. In previous experiments, in a heavily infected orchard, no ascospores were ever recovered with a Burkhardt volumetric spore trap (Gottwald, unpublished). If wind were a major factor in inoculum dispersal, one would expect to find numerous ascospores on spore trap tapes. Continuous wetting of the stromata resulted in a heavy discharge, and subsequent rain aided in water splash dispersal of the inoculum. However, free moisture can at times be deleterious to spore trapping; during trapping period 7, several slides were washed free of petroleum jelly. We believe that this caused the relatively low SDI for trapping period 7. Many more spores undoubtedly were released during this period but were washed from the trap slides.

Ascospore discharge also occurred even during periods of relatively little rain, i.e., periods 4 and 9 (Fig. 2). Spore discharge has been observed in the field almost immediately following a thorough wetting of the stromata which causes perithecia ostioles to open. The contents of the perithecia, under hydraulic pressure caused by imbibition of water, ooze out. Infection is undoubtedly less

Fig. 2. Correlation of meteorological data with ascospore discharge from Anisogramma anomala perithecia in cankers on European filbert trees in an orchard in Clark County, Washington. Ascospores were trapped on Vaseline-coated slides exposed above the cankers for periods of 5-20 days during the period from 25 November 1978 to 3 April 1979. A spore discharge index (SDI) of 0 to 2 was used with 0 = no spores, 1 = few scattered spores usually in groups of eight, and 2 = numerous spores found clumped together.

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frequent during these drier discharge periods due to the lack of free moisture necessary to carry the inoculum into the galled buds serving as the infection courts.

Although spore discharge may begin as early as November and continue through May of the succeeding year, the period of susceptibility of *Corylus avellana* is much shorter. Bud scales of mite-galled buds do not expand significantly, exposing the susceptible interior of the buds to germinating ascospores, until mid-February.

Although *Phomopsis* has been reported to be the imperfect state of a few genera of related ascomycetes such as several *Diaporthe* species (13), it has never been reported to be connected to *A. anomalae*. No infections resulted from the 500 individual inoculations of 100 isolates of *Phomopsis* originally taken from *Anisogramma*-diseased filbert wood. Therefore, it is highly unlikely that *Phomopsis* is the conidial stage of *A. anomalae* or is involved in the canker disease. Although *Phomopsis* was easily recovered from filbert tissue infected by *A. anomalae*, it is very likely a saprophyte inhabiting dead filbert tissue or an epiphyte existing in the periderm of healthy as well as infected filbert trees.

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**Ecology and Epidemiology**

**Disease Increase and the Dynamics of Spread of Canker Caused by *Anisogramma anomalae* in European Filbert in the Pacific Northwest**

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**ABSTRACT**


In southwestern Washington, eastern filbert blight, which is caused by *Anisogramma anomalae* (Diaportheaceae), was first discovered in 1973. The rate of disease increase and the geographical pattern of spread from the original focus of infection were studied. Increase in length of individual cankers averaged 31.7 cm/yr. Regression analyses of disease progress curves gave disease increase values of $r = 1.085$ and $r = 1.236$ unit per year within single trees (treated as independent epidemics) and disease increase within orchards, respectively. Within the area presently affected the original focus was determined to be a group of five orchards in the northeast quadrant from which the disease spread south and west to 44 additional orchards. Inoculum dispersal over long distances is infrequent; therefore, the southernmost diseased plantings pose the greatest threat of disease spread into orchards farther south in the main filbert-growing areas of Oregon.

*Additional key words: disease survey, Corylus avellana.*

In western Washington, eastern filbert blight, which is caused by *Anisogramma anomalae*, was first discovered in 1973 (5). Since that time 49 infected orchards have been found. Incidence of this disease within an individual orchard may be as high as 100%, and in a few cases, entire orchards have been killed by the disease. The fungus enters the tree primarily through galled buds infected with the eriophid mite *Phytophthora avellanea* Nal (3,7). Wounds which occur during the dormant season, when callus formation is slow, may remain receptive to infection by *A. anomalae* for long periods...