Infection of European Hazelnut by *Anisogramma anomala*: Site of Infection and Effect of Host Developmental Stage

K. B. Johnson, J. N. Pinkerton, S. M. Gaudreault, and J. K. Stone

First, third, and fourth authors: Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 92331-2902; second author: USDA ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330.

Research supported in part by the USDA Agricultural Research Service and Oregon Agricultural Experiment Station Specific Cooperative Agreement No. 58-5358-190, and the Oregon Hazelnut Commission.

Technical paper 10,510 of the Oregon Agricultural Experiment Station.

Accepted for publication 13 September 1994.

ABSTRACT

Johnson, K. B., Pinkerton, J. N., Gaudreault, S. M., and Stone, J. K. 1994. Infection of European hazelnut by *Anisogramma anomala:* Site of infection and effect of host developmental stage. Phytopathology 84:1465-1470.

The concentration of ascospores of *Anisogramma anomala* required to infect 50% of spray-inoculated shoots of European hazelnut (*Corylus avellana*) was 7×10^4 and 2×10^5 spores per milliliter for seedlings of cvs. Ennis and Royal, respectively. Placement of *A. anomala* ascospores (5×10^6 per milliliter) on the shoot internode immediately below the apical meristem resulted in 79% infection of inoculated seedlings. In contrast, ascospores applied onto the second, third, or fourth shoot internode below the meristem resulted in 32, 9, and 6% infection of seedlings, respectively. Similarly, incidence of infection after inoculation of the expanding leaf nearest the apical meristem averaged 64%, but only 19, 7, and 2% when ascospores were applied onto the second, third, or fourth leaf from the meristem, respectively. Ascospores spray-inoculated (1×10^5 spores per milliliter) onto vegetative buds and shoots at various stages of development resulted in infection of 0% of dormant buds, 63% of buds with leaf tips emerged, 79% of buds with a full leaf emerged, 88% of elongating shoots with two to four nodes, 29% of elongating shoots with eight to ten nodes, and 0% of shoots that had stopped adding new growth late in the season. Hazelnut buds infested with an eriophyid bud mite, *Phytoptus avellanae,* were not more susceptible to infection by *A. anomala* than noninfested buds as had been reported previously.

Additional keyword: eastern filbert blight.

Eastern filbert blight, caused by *Anisogramma anomala* (Peck) E. Müller in E. Müller and Arx, is a serious disease of European hazelnut, *Corylus avellana* L. (1,2,4,13). The pathogen, apparently an obligate parasite (4,13,14), colonizes secondary xylem and phloem layers of hazelnut branches, usually producing a canker 13–15 mo after infection (5,13). The disease spreads by perennial expansion of cankers (6) and as a result of new infections by ascospores that are produced in stromata associated with cankers on living wood (4,5,13). Canker expansion girdles branches and limbs, resulting in canopy dieback and death of trees in 4–10 yr (2,6,10).

Ascospores, the only spore produced by *A. anomala* (4), are released from perithecia during and after rain from fall to late spring (4,9). Based on artificial inoculation experiments, Gottwald and Cameron (4,5) concluded that dormant, vegetative buds infested with and galled by the eriophyid mite, *Phytoptus avellanae* Nal., were a principal site of infection by *A. anomala.* They reasoned that *P. avellanae* facilitated entry of *A. anomala* by wounding bud tissue during feeding and by creating a loose, open arrangement of galled bud scales in late winter. In contrast, Stone et al (14) infected European hazelnut by applying ascospores onto actively growing, non-mite-infested, vegetative shoots. Their data indicated that European hazelnut was susceptible to infection by

ascospores of *A. anomala* only after vegetative buds had resumed active growth in spring.

The infection biology of *A. anomala* is of practical concern to commercial European hazelnut growers in western Oregon. Eastern filbert blight was first reported in southwest Washington in 1970 (2,3) and in Oregon in 1986 (10). Most orchards in the vicinity of the initial detections have been destroyed as a result of the disease (6,10). Since its introduction, hazelnut growers have attempted to control eastern filbert blight with therapeutic pruning and protective fungicides (7,10). Control of the disease, however, particularly with fungicides, has been limited by the conflicting reports as to how *A. anomala* gains entry to its host.

The objective of this study was to further characterize infection of European hazelnut by *A. anomala*. Reported are the relationship between ascospore concentration and infection of vegetative shoots, the primary site of infection on vegetative shoots, the effect of the developmental stage of bud and shoot development on susceptibility to infection, and a reexamination of the effect of bud infestations of *P. avellanae* on host susceptibility.

MATERIALS AND METHODS

Preparation of inoculum and source of trees. Ascospore suspensions were obtained by dissecting whole perithecia from mature stromata of *A. anomala* on European hazelnut twigs, which had been collected the previous fall and stored frozen at −10 C in polyethylene bags. Perithecia were excised from the twigs with a scalpel and crushed with a mortar in sterile distilled water. The resulting suspension was filtered through three layers of cheesecloth; the desired concentrations were obtained with the aid of a hemacytometer.

Experiments were performed on hazelnut seedlings and on clonal cultivars. Seedlings were obtained by germinating hazelnut kernels collected from open-pollinated trees of the susceptible cvs. Royal or Ennis. The collected nuts were stored at 4 C. To enhance germination, kernels were removed from their shell, soaked overnight in 0.004% gibberellic acid (ProGibb, Abbott Laboratories, North Chicago, IL), and then planted in moist vermiculite. At an age of 5–6 wk, seedlings were transplanted into 5-cm^3 plastic pots that contained a commercial growth medium (33% peat, 33% bark dust, and 33% pumice by volume). Two-year-old trees of clonal cultivars were obtained in a dormant, bare root condition from commercial nurseries and planted in 7-L plastic pots that contained the same growth medium used for the seedlings. Growth conditions for seedlings and clonal cultivars are described below.

Inoculum concentration. Five- to six-week-old hazelnut seedlings were inoculated with *A. anomala* at concentrations of 1×10^4, 5×10^4, 1×10^5, 5×10^5, 1×10^6, or 5×10^6 ascospores per milliliter. Ascospore suspensions were applied to runoff onto shoots with a hand-held pump sprayer. After inoculation, the seedlings were placed in an intermittent mist chamber (fine mist for 30 s every 10 min during daylight hours, 20–26 C) for 0, 3, or 7 days. The experiment was conducted in 1990 and 1991 with seedlings of cv. Royal, and in 1991 with seedlings of cv. Ennis. A group of 12 seedlings composed an experimental unit; each inoculation date constituted a replication. Royal seedlings were inoculated on 8 February and 7, 16, and 22 March 1990, and on 24 April, and 2, 14, and 17 May 1991; Ennis seedlings were inoculated on 3 and 10 December 1991 (two replications on each date). After the mist treatment, seedlings were transplanted to 12-cm^3 plastic pots, and then grown in an unheated greenhouse for 12–18 mo (temperature range −10–35 C). During the winter, the pots were covered with 5–10 cm of sawdust to insulate the roots.

Incidence of infection was evaluated in May or June, 12–18 mo after inoculation. The trees were first inspected for stromata of *A. anomala*. On trees without stromata, 30% of the bark on the lower stem was stripped from each seedling with a knife to expose the phloem. The phloem was inspected for a chocolate brown–colored necrosis, which is indicative of infection by *A. anomala* (8,13). Freehand sections of secondary xylem located just below the necrotic phloem were made with a razor blade from all trees that showed the symptom. The sections were stained in 0.05% trypan blue in lactophenol and examined microscopically for characteristic hyphae of *A. anomala* (13).

Analysis of variance (ANOVA) (12) was used to test the effect of inoculum concentration and mist duration on the incidence of infection. Incidence data were transformed to arcsine $\sqrt{x}$ to reduce nonhomogeneity of within-treatment variances. Probit values (16) of the mean incidence of infection in seedlings misted for 3 or 7 days were regressed on the logarithm of ascospore concentration. The concentration of ascospores required to infect 50% of inoculated shoots was interpolated from the estimated regression equations.

Site of infection. Ascospores of *A. anomala* were inoculated onto specific stem internodes of 5- to 6-wk-old hazelnut seedlings. The first internode was defined as stem tissue from the apical meristem to the youngest expanding leaf, the second stem internode was between the youngest and second youngest leaves, and so on to the fourth internode. Inoculated internodes were isolated from other tissues by applying a latex, tree-wound sealant (Farwell Industries, Wenatchee, WA) around the stem at adjacent nodes with a small brush. Approximately 25 μl of an ascospore suspension (5×10^6 spores per milliliter) was applied to each internode with a camel hair brush and allowed to dry. Inoculated plants were positioned horizontally on plastic trays covered with moist paper towel, enclosed in plastic bags, and incubated for 5 days in a constantly lighted growth chamber (18 C, fluorescent, photosynthetic photon flux density = 40–60 $\mu E/m^2/s$). After incubation, the trees were transplanted in 25-cm^3 pots and watered without wetting the foliage for at least 4 wk. The trees were maintained in a heated greenhouse (20–26 C) for 3 mo and then in a nonlighted cold room (6 C) for 4 mo to induce dormancy. After the cold room treatment, the trees were returned to the greenhouse until external symptoms of disease (stromata) developed (3 mo).

The experiment was conducted in 1991 and again in 1992. Royal seedlings were inoculated on 12, 13, 18, and 27 June 1991, and on 5, 13, 20, 27 May and 3 and 10 June 1992. Ennis seedlings were inoculated on 5, 7, and 14 June 1992. Twelve seedlings composed an experimental unit; each inoculation date constituted a replication.

In 1992, the leaf nearest to the apical meristem (first leaf), the second leaf, or the third leaf was inoculated on the same dates as the stem internode treatments. Leaves were isolated from other tissue by applying the latex tree-wound sealant around the petiole, and then inoculated and incubated as described above.

All Royal seedlings were evaluated microscopically for infection prior to placement in the cold room in September and again the following April. Freehand sections of xylem were sampled with a razor blade from the inoculated internode or from stem tissue adjacent to the inoculated leaf. The sections were stained with 0.05% trypan blue in lactophenol and examined for hyphae of *A. anomala* (13). In April, incidence of stromata of *A. anomala* and the incidence of necrosis in the phloem also were recorded. Only incidence of stromata and phloem necrosis were recorded on Ennis seedlings.

ANOVA (12) was used to test if stem internode or leaf position significantly affected incidence of stromata, of phloem necrosis, and of *A. anomala* hyphae in xylem 3 and 9–10 mo after inoculation. The arcsine $\sqrt{x}$ transformation was applied to the data.

Effect of host development. In 1992, the effect of the developmental stage of hazelnut buds and shoots on susceptibility to *A. anomala* was evaluated on commercially grown hazelnut cvs. Barcelona, Casina, Daviana, Ennis, Hall's Giant, Tonda di Giffoni, and Willamette. The cultivars were grown and maintained in 7-liter plastic pots in an unheated shadehouse. Inoculated developmental stages (and range of inoculation dates) were: dormant buds (15 February), buds with a partial leaf emerged (26 February to 13 March), buds with a leaf fully emerged (13 March to 14 April), buds with multiple leaves emerged (27 March to 24 April), elongating vegetative shoots with two to four nodes (21 April to 3 May), elongating shoots with eight to ten nodes (10 to 13 July), and woody shoots that had stopped adding new

growth late in the season (11 to 18 September). Dates of inoculation were varied among the cultivars to achieve uniformity in the stage of development at the time of inoculation. The concentration of *A. anomala* ascospores (1×10^5 or 1×10^6 spores per milliliter) was an additional experimental factor. For each treatment combination, 12 buds or shoots on each of four trees were sprayed to runoff with a hand-held pump sprayer. After inoculation, inoculated buds or shoots were enclosed in plastic bags and the trees were incubated for 7 days in a heated greenhouse (20–26 C). After this period, the bags were removed and the trees were returned to the shadehouse.

Disease was evaluated in June and July of 1993. For each inoculated bud or shoot, the incidence of stromata of *A. anomala* was recorded, or the bark was stripped in the vicinity of the inoculation site to evaluate the incidence of phloem necrosis. Microscopic verification of *A. anomala* hyphae in xylem was limited to cankers where the margin of the phloem necrosis was not sharply delineated. ANOVA (12) was used to test if cultivar, developmental stage, or inoculum concentration affected incidence of disease. Incidence data were transformed to arcsine $\sqrt{x}$. Preliminary analysis found no significant effect of inoculum concentration ($P > 0.05$) but the cultivar × developmental stage interaction was significant ($P < 0.0001$). Consequently, to evaluate the effect of developmental stage on incidence of infection, ANOVA procedures were performed separately on each cultivar with data pooled over inoculum concentration.

Effect of big bud mite. The predisposing effect of the eriophyid bud mite, *Phytoptus avellanae,* on the susceptibility of vegetative hazelnut buds to infection by *A. anomala* was evaluated by applying ascospore suspensions onto healthy and mite-galled buds at 1-mo intervals during the winters and springs of 1990 and 1991. In April of 1989, 40 2-yr-old trees of cultivar Daviana were infested with *P. avellanae* by taping several mite-galled buds that had been collected in a commercial orchard onto elongating shoots. These trees were then maintained in an unheated shadehouse for 9–13 mo. In 1990, four newly galled buds on each of ten mite-infested trees and six healthy buds on each of ten noninfested trees were inoculated on 26 January (healthy buds dormant; mite-galled buds swollen), 24 February (healthy buds dormant; mite-galled buds swollen and bud scales loose), 24 March (healthy buds with a leaf partially emerged; mite-galled buds swollen and bud scales open), or on 24 April (healthy shoots with two to four nodes; mite-galled buds were swollen and bud scales were open). On each date, buds or shoot tips were inoculated by applying ascospores (1×10^5 spores per milliliter) with a camel hair brush. After inoculation, the trees were incubated 7 days in an intermittent mist chamber constructed in the shadehouse (fine mist for 30 s every 10 min during daylight hours, daily mean temperature 5–15 C). Inoculated trees were maintained in the shadehouse until evaluation. In June 1991, each inoculated bud was evaluated for stromata of *A. anomala* and for phloem necrosis. The experiment was repeated in 1991 with 3-yr-old trees of cv. Daviana that were infested with *P. avellanae* in the spring of 1990. Inoculation dates were 24 January (healthy buds dormant; mite-galled buds swollen), 24 February (healthy buds dormant; mite-galled buds swollen and bud scales loose), or 30 March (healthy buds with a leaf partially emerged; mite-galled buds swollen and bud scales open). After inoculation, each tree was covered with a plastic bag and incubated in a greenhouse for 7 days (20–26 C). After incubation, the trees were maintained in the shadehouse until June 1992 when the incidence of stromata of *A. anomala* and phloem necrosis was evaluated. Data were summarized by computing a mean and standard error for each bud type on each inoculation date.

RESULTS

Inoculum concentration. Royal seedlings that received a mist treatment 3 days postinoculation averaged 52% infection compared to 3% when trees were not misted (Table 1). There was no significant difference ($P > 0.05$) in infection incidence among mist treatment of 3 or 7 days. At the highest inoculum concentration (5×10^6 spores per milliliter), incidence of infection averaged 95 and 77%, respectively, in Royal and Ennis seedlings that received a mist treatment (Table 1). In general, incidence of infection in misted Royal seedlings increased linearly with logarithm of inoculum concentration ($R^2 = 0.88$), but this trend was not as pronounced in Ennis seedlings ($R^2 = 0.60$) (Fig. 1). The estimated inoculum concentration at which 50% of misted seedlings became diseased was 7×10^4 ascospores per milliliter

TABLE 1. Effect of inoculum concentration[a] of ascospores of *Anisogramma anomala* on infection of 5- to 6-wk-old European hazelnut seedlings misted for 0, 3, or 7 days postinoculation[b]

Year/cultivar[c]	Inoculum concentration (ascospores/ml)	Mist duration: 0 days		3 days		7 days	
		% infected[d]	% with stromata[e]	% infected[d]	% with stromata[e]	% infected[d]	% with stromata[e]
1990/Royal	5×10^4	0	0	5 ± 6	3 ± 3	0	0
	5×10^5	3 ± 3[f]	3 ± 3	70 ± 25	35 ± 19	60 ± 16	32 ± 10
1991/Royal	1×10^4	0	0	2 ± 1	2 ± 1	2 ± 1	0
	5×10^4	2 ± 1	2 ± 1	33 ± 4	23 ± 1	42 ± 9	30 ± 5
	1×10^5	0	0	52 ± 1	44 ± 5	50 ± 2	42 ± 4
	5×10^5	2 ± 1	2 ± 1	70 ± 9	47 ± 5	85 ± 5	53 ± 3
	1×10^6	8 ± 2	8 ± 2	83 ± 4	26 ± 10	75 ± 9	48 ± 10
	5×10^6	5 ± 2	6 ± 3	98 ± 1	63 ± 2	92 ± 2	52 ± 1
1991/Ennis	1×10^4	...[g]	...	23 ± 15	19 ± 14	30 ± 17	26 ± 19
	5×10^4	...	...	54 ± 8	29 ± 10	73 ± 10	46 ± 6
	1×10^5	...	...	40 ± 21	27 ± 14	56 ± 9	25 ± 11
	5×10^5	...	...	50 ± 31	21 ± 14	65 ± 16	25 ± 8
	1×10^6	...	...	71 ± 11	50 ± 11	90 ± 6	25 ± 9
	5×10^6	...	...	77 ± 15	31 ± 6	77 ± 14	44 ± 8

[a] Ascospore suspensions were applied with a hand-held pump sprayer to runoff.
[b] In a greenhouse, mist was applied intermittently for 30 s every 10 min during daylight; temperature of the mist chamber was approximately 20 C.
[c] Individual replications of the 1990 experiment were initiated on 8 February and 7, 16, and 22 March. In 1991, replications with Royal seedlings were begun on 24 April, and 2, 14, and 17 May, and on 3 and 10 December for Ennis seedlings. On each date, 12 seedlings were inoculated for each spore concentration and mist duration combination.
[d] Percentage of trees that developed stromata of *A. anomala* or a localized necrosis of the phloem 12–17 mo after inoculation. On trees with only phloem necrosis, infection was confirmed by microscopically examining freehand sections of young xylem for hyphae of the pathogen.
[e] Percentage of trees on which stromata of *A. anomala* developed.
[f] Standard error of the mean.
[g] Treatment was not evaluated.

for seedlings of Ennis and 2×10^5 spores per milliliter for seedlings of Royal. Stromata of *A. anomala* developed on 60% of the seedlings in which infection was confirmed microscopically. There was no effect of inoculum concentration or mist duration on this proportion.

Site of infection. For Royal seedlings, the incidence of *A. anomala* in xylem, of stromata of *A. anomala*, and of phloem necrosis increased with the proximity of the inoculated internode to the apical meristem (Table 2). Sixty-nine percent of seedlings inoculated at the first internode became diseased compared with 22, 4, and 1% inoculated at the second, third, and fourth internodes, respectively. Detection of hyphae of *A. anomala* in Royal seedlings increased only slightly from 3 to 12 mo after inoculation (Table 2), and the incidence of phloem necrosis within an inoculated internode was highly correlated ($r = 0.99$) with incidence of hyphae in xylem tissue sampled from the same internode. Infection of Ennis seedlings also increased with proximity of the inoculated internode to the apical meristem (Table 2). Compared with Royal seedlings, however, a greater proportion of Ennis seedlings were infected after inoculation at the third or fourth internodes distal to the shoot apex.

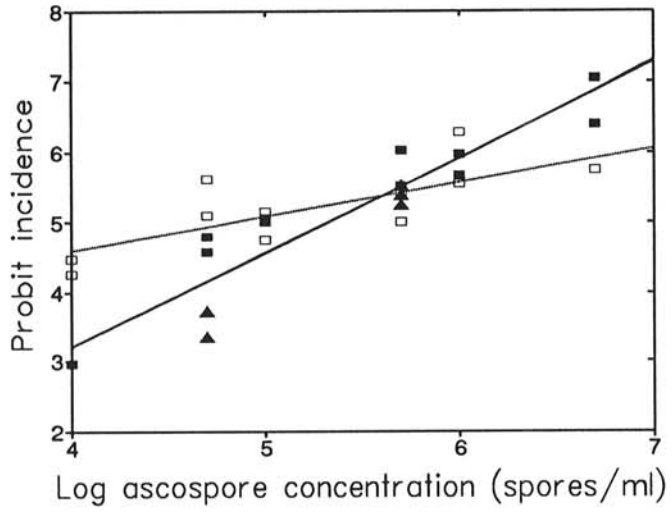

Fig. 1. Regression of the probit of incidence of eastern filbert blight in European hazelnut seedlings on the logarithm of *Anisogramma anomala* ascospore concentration (spores per milliliter). Thick line represents results from experiments with seedlings of cv. Royal ($y = -2.19 + 1.35x$, $R^2 = 0.88$); closed squares and closed triangles represent the 1990 and 1991 experiments, respectively (see Table 1). Thin line and open squares represent results from 1991 experiment with seedlings of cv. Ennis ($y = 2.66 + 0.49x$, $R^2 = 0.60$).

Results of leaf inoculations were similar to the internode inoculations in that the incidence of *A. anomala* hyphae in xylem, of stromata of *A. anomala*, and of phloem necrosis increased with the proximity of the inoculated leaf to the apical meristem. In Royal seedlings, for example, inoculation of the first, second, and third leaf resulted in 38, 8, and 0% infection, respectively (Table 2). Infection of leaf-inoculated Royal seedlings occurred at about half the rate of corresponding stem internode inoculations, but incidences of infection among corresponding inoculated leaves and stem internodes of Ennis seedlings were similar (Table 2).

Developmental stage. Inoculation of *A. anomala* ascospores on dormant buds in late winter (15 February) or onto shoots that had stopped growing in late summer (11–18 September) did not result in successful infection on any of the seven hazelnut cultivars (Table 3). In contrast, all cultivars became infected if inoculations were made during periods of active bud or shoot growth. Incidences of infection averaged over cultivar were 63, 79, 88, 77, and 29% at the developmental stages of partial leaf emergence, full leaf emergence, multiple leaves emerged, elongating shoots with two to four nodes, and elongating shoots with eight to ten nodes, respectively. Except for cvs. Tonda di Giffoni and Casina, high incidences of infection ($\geq$ 79%) were obtained at the developmental stages of full leaf emergence, multiple leaf emergence, and elongating shoots with two to four nodes (Table 3). The highest incidences of infection on cvs. Tonda di Giffoni (72%) and Casina (85%) occurred at the stage of multiple leaf emergence. All cultivars showed significant reductions ($P < 0.05$) in infection when elongating shoots with eight to ten nodes were compared with elongating shoots with two to four nodes.

Effect of big bud mite. Infection of either healthy or mite-galled buds did not occur when the trees were dormant in either

TABLE 2. Effect of inoculation site[a] on vegetative shoots of 5- to 6-wk-old European hazelnut seedlings on incidence of infection by ascospores[b] of *Anisogramma anomala*

Year/cultivar[c]	Position from apical meristem	Stem internode				Leaf		
		% infected at 3 mo[d]	% infected at 10–12 mo[d]	% with phloem necrosis	% with stromata[e]	% infected at 10–12 mo[d]	% with phloem necrosis	% with stromata[e]
1991/Royal	1	48 ± 14[f]	48 ± 8	50± 13	19 ± 6	...[g]	...	...
	2	4 ± 5	6 ± 12	6 ± 7	4 ± 3	...	...	...
	3	...	0	2 ± 3	0	...	...	...
	4	...	0	0	0	...	...	...
1992/Royal	1	85 ± 4	89 ± 5	90 ± 4	61 ± 10	38 ± 6	33 ± 6	13 ± 4
	2	20 ± 7	37 ± 9	29 ± 8	13 ± 5	8 ± 5	3 ± 3	0
	3	0	8 ± 3	7 ± 3	6 ± 3	0	0	0
	4	...	1 ± 1	1 ± 1	1 ± 1	...	...	...
1992/Ennis	1	...	...	97 ± 4	80 ± 7	...	90 ± 12	57 ± 4
	2	...	...	52 ± 35	33 ± 25	...	30 ± 7	27 ± 11
	3	...	...	19 ± 10	17 ± 11	...	13 ± 16	10 ± 12
	4	...	...	17 ± 15	17 ± 15	...	3 ± 4	3 ± 4

[a] Specific inoculation sites were stem internodes or young leaves numbered sequentially beginning at the apical meristem of the shoot.
[b] A suspension 1×10^6 ascospores per milliliter was applied to the inoculation site with a camel hair brush. After inoculation, the plants were incubated in a horizontal position on trays sealed inside a plastic bag and maintained at 18 C within a constantly lighted growth chamber for 5 days.
[c] Individual replications of the 1991 experiment were initiated on 12, 13, 18, and 27 June. In 1992, replications with Royal seedlings were inoculated on 5, 13, and 27 May and 3 and 10 June; and on 5, 7, and 14 June 1992 for Ennis seedlings. On each date, 12 seedlings were inoculated for each specific inoculation site.
[d] At 3 mo and 10–12 mo after inoculation, infection by *A. anomala* was determined by microscopically examining freehand sections of young xylem sampled from near the inoculation site for hyphae of the pathogen.
[e] Percentage of trees on which stromata of *A. anomala* developed on the main stem.
[f] Standard error of the mean.
[g] Treatment was not evaluated.

year (Table 4). In 1990, 21–29% of healthy and mite-galled buds became diseased when ascospores were applied after the resumption of growth in spring (Table 4); differences in infection frequency among healthy and mite-galled buds were not significant ($P > 0.05$). In 1991, 62% of healthy buds inoculated on 24 March (partial leaf emergence) became diseased but no infection was detected in mite-galled buds inoculated on the same date (Table 4).

DISCUSSION

Our results support the finding of Stone et al (13) that actively growing, vegetative buds and shoots of European hazelnut are susceptible to infection by ascospores of *A. anomala*. The data also refute the reports by Gottwald and Cameron (4,5,6) that the eriophyid bud mite, *P. avellanae,* has a major role in predisposing European hazelnut to infection by ascospores of *A. anomala*. Their conclusions were based on an inoculation study (5) in which ascospores of *A. anomala* were injected with a hypodermic needle into healthy or mite-galled vegetative buds during the months of December, January, or February. Although there was no statistical difference in disease incidence between the healthy and infested buds (5), they stated that galling of bud tissues by *P. avellanae* facilitated invasion of *A. anomala* by creating feeding wounds and a loose arrangement of bud scales. They did not consider healthy buds to be a site of infection because the scales are normally unwounded and too tightly appressed in winter to allow efficient spore penetration (5). Our experiments with *P. avellanae* demonstrated that neither its presence nor the effects of its presence (galling) enhance infection by *A. anomala*. In addition, the seedlings and trees used in our other experiments were not infested with *P. avellana*. Thus, as Stone et al (13) concluded, it is unlikely that this mite is significantly involved with the infection biology of this pathogen.

The site of infection experiments showed conclusively that young, vegetative tissues are susceptible to infection by *A. anomala*. The tissues most susceptible were the youngest stem internode and expanding leaf closest to the apical meristem. Under greenhouse conditions, the seedlings used in the experiments grew at a rate of about one node per week; thus, individual stem internodes or leaves were susceptible to infection for only a short period of time (2–3 wk). Two characteristics of young tissue that may account for its susceptibility are an immature epidermis and dense pubescence. Electron micrographs of young stem internodes (11) showed that ascospores of *A. anomala* adhered most frequently near the bases of trichomes, and that germ hyphae penetrated directly into underlying parenchyma before the epidermis matured. In addition, the dense pubescence on stem internodes proximal to the meristem may help to maintain a humid microclimate, which is favorable for ascospore germination and growth (J. Pinkerton, *unpublished data*).

The site of infection experiments also provided comparative results on the methods and evaluation criteria that we have used to study eastern filbert blight. Under field conditions, the minimum time from infection to development of external symptoms (stromata) is 12 mo (5,13). In contrast, microscopic detection of *A. anomala* in hand sections of xylem resulted in an accurate

TABLE 3. Effect of developmental stage of vegetative buds and shoots on seven European hazelnut cultivars on infection[a] by ascospores[b] of *Anisogramma anomala*

	Developmental stage at time of inoculation							
Cultivar	Dormant	Partial leaf emerged	Full leaf emerged	Multiple leaves emerged	Shoot elongation (2–4 nodes)	Shoot elongation (8–10 nodes)	Shoot growth ceased	LSD[c]
Barcelona	0	81 ± 5[d]	78 ± 11	96 ± 2	80 ± 7	31 ± 5	0	16
Casina	0	64 ± 9	74 ± 7	85 ± 4	58 ± 5	27 ± 4	0	15
Daviana	0	93 ± 3	91 ± 5	97 ± 3	80 ± 7	26 ± 8	0	14
Ennis	0	82 ± 4	97 ± 2	90 ± 4	90 ± 6	57 ± 6	0	2
Tonda di Giffoni	0	31 ± 6	26 ± 9	72 ± 6	61 ± 6	7 ± 3	0	16
Hall's Giant	0	58 ± 7	95 ± 3	79 ± 9	80 ± 6	22 ± 8	0	15
Willamette	0	33 ± 7	94 ± 14	96 ± 2	92 ± 3	34 ± 9	0	14
Average	0	63	79	88	77	29	0	

[a] Disease was evaluated in June or July of the year following inoculation by determining the incidence of stromata of *A. anomala* or phloem necrosis, which is a localized symptom indicative of infection by *A. anomala* (see Table 2).
[b] Twelve buds or shoots on each of four trees of each cultivar were sprayed with a suspension of 1×10^5 or 1×10^6 ascospores per milliliter. After inoculation, trees were covered with a plastic bag, incubated within a greenhouse at 20–26 C for 7 days, then returned to an outdoor shadehouse. The effect of inoculum concentration was not significant ($P > 0.05$).
[c] Fisher's protected LSD at $P = 0.05$.
[d] Standard error of the mean.

TABLE 4. Effect of bud infestations of the eriophyid mite, *Phytoptus avellanae*, on susceptibility of vegetative buds of European hazelnut to infection by ascospores[a] of *Anisogramma anomala*

	Healthy buds			Mite-infested buds		
Inoculation date	Bud development stage	No. of buds evaluated	% infected[b]	Bud development stage	No. of buds evaluated	% infected[b]
1990						
Jan 26	Dormant, scales normal	60	0	Bud scales swollen and galled	40	0
Feb 24	Dormant, scales normal	60	0	Buds scales loose and galled	40	0
Mar 24	Partial leaf emerged	60	29 ± 11[c]	Buds scales open and galled	40	21 ± 5
Apr 24	Shoot elongation	60	23 ± 9	Buds scales open and galled	40	22 ± 6
1991						
Jan 24	Quiescent, scales normal	69	0	Bud scales swollen and galled	78	0
Feb 24	Quiescent, scales normal	35	0	Buds scales loose and galled	75	0
Mar 30	Partial leaf emerged	21	62 ± 13	Bud scales open and galled	33	0

[a] A suspension of 1×10^5 ascospores per milliliter was applied to buds with a camel hair brush.
[b] Disease was evaluated on June of the year following inoculation by determining the incidence of stromata of *A. anomala* or phloem necrosis, which is a localized symptom indicative of infection by *A. anomala*.
[c] Standard error of the mean.

assessment of infection at 3 mo after inoculation (Table 2). Accuracy of the freehand sectioning technique was dependent on knowing which stem internode was most susceptible to infection at the time of inoculation. The brightly-colored tree-wound sealant applied to isolate inoculated stem internodes from surrounding tissues remained on the stems during the incubation period and served as a marker of the probable site of infection. The cold period, to which all inoculated plants were subjected during incubation (either naturally or artificially), was necessary to initiate development of stromata of *A. anomala* and phloem necrosis (14). Compared with a natural dormancy period, the artificial 4-mo cold room treatment imposed on Royal seedlings reduced the time to development of stromata and phloem necrosis by about 2 mo. The symptom of phloem necrosis, observed by stripping the bark from woody stems, was consistently associated with seedlings in which hyphae of *A. anomala* were detected. Conversely, development of stromata of *A. anomala* occurred on only 60% of infected seedlings, and thus was the least reliable of methods used to confirm infection. Many, but not all, of the infected seedlings that failed to develop stromata died before the end of the incubation period, apparently from the stress of disease. For infected seedlings that remained alive, failure to produce stromata was consistent with a field study (13) in which some naturally infected trees (10%) did not produce stromata until 24–27 mo after inoculation.

Stone et al (13) established that a postinoculation mist treatment increased incidence of infection by *A. anomala* on hazelnut seedlings. The inoculum concentration experiments in the present study confirmed that infection is enhanced by this treatment. However, enclosing inoculated plants in plastic bags was equally effective, and we now use this treatment preferentially to avoid potential redistribution of ascospores placed on shoot tips.

Ascospore concentrations in applied inoculum in excess of 5×10^4 spores per milliliter were necessary to ensure a reasonable chance of infection (Table 1). This effective concentration of ascospores is relatively high compared with many other fungal pathogens (15). Nonetheless, in moderately to severely diseased orchards, we have routinely measured average daily ascospore releases in the range of 10^5–10^7 ascospores/m^2 of trap surface in rain caught under hazelnut canopies during March and April (7,9,13).

The qualitative effects of the developmental stage of European hazelnut on susceptibility to *A. anomala* were consistent across seven cultivars. No cultivar became diseased when ascospores were applied to dormant buds in mid-February, or when ascospores were applied to woody shoots that had stopped growing in late summer. All cultivars became diseased if buds or shoots were growing actively. Overall, the most susceptible developmental stages were from full leaf emergence (mid- to late March) to elongating shoots with two to four nodes (late April to early May). Among cultivars, quantitative differences in the incidence of infection may correspond to the level of resistance to the disease. Cultivars Casina and Tonda di Giffoni, which had the lowest incidences of infection in this study, were found previously (8) to be more resistant to eastern filbert blight than cvs. Barcelona, Daviana, and Ennis, which had the highest incidences of disease in Table 3.

Results of the development stage experiment confirm the conclusion of Johnson et al (1993) that three to five fungicide treatments applied on a 2–3 wk schedule beginning at bud break may be necessary to control eastern filbert blight in susceptible European hazelnut cultivars. In Oregon, releases of ascospores occur frequently in March and April, but begin to decline in May and usually stop completely in summer (9). Consequently, once European hazelnut has resumed active growth in spring, the temporal availability of inoculum within an orchard is probably more limiting to the establishment of new infections than is the availability of susceptible tissue.

LITERATURE CITED

1. Barss, H. P. 1930. Eastern filbert blight. Calif. Agric. Dep. Bull. 19:489-490.
2. Cameron, H. R. 1976. Eastern filbert blight established in the Pacific Northwest. Plant Dis. Rep. 60:737-740.
3. Davidson, A. D., and Davidson, R. M. 1973. *Apioporthe* and *Monchaetia* canker reported in Western Washington. Plant Dis. Rep. 57:522-523.
4. Gottwald, T. R., and Cameron, H. R. 1979. Studies on morphology and life history of *Anisogramma anomala.* Mycologia 71:1107-1126.
5. Gottwald, T. R., and Cameron, H. R. 1980. Infection site, infection period, and latent period of canker caused by *Anisogramma anomala* in European filbert. Phytopathology 70:1083-1087.
6. Gottwald, T. R., and Cameron, H. R. 1980. Disease increase and the dynamics of spread of canker caused by *Anisogramma anomala* in European filbert in the Pacific Northwest. Phytopathology 70:1087-1092.
7. Johnson, K. B., Pscheidt, J. W., and Pinkerton, J. N. 1993. Evaluation of chlorothalonil, fenarimol, and flusilazole for control of eastern filbert blight. Plant Dis. 77:831-837.
8. Pinkerton, J. N., Johnson, K. B., Mehlenbacher. S. A., and Pscheidt, J. W. 1993. Susceptibility of European hazelnut clones to eastern filbert blight. Plant Dis. 77:261-266.
9. Pinkerton, J. N., Johnson, K. B., and Pscheidt, J. W. 1990. Ascospore discharge of *Anisogramma anomala* under field and controlled conditions. (Abstr.) Phytopathology 80:1031.
10. Pinkerton, J. N., Johnson, K. B., Theiling, K. M., and Griesbach, J. A. 1992. Distribution and characteristics of the eastern filbert blight epidemic in western Oregon. Plant Dis. 76:1179-1182.
11. Pinkerton, J. N., Stone, J. K., Nelson, S. S., and Johnson, K. B. 1993. Site and mode of infection of *Anisogramma anomala* in European hazelnut (*Corylus avellana*). (Abstr.) Phytopathology 83:1391.
12. SAS Institute Inc. 1988. SAS/STAT users guide, Release 6.03 edition. SAS Institute Inc., Cary, NC.
13. Stone, J. K., Johnson, K. B., Pinkerton, J. N., Pscheidt, J. W. 1992. Natural infection period and susceptibility of vegetative seedlings of European hazelnut to *Anisogramma anomala.* Plant Dis. 76:348-352.
14. Stone, J. K., Pinkerton, J. N., and Johnson, K. B. 1994. Axenic culture of Anisogramma anomala: Evidence for self-inhibition of ascospore germination and colony growth. Mycologia 86:674-683.
15. Van der Plank, J. E. 1975. Principles of Plant Infection. Academic Press, New York.
16. Zadoks, J. C., and Schein, R.D. 1979. Epidemiology and Plant Disease Management. Oxford University Press, New York.