

Phomopsis Canker of European Black Alder Found in Kentucky Seed-Production Areas

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ABSTRACT

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Phomopsis alnea caused basal stem cankers on European black alder (*Alnus glutinosa*) growing in two Kentucky seed-production areas. Fall surveys indicated that cankers affected 52 and 35% of the stems at the two sites. Mortality was 17 and 11%, respectively. Wound inoculation of moisture-stressed seedlings in the greenhouse resulted in cankers from which *P. alnea* was later recovered. The fungus inhibited normal callus tissue formation in all inoculation treatments and caused one canker that was actively expanding 23 wk after inoculation.

European black alder (*Alnus glutinosa* (L.) Gaertn.) is a naturalized tree in some areas of eastern Canada and the northeastern United States (1). Biomass plantations of domestic alder species have produced fiber in two or more cutting cycles under coppice management (2), and this system may apply to European black alder. More commonly, black alder is interplanted with other commercial tree species on the dry adverse sites common to mine spoil in Kentucky. It is considered a valuable site-rehabilitating tree of short life and limited product value. Stands are established by planting 1-0 bareroot nursery stock.

To overcome dependence on outside sources of seed for nursery production of black alder, the Kentucky Division of Forestry established seed-production areas near Gilbertsville, KY, and in the Pennyriple State Forest in 1978. Phenotypically superior selections from seedbeds at the John P. Rhody Nursery (Kentucky Division of Forestry) were

planted on old fields. Early survival was nearly 100% at both sites, but dieback and mortality were evident in a few seedlings by the end of 1978. Symptoms did not appear to be related to outplanting shock. By midsummer 1980, stem mortality was common in both plantings. Close examination revealed sunken basal cankers with abundant pycnidia of *Phomopsis* spp. present on many dying and dead trees.

A survey was needed to determine the cause and extent of the condition. This paper reports the occurrence of previously unreported disease of European black alder and the results of the surveys conducted in 1981.

MATERIALS AND METHODS

Survey. A complete survey of the Gilbertsville planting was made in February 1981 and again in September. The Pennyriple planting was surveyed only in September. Trees were examined for cankers and any signs of causal agents and for resprouting from the root collar of killed stems.

Pathogenicity tests. Preliminary inoculations of vigorously growing, unwounded, succulent stem tissue with conidia of the *Phomopsis* spp. isolate did not result in cankers. Three wound-inoculation techniques were then tested on moisture-stressed seedlings. Attempts were made to establish moisture-stress conditions shown by Schoeneweiss (4) to predispose woody ornamentals to attack by canker-causing pathogens commonly associated with diseased stressed plants. Potted (pots 20 × 25 cm) 1-0 *A. glutinosa* seedlings (0.75 cm minimum basal stem caliper) were grown in a greenhouse for about 2 mo. Actively growing seedlings were continuously stressed for 3 wk

before inoculation by withholding moisture until the foliage wilted and partial defoliation occurred. Water was then applied in sufficient quantity (about 150 ml) to moisten the soil without alleviating wilt symptoms for more than 2-3 hr. Many remaining leaves showed marginal foliar necrosis at the time of inoculation.

Three wound-inoculation techniques (treatments) were used to evaluate the pathogenicity of the *Phomopsis* isolate. A 3-wk-old single-spore isolate was used in all treatments. Treatment one consisted of a suspension of 5×10^6 *Phomopsis* conidia per milliliter of sterile deionized water (SDW) introduced into three syringe needle wounds through the bark to the xylem on newly formed stem tissue. It is estimated that 1.5×10^5 spores were inoculated into each seedling.

Treatment two involved slicing through the bark and cambium to the surface of the xylem with a sterile scalpel in a "T" configuration on suberized tissue 10 cm above the ground line and introducing a 3-mm-square block of 2% V-8 agar (V8A) supporting mycelium and at least one pycnidium of the fungus under the bark.

Treatment three consisted of splitting the stem longitudinally for about 4 cm on suberized tissue 10 cm above the ground line and inserting a 2% V8A block colonized by the *Phomopsis* isolate and supporting at least one fruiting body to expose all available stem tissue types to the fungus. Control trees consisted of SDW introduced into wounds for treatment one and sterile 2% V8A blocks for treatments two and three. The wounds produced in treatments two and three were sealed with Parafilm immediately after inoculation. Four control and 10 inoculated seedlings were used in each treatment. All trees were incubated in the greenhouse under continued moisture stress for 6 wk, when the Parafilm seals were removed and the trees moved to a shadehouse under a polyethylene tarp to prevent rain watering. Twelve weeks after inoculation, all inoculation and control wound sites were photographed and one-half of all treatments were harvested and stem tissue cultured on 2% V8A. After leaf fall (23 wk after inoculation), the remaining trees were harvested and cultured.

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RESULTS

Survey. Multiple cankers were common on all survey dates and most were found on the main stem within 0.5 m of the ground and on root collar sprouts

appearing after main stem death. Canker margins were discrete, with dark faces and intact bark (Fig. 1A). Dark pycnidia were often observed fruiting on the canker (Fig. 1B). Cankers placed in moist

chambers for 1 wk produced pycnidia with alpha- and beta-conidia, typical of *Phomopsis* spp. Host tissue placed on 2% V8A produced cultures identical to single alpha-conidia isolates from the pycnidia found on host material. The fungus was identified as *P. alnea* (Sacc.) Hoehn. by F. A. Uecker, Research Mycologist, USDA, ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705.

The February survey of the Gilbertsville area showed that 56.5% of the original 540 trees had stem cankers and 24.7% were dead and cankered (Fig. 2), as indicated by sunken areas near the ground line and the presence of *P. alnea* fruiting bodies. Perennial stem cankers with killed callus tissue were absent. Missing trees accounted for 6.3% of the original planting. Cankers were present on all sides of the main stem and were never higher than 1.25 m above the ground. Branch cankers were uncommon. Canker-wound associations were not evident, in spite of the presence of several large basal wounds caused by mowers.

Some recovery in the Gilbertsville planting was noted during the September survey (Fig. 2). There was a 35% decline in the number of dead cankered trees and a slight increase (8.4%) in the number of live healthy trees. The increase in the number of missing trees over the growing season was due to the deterioration of small dead cankered trees that were standing in February. The total number of cankered trees dropped by 33, a decline of 4.7% based on the number of trees available for survey on each date. Canker-free root collar sprouts produced after cankered stems died accounted for most of the increase in the number of healthy trees. Some root collar sprouts of cankered trees were newly infected, but all callus tissue appeared healthy. Only one canker was completely closed by callus tissue.

Disease incidence and mortality at the Pennyriple site were less than at Gilbertsville. Basal stem cankers were present on 34.9 and 51.8% of the stems, respectively, whereas canker-associated mortality was 11.9 and 16.6%, respectively (Fig. 2). Damage by deer and from hand tools used for weed control was common, but cankers were not associated with this damage. Root collar sprouts were observed at the bases of some dead stems that had previously been cankered.

Inoculations. Inhibition of callus formation was the primary symptom observed with all inoculation techniques and was most pronounced in treatment two (Fig. 3). Wounds on 42% of all control seedlings were closed by callus tissue 12 wk after inoculation, whereas all wounds on inoculated seedlings were still open. After 23 wk of incubation, all remaining wounds on control seedlings were closed, but only 20, 0, and 60% of the remaining inoculated seedlings in treatments one, two, and three, respective-

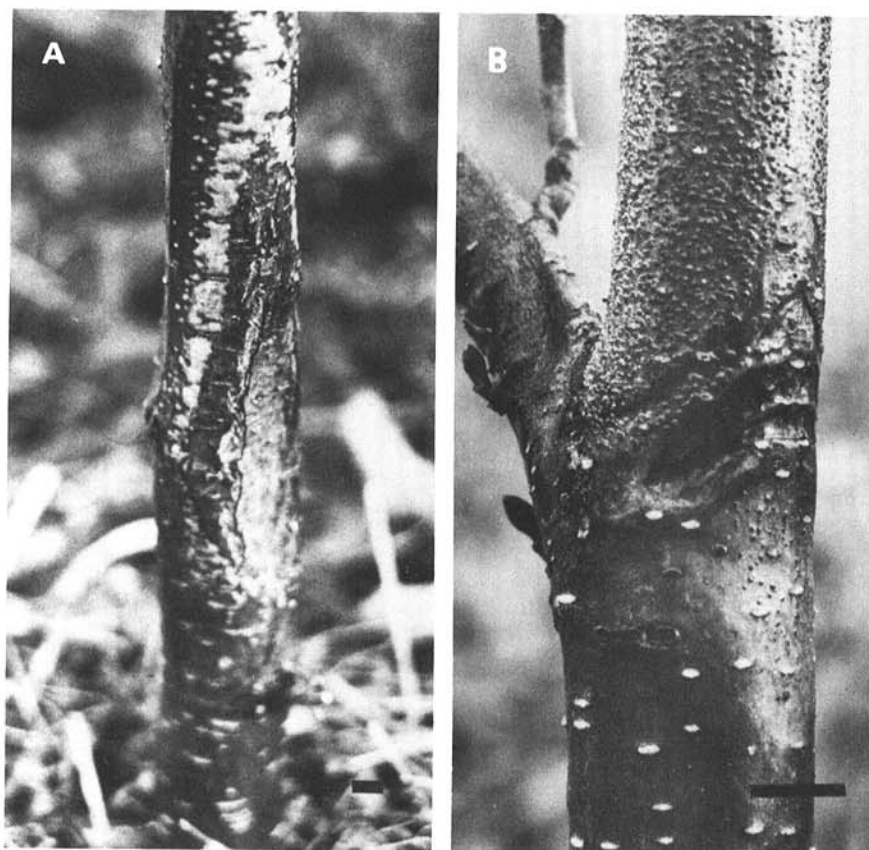


Fig. 1. (A) Basal canker on *Alnus glutinosa*. (B) Girdling stem canker with pycnidia on small diameter stem of *A. glutinosa*. Scale bar = 1 cm.

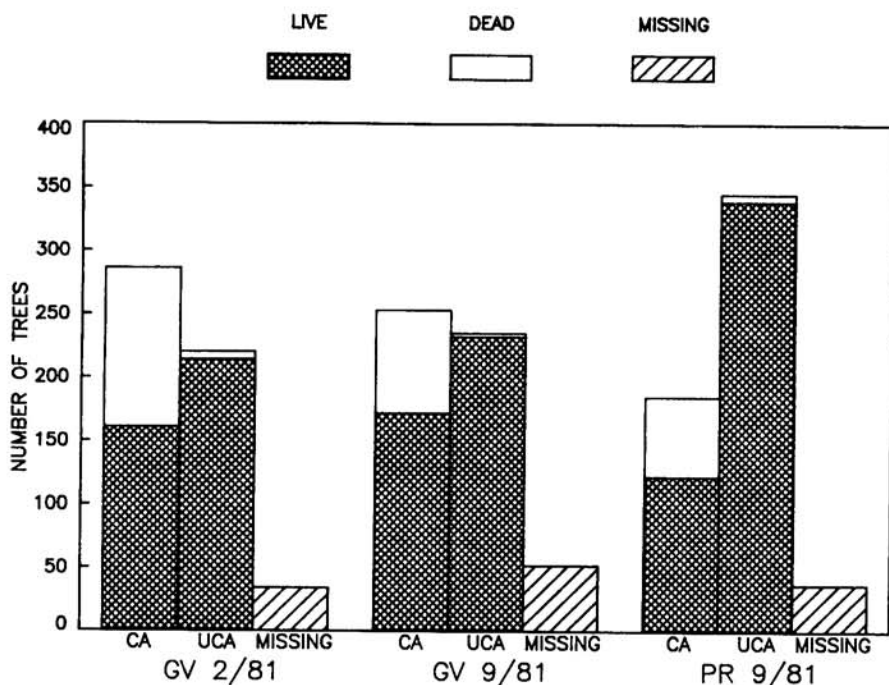


Fig. 2. Number of trees cankered by *Phomopsis alnea* (CA), uncankered (UCA), and missing in European black alder seed production areas at Gilbertsville (GV) and Pennyriple (PR), KY. Surveys were conducted in February and September 1981. The Gilbertsville and Pennyriple sites were planted with 540 and 567 trees, respectively.

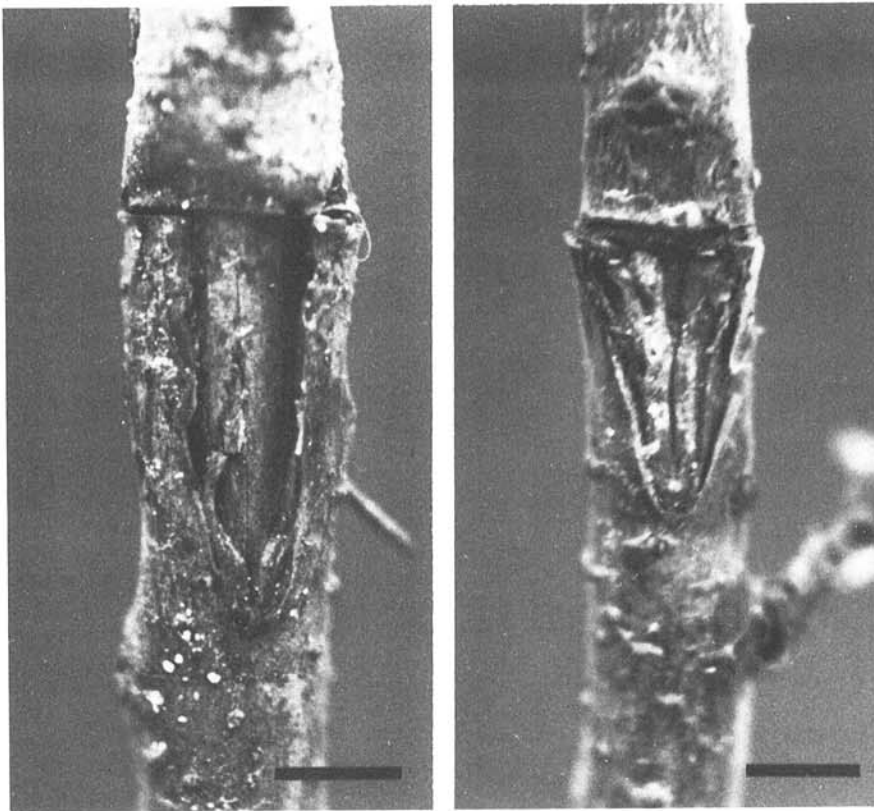


Fig. 3. Stems of *Alnus glutinosa* (left) inoculated (treatment two) with *Phomopsis alnea* and (right) control. Callus inhibition was consistent in all wound-inoculation treatments and at both observation dates. Scale bar = 1 cm.

ly, had closed wounds. Only one inoculated seedling (treatment two) showed necrosis of phloem extending beyond the inoculation zone, characteristic of stem cankers in the field. *P. alnea* was recovered from cankered tissue of this seedling 7.5 mm distal from the inoculation point. Xylem discoloration extending several millimeters radially into the stem was observed in inoculated seedlings of treatments two and three, but only surface xylem discoloration at the wound site was evident in the controls of those treatments.

Pycnidia of *P. alnea* containing viable conidia were present in all wound-inoculation treatments regardless of wounding technique and isolation date. Fungus isolates identical to *P. alnea* were recovered from all wound-inoculated seedlings, but control seedlings yielded no pathogenic fungi. *P. alnea* was occasionally recovered from the discolored

xylem subtending the surface of the inoculation zone, indicating active colonization by the fungus.

DISCUSSION

This is the first report of *Phomopsis* canker of European black alder (3,5,6). *P. alnea* appears to be a weak canker pathogen on this species. Trees infected in the field developed annual cankers of restricted size, involving a portion of the circumference on sapling-sized trees. Small diameter saplings, however, may develop girdling cankers that sometimes result in tree mortality. Usually, girdled trees sprout from the root collar.

Although only one of 30 wound-inoculated moisture-stressed seedlings produced a canker similar to those observed on naturally infected trees, callus inhibition was consistent in all inoculation treatments. Discoloration and colonization of stem xylem tissue and

the presence of *P. alnea* pycnidia represent further supporting evidence of pathogenicity. The production of stem cankers on artificially inoculated trees with characteristics similar to those observed in the field may be possible under different conditions.

Various black alder provenances show different levels of growth, stem form, and survival (1). If different seed sources are differentially susceptible to the disease and the seedlings in our inoculation tests represent relatively disease-resistant stock, the pathogenicity test results we have reported are not representative of the overall susceptibility of European black alder to *Phomopsis* canker.

No evaluations were made concerning disease control options. If *P. alnea* inoculum remains available and conditions favorable for disease development occur, a natural screening for disease resistance may result from survival of canker-free trees in the seed-production area. The effectiveness of natural screening is determined by the heritability of disease resistance.

Deterioration of alder established on mine spoil has been observed by the second author in Kentucky and by others elsewhere (1). Surveys of alder plantings on strip-mined land in the eastern and central United States should be considered to determine the extent and severity of the problem and the role of *Phomopsis* canker in the decline of European black alder plantings.

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