Research Reports

Bleeding Canker of European Beech in Southeastern New York State: *Phytophthora* Species, Spatial Analysis of Disease, and Periodic Growth of Affected Trees[1]

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Abstract

The epidemiology of bleeding canker, a *Phytophthora*-associated disease, on European beech remains unclear. Pathogen surveys as well as dendrological and spatial point pattern analyses (SPPA) were conducted to identify factors contributing to disease progress on beech at the Green-Wood Cemetery (GWC) and Planting Fields Arboretum (PFA) in southeastern New York State. *Phytophthora pini* was the predominant *Phytophthora* isolated from cankers as well as soil under asymptomatic and diseased (canker bearing) European beech at each site. No significant differences existed between asymptomatic and *Phytophthora*-infected trees according to diameter breast height, elevation, and the *Phytophthora* spp. The radial growth (25-yr chronology for 1986 to 2010) of infected European beech at GWC and PFA, however, was significantly less when compared to asymptomatic beech; yet, residual growth was similar among the latter cohorts, providing no evidence for the instigatory effect(s) of environmental stressors on disease progress. SPPA demonstrated all beech at GWC and PFA were planted in non-random aggregates, whereas the distribution of diseased European beech did not deviate from random. Collectively, results indicated bleeding canker is a slow, chronic disease and the overland tree-to-tree spread of *P. pini*, and accompanying *Phytophthora* spp., is rare or does not occur at GWC or PFA.

Index words: beech decline, dendrochronology, *Fagus*, K-function, plant disease, soil.

Species used in this study: European beech (*Fagus sylvatica* L.).

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Significance to the Horticulture Industry

Phytophthora species are multi-host plant pathogens of herbaceous and woody plants, including European beech, and are among the most ecologically and/or economically damaging pathogens in forests, nurseries, and urban landscapes, worldwide. The aim of the present study was to elucidate the epidemiological factors *in situ* permitting the spread and intensification of bleeding canker on European beech, a disease caused by at least five *Phytophthora* species, and presently associated with the mortality of countless mature (>100 yrs old) beech in arboreta, public gardens, and private landscapes in New York State and elsewhere in the United States. *Phytophthora pini*, formerly classified phylogeneti-

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cally as *P. citricola* taxon I, was most often cultured from necrotic lesions on the aboveground portion of European beech well as the rhizosphere of asymptomatic and diseased trees. The primary infection of European beech by *Phytophthora* spp., particularly *P. pini*, is restricted to fine roots and the tree-to-tree movement of these pathogens likely is confined to soil, if and when it occurs. *Phytophthora* infection likely is also a predisposing factor, increasing the susceptibility of infected beech to abiotic and/or biotic stress that further hasten reductions in tree vigor, and hence, the longevity of affected trees. Future studies should focus on examining the spatial interactions between additional tree-site conditions (e.g., soil architecture and chemistry) and the below-ground population biology of *P. pini* (genotypic diversity and inoculum density). The phosphite fungicide AGRI-FOS® appears to be an effective, therapeutic rather than curative chemical control of bleeding canker on European beech.

Introduction

European beech was introduced to North America during Colonial times, adding Old World charm to New World gardens and landscapes. The species thrives with minimal supplemental care and is relatively free from damaging insect pests or diseases (Johnson and Lyon 1976, Sinclair and Lyon 2005). Furthermore, when individual trees are grown without nearby competition, the lower branches often become so massive as to rival the main stem for prominence. Consequently, European beech has become a dominant tree at historic sites, arboreta, college campuses, public gardens, and residential estates in the northeastern United States (Dirr 1998). Moreover, numerous 200+ year-old trees have added value as witness trees because of their presence during significant events in American history, such as the War of Independence from 1775 to 1783.

In the past 70 years, and with increasing frequency since the mid-1980s, an abrupt (and at first, inexplicable) decline and death of mature European beech caused great concern among municipal foresters, arborists, and tree health specialists in the northeastern U.S. Tree death often occurred in as few as one or two years after the first appearance of conspicuous crown symptoms, which included rapid wilting of foliage and chronic branch dieback. Affected beech often were in sites seemingly favorable for growth because the trees in question were greater than 80 years old, and asymptomatic trees of less and/or similar age were often growing nearby [e.g. within 200 m (656 ft)]. In 1998, with the number of reports from the field of declining European beech seemingly increasing and results of a northeast U.S. regional survey suggesting disease incidence in excess of 40% at many sites (G. Hudler, unpublished), we began a more thorough assessment of the problem across a large geographic area including site(s) in Connecticut, Maryland, Massachusetts, New York State (NYS), and Pennsylvania. One of our first discoveries was that trees in the earliest stages of disease with little evidence of branch dieback or leaf chlorosis consistently had bleeding cankers as the first symptoms of impending tree decline (Jung et al. 2005). Subsequent analyses of bark from the cankered areas via *Phytophthora*-specific ELISA tests and isolation of putative pathogens to selective culture media indicated that taxa in the genus *Phytophthora* de Bary (Pythiaceae) were associated with, and likely responsible for bleeding cankers on European beech in incipient as well as advanced stages of decline. Completion of Koch's postulates on greenhouse-grown seedlings and one 60-year-old landscape tree further confirmed the infectivity and virulence of *P. cactorum* (Leb. & Cohn) Schröeter and *P. citricola* Sawada isolated previously from diseased beech (Nelson et al. 2010, Weiland et al. 2010).

The genus *Phytophthora* consists of numerous soil-borne root and canker pathogens that are among the most destructive diseases of agricultural crops, nursery plants, and forest trees worldwide (Brasier and Jung 2003, Brasier and Jung 2006, Erwin and Ribeiro 1996, Hansen et al. 2012). Moreover, *Phytophthora*-associated beech decline, a disease hereafter referred to as bleeding canker, is not new. Day (1938, 1939) first reported characteristic symptoms of bleeding canker on European beech and sweet chestnut (*Castanea sativa* L.) in Europe, attributing aboveground symptoms to either *P. cambivora* (Petri) Buism or *P. cinnamomi* Rands. He also reported *P. syringae* Kleb. infected the roots of declining trees. Thereafter, Pirone (1942) and Caroselli (1953) found *P. cactorum* inciting similar cankers on European beech as well as a numerous other hardwoods in the northeast U.S. More recently, Jung and Blaschke (1996), Jung et al. (2003), and Motta and Annesi (2003) reported that in addition to the aforementioned species, *P. citricola*, *P. pseudosyringae* T. Jung & Delatour, *P. gonapodyoides* (Petersen) Buisman, and a yet-to-be described species could also be isolated from the margins of bleeding cankers of beech. Jung et al. (2005) then found that a putative pathogen, tentatively identified as *P. inflata* Caroselli & Tucker [perhaps part of the *P. citricola* complex (Kong et al. 2003)] was also associated with declining trees in sites throughout NYS. A more thorough survey by Nelson et al. (2010) in NYS and adjacent states revealed that at least one symptomatic tree could be found in every site where ten trees were visible from an at-grade vantage point, and in some sites, the incidence of symptomatic trees was as high as 50%. Following attempts to grow the pathogen from diseased bark onto selective media (Nelson et al. 2010), two-thirds of the cankers yielded isolates in the genus *Phytophthora*, but confirmation of the identity of one or more putative pathogens was confounded by newly emerging nucleic acid sequence data that challenged previous assumptions about individual species and species complexes.

Oudemans et al. (1994) concluded that *P. citricola*, in particular, was actually a species complex consisting of as many as five distinct taxa. Delimitation via single-stranded conformation polymorphisms (SSCP) of ribosomal DNA (Kong et al. 2003) pared the complex to four taxa and with further morphological analysis, eventually to three (Gallegly and Hong 2008). Shortly thereafter, *P. citricola* taxon II was described as *P. plurivora species novum* (Jung and Burgess 2009) and *P. citricola* I was resurrected to *P. pini* Leonian (Hong et al. 2011); the latter a species originally described by Leonian in 1925 as a pathogen of pine roots in northern Minnesota (Leonian 1925). Identity of the third species, presently *P. citricola* III, has not been determined.

Integration of taxonomic work by colleagues with our own field and laboratory observations (Nelson et al. 2010, Weiland et al. 2010), leave us to conclude that the species of *Phytophthora* most often associated with bleeding cankers on European beech in the northeastern U.S. are *P. pini* (65% recovery) followed by *P. cactorum* (28% recovery) and occasionally other species. One concern among the tree care community, that the seemingly rapid escalation in bleeding canker incidence since the mid-1990s was due to the ap-

pearance of a new exotic pathogen, perhaps *P. ramorum* (Rizzo et al. 2002, 2005; Grünwald et al. 2012), was allayed as hundreds of microscopic examinations of cultures and analyses of definitive nucleic acid sequences failed to support said concerns.

Little epidemiological information exists for *Phytophthora* on European beech in N. America. There are several chronic obstacles that plagued any effort to do epidemiological research with large numbers of large trees on private land. First, trees often are in urban or suburban landscapes with individual trees or copses of less than four trees at each site independently managed by the landowner or a tree care professional(s) hired to provide plant health maintenance on that particular property. Second, the trees are widely dispersed geographically and generally occur on sites with different soils, site histories, and management regimes. Third, historical records for most trees were poor, limited to the memory of the current landowner who rarely had information beyond the most recent decade. Fourth, inasmuch as European beech is not native to N. America, trees were transplanted from unknown origins (sometimes from overseas) to their current site(s) as bare root or balled saplings. In either case, these trees had been grafted onto rootstock also of unknown origin with whatever soil and associated microbes comprised the rhizosphere.

With these limitations in mind, we evaluated two somewhat unique populations of European beech in southeastern New York, each with over 40 European beech trees > 80 years old and under single-owner management. Our objectives were to (1) determine which taxa of *Phytophthora* were most abundant in symptomatic trees and in rhizospheres of asymptomatic and diseased trees, (2) to determine via growth ring analysis whether the incidence of disease was reflected in the most recent periodic growth series of affected beech, and, (3) determine whether diseased trees occurred in predictable clusters or were randomly distributed through portions of the sites where beech had been planted.

Materials and Methods

Study sites. The two sites, approximately 43 km (27 miles) apart, were established on Long Island, NY. The first was the 193 ha (478 A) Green-Wood Cemetery (GWC; Brooklyn, Kings County), a former Revolutionary War battlefield converted to a formal cemetery in 1838. Inspection of historical photography and tree maintenance records indicated that the first cohort of beech likely was planted in the late 19th to early 20th-century with subsequent cohorts installed periodically over the last 75 years. A 2005 tree inventory counted 201 European beech within the cemetery including 189 standing trees and 12 stumps. In 2009, at the beginning of present study, 155 beech were identified on the cemetery grounds (Fig. 1) with nearly 60 trees of approximately100 years of age or older. Tree maintenance records showed also that at least 30 additional trees from the over-100-year cohort had died following the appearance of bleeding cankers or undetermined causes and storm damage. All trees with bleeding cankers were treated in 2008 with the phosphite fungicide AGRI-FOS® (Agrichem/Liquid Fertiliser PTY. LTD, Queensland, Australia) plus the surfactant PENTRA-BARK® (Quest Product Corp., Linwood, KS) via low pressure, bark drench from ground level to 2.25 m (7.4 ft) high. Soils at GWC were derived from glacial till and characteristic of the Montauk-Forest Hills and Flat Bush-Riverhead series: well-drained; texture silt to sandy loam (or loamy sand); and,

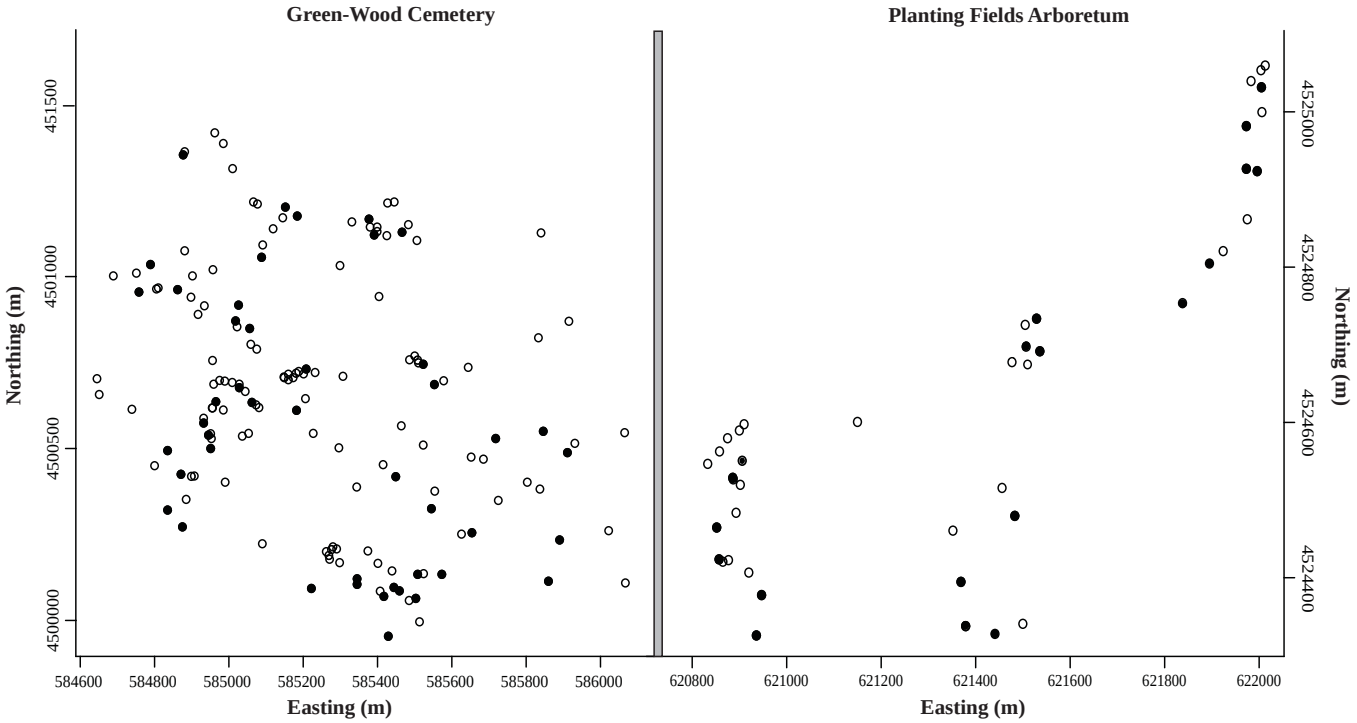

Fig. 1. Spatial distribution (UTM: Northing and Easting) of European beech within the Green-Wood Cemetery (Left) and Planting Fields Arboretum (Right) on Long Island, New York. Open circles and solid circles represent asymptomatic beech and diseased trees with bleeding canker, respectively.

moderately to strongly acidic (New York City Soil Survey Staff 2005).

The second site was at Planting Fields Arboretum (PFA) near Oyster Bay (Nassau Co.); PFA was formerly the 166 ha (400 A) private estate of the Coe family and deeded to the State of New York Trustees in 1955 and established as a state arboretum in 1971. Forty-three (n= 43) beech located within PFA were examined (Fig. 1). Soils under the trees were of the Plymouth series consisting of glacial outwash parent material and deep, excessively well-drained and sandy loam soils (Wulforst 1987). The majority of European beech at Planting Fields were transplanted to the site by the Coe family, including the legendary Fairhaven Beech, which was transplanted in winter 1915 [a specimen approximately 15 m (50 ft) tall and weighing 25,400 kg (5,600 lb) at time of transport] from Fairhaven, Massachusetts. The tree survived 69 years post-transplant until it succumbed, purportedly, to bleeding canker in 1984.

Sampling and isolation procedures and identification of Phytophthora species. European beech at GWC and PFA were systematically examined in August to November 2010 and July 2011, respectively. The following measurements and observations were taken and recorded for each tree per site: diameter breast height (dbh), presence and location of bleeding cankers (branch, main stem, and/or root flare), health status [0 = asymptomatic, no aboveground symptoms of disease (e.g., crown dieback, bleeding cankers, etc.); 1 = diseased, aboveground symptoms present including bleeding cankers], geographic position [Universal Transverse Mercator (UTM)], and elevation.

For diseased trees, bark plates (*c*. 4–25 cm^2 [*c*. 0.6–4 in^2]) including the phloem were collected from the outer edge of necrotic lesions and actively bleeding cankers with a mallet and sanitized chisel, transported to the laboratory in a picnic cooler with added ice, and stored at 4.0C (39.2F) for 12–18 h until processing. From each plate, 24 to 36 small pieces (*c*. 2 to 3 mm^2 [*c*. 0.003 to 0.005 in^2]) from the inner bark were removed and transferred directly onto selective PARPH-V8 agar [6 pieces/Petri dish; (Ferguson and Jeffers 1999)]. An additional 10–20 pieces of inner bark, of approximately equal size to those used for direct plating, were soaked in sterile distilled water for 36 to 72 h, blotted dry on sterile filter paper, and plated to PARPH-V8. Water within the flooded plates was decanted and replaced approximately every 12 h to remove tannins and minimize bacterial contamination. Plated samples were incubated in an environmental chamber at 20C (68F) in complete darkness, and after 2 d, developing cultures were examined daily with a compound microscope for the presence of oogonia and/or coenocyctic, *Phytophthora*-like hyphae. Suspected *Phytophthora* isolates were transferred to clarified 10% V8 juice agar [cV8A (17)].

At each site, soil samples, including fine and coarse roots, were taken from the rhizosphere under each diseased beech, as well as from under an equal number of asymptomatic trees. Samples were taken at four cardinal points at a distance of 1.0 to 1.5 m (3.3 to 4.9 ft) from the base of the main stem. Individual samples were procured by removing the upper organic horizon and collecting 500 g (~1 lb) soil to the depth of 30 to 35 cm (*c*. 12 to 14 in). Upon return to the laboratory, soils for each tree were bulked, mixed thoroughly, and assayed for the presence of *Phytophthora* spp. using a leaf baiting method described by Jung et al. (1996). Briefly, 3–6 d-old European beech and English oak (*Quercus robur* L.) leaflets were floated over 250 g of flooded soil (2.0 mL distilled water/g soil) for 3 to 10 d. Flooded soils were kept on a laboratory bench top (16:8 photoperiod, light:dark) at room temperature, and necrotic leaflets were examined microscopically for the presence of sporangia typical of *Phytophthora* spp. *Phytophthora*-infected leaflets were blotted dry, cut into small pieces, plated onto PARPH-V8, and incubated at 20C (68F) in complete darkness. Negative soil samples were dried at room temperature for 10 to 18 d and re-assayed using the same baiting technique.

Phytophthora spp. were identified according to morphology — colony pattern on culture media [cV8A, malt extract agar (MEA; BD Difco™; 45 $g \cdot L^{-1}$), potato dextrose agar (PDA; BD Difco™; 39 $g \cdot L^{-1}$)], hyphal swellings, and morphology and dimensions of sporangia, oogonia, and antheridia — and comparison to species descriptions (Erwin and Ribeiro 1996, Gallegly and Hong 2008, Hong et al. 2011). Isolates were also compared to *Phytopthphthora* spp. previously delineated to species using molecular analysis of rDNA (Nelson et al. 2010). Formation of sporangia for each isolate/species was stimulated by collecting five 5-mm (0.2 in) diameter agar plugs from the edge of a 5–7-d-old culture grown on cV8A. Plugs were placed into 10-cm (4 in) diameter Petri dishes, flooded with non-sterile 1.5% soil extract water [15 g (0.3 lb) *Phytophthora*-free field soil·L^{-1} sterile distilled water], and kept under continuous fluorescent light at 20C (68F). After 24 h, the plugs were rinsed twice with sterile distilled water, flooded with soil extract water, and incubated for an additional 24 to 48 h. Oogonia of heterothallic species were produced by crossing known mating-type testers on cV8A. Stock cultures of the *Phytophthora* spp. isolated from bark and soil samples in this study were maintained on corn meal agar (CMA; BD BBL™; 18 $g \cdot L^{-1}$; subcultured every 4 wk to fresh CMA) and deposited for storage at the Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY.

ELISA tests. In parallel with the isolation procedure, the presence of *Phytophthora* spp. was assessed in all bark panels removed from necrotic lesions and bleeding cankers using a commercial, double-antibody sandwich enzyme linked immunosorbent assay (DAS ELISA) kit (*Phytophthora* PathoScreen® Kit, Agdia Inc., Elkhart, IN). For each bark plate, finely ground samples of the inner bark (0.1 g) were removed using 225-grit sandpaper, added to the kit-provided extraction buffer (GEB2; 27.9 g·500 mL^{-1} sterile, Milli-Q water) in sterile 1.5-mL centrifuge tubes, and macerated. The ELISA was performed following the manufacturer's instructions, and, samples including positive and negative (GEB2) controls were tested in duplicate in each plate. After the final incubation step, the optical density (OD) for each well was measured in a spectrophotometer at 405 nm. The threshold for a positive reaction was ≥ 2× OD of the negative control.

Radial growth increment. The growth of *Phytophthora*-infected and disease-free European beech was compared at GWC and PFA. Using disease survey data (including ELISA results) from the respective sites, ten pairs consisting of one asymptomatic and one diseased beech were selected according to distance [< 50 m (164 ft) between paired trees] and similarity in dbh (< 10% difference) as well as site conditions

(aspect, slope). Trees were paired by proximity and dbh to reduce variation in growth attributable to differences in site characteristics and tree age. For each tree, one core at breast height [1.4 m (4.5 ft), uphill side] was removed with an increment borer [5 mm (0.2 in) diameter; Haglöf Inc., Madison, MS] and the resulting wound was filled with LacBalsam® (GmbH & Co., Minden, Germany). Although extracting two cores per tree is standard practice in dendrochronology and dendroclimatology studies (Speer 2010), a single-core per tree was taken at each site here per the request of the grounds management team to minimize the creation of entry points for pests and pathogens as well as reduce the aesthetic impacts on the trees sampled. All cores were placed into individually-labeled plastic straws with slits for ventilation and bundled in newspaper for transport to the laboratory.

Core samples were prepared for microscopic examination as described by Phipps (1985) and Stokes and Smiley (1996): air dried at room temperature for 7 d; glued into routed, solid wood-mounts with the cross-sectional view facing up using white water-soluble glue and tightly-woven string; and, left at room temperature for an additional 24 h to permit the glue to dry. Each core was sanded sequentially using 125-, 150-, 220-, 400-, and 800-grit sandpaper and cleaned with an airbrush. Tree-ring widths (radial increments) were obtained with 0.01 mm precision using a semi-automated measuring platform (LINTAB-III, Frank Rinn S.A., Heidelberg, Germany) beneath a stereomicroscope with cross hairs and the analysis software Corina Desktop version 2.12 (Brewer et al. 2010). The differentiation between earlywood and latewood according to color and vessel size was present in all cores examined consisting of 30 to 41 annual growth rings.

Spatial analyses. The geographic distributions (i.e., random or clustering) of *Phytophthora*-infected beech at GWC and PFA were analyzed separately in R ver. 2.13.1 (R Development Core Team 2011) using spatial point pattern analysis (SPPA), Ripley's *K*-function (Ripley 1977, Ripley 1981), combined with Monte Carlo (MC) simulations to discriminate aggregation and dispersion of diseased trees per site independent of the combined/overall distribution pattern of European beech [asymptomatic and infected; (Diggle 2003)]. The *K*-function quantifies the second-order property of a point process, interactions between points, by counting the expected points within Euclidean distance t of an arbitrary point within the fixed-area of interest (e.g., GWC or PFA). For SPPA, a circle of radius t was centered on each point (tree) and the number of neighboring points within the circle was counted. The mean number of points per unit area [density (λ)] was therefore defined as individual points n divided by area A ($\lambda = n / A$). The function $\lambda K(t)$ estimated the expected number of additional points within radius t of an arbitrary point within the area of interest. If the points were randomly distributed (i.e., Poisson distribution), the expected value of $K(t)$ would equal the area of a circle of radius t ($K(t) = \pi t^2$). In contrast, a $K(t)$ value greater than the area of a circle of radius t ($K(t) > \pi t^2$) would indicate aggregation, clustering. The unbiased estimator for $K(t)$ for an observed spatial pattern was

$$\hat{K}(t) = N^{-2} A \sum_{i} \sum_{j \neq i} w_{ij}^{-1} I_t(u_{ij})$$

where N is the number of points (trees) in the area of interest (A); u_{ij} is the distance between points i and j; w_{ij} is the weighting factor (i.e., the proportion of the circumference of a circle centered at point i with radius u_{ij} within *A)* to account for edge effects; and $I_t(u_{ij})$ is the counter variable, equal to 1 if $u \leq t$ and 0 if $u > t$; and, the summation is over all pairs of points [i.e., trees (Ripley 1977, 1981)].

Separate $K(t)$ estimates with radius u_{ij} = 50 m (164 ft) were analyzed in parallel to compare the spatial pattern of *Phytophthora*-infected beech versus the overall distribution of European beech at GWC as well as PFA. Monte Carlo simulations (1000 runs per simulation) of the distribution of 45 and 19 randomly chosen points without replacement, corresponding to the number of infected trees per site, were compared to the observed distribution of infected trees at GWC and PFA, respectively. Similarly, to determine the overall distribution of European beech (i.e., random or aggregated) at each site, MC simulations (1000 runs per simulation) of the distribution of 155 and 43 randomly chosen points without replacement were compared to the observed spatial distribution of asymptomatic and infected trees within the GWC and PFA. A ninety-five percent (95%) confidence envelope of spatially random distributions was calculated for each MC simulation.

Statistical analyses. For each site, the individual relationships of *Phytophthora*-infection (presence/absence) with dbh and elevation were evaluated separately using nominal logistic regression and one-way analysis of variance (1-way ANOVA). The dbh for seven *Phytophthora*-infected trees at PFA were not obtained as each tree was multipodal beginning below 1.4 m (4.5 ft). The number of samples included in the ANOVA for dbh between asymptomatic and infected beech, therefore, was reduced to 24 and 12, respectively.

The presence of *Phytophthora* spp. (count data), regardless of species, in the rhizosphere of asymptomatic and diseased trees was compared using contingency table analysis. Similarly, contingency analysis was also utilized to compare the location of bleeding cankers among diseased trees (branch, mainstem, and/or root flare) as well as to determine whether a relationship existed between the presence of *Phytophthora* spp. within rhizosphere soil and the incidence of cankers on aboveground tree parts.

The radial growth increment (25-yr series; 1986 to 2010) for *Phytophthora*-infected and asymptomatic beech was examined via two-way (2-way) ANOVA using presence/absence of *Phytophthora* infection, study site, and the interaction between *Phytophthora*-infection and site as dependent variables. Mean growth differences by health status and site were determined using post priori contrast comparisons ($\alpha = 0.05$), while a Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$) was utilized post hoc to compare differences of the mean 25-yr growth increment among infection status-site combinations. Ninety-five percent (95%) confidence intervals ($\alpha = 0.05$) in lieu of standard deviations were also calculated to demonstrate differences in mean growth by each of the dependent variables described previously.

In order to examine trends in overall growth between asymptomatic and diseased trees, the radial measurements per core were standardized to correct (detrend) for site, health status, and possibly, age-related growth. To do this, the growth series for each tree was fitted separately using linear regression and the residuals [increment width (mm) – mean increment (mm) per series] were used as the standardized radial increments. The standardized, periodic growth (5-yr

Table 1. Presence of cankers, ELISA results, and comparison of mean[z] diameter and elevation between beech with (infected) and without cankers at the Green-Wood Cemetery (GWC) and Planting Fields Arboretum (PFA)[y].

Site	Trees (n)	Cankers (% trees)		Mean diameter breast height (dbh, cm)		Mean elevation (m)	
		Absent	Present (Positive ELISA)	Without cankers (CI)	Infected (CI)	Without cankers (CI)	Infected (CI)
GWC	155	71	29 (100)	98.6 (± 5.2)	103.8 (± 6.5)	38.4 (± 2.1)	35.3 (± 3.0)
PFA	43	56	44 (100)	121.7 (± 14.9)	135.7 (± 10.2)	45.2 (± 6.8)	48.6 (± 8.2)

[z]95% percent confidence intervals (CI) were computed for comparison of mean differences.

[y]At both sites, no significant differences were observed in mean dbh and mean elevation between between canker-free and trees with cankers (infected) based on 1-way ANOVA.

increments) across the 25-yr series between asymptomatic and *Phytophthora*-infected beech was then compared using a 2-way ANOVA; mean differences by health status-site combinations among the 5-yr periodic growth increments were determined using a Tukeys' HDS.

Results and Discussion

Tree diameter, elevation, and ELISA. Of the 155 beech at the GWC, 45 (29%) had bleeding cankers (necrotic lesions of the inner bark) and at least one canker per diseased tree yielded a *Phytophthora*- positive ELISA (Table 1). Likewise, 19 of 42 trees (44%) examined at PFA had one or more bleeding cankers that were ELISA positive. However, no statistical difference existed between asymptomatic and diseased European beech at the GWC and PFA with regards to mean dbh or elevation (Table 1). The mean dbh for asymptomatic and diseased trees in the GWC was 98.6 cm [38.9 in; range = 13.4 to 170.7 cm (5.3 to 67.2 in)] and 103.7 cm [40.8 in; range = 11.7 to 156.5 cm (4.6 to 61.6 in)], respectively. European beech in PFA without aboveground symptoms of bleeding canker averaged 121.7 cm in dbh [47.9 in; range = 27.9 to 163.8 cm (11.0 to 64.5 in)] compared to 135.7 cm (53.4 in) for diseased trees [range = 103.1 to 162.6 cm (40.6 to 64.0 in)]. The mean elevation of asymptomatic beech in the GWC was slightly higher [38.4 m (126.0 ft)] when compared to symptomatic beech [35.3 m (115.8 ft)]; in contrast, diseased trees at PFA generally were positioned topographically at higher elevations [48.6 m (159.4 ft)] than asymptomatic trees [45.2 m (148.3 ft)].

Phytophthora *spp.: cankers and soil baiting. Phytophthora* species were isolated frequently from cankers as well as soils under asymptomatic and diseased European beech at the GWC and PFA (Table 2). Three different species were isolated, with varied frequency, from cankers on diseased trees in the GWC with *P. pini* (67%) being the most frequently isolated species followed by *P. cactorum* (18%) and *P. cambivora* (4%; A1 mating-type only). *Phytophthora pini* also was most frequently isolated at PFA, being recovered from cankers on 63% of *Phytophthora*-infected beech whereas *P. cactorum* was recovered less frequently (16%) and *P. cambivora* was not isolated from cankers sampled in PFA.

There was no statistical relationship between *Phytophthora* species and the above ground location of bleeding cankers, branches, main stem, and/or root flares, on European beech at either study site. The occurrence of bleeding cankers by tree part, however, differed significantly at both the GWC ($\chi^2 = 103.2$, df = 9, $P < 0.001$) and PFA ($\chi^2 = 36.3$, df = 9, $P < 0.001$). At each site, cankers were most often found only on the main stem (43% GWC and 47% PFA trees) or in combination on the main stem and root flares (33% GWC and 42% PFA trees). Aerial cankers on branches within the canopy of individual trees were rarely observed (9% GWC and 11% PFA trees); but when evident, they accompanied

Table 2. Isolation of *Phytophthora* spp. from bleeding cankers (cankers) as well as the rhizosphere soil (soil) of European beech with and without cankers in the Green-Wood Cemetery (GWC) and Planting Fields Arboretum (PFA).

Site / Material	Positive *Phytophthora* spp. isolation[z] (% trees)				*Phytophthora* spp. combinations[z] (% trees)				
	PCT	PCM	PGN	PIN	PCT, PCM, PIN	PCT, PIN	PCM, PIN	PGN, PIN	*Phytophthora*-free[y] (% trees)
GWC[x]									
Cankers	18	4		67					11
Soil									
Without cankers	4	2	2	33		13	4	2	38
With cankers	4	4		27		22	13	2	22
PFA[x]									
Cankers	16			63		5			16
Soil									
Without cankers	11	5		42		5			37
With cankers	5	11		37	5				42

[z]PCT = *Phytophthora cactorum*, PCM = *P. cambivora*, PGN = *P. gonapodyides*, PIN = *P. pini*.

[y]Trees or rhizosphere soil yielding no *Phytophthora* spp.

[x]Site material (total number of trees): GWC cankers (45) and soil (45 without cankers, 45 cankered); PFA cankers (19) and soil (19 without cankers, 19 cankered).

cankers on the main stem and root flares. European beech with only root flare cankers, however, were not found at either the GWC or PFA.

Although the incidence of bleeding cankers differed significantly by tree part, no association existed between the health status of European beech (i.e., asymptomatic versus *Phytophthora*-infected trees, according to ELISA and culture results) and the isolation of *Phytophthora* spp. from rhizosphere soil (Table 2). At GWC, *Phytophthora* species were isolated from soil beneath infected (78%) and asymptomatic beech (62%) at the GWC ($\chi^2 = 2.62$, df = 1, $P = 0.1059$). The assemblage of *Phytophthora* spp. from soils of asymptomatic and diseased beech at GWC included *P. cactorum*, *P. cambivora* (A1 mating-type), *P. gonapodyides*, and *P. pini* with the latter species being most often recovered (Table 1). *Phytophthora gonapodyides* was not baited from soils of neither asymptomatic nor infected trees at PFA; however, as with the GWC soils, *Phytophthora* spp. were isolated consistently at PFA from soils under asymptomatic (63%) and diseased trees (74%; $\chi^2 = 0.49$, df = 1, $P = 0.4844$). *Phytophthora pini* was also isolated most frequently via soil baiting, regardless of the tree health status, followed by *P. cactorum* and *P. cambivora* (A1 mating-type; Table 2). Baiting assays (×2) of rhizosphere soil under asymptomatic and diseased beech at GWC were *Phytophthora* negative 38 and 22% of the time, respectively. Likewise, two independent baiting assays also failed to recover a single *Phytophthora* spp. from 37 and 42% of soils collected in PFA under asymptomatic and *Phytophthora*-infected trees, respectively.

Radial growth of asymptomatic and Phytophthora-infected beech from 1986 to 2010. Analysis of the raw (non-standardized) growth data 1986 to 2010 revealed significant mean differences (2-way ANOVA $F_{2, 996} = 192.5$, $P < 0.0001$) among asymptomatic and *Phytophthora*-infected trees at both study sites with health status (contrast comparison $F_{1, 996} = 571.7$, $P < 0.0001$) and the interaction between health status and site (constrast comparison $F_{1, 996} = 5.0$, $P = 0.0254$) contributing significantly as effects. Site alone (GWV or PFA), however, had no influence statistically on mean radial growth (contrast comparison $F_{1, 996} = 0.6$, $P = 0.4270$). The relationship of radial growth from 1986 to 2010 between asymptomatic and infected European beech as well as between site and health status is illustrated in Fig. 2. Over the 25-yr chronology, the mean radial increment for *Phytophthora*-infected trees [2.22 mm (0.09 in)] was significantly less (contrast comparison $F_{1, 996} = 571.7$, $P < 0.0001$) than those measured for beech expressing no visible symptoms of disease [4.59 mm (0.18 in)]. Moreover, infected trees at both sites grew significantly slower than their asymptomatic counterparts (contrast comparison $F_{1, 996} = 518.9$, $P < 0.0001$). The mean radial growth of infected trees was 2.37 mm (0.09 in) for the GWC and 2.07 mm (0.08 in) for PFA whereas the mean growth of asymptomatic beech in the GWC and PFA was 4.67 (0.18 in) and 4.52 mm (0.18 in), respectively.

Standardizing (centering) the radial increments across the 25-yr chronology (1986 to 2010) effectively eliminated the influence of site and health status (Fig. 3), providing a less confounded growth chronology for all European beech examined as well as insight into the responsiveness of these trees to environmental stress. Between 1986 and 2010, the trajectories of standardized radial growth for asymptomatic and diseased trees at the cemetery and arboretum were similar, differing in most years by response (i.e., difference away from the mean 25-yr radial increment) as opposed to direction. A closer, more refined examination of the standardized radial growth by 5-yr periodic increments (Fig. 4), however, revealed that the mean growth among all trees was significantly greater for years 1986 to 1991 [0.36 mm (0.1 in); Tukey's HSD $P \leq 0.009$] compared to 1996 to 2000 [–0.09 mm (< –0.01 in)], 2001 to 2005 [0.01 (< 0.01 in)], and 2006 to 2010 [–0.55 mm (–0.22 in)]. Likewise, the mean radial growth for all trees in years 2006 to 2010 was significantly less (Tukey's HSD $P < 0.0001$) than that observed across the preceding four periodic increments that spanned 1986 to 2005. Although all European beech at each site followed statistically the same general growth pattern between 1986 and 2010, *Phytophthora*-infected trees at PFA were notably less responsive to their environment(s) as, in comparison to asymptomatic trees at PFA, year-to-year variability in mean radial was limited. For PFA-infected trees, the mean standardized growth was also less than zero in three of four 5-yr periodic increments and the pattern of annual growth often differed when compared to asymptomatic trees at PFA as well as all trees examined in the GWC (Fig. 4). Moreover, no disturbance in the growth chronologies indicative of the onset of disease was present for beech growing at either site. The raw radial growth of *Phytophthora*-infected beech was reduced consistently compared to asymptomatic beech at the GWC and PFA, and the year-to-year standardized growth between infected and asymptomatic trees was similar.

As noted previously, all *Phytophthora*-infected European beech at GWC were treated in 2008 with the phosphite fungicide AGRI-FOS®. Interestingly, one-year after application, a discernible difference in the mean standardized radial increment for 2009 was observed between asymptomatic [–1.19 mm (–0.0005 in)] and infected trees [–0.82 mm (–0.003 in)] with the latter trees having improved growth (Fig. 3).

Spatial analysis of disease. The spatial distributions of all European beech at the GWC and PFA showed non-random, strongly aggregated distributions over each site (Fig. 5A, B). Ripley's K-function [K(t)] values for all trees at GWC, and PFA, exceeded the 95% confidence envelope of random distribution at all distances, indicating a strong aggregated distribution of trees. However, despite the aggregated distribution of all European beech at GWC and PFA, the spatial distribution of *Phytophthora*-infected beech, trees with cankers yielding positive ELISA and/or *Phytophthora* isolates, at both sites were not significantly different from random (Fig. 5C, D). The K(t) values for infected trees at GWC as well as PFA were well within the 95% confidence envelope of random distribution at all distances. Although beech were planted at GWC and PFA in spatially definable groups as one would expect for landscape design purposes, the occurrence of disease on European beech at both sites was spatially uncorrelated.

During the last decade, the involvement of *Phytophthora* species in the decline and mortality of fagaeceous trees has been demonstrated to occur throughout beech- and/or oak-dominated forest ecosystems of Europe (Balci and Halmschlager 2003a, Balci and Halmschlager 2003b, Belisario et al. 2006, Brown and Brasier 2007, Moreira and Martins 2005, Jönsson et al. 2005, Jung 2009, Motta and Annesi. 2003, Vettraino 2002) and N. America (Balci et al. 2007, Balci et al. 2010, Grünwald et al. 2012, Hansen 2008, Nagle

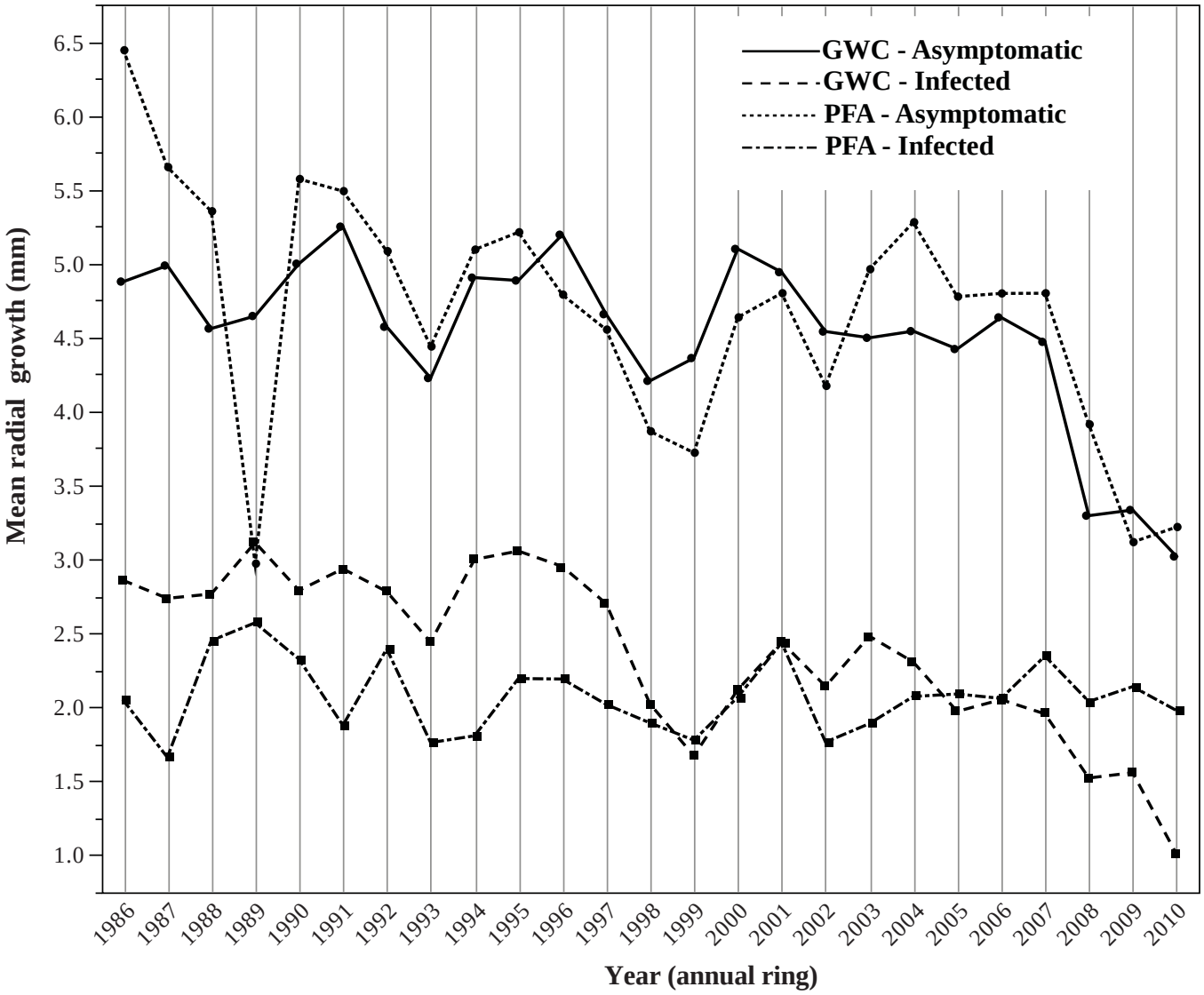

Fig. 2. Tree ring analysis, 1986 to 2010. Mean radial growth (mm) of European beech at the Green-Wood Cemetery (GWC) and Planting Fields Arboretum (PFA) by health status [asymptomatic versus *Phytophthora*-infected (infected)].

et al. 2010, Rizzo et al. 2002, 2005). The possible role of *Phytophthora* in causing bleeding canker of European beech in urban forests and ornamental landscapes of New York and elsewhere in the northeastern U.S. has also been sufficiently established (Jung et al. 2005, Nelson et al. 2010, Weiland et al. 2010). The results presented here, however, demonstrated that there is no clear association between the presence of *Phytophthora* spp. in rhizosphere soil and the incidence of diseased European beech at two sites in southeastern NYS (GWC and PFA). *Phytophthora pini* was the most frequently isolated species from cankers and rhizosphere soil of infected beech whereas *P. cactorum* and *P. cambivora* were found less frequently in cankers or soils under affected trees. However, although isolates of the aforementioned species reportedly cause considerable damage to root systems of young beech under controlled conditions (Fleischmann 2002, Jung et al. 2005, Nelson et al. 2010, Weiland et al. 2009, 2010), we found no significant difference in the frequency of isolation for *P. pini*, or all three species collectively, from rhizosphere soil between infected and asymptomatic European beech at GWC as well as PFA. The absence of a distinct association between *Phytophthora* spp. and tree health has also been reported by Hansen and Delatour (1999) and Jung et al. (2000) as well as others (Balci et al. 2010, Camy 2003, Delatour et al. 2000, Moreira and Martins 2005), suggesting the interplay between causal factors of tree decline or mortality, including *Phytophthora* spp., likely vary by site and/or region (Jönsson 2006, Jung et al. 2000, Manion 2003, Thomas et al. 2002).

Infection by *Phytophthora* spp., particularly *P. pini*, is not the only causal or contributing agent associated with bleeding canker of European beech in southeastern NYS and likely elsewhere in the eastern U.S. (Jung et al. 2005, Nelson et al. 2010). The random distribution of *Phytophthora*-infected trees combined with the presence of *Phytophthora* spp. within the soil of asymptomatic European beech at both GWC and PFA suggest that other factors, both abiotic and/or biotic, likely are involved in the manifestation of bleeding cankers that may or may not eventually lead to host death. Soil moisture and chemistry, tree age and vitality, winter injury, and/or extreme meteorological events such as drought

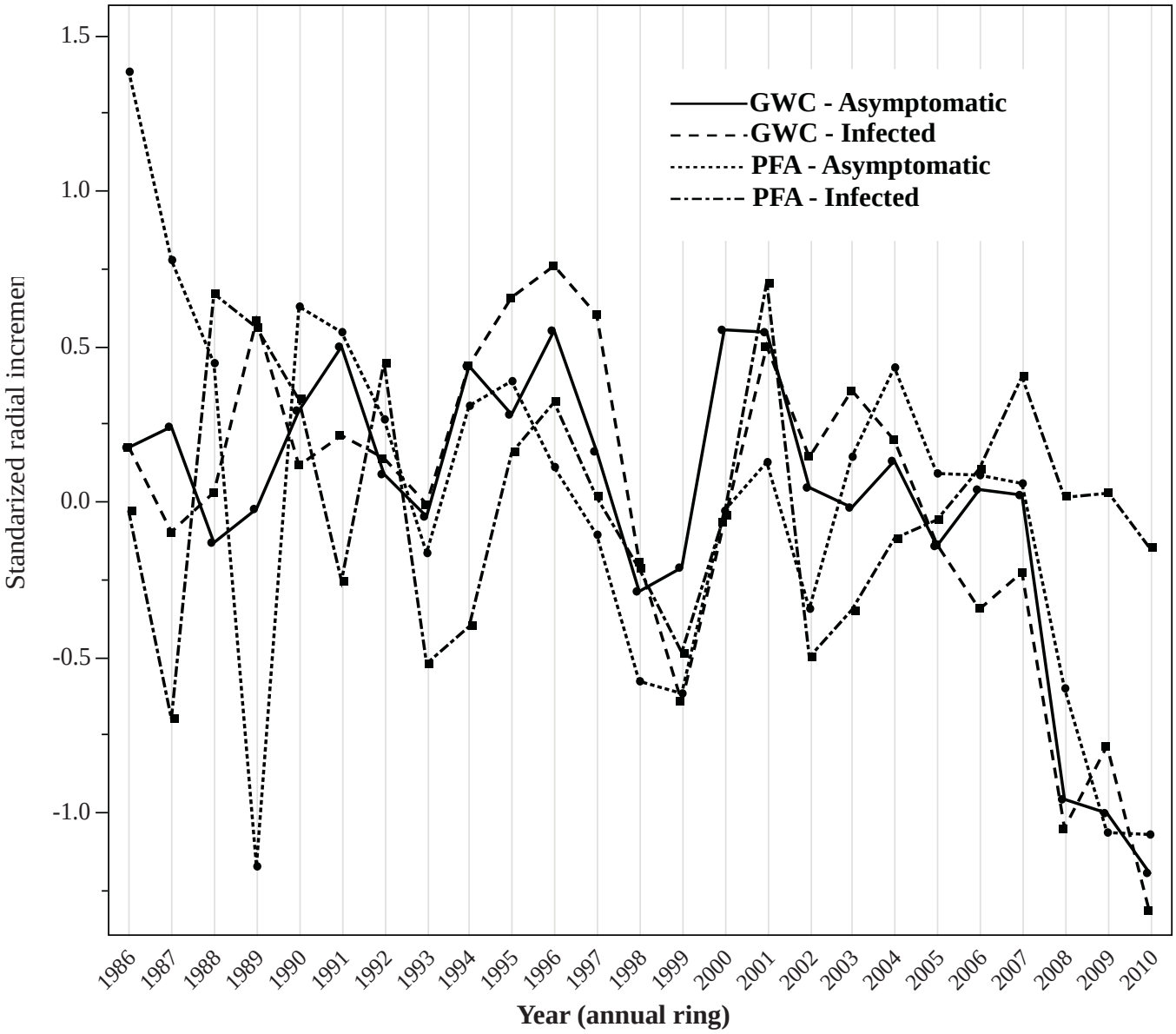

Fig. 3. Tree ring analysis, 1986 to 2010. Mean standardized radial growth (mm) of European beech at the Green-Wood Cemetery (GWC) and Planting Fields Arboretum (PFA) by health status [asymptomatic versus *Phytophthora*-infected (infected)].

and temporary flooding previously have been emphasized as possible inciting or contributing factors to *Phytophthora*-associated tree decline (Balci et al. 2010, Erwin and Ribeiro 1996, Jönsson et al. 2005, Jung 2009, Jung et al. 2000, Maurel et al. 2001, Thomas et al. 2002). Analysis of unstandardized radial increments of asymptomatic and infected beech clearly demonstrated that the growth of *Phytophthora*-infected trees at GWC and PFA was significantly less in comparison to their asymptomatic counterparts between 1986 and 2010. We were, however, unable to identify an inciting disturbance within the growth chronologies of infected beech and accordingly, all trees at the GWC and PFA followed the same general growth pattern and response to prevailing environmental conditions between 1986 and 2010. These results are noteworthy because the involvement of *Phytophthora* in the decline and mortality of European beech at both sites as well as elsewhere on Long Island was first suspected in the early-1990s; thereby, suggesting that chronic growth reductions imposed by *Phytophthora* infection of the roots likely were affecting tree vigor at least 10 yr prior to the outward expression of disease, particularly the appearance of bleeding cankers. Therefore, bleeding canker at GWC and PFA is a slow and chronic disease, and *Phytophthora*-infection likely is a predisposing factor, increasing the susceptibility of infected trees to opportunistic pathogens, pests, and/or meteorological stressors that further hasten reductions in tree vigor and increase disease severity (Jönsson 2006). However, information regarding the epidemiological factors, such as mode of spread and pathogenesis, that influence and/or modify the parasitic relationship between *Phytophthora* and European beech is required to test this hypothesis.

Because the life histories of *Phytophthora* spp. are critical in shaping the temporal and spatial pattern of disease (Balci et al. 2010, Martins et al. 2007, Ristaino and Gumpertz 2000, Vannini et al. 2010), we compared the distribution of *Phytophthora*-infected trees to all trees at the GWC and

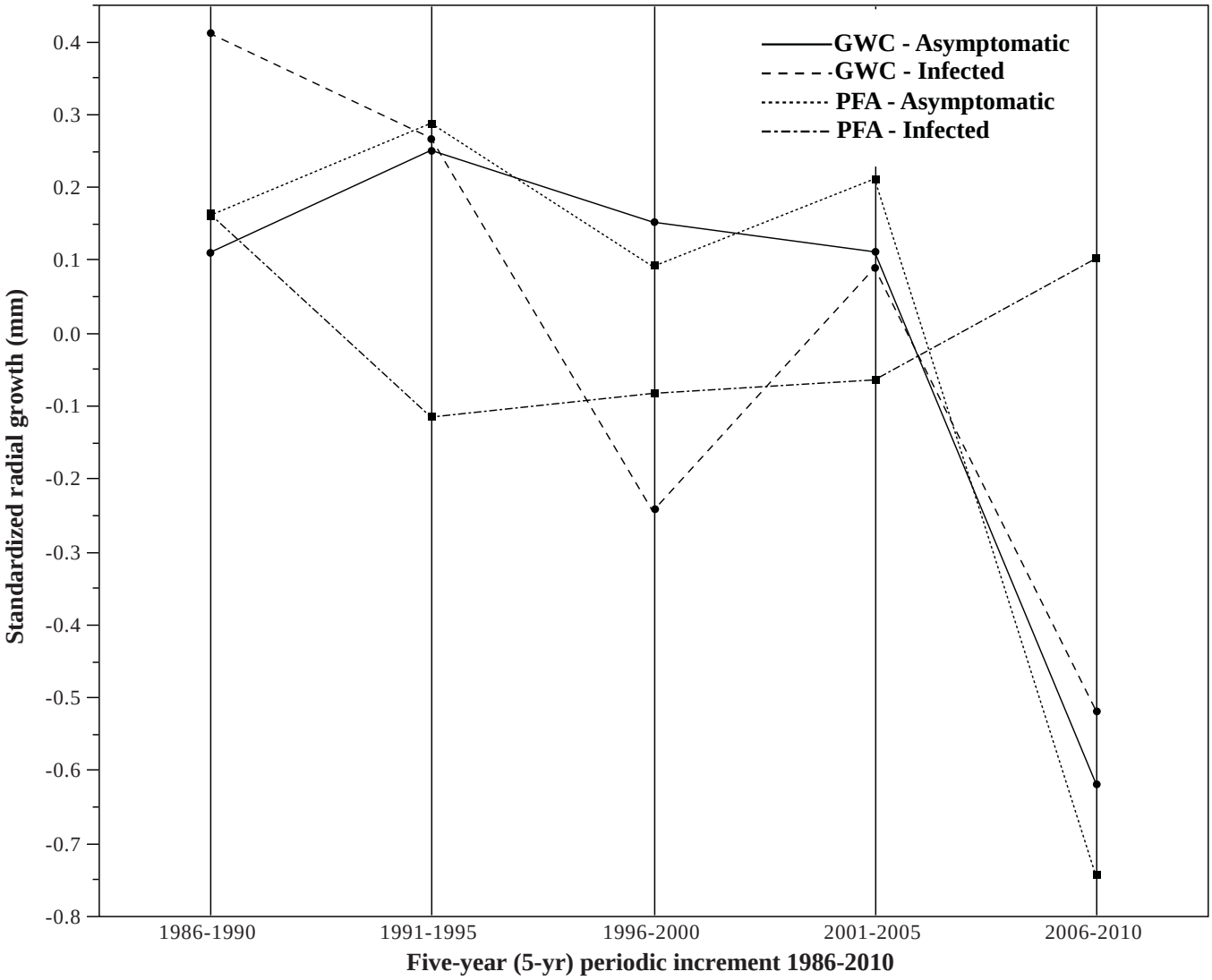

Fig. 4. Tree ring analysis, 1986 to 2010. Mean standardized radial growth (mm) by five-year periodic increments for European beech at the Green-Wood Cemetery (GWC) and Planting Fields Arboretum (PFA) according to health status [asymptomatic versus *Phytophthora*-infected (infected)].

PFA using SPPA and the Ripley's *K*-function (Diggle 2003, Ripley 1977, Ripley 1981). The spread of soil-borne *Phytophthora* spp. is largely contingent on either the movement of infested soil via human activities or, in the presence of free soil moisture, the production and dispersal of zoospores (Erwin and Ribeiro 1996). The spatial aggregation (non-random clustering) of disease to discrete foci or gradients in *Phytophthora*-infested sites, therefore, would indicate the involvement of effective dispersal mechanisms such as moving surface water or wind-driven rain (Jönsson 2006, Jung and Blaschke 2004, Ristaino and Gumpertz. 2000, Vannini et al. 2010). However, at GWC and PFA, the spatial distribution of asymptomatic and diseased European beech conflicted: asymptomatic trees were found in aggregated plantings whereas diseased trees were distributed randomly amongst asymptomatic trees often less than 20 m away. The lack of solitary or multiple disease centers indicates that natural soil drainages, slope and elevation differences among trees, and human activities (e.g., the introduction of *Phytophthora*-infected plant material) likely are not responsible for, or contributing strongly to, the disease problems at GWC and PFA. These results suggest that primary infection likely is restricted to fine roots and tree-to-tree movement is limited to soil, if and when it occurs. A similar situation was reported by Vannini et al. (2010) examining the involvement of *P. cambivora* in inciting ink disease on sweet chestnut. They characterized disease progress in chestnut forests and attributed the slow, deterioration of chestnut health to localized infection of the fine roots particularly on dry sites. The soils at GWC and PFA in the present study were also characterized as well drained and excessively well-drained, respectively, owing largely to the high amount of sand in most Long Island soil profiles.

However, the role of soil inoculum in the dissemination and survival of *P. pini*, *P. cactorum*, *P. cambivora*, the three most frequently encountered species in this study, under European beech in N. America is unknown. Moreover, until recently *P. pini* was classified taxonomically as *P. citricola* sensu lato (Hong et al. 2011). This species is considered to be widely distributed in soil and aquatic environments in North

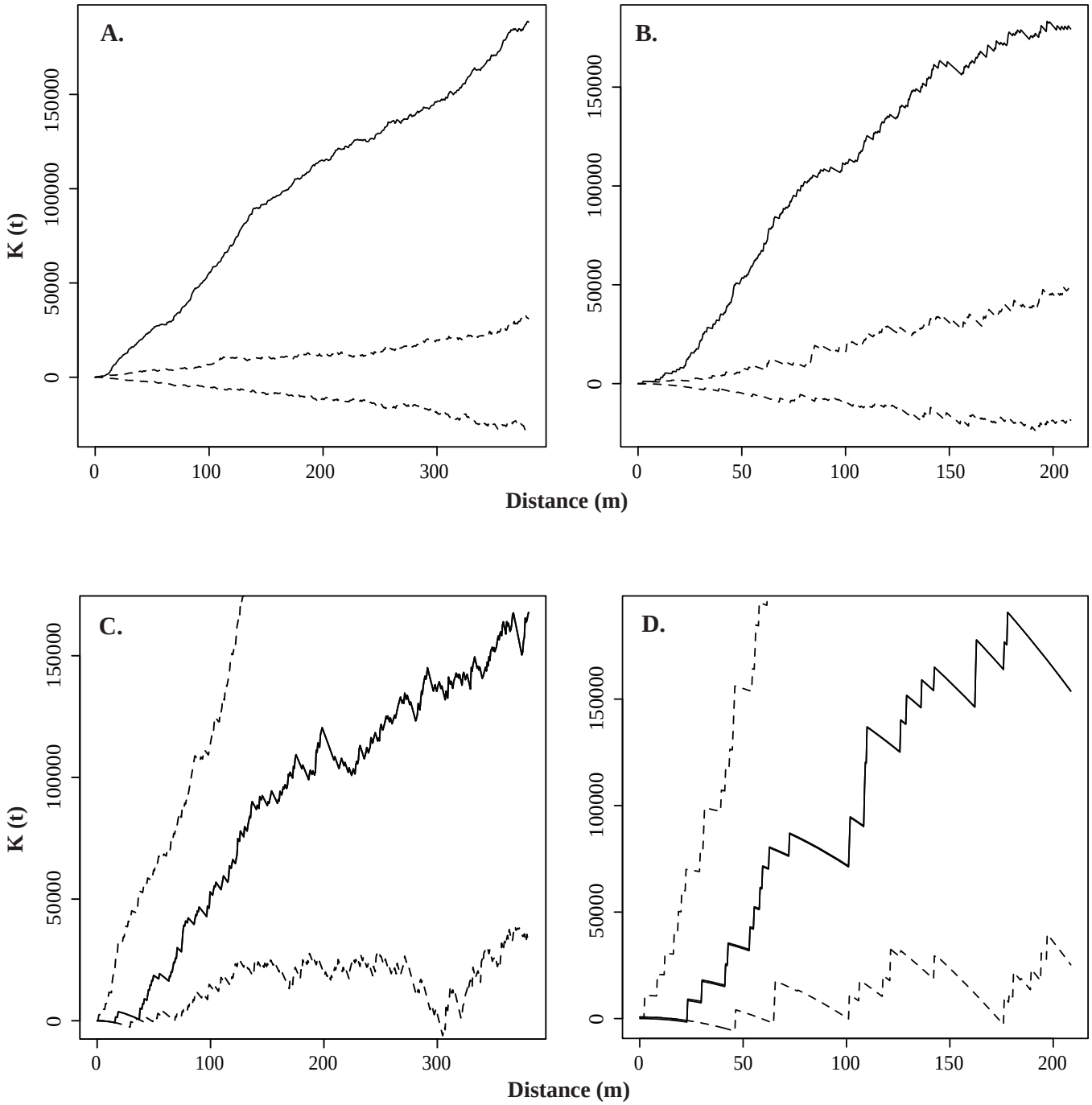

Fig. 5. Ripley's K-function [K(t)] values and univariate distributions of European beech: All trees at Green-Wood Cemetery (A) and Planting Fields Arboretum (B), and, diseased trees at Green-Wood Cemetery (C) and Planting Fields Arboretum (D). Black solid lines indicate actual K(t) values; black dashed lines indicate 95% confidence envelopes for the pattern expected from a random distribution of beech and a random distribution of cancors given over the existing beech distribution.

America (Hong et al. 2011, Jung and Burgess 2009) and its host range includes multiple genera in addition to *Fagus*. Similarly, *P. cactorum* and *P. cambivora* are also widely distributed throughout temperate regions of N. America, and both are pathogenic on numerous plant species (Erwin and Ribeiro 1996, Farr and Rossman 2013). The extensive host-ranges and ubiquitous distributions of these pathogens in forest and urban landscapes suggest that their involvement in bleeding canker of European beech will continue, necessitating the long-term monitoring as well as chemical and cultural management of high-valued trees (Weiland et al. 2009).

The effectiveness of phosphite fungicide(s) on eradicating *Phytophthora* spp. appears to be limited as *P. pini* was isolated successfully from 67% of infected beech at GWC previously treated with AGRI-FOS® in 2008. Therefore, the phosphite fungicide likely provides a therapeutic rather than a curative management option as suggested by Weiland et al. (2009). However, a noticeable short-lived increase in the standardized radial growth of *Phytophthora*-infected trees

at GWC was observed one year after fungicide application in 2009 (Fig. 3) whereas asymptomatic trees not treated with fungicide at GWC exhibited less growth. Refining the frequency of application likely will be required to improve the long-term efficacy of phosphite fungicides in the management of bleeding canker.

Phytophthora pini is the most common *Phytophthora* spp. associated with cankers and rhizosphere soil of diseased European beech at two sites on Long Island, NY. However, no statistical association was found between the presence of *P. pini*, in soil and/or *Phytophthora*-infected European beech. Future research should be undertaken to further elucidate the ecologies and population biology of *Phytophthora* spp. under asymptomatic and diseased European beech as well as to determine the influence of soil conditions on disease severity and inoculum spread.

Literature Cited

Balci, Y., S. Balci, J. Eggers, W.L. MacDonald, J. Juzwik, R.P. Long, and K.W. Gottschalk. 2007. *Phytophthora* spp. associated with forest soils in eastern and north-central U.S. oak ecosystems. Plant Dis. 91:705–710.

Balci, Y. and E. Halmschlager. 2003a. Incidence of *Phytophthora* species in oak forests in Austria and their possible involvement in oak decline. Forest Pathol. 33:157–174.

Balci, Y. and E. Halmschlager. 2003b. *Phytophthora* species in oak ecosystems in Turkey and their association with declining oak trees. Plant Pathol. 52:694–702.

Balci, Y., R.P. Long, M. Mansfield, D. Balser, and W.L. MacDonald. 2010. Involvement of *Phytophthora* species in white oak (*Quercus alba*) decline in southern Ohio. Forest Pathol. 40:430–442.

Belisario, A., M. Maccaroni, and M. Vettorazzo. 2006. First report of *Phytophthora cambivora* causing bleeding cankers and dieback on beech (*Fagus sylvatica*) in Italy. Plant Dis. 90:1362.

Brasier, C.M. and T. Jung. 2003. Progress in understanding *Phytophthora* diseases of trees in Europe. p. 4–18 *In*: J.A. McComb, G.E.S.J. Hardy, and I. Tommerup (eds.), *Phytophthora* in Forests and Natural Ecosystems. Proc. 2nd Int. Mtg IUFRO Work Party 7.02.09 Meeting, Albany, Western Australia. Murdoch University Press, Perth, Australia.

Brasier, C.M. and T. Jung. 2006. Recent developments in *Phytophthora* diseases of trees and natural ecosystems in Europe. p. 5–16 *In*: C.M. Brasier, T. Jung, and W. Oßwald (eds.), Progress in Research on *Phytophthora* Diseases of Forest Trees. Proc.3rd Int. IUFRO Working Party 7.02.09 Mtg. Forest Research, Farnham, Surey, UK. Freising, Germany.

Brewer, P.W., K. Sturgeon, L. Madar, and S.W. Manning. 2010. A new approach to dendrochronological data management. Dendrochronologia 28:131–134.

Brown, A.V. and C.M. Brasier. 2007. Colonization of tree xylem by *Phytophthora ramorum*, *P. kernoviae* and other *Phytophthora* species. Plant Pathol. 56:227–241.

Camy, C., C. Delatour, and B. Marcais. 2003. Relationships between soil factors, *Quercus robur* health, *Collybia fusipes* root infection and *Phytophthora* species. Ann. For. Sci. 60:419–426.

Caroselli, N.E. 1953. Bleeding canker disease of hardwoods. Bartlett Tree Research Laboratories, Scientific Tree Topics 2:1–6.

Day, W.R. 1939. Root-rot of sweet chestnut and beech caused by species of *Phytophthora* II. Inoculation experiments and methods of control. Forestry 13:46–58.

Day, W.R. 1938. Root-rot of sweet chestnut and beech caused by species of *Phytophthora* I. Cause and symptoms of diseases: Its relation to soil conditions. Forestry 12:101–116.

Delatour, C., M.L. Desprez-Lousteau, and C. Robin. 2000. Pathogenicity of *Phytophthora* species on oaks. p. 102–104 *In*: E.M. Hansen and W. Sutton (eds.), *Phytophthora* Diseases of Forest Trees. Proc. 1st Int. Mtg. *Phytophthora*s in forest and wildland ecosystems, IUFRO Working Party 7.02.09 Meeting. Forest Research Laboratory, Oregon State University, Corvallis, OR. Grants Pass, OR.

Diggle, P.J. 2003. Statistical Analysis of Spatial Point Patterns. Second edition. Oxford University Press, Inc., New York, NY. 159 pp.

Dirr, M.A. 1998. Manual of Woody Landscape Plants. Fifth edition. Stipes Publishing L.L.C., Champaign, IL. 1187 pp.

Erwin, D.C. and O.K. Ribeiro. 1996. *Phytophthora* Diseases Worldwide. APS Press, St. Paul, MN. 562 pp.

Farr, D.F. and A.Y. Rossman. 2013. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. http://nt.ars-grin.gov/fungaldatabases/. Accessed May 20, 2014.

Ferguson, A.J. and S.N. Jeffers. 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Dis. 83:1129–1136.

Fleischmann, F., D. Schneider, R. Matyssek, and W.F. Oßwald. 2002. Investigation on net CO_2 assimilation, transpiration and root growth of *Fagus sylvatica* with four different *Phytophthora* species. Plant Biology 4:144–152.

Gallegly, M.E. and C. Hong. 2008. *Phytophthora*: Identifying Species by Morphology and DNA Fingerprints. APS Press, St. Paul, MN. 168 pp.

Grünwald, N.J., M. Garbelotto, E.M. Goss, K. Heungens, and S. Prospero. 2012. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. Trends Microbiol. 20:131–138.

Hansen, E.M. 2008. Alien forest pathogens: *Phytophthora* species are changing world forests. Boreal Environ. Res. 13:33–41.

Hansen, E.M. and C. Delatour. 1999. *Phytophthora* species in oak forests of north-east France. Ann. For. Sci. 56:539–547.

Hansen, E.M., P.W. Reeser, and W. Sutton. 2012. *Phytophthora* beyond agriculture. Ann. Rev. Phytopathol. 50:359–378.

Hong, C., M.E. Gallegly, P.A. Richardson, and P. Kong. 2011. *Phytophthora pini* Leonian resurrected to distinct species status. Mycologia 103:351–360.

Johnson, W.T. and H.H. Lyon. 1976. Insects that Feed on Trees and Shrubs. Cornell University Press, Ithaca, New York. 463 pp.

Jönsson, U. 2006. A conceptual model for the development of *Phytophthora* disease in *Quercus robur*. New Phytol. 171:55–68.

Jönsson, U., T. Jung, K. Sonesson, and U. Rosengren. 2005. Relationships between health of *Quercus robur*, occurrence of *Phytophthora* species and site conditions in southern Sweden. Plant Pathol. 54:502–511.

Jung, T. 2009. Beech decline in Central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. Forest Pathol. 39:73–94.

Jung, T., H. Blaschke, and P. Neumann. 1996. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. Eur. J. Forest Pathol. 26:253–272.

Jung, T., H. Blaschke, and W. Oßwald. 2000. Involvement of *Phytophthora* species in Central European oak decline and the effect of site factors on disease. Plant Pathol. 49:706–718.

Jung, T. and M. Blaschke. 2004. *Phytophthora* root and collar rot of alders in Bavaria: distribution, modes of spread and possible management strategies. Plant Pathol. 53:197–208.

Jung, T. and M. Blaschke. 1996. *Phytophthora* root rot in declining forest trees. Phyton (Austria) (Special issue) 36:95–102.

Jung, T. and T.I. Burgess. 2009. Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytopthora plurivora* sp. nov. Persoonia 22:95–110.

Jung, T., G.W. Hudler, H.M. Griffths, F. Fleischmann, and W. Oßwald. 2005. Involvement of *Phytophthora* spp. in the decline of European beech in Europe and USA. The Mycologist 19:159–166.

Jung, T., J. Nechwatal, D.E. Cooke, G. Hartmann, M. Blaschke, W.F. Oßwald, J.M. Duncan, and C. Delatour. 2003. *Phytophthora psuedosyringae* sp. nov., a new species causing root and collar rot od deciduous tree species in Europe. Mycol. Res. 107:772–789.

Kong, P., C.X. Hong, P.A. Richardson, and M.E. Gallegly. 2003. Single-stranded-conformation polymorphism of ribosomal DNA for rapid species differentiation in genus *Phytophthora*. Fungal Genet. Biol. 39:238–249.

Leonian, L.H. 1925. Physiological studies on the genus *Phytophthora*. Am. J. Bot. 12:444–448.

Manion, P.D. 2003. Evolution of concepts in forest pathology. Phytopathology 93:1052–1055.

Martins, L., J. Castro, W. Macedo, C. Marques, and C. Abreu. 2007. Assessment of the spread of chestnut ink disease using remote sensing and geostatistical methods. Eur. J. Plant Pathol. 119:159–164.

Maurel, M., C. Robin, G. Capron, and M.L. Desprez-Lousteau. 2001. Effects of root damage associated with *Phytophthora cinnamomi* on water relations, biomass accumulation, mineral nutrition and vulnerability to water deficit of five oak and chestnut species. For. Pathol. 31:353–369.

Moreira, A.C. and J.M.S. Martins. 2005. Influence of site factors on the impact of *Phytophthora cinnamomi* in cork oak stands in Portugal. For. Pathol. 35:145–162.

Motta, W. and T. Annesi. 2003. A new *Phytophthora* spp. causing a basal canker on beech in Italy. Plant Dis. 87:1005.

Nagle, A.M., R.P. Long, L.V. Madden, and P. Bonello. 2010. Association of *Phytophthora cinnamomi* with white oak decline in southern Ohio. Plant Dis. 94:1026–1034.

Nelson, A.H., J.E. Weiland, and G.W. Hudler. 2010. Prevalence, distribution, and identification of *Phytophthora* species from bleeding canker on European beech. J. Environ. Hort. 28:135–149.

New York City Soil Survey Staff. 2005. New York City Reconnaissance Soil Survey. U.S. Dept. Ag., Natural Resources Cons. Ser., Staten Island, NY. 55 pp.

Oudemans, P., H. Forster, and M.D. Coffey. 1994. Evidence for distinct isozyme subgroups within *Phytophthora citricola* and close relationships with *P. capsici* and *P. citrophora*. Mycol. Res. 98:189–199.

Pirone, P.P. 1942. A new disease of sweet gum. Amer. Forests 48:130–131.

Phipps, R.L. 1985. Collecting, preparing, crossdating, and measuring tree increment cores. U.S. Geological Survey, Water-Resources Investig. Rep. 85-4148. 55 pp.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Accessed on June 18, 2013. http://www.R-project.org/.

Ripley, B.D. 1977. Modeling spatial patterns. J. R. Stat. Soc. — Series B: Statistical Methodology 39:172–212.

Ripley, B.D. 1981. Spatial Statistics. John Wiley & Sons, Inc., New York, NY. 272 pp.

Ristaino, J.B. and M.L. Gumpertz. 2000. New frontiers in the study of dispersal and spatial analysis of epidemics caused by species in the genus *Phytophthora*. Ann. Rev. Phytopathol. 38:541–576.

Rizzo, D.M., M. Garbelotto, J.M. Davidson, G.W. Slaughter, and S.T. Koike. 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. Plant Dis. 86:205–214.

Rizzo, D.M., M. Garbelotto, and E.M. Hansen. 2005. *Phytophthora ramorum*: integrative research and management of an emerging pathogen in California and Oregon forests. Ann. Rev. Phytopathol. 43:309–335.

Sinclair, W.A. and H.H. Lyon. 2005. Diseases of Trees and Shrubs. Second edition. Cornell University Press, Ithaca, NY. 660 pp.

Speer, J.H. 2010. Fundamentals of Tree-ring Research. The University of Arizona Press, Tucson, AZ. 333 pp.

Stokes, M.A. and T.L. Smiley. 1996. An Introduction to Tree-ring Dating. The University of Arizona Press, Tucson, AZ. 73 pp.

Thomas, F.M., R. Blank, and G. Hartmann. 2002. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. Forest Pathol. 32:277–307.

Vannini, A., G. Natili, N. Anselmi, A. Montaghi, and A.M. Vettraino. 2010. Distribution and gradient analysis of ink disease in chestnut forests. Forest Pathol. 40:73–86.

Vettraino, A.M., G.P. Barzanti, M.C. Bianco, A. Ragazzi, P. Capretti, E. Paoletti, N. Luisi, N. Anselmi, and A. Vannini. 2002. Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. Forest Pathol. 32:19–28.

Weiland, J.E., A.H. Nelson, and G.W. Hudler. 2009. Effects of mefenoxam, phosphonate, and paclobutrazol on in vitro characteristics of *Phytophthora cactorum* and *P. citricola* and on canker size of European beech. Plant Dis. 93:741–746.

Weiland, J.E., A.H. Nelson, and G.W. Hudler. 2010. Aggressiveness of *Phytophthora cactorum*, *P. citricola* I, and *P. plurivora* from European beech. Plant Dis. 94:1009–1014.

Wulforst, J.P. 1987. Soil survey of Nassau County, New York. U.S. Dept. Ag., Soil Conversation Ser., Cornell University Agricultural Station Report. 167 pp.