A Canker Disease of Seedlings and Saplings of *Tetragastris panamensis* (Burseraceae) Caused by *Botryosphaeria dothidea* in a Lowland Tropical Forest

Gregory S. Gilbert, Postdoctoral Fellow, Smithsonian Tropical Research Institute, P.O. Box 2072, Balboa, Ancon, Republic of Panama; and **Diane De Steven,** Associate Professor, Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee 53201

ABSTRACT

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Infection by *Botryosphaeria dothidea* was associated with stem canker and dieback of seedlings and small saplings of *Tetragastris panamensis* (Burseraceae) in the lowland tropical moist forest of Barro Colorado Island, Panama. Experimental inoculations in the field resulted in canker formation, reduced plant growth, and increased plant mortality. However, canker development was not a good indicator of the likelihood of mortality, as many inoculated plants died without forming cankers, and plants with cankers that resulted from either natural or experimental infection suffered less mortality than did canker-free plants.

Additional keywords: *Fusicoccum aesculi*, tropical rain forest

Tropical regions harbor a greater diversity of plant diseases than do their temperate counterparts (8), and there is growing evidence that plant diseases have significant effects upon the population dynamics and spatial distribution of trees in old-growth tropical forests (1,5). Tropical trees suffer from a range of fungal pathogens (3, 4,8), but the true diversity and activity of plant pathogens in natural forest ecosystems in the tropics is still being discovered.

During a field study of seedling and sapling demography of several neotropical tree species in the old-growth moist tropical forest in Panama (2), the second author noted a potentially ecologically important stem canker disease on juveniles of the common hardwood tree *Tetragastris panamensis* (Engl.) Kuntze (Burseraceae). In addition to displaying stem cankers, leading stems often became brittle and died back. Young saplings often were able to re-sprout from below the dieback zone, but smaller seedlings would frequently die. In this report, we describe the isolation of *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not. (anamorphic state *Fusicoccum aesculi* Corda) from symptomatic trees, and the effect of experimental fungal inoculation on plant growth and mortality. Additionally, we present observations about the occurrence of the disease under natural conditions.

Corresponding author: G. S. Gilbert, c/o STRI, P.O. Box 2072, Balboa, Ancon, Republic of Panama; FAX (011)(507) 272-3065.

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MATERIALS AND METHODS

Study site. The study was conducted in the Barro Colorado Nature Monument (BCNM), a forest reserve located in the Panama Canal and administered by the Smithsonian Tropical Research Institute (STRI). The BCNM consists of Barro Colorado Island (BCI) and adjacent mainland peninsulas. The reserve area is covered by semi-evergreen tropical moist forest, receives approximately 2,600 mm of rain per year, and has a 4-month dry season between December and April. The initial observations of canker disease and the isolation of associated fungi were from an old-growth forest on BCI. Subsequent pathogenicity testing was conducted in a secondary-growth forest on nearby Gigante Peninsula.

Isolations. On 3 January 1992, two *T. panamensis* seedlings with several cankerous growths on their stems were collected from a long-term study area near BCI trail marker Zetek 9.0. The stems were washed in tap water, surface sterilized for 2 min in 70% alcohol, briefly flamed, aseptically cut into numerous small pieces, and placed on acidified potato dextrose agar. A fungus exhibiting uniform colony morphology was consistently isolated from the stem sections near all the cankers, and one subculture, G0030, was used for pathogenicity testing.

Pathogenicity tests. In order to verify the isolated fungus as the cause of the cankers and to determine the effect of fungal infection on host plant survival, a field inoculation experiment was conducted on naturally growing *T. panamensis* seedlings and young saplings. To prepare the inocula, the fungus was grown on 2% malt extract agar (MEA) under ambient laboratory conditions (approximately 22°C). When mycelium established on the plates (after 2 days), the surface of the plate was sprinkled with approximately 100 sterile pieces of wooden toothpick (each about 3 × 1 mm). For controls, toothpicks were also placed on noninoculated plates of MEA. The fungus colonized the toothpicks after 1 week.

In October 1992 (mid-rainy season), seven 4-m^2 experimental plots were established in a dense stand of apparently healthy *T. panamensis* seedlings and young saplings on the Gigante Peninsula (initial plant heights: range = 12.5 to 54.4 cm, mean = 24.0 cm, standard deviation = 5.9). In three plots selected at random, all plants (n = 76) were inoculated with isolate G0030. In three other plots, all plants (n = 66) were inoculated as a "wound control." The plants were inoculated by aseptically cutting a small incision at an oblique angle, just penetrating the bark of the stem 2 cm from the soil line and inserting one appropriate toothpick inoculum into the wound. Toothpicks were removed after 1 week. In the seventh plot, all plants (n = 20) were left unmanipulated to serve as a "no-treatment control." Each plant was marked with a numbered bird band to permit monitoring of individual symptoms, growth, and survival. Plants were inspected regularly for mortality or symptoms of infection, and the presence or absence of cankers and number of leaves per plant were recorded 3, 5, 7, 13, 19, and 27 months after inoculation. Plant height was also measured after 3, 13, 19, and 27 months.

At the 7-month census, one cankered plant from the G0030 treatment was sacrificed in order to reisolate the pathogen, using the same procedures described above. After 27 months, two additional plants from each treatment were harvested; tissue removed aseptically from inside the stems near the wound sites was placed on MEA to determine what fungi were associated with cankered or callused wounds.

Chi-square tests were used to determine the following: (i) whether incidence of cankers was greater in G0030-inoculated than in wound-control plants; (ii) whether G0030-inoculated plants suffered greater mortality than did wound-control plants; and (iii) whether presence of a canker in one census affected the probability of survival to subsequent censuses. Student's t

test was used to determine whether the growth of surviving plants (plant height or number of leaves) differed between the G0030-inoculated and wound-control treatments. Tests were performed using STATISTICA/Mac (Statsoft, Inc., Tulsa, OK).

Natural disease occurrence. Field observations were derived from an ongoing study of *T. panamensis* seedling and sapling demography. In this study, recruitment, growth, and mortality of all individuals <1 cm diameter at breast height (1.3 m) have been monitored since 1987 in 10 4 × 16 m transects, with each transect centered on an adult *Tetragastris* (2). The incidence of stem cankers, stem dieback, and mortality were summarized over the years 1987 to 1994 for the multi-aged population of plants first marked in 1987 (plants more than 1 year old).

RESULTS

Isolation and identification. Only *Botryosphaeria dothidea* was isolated consistently from naturally cankered plants. The fungus was found both inside the canker and up to 1 cm away (but not farther). In culture on MEA, rapidly growing colonies are initially light brown, turning darker with age, with much submerged mycelial growth and a finely felty surface. Numerous solitary black, globose pycnidia (0.4 to 0.6 mm in diameter) produced masses of hyaline conidia 6 to 7 × 16 to 21 µm (mean = 6.6 × 19 µm), somewhat pointed at both ends. Holoblastic conidiophores were hyaline and unbranched, and lined the inner wall of the conidioma. In cankered plants, shaving away bark from around cankers exposed conidiomata that were uni- or multiloculate, 0.5 to 1 mm in diameter, and immersed to erumpent. The teleomorphic state was not observed on plants or in culture. Identity of *B. dothidea* was confirmed by B. C. Sutton of the International Mycological Institute (IMI #369198), who suggested that the anamorphic state of our isolate (*Fusicoccum aesculi*) is morphologically different from that of *B. ribis* Gross. & Duggar, which some authors consider to be a synonym of *B. dothidea.*

Symptoms and pathogenicity. *T. panamensis* plants inoculated with *B. dothidea* G0030 usually developed cankers and/or died. Cankers first appeared soon after the beginning of the dry season, 3 months after inoculation. The symptoms of pronounced swelling and roughening or cracking of the bark were identical to those on the plants from which the original isolates were obtained. In contrast, wounds on nearly all wound-control plants were completely closed by a smooth, reddish callous tissue, and the plants appeared healthy.

At the time of inoculation, none of the field plants had cankers. By 5 months after inoculation, 23% of the G0030-inoculated plants had cankers, whereas only 1 wound-control plant (2%) showed a canker. By the end of the study after 27 months, 80% (n = 76) of the inoculated plants versus 14% (n = 66) of the wounded controls had at some time during the study shown canker symptoms ($\chi^2 = 62.7$, $P < 0.0001$). Most of the wound-infected plants that developed cankers did so more than 18 months after inoculation, and may represent secondary spread of the pathogen from inoculated plants. None of the no-treatment controls (n = 20) developed canker symptoms. Many of the plants that survived to 27 months showed a slight roughening of the bark of the leading shoot, but there was no significant difference in incidence of this symptom between G0030-inoculated plants and wound-control plants (40 and 39%, respectively, $\chi^2 = 1.50$, $P > 0.2$). Inoculation with G0030 in the greenhouse often resulted in dieback of the leading stem without prior canker formation (n = 6).

A fungus morphologically identical to G0030 was isolated from all sections of the stem up to 1 cm from the canker of the G0030-inoculated seedling that was sacrificed after 7 months. The fungus was also recovered from greenhouse-inoculated seedlings that died back without forming cankers. After the 27-month census, G0030-like fungus was again isolated from tissue beneath the canker in one of two G0030-inoculated plants, but not from tissue beneath the callus of two nonsymptomatic wound-control plants.

Experimental host plant mortality and growth. Mortality was threefold greater among plants inoculated with the fungus than among wound-control seedlings. Although the percent mortality was always greater for inoculated than for control treatments (Fig. 1), the difference was statistically significant beginning with month 13 ($\chi^2 = 8.73$, $P < 0.003$), and remained significant for subsequent censuses. Two of 20 no-treatment control plants had died by month 27, which is similar to the mortality rate for wound controls (10 versus 11%).

Canker formation was not a good indicator of whether an individual plant was likely to die in coming months, as many plants died without external disease symptoms. For each census beginning with month 5, plants that were cankered versus "nonsymptomatic" (combining wound-control and inoculated groups) at that census were compared for their mortality at each subsequent census (e.g., for cankered versus nonsymptomatic plants alive in month 5, the mortality at months 7, 13, 19, and 27). There was no correlation between canker status in one census and survival to later censuses; one of the 10 possible comparisons was statistically significant (P = 0.04), but was not significant after Bonferroni adjustment of significance probability for multiple tests. In similar analyses using only cankered versus nonsymptomatic G0030-inoculated plants, none of the comparisons was statistically significant (P > 0.20 for all tests). However, overall mortality among G0030-inoculated plants that ever developed cankers was lower than the mortality rate among never-cankered plants; 22% of plants that showed canker symptoms at any time between 5 and 19 months had died by month 27 (n = 55), compared with 67% mortality among plants that had never shown cankers (n = 21) (χ^2 = 13.6, $P \leq 0.0002$). Among

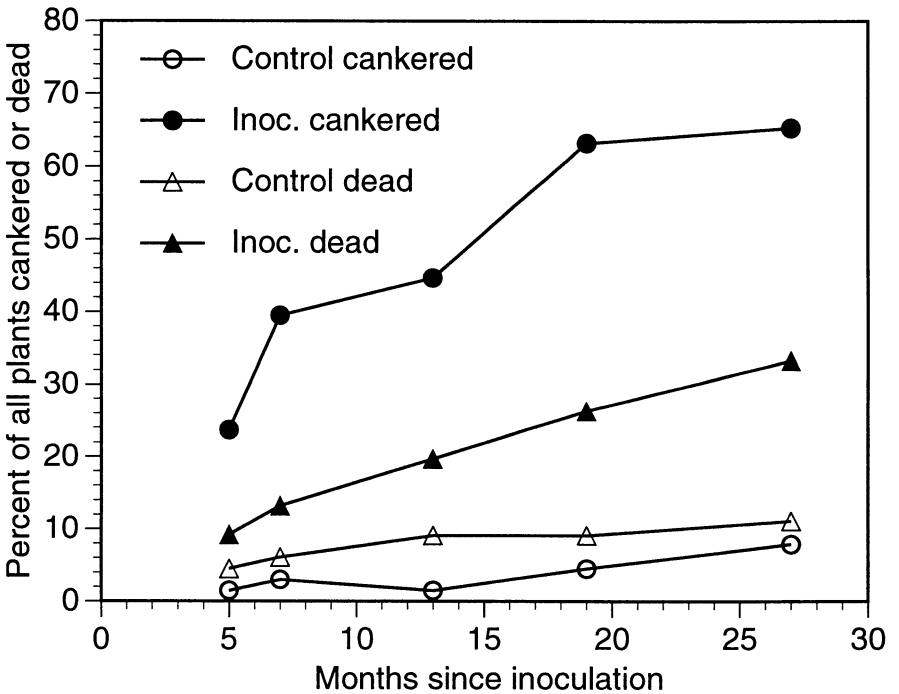

Fig. 1. Percentage of *Tetragastris panamensis* plants in wound-control treatment (n = 66), or inoculated with *Botryosphaeria dothidea* G0030 (n = 76), that were found dead or cankered during each census over 27 months after inoculation.

wound-control plants, 17% (n = 60) of never-cankered plants and none (n = 6) of cankered plants had died by month 27.

Inoculation with G0030 limited plant growth to 59% of that of controls by the end of the study. Among those plants that survived 27 months, wound-control plants grew 3.9 cm (standard deviation = 3.1, n = 54) whereas wound-inoculated plants grew only 2.3 cm (standard deviation = 1.4, n = 50) (t = 3.4, $P \leq 0.001$). Height growth of wound-control plants and no-treatment control plants was not statistically different ($P > 0.18$). Number of leaves gained or lost did not differ statistically between surviving control and inoculated plants ($P > 0.09$).

Natural disease incidence and host plant demography. Over the 7 years of demographic study of *T. panamensis* on BCI, at least 7% of a multi-aged seedling and sapling population developed stem cankers, whereas a much greater percentage (31%) was affected by stem dieback (with or without cankers) (Table 1). Dissection of several plants with brittle and dead leading stems revealed that the affected portion of each stem had become walled off at the base by a lignified tissue layer; any re-sprouting occurred from beneath this layer. Of stems with cankers (n = 18), 72% also suffered from dieback; however, of stems showing dieback (n = 69), only 19% also showed cankers. Incidence of both cankers and dieback increased with increasing plant size. Because new seedling recruitment is irregular and new seedlings have high post-germination mortality (2), it is difficult to assess the initial incidence of canker disease and dieback. However, a large seedling cohort that recruited in 1993 at the BCI Zetek 9.0 site (cf. Materials and Methods) had 7% cankers and 13% dieback (n = 68) by 1994, which indicates that natural infection can begin as early as 1 year after germination (and perhaps earlier).

Cankers were more common near to parent trees. Whereas only 46% of all stems first marked in 1987 (n = 242) occurred within the first 4 m of the 16-m transect length (an area situated beneath the tree crowns), 73% of all cankered plants (n = 18) were found beneath parental tree crowns ($\chi^2 = 5.4$, $P \leq 0.02$). However, dieback symptoms (with or without cankers) were randomly distributed with respect to the parental crown, with 46% of dieback-symptomatic plants found beneath the tree crowns.

In general, *T. panamensis* mortality rates decline with increasing plant size (2). This pattern was evident in both symptomatic and apparently nonsymptomatic plants (i.e., plants showing neither cankers nor dieback) (Table 1). However, there was no significant difference in mortality between symptomatic plants and nonsymptomatic plants in either the smallest ($\chi^2 = 2.02$, $P > 0.15$) or the largest ($\chi^2 = 1.96$, $P > 0.16$) size class. Mortality of nonsymptomatic plants in the middle size class (50 to 100 cm) was actually significantly greater than that of affected plants ($\chi^2 = 5.15$, $P < 0.02$), a result paralleling that observed for the experimentally infected plants.

DISCUSSION

Taken together, the results from proof-of-pathogenicity testing, experimental inoculation, and field observations indicate that *Botryosphaeria dothidea* causes stem canker formation, stem dieback, and mortality in young *Tetragastris panamensis*. Infected plants also suffer significant reductions in stem height growth.

Botryosphaeria dothidea (usually recognized by its anamorphic state *Fusicoccum aesculi*) is known to opportunistically attack more than 100 genera of primarily woody plants throughout temperate and tropical areas (7), although it has not previously been reported on *Tetragastris*. Infection and disease development are often related to environmental stresses such as drought (6) or to hosts already weakened by other pathogens. Symptoms range from necrosis and dieback to cankers with callus ridges, and symptoms often develop only after long incubation periods or in association with abiotic stresses (7). These patterns are consistent with our observations that dieback or cankers developed 3 months after inoculation, coincident with onset of the dry season.

Although infection by *B. dothidea* appears related to both cankers and seedling death, external symptoms are not necessarily good indicators of infection or the probability of mortality. In fact, both the inoculation study and observations of naturally occurring plants suggest a trend toward death rates higher for nonsymptomatic plants than for symptomatic plants. Against the background of other nonselective mortality factors, a hypothesis for this difference is within-population diversity of host-pathogen interactions. In some cases, infection may result in rapid death, without producing other external symptoms. In other cases, the host may tolerate the infection to varying degrees, and either die back and re-sprout or form a canker as part of a defense response. Thus, individuals capable of mounting a defense that leads to canker formation might be among the most resistant individuals in the population. Whether such a diversity of host responses reflects genotypic variability in the host or pathogen populations, or is also influenced by environmental factors (e.g., drought stress), would require further study. The greater incidence of canker formation beneath the crowns of parent trees than farther away, in contrast to the spatially random distribution of dieback symptoms, may indicate an environmental component to disease development. Regardless of the origin of this spatial variability, the occurrence of pathogen-induced mortality without prior external disease symptoms presents logistical problems for efficient, nondestructive assessment of disease incidence in natural host populations.

It is possible that dieback symptoms observed in the field may sometimes be caused by organisms other than *B. dothidea*. Other potentially pathogenic organisms have been isolated from dead or diseased seedlings of *T. panamensis*, but although experimental inoculations have led to the development of stem lesions and leaf necrosis, to date none besides *B. dothidea* have produced the shoot dieback or stem cankers described here (J. Davidson and G. S. Gilbert, unpublished data).

The ecological significance of this disease in the population dynamics of *Tetragastris panamensis* is not yet known. In particular, additional studies of variation in susceptibility within the host population, variability within the population of *B. dothidea*, interactions with other disease-causing organisms, and large-scale spatial distribution are needed. However, the substantial increase in mortality rates and the strong reduction in growth of infected plants suggest that this disease has the potential to be an important factor in regulating populations of *T. panamensis* in tropical forests.

Table 1. Incidence of plants showing cankers alone or stem dieback (with or without cankers) from natural infections of *Botryosphaeria dothidea*, and mortality of symptomatic (with cankers or dieback) or asymptomatic plants, from 1987 to 1994 in a multi-aged cohort of *Tetragastris panamensis* seedlings and saplings

Height class (cm)	n	Stems cankered (%)	Stems symptomatic (cankered + dieback) (%)	Mortality of symptomatic stems (%)	Mortality of nonsymptomatic stems (%)
<50	170	4	23	67	78
50 to 100	44	7	39	18	52
100 to 300	27	30	67	11	33
Total	241	8	31	42	71

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