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A Nasute Termite and a Buprestid Beetle Enhance Necrotrophy of *Cytospora* canker of Puerto Rican Red Mangroves

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Abstract

Rhizophora mangle L., cankers, dead branches and trunks, and as much as 32% mortality, were consistently associated with *Cytospora rhizophorae* in the Bahía Fosforecénte and in other plots in southwest Puerto Rico. The presence of this imperfect fungus, an agent of the cytospora canker disease, correlates with proximity to the arboreal nests of the "higher" termite *Nasutitermes costalis* (Isoptera: Nasutitermidae). Isopterans and other insects enhance mortality and decline of red mangrove forests presumably by the role they play in distribution of *Cytospora*. High incidence of the termite *N. costalis*, 40%, was detected in injured red mangroves. *C. rhizophorae*, causes cankers when inoculated into healthy red mangrove seedlings and has been isolated from *N. costalis* exoskeletons, fecal cement-covered paths and from active arboreal nests. It is hypothesized that *C. rhizophorae*, a food source for *N. costalis*, is carried and disseminated by these termites. Spores that enter branch and root wounds germinate and form canker-weakened trees. The termite-mangrove association increases the probability that the *C. rhizophorae*-infected mangroves die prematurely.

Keywords: *Cytospora* canker, nasutes, termite, red mangrove, Puerto Rico

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1. Introduction

Cytospora rhizophorae cankers, dieback and mortality in Puerto Rican red mangrove forests was reported by Tattar et al. (1994). This fungus was consistently associated with cankers and dieback in a field study conducted over a two-year period (Wier et al., 2000). *C. rhizophora,* a halotolerant fungus (Kohlmeyer and Kohlmeyer, 1971), was demonstrated to be a pathogen of *Rhizophora mangle* that causes cankers and mortality in as many as a third of all trees in inoculation experiments. Viable conidia of *C. rhizophorae* are apparently carried by seawater, grow in wounded roots and submerged branches of *R. mangle* and cause necrosis. A preliminary experiment tested the pathogenicity of *C. rhizophorae* on *R. mangle,* where one red mangrove seedling was inoculated with conidia of *C. rhizophorae* (Kohlmeyer, 1980). After three months there were no signs of infection and Kohlmeyer hypothesized that "*C. rhizophorae* is unable to infest healthy plants and grows only on weakened or greatly damaged *Rhizophora.*" Wounded tissue was required for the *R. mangle* infection by *C. rhizophorae* and that as many as one-third of *R. mangle* inoculated with *C. rhizophorae* died within seven months (Wier et al., 2000).

This study investigated: (1) the proximity of *N. costalis* termite arboreal nests to red mangrove decline and mortality, (2) insects and other invertebrates in red mangrove dead branches (stubs), cankers and dead wood, (3) if wounds are needed for infection of red mangrove stems and roots, i.e., an etiology of *Cytospora* canker of *R. mangle,* and (4) association of *C. rhizophorae* with termites and termite colonies commonly found on red mangrove.

2. Materials and Methods

Field plots

Eight plots, 15 by 30 m, were established in a variety of red mangrove environments along a 20-km coastline of southwest Puerto Rico near La Parguera (GPS N 23°03.724, E 121°38.415). A 'V' transect, that consisted of two 20 m transects joined at one end, was taken through each plot, sampling the closest tree to each 1 meter point, excluding trunks less than 2 cm in diameter. Each tree in the sample was investigated for (a) incidence of covered trails and arboreal nests of *N. costalis* and (b) a dieback rating score (Newbanks and Tattar, 1977) based on the severity of dieback symptoms. Healthy trees were scored as '1' and trees with severe dieback as '5' (Table 1), and stem diameter was measured at 1.4 m above ground. The Bahía Fosforecénte, with the highest dieback ratings of all plots in the region, was selected for further investigation.

Table 1. *N. costalis* and dieback rating in eight *R. mangle* forest plots in southwest Puerto Rico.

Plot #	Population	Incidence of *N. costalis*[a]	Dieback rating[b]	*R. mangle* stem diameter (cm) at 1.5 m height[b]
1	Boqueron West	7	1.2±1.2	10.8±2.3
2	Boqueron East	10	0.5±0.8	13.0±2.2
3	Matt I	8	1.5±1.3	13.5±2.4
4	Cayo Bayo	8	1.5±1.3	13.5±2.4
5	Enrique	0	0.3±0.6	11.4±2.8
6	Caracoles	1	0.9±1.1	12.0±1.9
7	Bahía Fosforecénte	16	2.6±1.6	8.0±1.7
8	Bahía Montalvo	13	2.0±1.2	14.0±2.1

[a]40 trees sampled from each plot. [b]Mean and standard deviation.

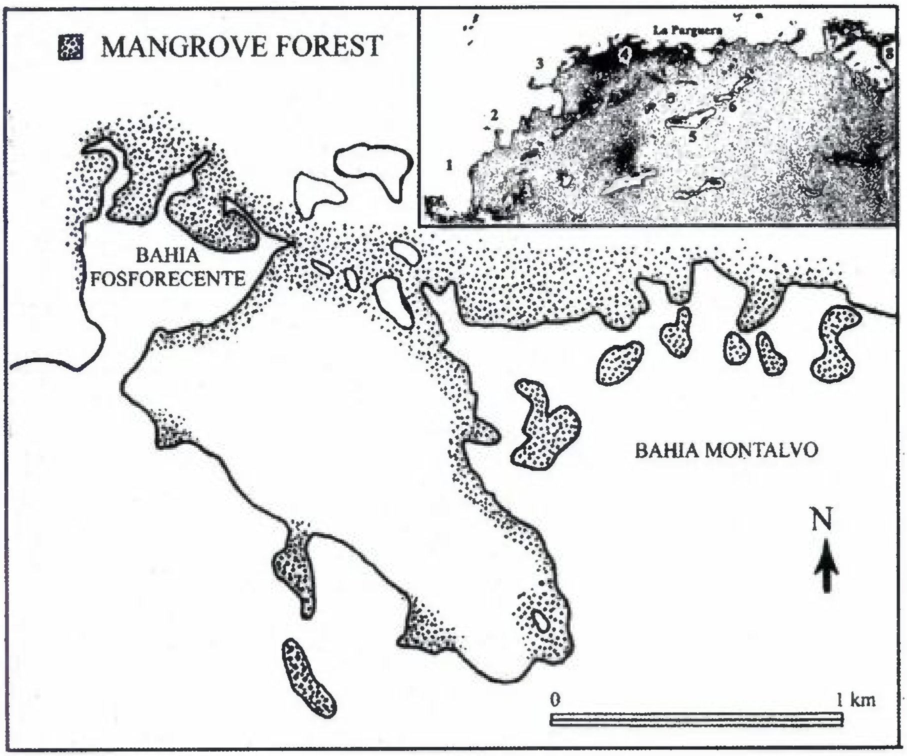

Figure 1. Study localities, southwest Puerto Rico.

Six active *Nasutitermes costalis* arboreal nests, >50 cm in diameter by ~80 cm in height were selected at random on the shore of the Bahía Fosforecénte. The trees around each nest were surveyed for dieback using two perpendicular transects of 15 meters each that intersected the nests at mid-transect. The two transects were taken through each nest, sampling the closest tree to one meter intervals. Trees fewer than 4 cm in diameter were excluded. Similar quantitative measures of each tree in the sample were taken. Data on nest size, its height in tree, were also recorded.

Red mangroves

All trees used in the inoculation experiments were between 12 and 28 cm in diameter. Trees were selected randomly along a 200 m coastline in the Bahía Fosforecénte (Fig. 1).

Arthropod survey

In a 10 by 15m plot established in Bahía Fosforecénte the invertebrates found in wounds, cankers and dead branches were collected and fixed in 70 percent ethanol. The location of each arthropod in the wound, canker or branch stub was noted. The plant tissue where each specimen was collected was recorded as either living or necrotic. All animals or arthropods collected were identified in our laboratory or sent out for identification by Rudi Scheffran, Ft. Lauderdale, Florida, and Sean Werle, Department of Biology, University of Massachusetts, Amherst.

Root and branch wounding

In the Bahía Fosforecénte plot, 15 roots of approximately 2–4 cm in diameter were selected from 15 individual mature red mangroves (diam = 8 cm). Each root was cut at 30 cm above the sea level at low tide, completely separated from the stem and tagged for identification. At the same site, 15 roots of approximately 2–4 cm in diameter were selected from another 15 mature mangroves. Each root was "girdled" also at 30 cm above the sea level at low tide: a cut was made and a 6 cm strip of bark around each root was completely removed.

Adjacent to the root wounding experiments, branch wounding was conducted on another set of individual trees. Ten branches from 10 mature mangroves, approximately 4–10 cm in diameter were cut. A 25 cm branch stub remained after separation from the stem. Another 10 branches of approximately 4–10 cm in diameter from 10 different mature mangroves were girdled 25 cm from the tree stem in the same manner as described for the roots. All branches, tagged for

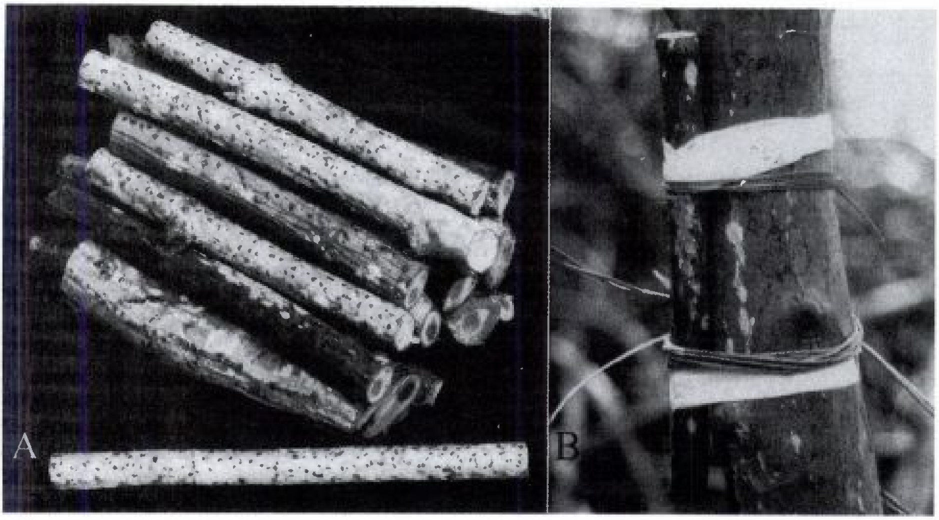

Figure 2. Inoculation of mature *R. mangle*. (A) *R. mangle* branch 30 cm sections cut from trees in the Bahía Fosforecénte heavily infected with *C. rhizophorae*. Orange conidia with cirrhi exude from subepidermal pycnidia. (B) Infected branch segment was tied and taped parallel to and in direct contact with healthy *R. mangle* at 1.4 m above the ground.

identification, were within 2–2.5 m from the sea level at low tide. Canker and other signs of *C. rhizophorae* were rated after 32 weeks.

C. rhizophorae inoculations

Fifteen mature, >12 cm diameter, healthy *R. mangle* (dieback rating 1) were selected for inoculation. Ten 30 cm branch sections were cut from trees elsewhere in the Bahía Fosforecénte that were infected with *C. rhizophorae* (Fig. 2A). Orange conidia-filled cirrhi exuded from subepidermal pycnidia in all branch sections. Sections were tied and taped with masking tape parallel to and in direct contact with one healthy *R. mangle* at 1.4 m above the ground (Fig. 2B). Uninfected 30 cm branch segments were tied to the five trees. Canker and other signs of *C. rhizophorae*, were scored after 32 weeks.

Isolation of C. rhizophorae from termites and termite fecal material

Neotermes mona, *Incisitermes* nr. *incisus*, *Procryptotermes corniceps* and *Nasutitermes costalis* were the four termite species present in the eight field plots. From thirty to fifty workers from each were collected, placed in individual vials and stored at 4°C in the laboratory. Termites, rinsed in 5 ml distilled water for 15 sec and dried on sterile filter paper, were placed on 2% Acidified Potato Dextrose Agar (APDA), DIFCO®, for growth of fungi. Termite fecal material, collected with forceps, from nests of each of the four termite species, placed in Fisherbrand Tin-Tie Sample Envelopes, Fisher Scientific, and were stored at 4°C. Fecal cement material collected from *N. costalis* nests and fecal-cement covered trails was rinsed in 10% sodium hypochlorite for 45 sec, dried on sterile filter paper, plated for growth on APDA and incubated for 2 days at 25°C. Fungal subcultures were examined microscopically. Pure isolates that showed characteristic slow growth and no conidial stages were further subcultured in an attempt to induce sporulation. Autoclaved discs of red mangrove hypocotyl tissue were placed onto APDA cultures of *C. rhizophorae* to induce formation of pycnidia.

3. Results

Red mangrove dieback and mortality and arboreal nests of Nasutitermes costalis

Mangrove dieback ratings increased with proximity to arboreal *N. costalis* nests. The highest incidence of *N. costalis*, 16 (n=40), was correlated with the highest dieback rating (Table 1). The smallest mean stem diameter of the populations surveyed, occurred in trees from the Bahía Fosforecénte. A direct correlation between *R. mangle* dieback rating and proximity to the arboreal nests of *N. costalis* was measured in the Bahía Fosforecénte (Fig. 3).

Invertebrate survey

Large and diverse populations of insects and other invertebrates inhabit the dying red mangrove habitat in Bahía Fosforecénte. The highest incidence of all the invertebrates found in the survey was *N. costalis* with 16 occurrences (Table 1). Ten of those incidences were fecal-cemented covered trails, and four were points on branch stubs where covered trails ended. At three of these four branch stubs *N. costalis* actively mined dead and callous canker tissue.

Procryptotermes corniceps was found in seven branch stubs. Young colonies numbered fewer than a hundred individuals at each branch stub. *P. corniceps* colonies were all in dead xylem and the branch stubs had been completely compartmentalized from the living trunk.

Colonies of *Incisitermes* nr. *incisus*, numbering fewer than 50–80 individuals, were found in two branch stubs and in one dead stem of *R. mangle.*

Neotermes mona, with colonies that ranged from 80–500 individuals, was

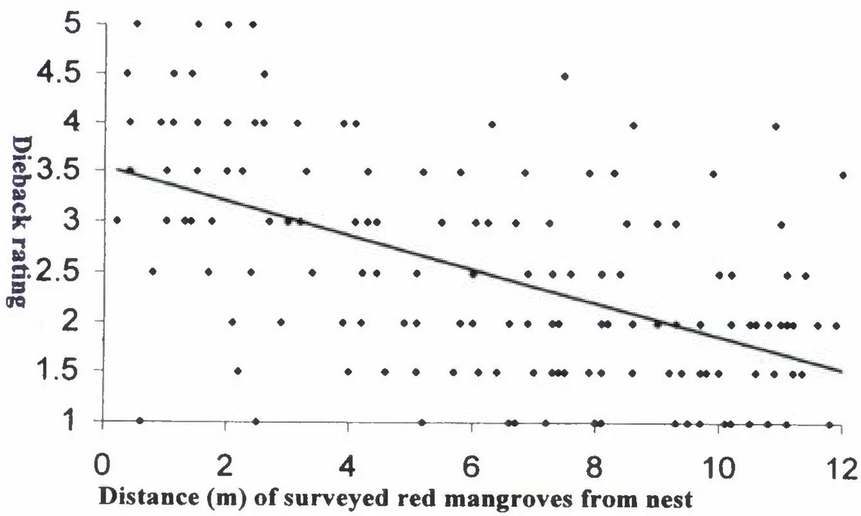

Figure 3. Distance of red mangrove dieback from *N. costalis* arboreal nests. Linear regression analysis.

found in eight instances in the survey plot but only in long dead and decaying stems.

Chrysobothris tranquebarica (Buprestidae; Coleoptera) larvae that had been oviposited at branch stub canker margins and bored into the underlying tissue were seen in six trees. Three of these *C. tranquebarica* were in disturbed living tissue where the inner cambium was being actively mined in close proximity to *N. costalis* end trails.

Psychonoctua personalis larvae (Cossidae; Lepidoptera) that had bored into branch xylem were found in two instances. In both cases the branches that contained the wood boring *P. personalis* were weak and starting to fracture.

Two ant species, *Camponotus kauri* (Formicinae; Formicidae) and *Crematogaster steinheili* (Myrmecinae; Formicidae), were identified. The carpenter ants, *C. kauri* were found in a dead rotting branch once and *C. steinheili* were identified inside decayed roots of red mangrove.

Root and branch wounding

Root and stem tissue samples for fungal isolations on APDA were taken after 32 weeks. Signs of *C. rhizophorae* were found in 6.6% of the roots that were girdled. In the root cut experiment 40% of the roots showed signs of *C. rhizophorae* and were confirmed infected via isolation of the fungus. No symptoms or signs of *C. rhizophorae* were found in either the branch girdling or the branch cut experiment nor was *C. rhizophorae* obtained from branch tissues cultures.

Inoculation of mature red mangrove stem and roots with C. rhizophorae

None of the trees in the inoculation experiment had developed any symptoms of *Cytospora* canker nor were there any signs of *C. rhizophorae* produced during the 32 week experiment. Nine of the ten infected branch segments remained attached to the test trees and all nine housed colonies of the ant *C. steinheili* at the end of the experiment. One infected branch segment, disturbed during the experiment was never recovered. None of the five trees that had healthy stem sections attached developed any sign of *C. rhizophorae.* All five of the uninfected branch segments were colonized by *C. steinheili.*

Isolation of C. rhizophorae from termites and termite fecal material

Of the fifty APDA cultures from *N. costalis* individuals, twelve had *C. rhizophorae* associated with their head and thoracic body parts. The other three termite species, *N. mona, Procryptotermes* spp. and *I. incisus,* yielded no isolations of *C. rhizophorae.* Four isolates of *C. rhizophorae* were cultured from the fifty fecal cement isolations that were taken from the *N. costalis* nests in Bahía Fosforecénte. Nine *C. rhizophorae* isolates were found in the fifty samples taken from *N. costalis* fecal cement covered trails. No isolates of *C. rhizophorae* were cultured from any of the fecal material or body parts taken from any of the other termite species.

The most common arthropod in Bahía Fosforecénte was the arboreal termite *N. costalis. Nasutitermes costalis* foraged in dead wood and mined dead xylem from cankers and woundwood interfaces. The larvae of *Chrysobothris tranquebarica* caused wounds and extensive burrows in woundwood around branch stubs. The burrows wander through both the phloem cortex and dead xylem. The observed close proximity of larvae, their burrows and *N. costalis* to wounded and dead xylem suggest that the *C. tranquebarica* larvae caused initial wounding to the red mangroves which then provided access of these tissues to the opportunistic *N. costalis.* Since the three other termite species were never in close proximity to living tissue or growing cankers but only in dead and decaying wood, they probably are not part of the etiology of *C. rhizophorae* canker.

C. rhizophorae was recovered from head and thoracic body portions in 12 out of 50 *N. costalis* bodies. Nest and fecal cement material also yielded *C. rhizophorae* but in lower frequency. The unsuccessful attempts to isolate *C. rhizophorae* from the other termites, all dry wood termites (Kalotermitid), for

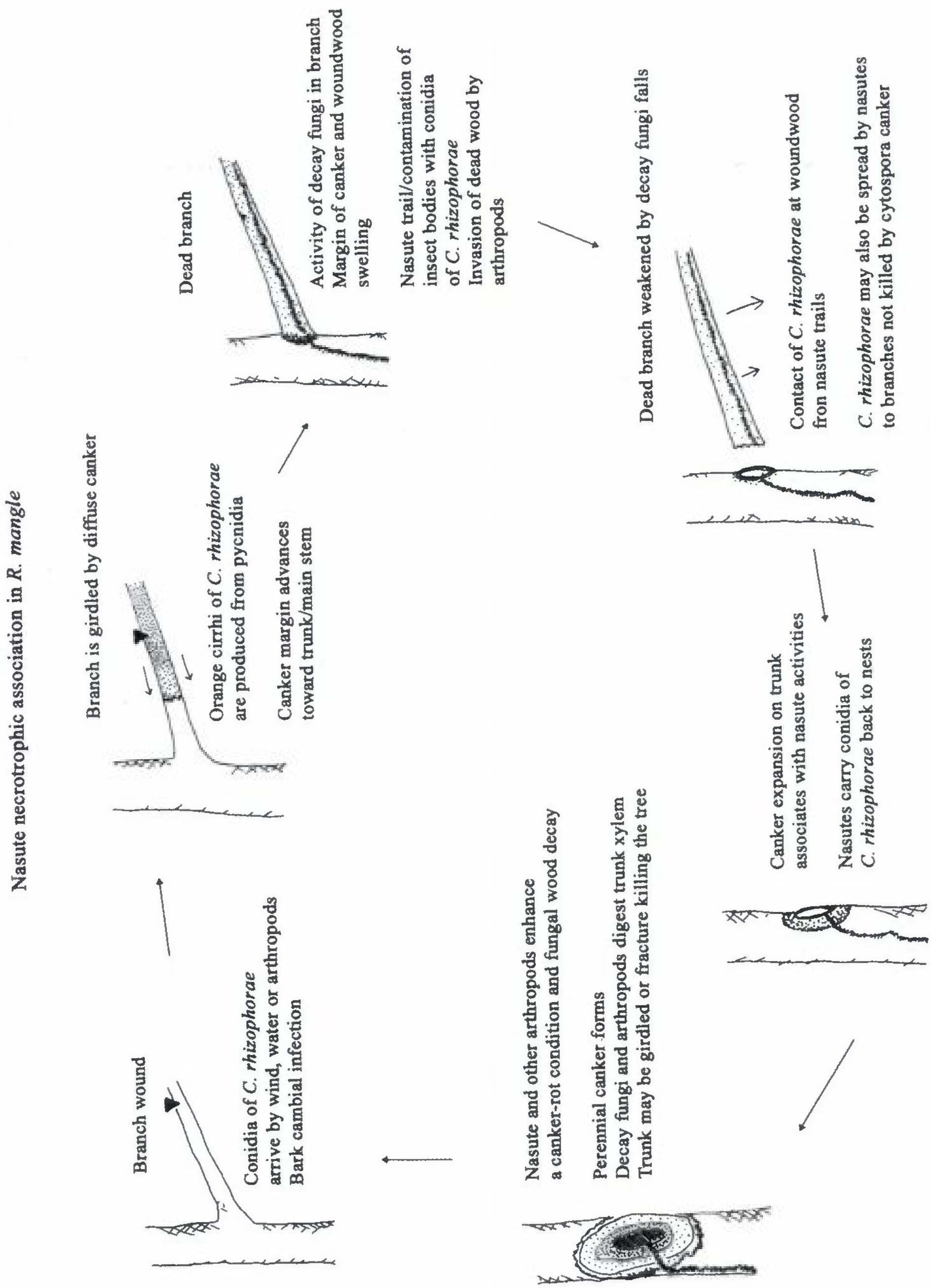

Figure 4. Proposed etiology of *Cytospora* canker of red mangrove.

unlike the arboreal *N. costalis* they tend to remain in their galleries and do not forage at night.

Active *C. rhizophorae* cankers with orange cirrhi exuded from erumpent pycnidia were removed and seen to be associated with fresh nasute fecal-cement covered trails. The trails ended in close proximity to active cankers.

4. Discussion

Dieback, mortality and incidence of *N. costalis* were strong correlated. Decreased red mangrove tree health is associated with proximity to *N. costalis* arboreal nests. Bahía Fosforecénte had the highest dieback rating, and the smallest mean stem diameter among eight plots surveyed in the region.

In their study of timber and living trees Brooks et. al (1941) stated that *N. costalis* was not a pest to *R. mangle* and never attacked living mangrove tissue. Our investigation, that showed *N. costalis* in Southwestern Puerto Rico frequently occupying the branch stub/woundwood interface and mining xylem beneath expanding cankers, is consistent with the work of Martorell (1941) and Harris (1971).

The wounded roots in contact with seawater carry halotolerant conidia of *C. rhizophorae*. Branch wounding as well as the mature tree inoculation experiments suggests that both a wound, as well as an inoculum source, are required for damage of *R. mangle* by *C. rhizophorae.*

A proposed developmental sequence of *Cytospora* canker of red mangrove based on the results of these investigations is shown in Fig. 4. In (1) a wound is caused mechanically or by arthropods at branch stubs or at the trunk. (2) Conidia of *C. rhizophorae* arrive by wind, watersplash or from an arthropod leads to an infection of bark cambial tissue. (3) A diffuse canker grows forming orange cirrhi from pycnidia of *C. rhizophorae* as the dieback occurs. The canker margin advances towards the main stem. (4) Nasutes and other

Figure 5. Progression of *Cytospora* canker on red mangrove. (A) Initial wound caused by macroinvertebrate in woundwood at branch stub. (B) *Cytospora rhizophorae* infection of branch stub from contact with macroinvertebrates. (C) *Nasutitermes costalis* mine rotting xylem out of branch stub and woundwood interface. (D) *N. costalis* covered trail leads from branch stub back to the arboreal nest. (E) Perenial canker forms. (F) Continued arthropod predation widens woundwood, exposes more xylem as *C. rhizophorae* progresses into trunk. (G) Trunk is almost girdled, *Cytospora* canker reaches inner xylem. Canker rot fungi activity increases. (H) Trunk of red mangrove is completely girdled and opportunistic arthropods and fungi enter.

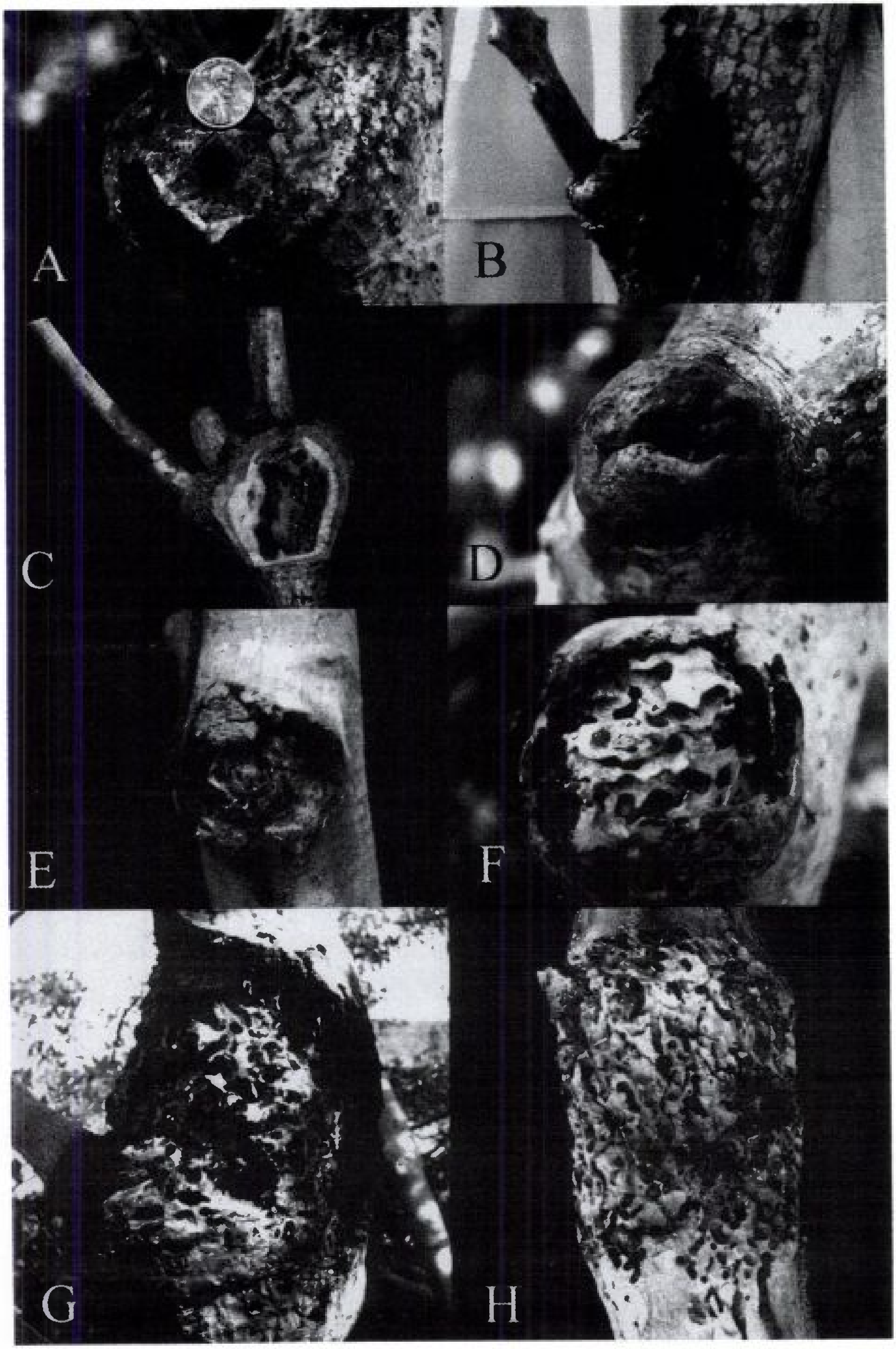

Figure 5. See legend on previous page.

arthropods invade the dead wood. This permits entry of decay fungi that degrade the inner xylem. Conidia of C. rhizophorae contact the arthropods and are carried off to other trees. (6) Massive activity at the branch stub/woundwood junction cause expansion of canker. (6) A perennial canker forms. Decay fungi and arthropods continue to digest xylem within the trunk. (7) The trunk becomes girdled or the structure of the tree is weakened sufficiently to cause breakage of the main stem. These stages are in Fig. 6.

Cytospora canker is endemic in Southwestern Puerto Rico and is enhanced by the creation of entry wounds by the buprestid beetle larvae of Chrysobothris tranquebarica and the introduction spores carried by [illegible]. The canker community, comprised of C. rhizophorae, buprestid beetle larvae, the [illegible] termites and other invertebrates, may impart some habitat benefits to the mangrove environment; as to whether the canker community seriously threatens the coastal mangroves is beyond the scope of this investigation.

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REFERENCES

[illegible], F.L., [illegible], A.M., [illegible], R.S., and [illegible] 1991. [illegible] Caribbean [illegible].

[illegible] 1971. [illegible] 2nd ed. [illegible] Publishers pp. [illegible].

Kohlmeyer, J. and Kohlmeyer, E. 1971. Marine fungi from tropical America and Africa. Mycologia 63: [illegible].

Kohlmeyer, J. 1969. Tropical and subtropical filamentous fungi of the Western Atlantic Ocean. Botanica Marina [illegible].

Martorell, L.F. A survey of the forest insects of Puerto Rico. Caribbean Forester [illegible].

[illegible], [illegible] and Tattar, T.A. [illegible]. The relationship between [illegible] and severity of [illegible]. Canadian Journal of Forest Research [illegible].

Tattar, T.A., Klekowski, E.J. Jr., and Stern, A.I. 1994. Dieback and mortality in red mangrove, Rhizophora mangle L., in southwest Puerto Rico. Arboricultural Journal 18: 419–429.

Wier, A.M., Tattar, T.A., and Klekowski, E.J. 2000. Disease of red mangrove, Rhizophora mangle, in Southwest Puerto Rico caused by Cytospora rhizophorae. Biotropica 32: [illegible].