Fungi associated with the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae)[1].

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Abstract. Insects have been shown to serve as vectors for a wide array of fungi. In some cases, insect-vectored fungi produce potent toxins in the host plant, which might create problems for the food industry. Recent findings indicate that the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) could be vectoring toxigenic fungi in coffee plantations.

Introduction

Based on then fossil record, it is known that insects have been associated with fungi for hundreds of millions of years (Borror *et al.* 1976; Brock 1974). These associations may range from casual to associations based on evolutionary benefits for the fungus, the insect or both (symbiosis).

An insect-fungus association may benefit the fungus in various ways. The insect may (1) move the fungus to a desirable location; (2) facilitate entry of the fungus into a host plant through damaging a host-derived barrier to the fungus (such as a seed hull); (3) serve as a host for the fungus, either in a pathogenic, commensal (benefitting one but not harming the other), or a mutualistic/symbiotic relationship. The association may also benefit the insect. For example, the fungus may serve as food for the insect. Fungi can be more nutritious than plant material due to relatively higher levels of proteins or sterols (Southwood 1973). The fungus may also modify a host material so it is more suitable for the insect. Appropriate modifications may include degrading the material to a suitably nutritious form (such as occurs for larvae of wood boring wood wasps; Kukor and Martin 1983) or detoxification of host-produced defensive compounds (Dowd 1992a). The insect, through carrying a toxin-producing fungus, may exclude other insect competitors from the food source provided the vector insect is resistant to the toxin (such as the sap beetle-toxigenic fungi relationship in maize, Dowd 1992b).

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Insect vectoring ability can be specifically defined by criteria provided by Leach (1940): (1) a close, although not necessarily constant, association of the insect with diseased plants must be demonstrated; (2) it must be demonstrated that the insect also regularly visits healthy plants under conditions suitable for the transmission of the pathogen; (3) the presence of the pathogen in or on the insect in nature or following visitation to a diseased plant must be demonstrated; (4) the disease must be produced experimentally by insect visitation under controlled conditions with adequate checks. However, even if all criteria are not fulfilled, the insect may still facilitate entry of the fungus, as discussed above (Dowd 1998). Although the vectoring criteria may only be critically and completely demonstrated for some insects for a particular plant-fungus relationship, many insect species may actually be involved in vectoring (Dowd 1998). Vectoring of fungi may be facilitated by sticky spore masses produced by a fungus (along with a feeding or odor attractant) (*e.g.,* Leach 1940), the presence of hairs on an insect that collect dry spores (much like bee hairs collect pollen) (*e.g.*, Juzwik and French 1983), or specific structures designed to house/collect the fungus, such as the mycangia of bark beetles (Scolytidae) (Beaver 1989; Berryman 1989).

Past reports of vector associations between insects and fungi have indicated significant economic losses in several instances. One of the earliest indications that insects vectored economically important fungal pathogens of concern was for ergot (Atanasoff 1920; Leach 1940 and references therein). Ergot is the sclerotia (a hard, long-term survival structure) of the fungus *Claviceps* (*paspalum, purpureum* or other species) that occurs in grain heads such as rye. In the times of Caesar and in the Middle Ages, outbreaks of ergot in grain often resulted in death of significant portions of regional populations of people or livestock due to the neurotoxic compounds present in the ergot sclerotia, which do not readily separate from the grain during threshing (Atanasoff 1920). Symptomatology of affected people was attributed to witchcraft or possession by evil forces due to the strange behavior caused by the active compounds, which include hallucinogens (Atanasoff 1920).

Other instances of economic consequences of insect vectored fungi have been of more recent concern. Although mycotoxin presence in crops such as maize is complicated, insects generally appear to be important contributors, and greatly reducing insect damage can greatly reduce mycotoxin levels (*e.g.* Anderson *et al.* 1975; Lillehoj *et al.* 1976; Smith and Riley 1992; Munkvold *et al.* 1997, 1999; Dowd *et al.* 1999). Levels of direct and indirect economic losses due to mycotoxins in maize in the U.S. have been estimated in the billion dollar range (Vardon 1998; USDA-ARS 1999). Many tree pathogens are vectored by insects such as sap beetles (*e.g.*, oak wilt disease; Juzwik and French 1983) or bark beetles (*e.g.*, Dutch elm disease; Leach 1940; Webber and Gibbs 1989). Even though economic losses are often hard to compute for these diseases due to variation in occurrence in "farmed" trees vs. ornamentals in populated areas, monetary losses due to Dutch elm disease have been estimated at billions of dollars (Karnosky 1979; Strobel and Lanier 1981). However, both the American chestnut and several native elms, both valuable as ornamentals and for commercial use, have been virtually eliminated in the U.S. due to pathogens carried by insects (Leach 1940).

The coffee berry borer

Hypothenemus hampei is a serious pest of coffee throughout the world (Le Pelley 1968). Endemic to Central Africa, it has now spread to most coffee growing regions, and has been reported in Uganda, Congo, Benin, Togo, Ivory Coast, Kenya, Nigeria, Angola, Ethiopia, Brazil, Colombia, Guatemala, Ecuador, Nicaragua, Honduras, Mexico, Malaysia, Indonesia, Sri Lanka, Jamaica, New Caledonia, India, and other countries.

The female adult bores a hole into the coffee berry where she deposits 20-50 eggs (Baker *et al*. 1992). Larvae feed on the endosperm, lowering the quality of the berry and possibly causing abscission of the fruit. There is a 10 to 1 female to male sex ratio, and females mate inside the berry; therefore, once they emerge, they are already inseminated and ready to deposit eggs into another coffee berry (Waterhouse and Norris 1989).

As part of a foreign exploration program aimed at finding biological control agents against CBB, coffee berries showing the characteristic hole bored by the female were collected in the field in Benin, Uganda, Cameroon, Honduras and Nicaragua, and brought into the laboratory. CBB's that emerged from the coffee berries were placed with their dorsum touching the surface of water agar in sealed Petri dishes, in order to promote growth of any fungal spores carried by the insect. The following fungi were isolated from *H. hampei*: *Aspergillus ochraceus, Aspergillus flavus, Aspergillus niger, Fusarium* sp., *Penicillium chrysogenum*, *Penicillium brevicompactum* and *Verticillium* sp., in addition to the fungal insect pathogens *Beauveria bassiana*, *Paecilomyces farinosus*, and *Paecilomyces lilacinus.* Many other fungi have also been isolated from CBB by other researchers throughout the world (Table 1).

A. ochraceus, as well as other members of the genus *Aspergillus*, are known to be important toxigenic fungi (Powell *et al.* 1994). For the coffee industry, the presence of ochratoxin A (produced by *A. ochraceus*) in coffee is a serious concern. Ochratoxin A has been reported in green coffee beans, roasted coffee, instant coffee, and coffee brews (Levi *et al.* 1974; Tsubochi *et al.* 1984; Micco *et al.* 1989; Studer-Rohr *et al.* 1994; Patel *et al.* 1997). CBB-infection rates with *A. ochraceus* in different countries is presented in Table 2. The isolation of *A. ochraceus* suggests that CBB might serve as a vector for this toxigenic fungus.

We hypothesize that the adult progeny emerging from coffee berries which were originally attacked by an *A. ochraceus*-contaminated female, could carry spores once they leave the berry. Thus, field studies should be conducted to determine if adult CBBs exiting coffee beans are carrying *A. ochraceus*, and if so, whether the fungus becomes established in a coffee berry once the female adult bores a hole to deposit her eggs. Plans aimed at managing ochratoxin levels in coffee should take into consideration the presence of *H. hampei* in the field, and its possible role as a mechanical vector for *A. ochraceus*.

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Table 1. Fungi isolated from coffee berry borers.

PATHOGEN	COUNTRY	REFERENCE
Aspergillus flavus	Benin India	Vega and Mercadier, unpubl. Kumar *et al.* 1994
Aspergillus niger	Benin	Vega and Mercadier, unpubl.
Aspergillus ochraceus	Benin, Uganda	Vega and Mercadier 1998
Fusarium spp.	Benin, Uganda	Vega and Mercadier, unpubl.
Fusarium avenaceum	India	Balakrishnan *et al.* 1995
Fusarium oxysporum	Colombia	Bustillo *et al.* 1998
Fusarium pallidoroseum	India	Kumar *et al.* 1994
Fusarium solani	Benin, Mexico	Rojas *et al.* 1999
Beauveria bassiana	Brazil	Averna-Saccá1930 Villacorta 1984 Jiménez-Gómez 1992
	Cameroon	Pascalet 1939
	Colombia	Bustillo *et al.* 1991, 1998 Varela and Morales 1996
	Ecuador	Klein-Koch *et al.* 1988 Klein-Koch 1990 Jiménez-Gómez 1992
	India	Balakrishnan *et al.* 1994
	Indonesia	Junianto and Sri-Sukamto 1995
	Ivory Coast, Togo	Vega and Mercadier, unpubl.
	Mexico	Méndez-López 1990 Barrera 1995
	Kenya, Togo, Mexico, Ecuador,	Bridge *et al.* 1990

	Guatemala, Indonesia, New Caledonia, Brazil, Sri Lanka, Jamaica	
	Brazil, Kenya, Nicaragua, Togo Mexico	Humber 1992
	Thailand	Varela and Morales 1996
Hirsutella eleutheratorum	Colombia	Bustillo *et al.* 1998
Metarhizium anisopliae	Colombia	Bustillo *et al.* 1998
Nomuraea rileyi	Brazil	Le Pelley 1968
Paecilomyces amoeneroseus	India	Kumar *et al.* 1994
Paecilomyces farinosus	Ivory Coast, Togo	Vega and Mercadier, unpubl.
Paecilomyces fumosoroseus	India	Balakrishnan *et al.* 1995
Paecilomyces javanicus	Java, Indonesia	Friederichs and Bally 1923 Samson 1974
Paecilomyces lilacinus	Benin	Vega and Mercadier, unpubl.
Penicillium brevicompactum	Benin, Uganda	Vega and Mercadier, unpubl.
Penicillium chrysogenum	Uganda	Vega and Mercadier, unpubl.
Verticillium sp.	Benin, Uganda	Vega and Mercadier, unpubl.
Verticillium lecanii	India	Balakrishnan *et al.* 1995

Table 2. Percent *A. ochraceus* infection rates in coffee berry borers from different countries.

COUNTRY	# insects	% infected
Benin	564[1]	17.4
	258	47.3
Uganda	636[1]	5.3
Cameroon	355	0.8
Honduras	163[2]	0.6
Nicaragua	14[2]	0

[1] See Vega and Mercadier 1998; numbers given for Benin are for two separate collections.

[2] Preliminary data; research in progress.