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Fungi Associated with Two *Vespula* (Hymenoptera: Vespidae) Species in the Eastern San Francisco Bay Area

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Abstract.—Fungi associated with two *Vespula* species were isolated from insects or nests from four sites in the eastern San Francisco Bay area. A total of twenty-two species representing six genera were recovered, including at least four facultative pathogens taken from dead insects. Fungi in the genera *Aspergillus* and *Penicillium* were most common. Successful fungal invasion of colonies usually occurs during late-season decline.

INTRODUCTION

A social insect colony presents a rich sequestration of resources susceptible to exploitation by non-colony organisms. Colonies of the honey bee *Apis mellifera* Linnaeus, the most widely studied social insect, are plundered by a variety of saprophytes, pathogens and predators ranging in size and complexity from viruses to bears (Morse, 1978). In colonies of vespine wasps, nutritive resources available for exploitation include structural paper, silk, the insects themselves, and their waste products.

The abundant literature concerning the organisms associated with, and presumably benefitting from, vespine colonies, is reviewed by Spradbery (1973) and Edwards (1980). Most emphasis is on arthropods. Although there is a modest number of references to vespine fungal associates, many are brief, mentioning little more than the co-occurrence of two organisms. Several original records have been cited repeatedly in subsequent works, inflating the literature in proportion to the actual number of specimens. Furthermore, since many fungi are facultative pathogens, also able to develop as saprophytes, a report of a vespine cadaver supporting fungal growth does not indicate the nature of the relationship between insect and fungus. Consequently our knowledge of fungal associates of the Vespinae is incomplete.

The earliest mention of fungal growth on a vespine is Gray's (1858) account of *Hymenostilbe sphecophila* (Ditmar) Petch on an adult of *Vespa crabro* Linnaeus. Additional records of this fungus, all on adult wasps, are provided by Smith (1882; 1884), Cooke (1892), Petch (1932; 1948), and Leatherdale (1970). Other fungi associated with vespine adults include *Cordyceps sphecocephala* (Klotzsch) (Cooke, 1892; Petch, 1932; Petch 1948; Poelt and Jahn, 1963; Leatherdale, 1970; Edwards, 1980); *Paecilomyces farinosus* (Dickson) Smith et Brown (Petch, 1932; Leatherdale, 1970; Kmitowa, 1982); *Beauveria tenella* Delacroix (Leatherdale, 1970); and

Beauveria bassiana (Balsamo) Vuillamin (Leatherdale, 1970; Thomas and Poinar, 1973). Unidentified fungi associated with adults are mentioned by Duncan (1939), Spradbery (1973), and Edwards (1980).

Fungi are less commonly noted to occur in association with larvae. Nakahara (1980) recovered a *Beauveria* species growing on larvae in a nest of *Vespula pensylvanica* (Saussure) on the island of Hawaii. Other records of unidentified fungi associated with larvae are found in Duncan (1939) and Akre and Reed (1981).

Old nest material commonly supports fungal growth. MacDonald (1977) found *Aspergillus* and *Penicillium* species growing on abandoned subterranean nests. Sagara and Kobayashi (1979) and Sagara *et. al.* (1985) found basidiocarps of *Hebeloma spoliatum* (Fries) Karst and *H. radicosum* (Fries) Ricken growing from abandoned ground nests of *Vespula flaviceps lewisii* Cameron. They speculated that the nitrogen-rich meconia provided nutrients otherwise lacking in the soil, thus allowing the mushrooms to develop to fruition at these specific sites. Other unidentified fungi, apparently involved in the decomposition of nest materials, have been noted by Spradbery (1973) and Edwards (1980).

Durrell (1965) proposed that hyphal ramifications of certain fungi may strengthen the paper of active nests by binding the fibers together, thus benefitting the wasps. He recovered *Aureobasidium pullulans* (deBary) Arnaud, *Fusarium roseum* Link, *Mucor varians* Povah, *Alternaria tenuis* Auct., *Stemphylium ilicis* Teng., and *Phoma* sp. from paper of an aerial *Vespula* (or more likely, *Dolichovespula*) nest. Additional support for this theory is provided by the findings of *Botrytis cinerea* Persoon in vespine nests by Acolat (1953) and Cymorek (1978), and of *Aureobasidium pullulans* by Edwards (1980).

Some yellowjacket species are significant economic pests (Akre and MacDonald, 1986). Because of the possible usefulness of fungi as biological control agents, we investigated those representatives that occur in association with two common Californian pest species, *Vespula vulgaris* (Linnaeus) and *V. pensylvanica*.

Materials and Methods

Fungi associated with *Vespula* wasps or their nests were collected from four east San Francisco Bay (California, U.S.A.) localities: Orinda (ORI; Contra Costa County), Berkeley (BER; Alameda County), Albany (ALB; Alameda County), and Tilden Park (TIL; Contra Costa County).

The ORI nests had housed active colonies during the early summer of 1983, but at the time of excavation no living adults were present and there were no immatures, alive or dead, in any of the cells. Fungi had grown and sporulated on the backs (tops) of some of the upper combs (Figure 1), in some cases covering nearly the entire comb. The nests were brought to the laboratory where fungal spores were inoculated onto Sabaraud dextrose agar + 0.2% yeast extract (Poinar and Thomas, 1984), or potato dextrose agar, in 100 × 15 mm Petri dishes. These standard artificial growth media were used to culture fungi throughout this study.

The BER (except BER–5), ALB, and TIL colonies were active immediately prior to excavation. Flying adults were removed by placing a funnel trap over the surface entrance hole of the tunnel leading to the nest. After removing the trap, the remaining wasps were anaesthetized by injecting ethyl ether into the hole before excavating the nest.

Intact nests were brought to the laboratory, where the envelope was removed and the combs separated. Combs containing brood, but free of adults, were installed in sealed plastic food containers and kept in an incubator at 28 ± 1.5°C. Larvae were reared using a modification of the methods of Parrish and Roberts (1983). Fungal growth was not evident on these combs at the time of installation. After one day, sporulating growth was apparent on a wall of a vacant cell of a comb of ALB–1. The capped cell opposite this cell wall contained a dead, fully formed adult worker that also supported some fungal growth. This adult and the portion of cell wall to which it was fused were removed from the comb and placed on growth medium. Combs from colonies BER–3 and BER–4 showed fungal growth after five days. Hyphae and spores were transferred from the combs to culture medium.

Fungi were also recovered from individual insects. A larva of colony BER–1 turned red one day after excavation. It was removed from the comb and placed on moist filter paper in a 30 mm Petri dish. It hardened, and after 5 days, a thin white fungal growth appeared over its surface.

Fifteen workers newly emerged from a comb of colony BER–2 were allowed to remain on the comb for up to 12 hours. All fifteen were transferred to a sterile 400 ml tissue culture flask, which was sealed and returned to the incubator. These wasps died after several days, and sporulating fungi issued from the cadavers after approximately 10 days (Figure 2).

Colony BER–5 was not excavated for this study. However, two adult workers partly covered with fungal growth (Figure 3) were recovered from moist leaf litter just outside the entrance hole.

To isolate fungi from the guts of larvae of colonies TIL–1 and BER–6, apparently healthy larvae were removed from cells of the excavated nests and placed on sterile filter paper in petri dishes. The next day larvae that had not defecated were individually surface-sterilized in two rinses of 70% EtOH. The gut was dissected out in sterile 0.7% NaCl saline solution and passed through four rinses of sterile distilled water. The gut was ground in sterile saline with mortar and pestle, and inoculum was transferred via loop to growth medium.

Wasps designated CAL–1 were dead adult workers supporting fungal growth, which were collected from an unspecified northern California locality and submitted to us for diagnosis.

Fungal cultures were examined with a stereo dissecting microscope. Sporulating portions were mounted in Guegen's medium for examination under a compound microscope.

Results and Discussion

Table 1 lists fungi recovered in this study. Because it was often difficult to distinguish between fungal growth arising from comb paper, silk, and meconia at the bottom of otherwise empty cells, these three sources of nutrition are collectively referred to as "comb" in the table.

Nearly all species of fungi recovered can be considered common saprophytic soil organisms (Thom and Raper, 1945; Raper and Thom, 1949; Barnett and Hunter, 1972; Samson, 1974). The occurrence of soil fungi in subterranean wasp nests is hardly surprising. Yellowjacket workers enlarge their nest cavity by removing soil pellets, and foragers must pass through a tunnel in the soil to return to the nest. The

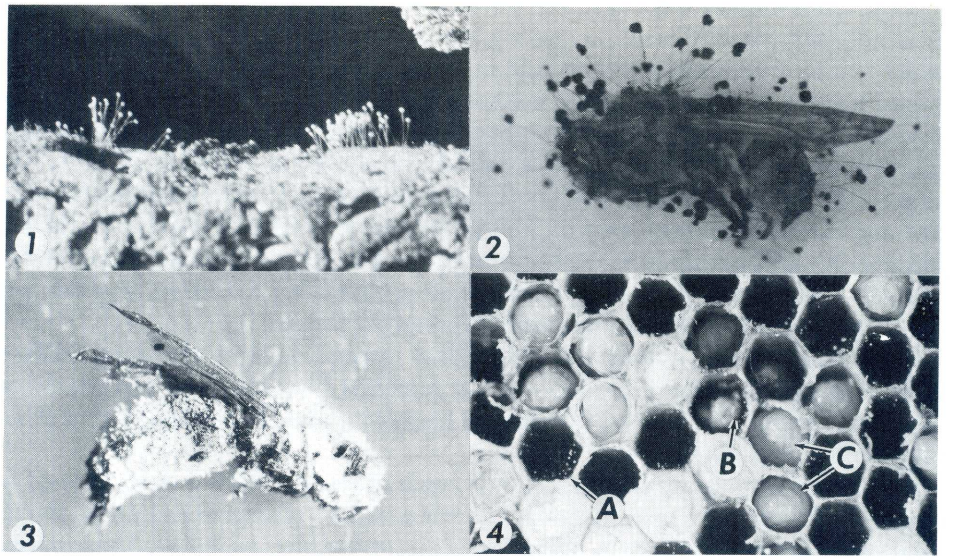

Figure 1. Sporulating heads of *Aspergillus oryzae* on back (top) of a *Vespula vulgaris* comb. Figure 2. Sporulating heads of *Aspergillus niger* on *V. vulgaris* adult. Figure 3. *Beauveria bassiana* spores on a *P. vulgaris* adult. Figure 4. Fungi on a *V. vulgaris* comb. Growth is present in empty cells (A) and those containing dead larvae (B), but absent in cells containing healthy larvae (C).

spread of fungal spores throughout the nest would thus seem inevitable. Some spores are ingested, but retain their viability, as indicated by the recovery of fungi from larval gut contents. Such spores, passed out with the voided meconium, would also account for the initiation of growth on this substrate.

Some of the saprophytic fungi that were recovered are also known to be facultative insect pathogens. Those recovered from insect cadavers during this study are implicated in this role. Two species, *Paecilomyces farinosus* and *Beauveria bassiana* attack many insects (Steinhaus, 1949), and have previously been associated with vespine adults (Leatherdale, 1970; Thomas and Poinar, 1973; Kmitowa, 1982). Both *Aspergillus flavus* Link and *A. niger* Bloch, which produce stonebrood in *Apis mellifera* (Morse, 1978), also have wide host ranges.

Although the flora varied between nests and sites, species of *Penicillium* and *Aspergillus* were the most common associates. Despite the single reference to these genera in the literature (MacDonald, 1977), their occurrence on old nests is probably widespread, and merely underreported. Members of the genus *Aspergillus*, which produce cellulolytic enzymes (Thom and Raper, 1945) would seem especially well adapted to derive nutrition from the structural paper of a nest.

Most subterranean colonies probably contain fungal spores, but these usually do not germinate and grow in active healthy colonies. The insects themselves may somehow inhibit fungal growth, as suggested by MacDonald (1977). In our rearing

Table 1. Fungi Associated with California Vespula Species.

Colony	Date	Species	Source	Fungus
CAL–1	6.ix.72	*pensylvanica*	Adult	*Paecilomyces farinosus* (Dicks.) Brown et Smith
ORI–1	21.ix.83	*vulgaris*	Comb	*Aspergillus restrictus* G. Smith
				Penicillium brevi-compactum Dierckx
ORI–2	21.ix.83	*vulgaris*	Comb	*Aspergillus niveus* Bloch
				Aspergillus niger van Tieghem
				Penicillium steckii Zaleski
				Penicillium stoloniferum Thom
ORI–3	21.ix.83	*vulgaris*	Comb	*Aspergillus oryzae* (Ahlberg) Cohn
BER–1	11.xi.83	*pensylvanica*	Larva	*Aspergillus terreus Thom*
BER–2	26.x.84	*vulgaris*	Adult	*Aspergillus niger*
				Aspergillus flavus Link
BER–3	26.x.84	*vulgaris*	Comb	*Aspergillus niger*
				Aspergillus niveus
				Aspergillus oryzae
				Aspergillus restrictus
				Cladosporium Link sp.
				Scropulariopsis brevicaulis (Sacchardo) Bainer
				Penicillium steckii
				Penicillium citrinum Thom
				Penicillium thomii Maire
			Larvae	*Aspergillus niveus*
				Aspergillus oryzae
				Aspergillus restrictus
				Penicillium steckii
				Penicillium citrinum
BER–4	8.xi.84	*vulgaris*	Comb	*Aspergillus oryzae*
				Penicillium steckii
BER-5	5.ix.85	*vulgaris*	Adult	*Beauveria bassiana* (Bals.) Vuill.
ALB–1	29.x.85	*pensylvanica*	Adult	*Penicillium corylophilum* Dierckx
TIL–1	16.x.86	*pensylvanica*	Larval Gut	*Penicillium restrictum* Gilman
				Penicillium phoenicium van Beyma
				Penicillium chermesinum Biorgue
				Penicillium decumbens series close to *P. citreo-viride* Biorgue
				Penicillium humili van Beyma
				Penicillium lanosum Westling
				Aspergillus wentii group
BER–6	16.xii.86	*vulgaris*	Larval Gut	*Penicillium aurantio-candidum* Dierckx

chambers, fungal growth was not visible in comb cells containing healthy larvae, even when growth and sporulation was abundant in adjacent cells (Figure 4).

When colonies enter late season decline, the depletion of the worker force reduces the efficiency of colony defense and sanitation, and regulation of temperature and humidity become irregular. Conditions within the nest then become more favorable for fungal spore germination and growth. With the overall health of the colony

weakened, facultative pathogens can overcome the defenses of live individuals, and saprophytes can spread over the abundant non-living material.

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