Microbial_cell densities under melaleuca and non-melaleuca environment

Thai Van and Min Rayamajhi

Introduction

Melaleuca quinquenervia trees are reputed source of respiratory problems due to presence of abundant volatile oils in leaves and pollens in inflorescences (Morton, 1962, Lockey *et al.*, 1981, Stanaland *et al.*, 1986), although there are no well established evidences. Morton (1966) reported that even a small amount of volatile oil can result in skin eruption in sensitive persons. Melaleuca trees flower several times a year (and produce tremendous amount of pollens, allegedly a mild respiratory allergen to which as much as 20% of human population in the area may suffer allergic reactions (Diamond *et al.*, 1991). Melaleuca flowering events occur several times a year (Meskimen, 1962) and monoculture stands produce abundant nectars during flowering season (Sanford 1988). Observations revealed tremendous amount of mold growth (personnel observation, Rayamajhi and Van) on nectar in inflorescences. Older melaleuca trees are known to produce multilayered papery bark on trunk and branches contributing ca 15-20% to stem volume (Wang 1984). These dead bark layers may be harboring saprophytic fungi and bacteria that sporulate under certain conditions and contribute to high spore counts in the air. No studies involving densities of microbial cells in air within and outside melaleuca infested areas have been reported in south Florida. In this study we attempt to assess the differences in microbial cell concentrations in the air in melaleuca and non-melaleuca stands.

Materials and Methods

Sites. Four spore trapping stations were identified in each of melaleuca and non-melaleuca areas in south Florida. Characteristics of these stations are presented in Table 1.

Table1. Description of spore trap stations south Florida.

Sites	Cover type	Other attributes	Location[2]
M1	DMM (75-100% melaleuca, mature)[1]	No undergrowth	Broward
M2	SDM (75-100% melaleuca, saplings)[1]	Remnant sawgrass	Broward
M3	DMM (75-100% melaleuca, mature)[1]	No undergrowth	Palm Beach
M4	SDM (75-100% melaleuca, saplings)[1]	Remnant sawgrass	Palm Beach
S1	Sawgrass (SDM within ca 100 m)	Mixed with other monocot	Broward
S2	Sawgrass clumps	Spotted by wax-myrtle	Broward
S3	Sawgrass clumps	Spotted with Typha grass	Broward
O1	Open Area (SDM within ca 200 m)	Edge of residential area	Palm Beach

[1] Cover type classification based on O'Hare and Dalrymple (1997).

[2] Counties in Florida, USA

Spore Trapping. In October 2002, we initiated this study. Since then we have been trapping microbial cells every 14-days in melaleuca and non-melaleuca stands listed in Table 1. Potato-dextrose-agar (PDA) and mycological agar (MA) (general microbial growth media) plates were used in trapping microbial cells in the air. A single stage Aerotech 6® microbial bioaerosol impaction sampler connected with Gast® Pump was used to draw spores on to the microbial media. The impaction sampler is held together by 3 spring clamps and sealed with 2 o-ring gaskets. The unit consisted of an inlet-cone, a jet classification stage and a base plate. The inlet-cone was equipped with an impactor stage containing 400 precision-drilled holes. When air was drawn through the sampler, multiple jets of air directed airborne particles toward the surface of the agar media placed on base plate. Gast® Pump was adjusted to draw 28.0 Liters of air per min^{-1}. One PDA or MA plate was placed on base plate, covered with impactor stage and sealed as described above. The gust pump was then run for 30 seconds to draw 14 Liters of air onto the media surface. Microbial cells contained in drawn air land on the media. The plate was then immediately removed, sealed, individually inserted in a zip-lock bag and placed in an ice-chest. This procedure was repeated three times each of PDA and MA. Thus each sampling spot had three replications per media. Plates were incubated at 27° C and the number of bacterial and fungal colonies were counted at 24-hour interval for 72 hours. Individual colony on a given media was assumed to be initiated by a single culturable cell (spore or fragmented hyphal cells), termed as colony forming unit (CFU). After 72 hours, morphologically similar colonies were identified and agar plugs from the margin of the colonies were transferred to new PDA plates to obtained reasonably pure representative colonies. These purified colonies were then incubated at 27° C for a period of 3 weeks or more to induce sporulation.

Data analyses. Data were analyzed using standard statistical procedure, SAS (1990). analysis of variance was performed to detect differences (P = 0.05) in CFUs among sites and months. Mean separations were performed using Waller-Duncan's multiple range t-test procedure.

Results

Maximum number of discernable colonies on both PDA and MA developed at 72-hour incubation period after which most colonies coalesced; these maximum numbers were used in our analysis. Fungal colonies were significantly abundant (P=0.05) on PDA (18.2 CFUs/14 L of air) than on MA (15.5 CFUs/ 14 L of air. However, the numbers of bacterial colonies were similar on both PDA (2.8 CFUs/14 L of air) and MA (2.4 CFUs/14 L of air).

The numbers of CFUs in melaleuca and non-melaleuca stands by month are presented in Table 1. The greatest (38.7 CFUs/14 L of air) and the least (8.2 CFUs/14 L of air) density of culturable fungal cells in the air in melaleuca stands were during the month of March and January of 2003 respectively. The greatest density (19.3 CFUs/14 L of air) of fungal cells in sawgrass stand was during the month of December 2002. Number of bacterial colonies in both melaleuca ((4.5 CFUs/14 L of air) and sawgrass (4.0 CFUs/14 L of air) stand was greatest in November 2002.

Table 1. Overall aerial concentration[1] of microbial cells measured every 2-week in two vegetation types in south Florida.

	Melaleuca stand[2]		Sawgrass stand[2]	
Months	Fungi	Bacteria	Fungi	Bacteria
October	15.0 (9.9)	2.8 (1.9)	11.1 (10.3)	2.5 (2.7)
November	14.8 (13.7)	4.5 (9.8)	12.0 (11.9)	4.0 (9.9)
December	21.9 (12.0)	1.9 (1.6)	19.3 (16.8)	2.8 (1.8)
January	8.2 (12.7)	2.0 (2.4)	11.8 (10.6)	2.8 (5.3)
February	19.7 (9.8)	3.0 (1.9)	11.1 (5.5)	1.9 (3.0)
March	38.7 (26.5)	2.5 (2.0)	16.8 (7.4)	1.2 (1.6)

[1] Values represent mean colony forming units (CFUs/14 L of air) developed on both PDA and MA based on 6-mo data.

[2] Value within parenthesis represent standard deviation of the mean.

The overall mean microbial CFUs in air have been presented in Table 2. Overall mean fungal CFUs in air during study period was higher in melaleuca stands compared to those in sawgrass stands and open areas. Among melaleucas, mature stand in Broward County had the highest concentration of fungal cells compared those in immature tree plots. Sawgrass stand located near (within ca 100 m aerial distance) melaleuca monoculture (site S1) had relatively more fungal cells in the air compared to the sawgrass stand located ca 3000 m from melaleuca monocultures. Concentrations of bacterial cells in the air in both melaleuca and non-melaleuca sites were not remarkably different.

Table 2. Aerial concentration[1] of microbial cells measured every 2-week in two vegetation types in south Florida.

	Microbial cells[2]	
Sites	Fungi	Bacteria
M1 (melaleuca, mature stand, Broward County)	23.8 a	2.1 a
M2 (melaleuca, immature stand, Broward County)	19.9 b	3.0 a
M3 (melaleuca, mature stand, Palm Beach County)	19.1 bc	2.8 a
M4 (melaleuca, immature stand, Palm Beach County)	17.0 bcd	3.3 a
S1 (sawgrass, Holiday Park, Broward County)	16.2 cd	2.9 a
S2 (sawgrass stand, Broward County)	14.5 de	1.8 a
S3 (sawgrass stand, Broward County)	12.1 e	2.1 a
O1 (open residential area, Palm Beach County)	11.9 e	2.9 a

[1] Values represent mean colony forming units (CFUs/14 L of air) developed on both PDA and MA based on 6-mo data.

[2] Values represent mean CFUs based on 6-mo data; values within column associated with same letter(s) are not significantly different from each other at P = 0.05 according to Waller-Duncan's t-tests.

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