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Colonization by *Cladosporium* spp. of painted metal surfaces associated with heating and air conditioning systems

Donald G. Ahearn[1], Robert B. Simmons[1], Katherine F. Switzer[1], Libero Ajello[2] and Duane L. Pierson[3]

[1]*Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, Georgia,* [2]*Emory University Eye Center, Atlanta, Georgia, and* [3]*NASA/Johnson Space Center, Houston, Texas, U.S.A.*

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SUMMARY

Cladosporium cladosporioides and *C. hebarum* colonized painted metal surfaces of covering panels and register vents of heating, air conditioning and ventilation systems. Hyphae penetrated the paint film and developed characteristic conidiophores and conidia. The colonies were tightly appressed to the metal surface and conidia were not readily detectable via standard air sampling procedures.

INTRODUCTION

Conidia of the form genus *Cladosporium*, particularly those of *C. cladosporioides* and *C. herbarum*, which are saprophytic on vegetation, are among the most common airborne fungal propagules [6,13]. Representatives of these species have been implicated frequently in the deterioration of outdoor paints [15]. The *Cladosporium* spp. and particularly the ascomycete *Hormoconis resinae* (syn. *C. resinae*) have broad enzymatic activities with capacities to metabolize diesel and jet fuels, creosote, and a variety of other hydrocarbon substrates [1,3,4]. Depending on the chemical composition of the colonized surfaces, fungi such as these not only cause problems of biodeterioration, but produce noxious odors that impact on the quality of indoor air [4]. Rarely, mention is made of the involvement of *Cladosporium* species in human mycoses such as keratitis and chromoblastomycosis [5,7], but there is frequent reference to their allergenic potential [8].

Although evaporative air cooler type systems may serve as amplification sites for indoor fungi [9], it is not clear whether the *Cladosporium* spp. recovered from indoor air of buildings with recirculating heating, ventilation, and air conditioning (HVAC) systems are derived from transient airborne conidia from outdoor habitats or from conidia produced in colonized niches of the HVAC systems (see [11]). During an investigation of the comparative densities of indoor and outdoor airborne fungi, we observed *Cladosporium* spp. associated with painted metal surfaces in indoor sites, but airborne conidia appeared in low densities. This report examines the sources of these fungi.

Correspondence: D.G. Ahearn, Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, GA 30302, U.S.A.

MATERIALS AND METHODS

Air samples were collected with a single-stage bioaerosol sampler (Model 10–880, Andersen Samplers, Atlanta, GA). The sampler was calibrated for a flow rate of 1.0 cubic foot/min. Samples were collected from five private residences in the southeastern U.S.A., four near metropolitan Atlanta, Georgia and one near Charleston, South Carolina. Multiple rooms in two large buildings subject to heavy public use also were examined. The air samples were collected before and after activation of the HVAC systems. Outside air samples were collected adjacent to each building within 30 min of the indoor sampling.

Mycological agar (Difco Laboratories, Detroit, MI) with and without 0.5% chloramphenicol was employed for the comparative isolation of fungi. Cells from representative colony types from each of the isolation agar plates were examined microscopically with the use of tape mounts. Colonies selected for further study were subcultured to various media such as potato dextrose and malt extract agars. Identification of the *C. cladosporioides* and *C. herbarum* was based on the diagnostic keys of *The Compendium of Soil Fungi* [4].

Swabs and acetate-tape stripping of various substrates

were employed in the isolation of fungi. In this latter procedure, the substrates, particularly the metal ducts and vents of HVAC systems, were stripped with clear acetate tape (Scotch Brand No. 600). Sections of tape smaller than a coverslip, which had been pressed onto a substrate with suspected fungi, were adhered (adhesive side up) to microscope slides with a drop of immersion oil. A drop of Lactophenol Cotton Blue stain was then placed on the center of the tape and a cover slip was applied and sealed with clear nail polish. We found also that the tape method could be used for collection and transport of samples to the laboratory for microscopic examination. The tape, after stripping of a surface, also was applied *lightly*, adhesive side down, to a clean glass slide. At the laboratory, the tape was peeled back from the slide and stained as above, or after partial peeling, a drop of stain was added and the tape was re-adhered to the slide over the stain for direct viewing with a light microscope.

Paint samples, about 1 cm^2, were removed from the metal surface of HVAC system mixing boxes with a sterile, single-edge razor blade and forceps. Enameled metal sample sections, approx. 1 cm^2, were removed with shears from the vanes of registers of the HVAC systems. Samples for scanning electron microscopy (SEM) were mounted with silver conducting paint directly onto aluminium stubs and sputter-coated with approx. 7 nm of gold/palladium. All specimens were examined in a JEOL JSM35 scanning electron microscope operated at 15 kV. Images were recorded on Polaroid Type 55 positive/negative film.

To test the survivability of conidia under varied ecological conditions, we placed metal sections of register vents colonized with fungi into vials without supplementation and lyophilized them in a Virtis freeze dryer (-25–$50\,^{\circ}C$, $<200\,\mu m$ pressure). The metal sections were removed from the lyophilization vial and surface-streaked onto isolation agar. Conidia from agar plates (10^6–10^8) were suspended in 1.0 ml water and in 20% casein and were used as controls.

RESULTS

C. cladosporioides and *C. herbarum* were isolated commonly from outdoor and indoor air samples, the vents of metal registers, and the interior of HVAC cover panels from most sites examined. In general, the highest airborne densities of the *Cladosporium* spp. propagules were found in outdoor air and in air samples collected in or near entrances to crawl spaces. Numbers of fungi at these sites were too numerous for accurate counts with the methods employed. For a few sites, even with sampling times of 30 s, all 400 air impact sites on the agar plates yielded mixed fungal colonies ($>14\,000$ cfu m^{-3}). *Cladosporium* spp. in these and the other air samples were estimated to constitute at least 30% of the total fungi isolated. At several private residences and in specific rooms of several large buildings the indoor airborne densities of the *Cladosporium* spp. were less than 35 cfu m^{-3}, but heavy growth of *Cladosporium* spp. could be recovered from swab cultures of painted metal surfaces of the HVAC systems. This low indoor distribution of <35 cfu m^{-3} was observed for samples collected within several meters of surfaces found to be colonized with the two *Cladosporium* spp. even after activation of the HVAC units (Table 1).

Tape-mount samples from register vents or from surfaces adjacent to coils or chill boxes revealed the presence of mature conidiophores and conidia mostly of the *Cladosporium* spp. Short hyphal segments and branched chains of conidia were regularly observed (Fig. 1). Occasional conidia of *Alternaria* spp., *Curvularia* spp. and

TABLE 1

Total airborne fungi and occurrence of *C. cladosporioides* and *C. herbarum* in air adjacent to indoor localities with colonized metal surfac

Sampling site	Number of samples	Total fungi		*Cladosporium* sp
		Indoor air No. m^{-3}	Adjacent outdoor air No. m^{-3}[b]	Indoor air[c] No. m^{-3}
R-1	6	120–1483	9650–10600	<35
R-2	9	<35–1116	350–14000+	<35
R-3	3	<35–120	<35–350	<35
B-1	45	<35–70	<35–450	<35

[a] Swab streaks of painted metal yielded confluent growth of cladosporia and microscopically observable conidiophores with conidia cladosporia were observed with tape mounts of the painted metal.

[b] Totals include estimated incidence of 30% *Cladosporium* spp.

[c] No more than one colony of *Cladosporium* spp. per isolation plate.

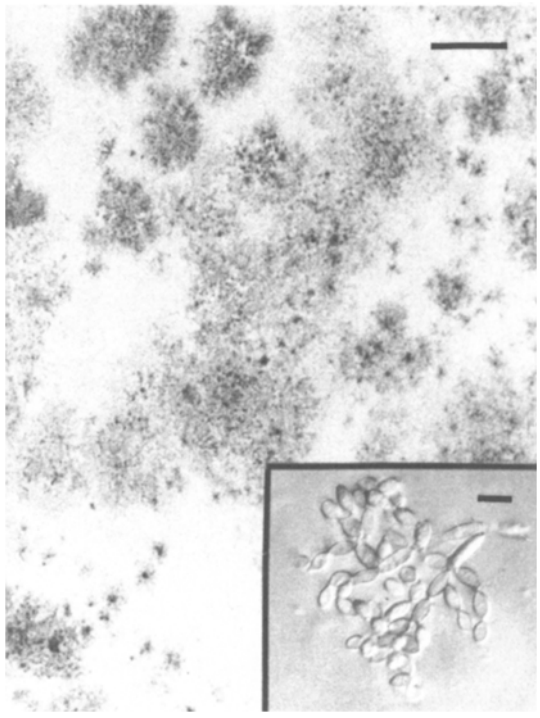

Fig. 1. Photograph of colonization of painted metal surfaces of mixing box on an air conditioner duct (bar = 1 cm); (inset) light micrograph from acetate tape mount from surface of metal demonstrating conidia of *Cladosporium* spp. (bar equals 10 μm).

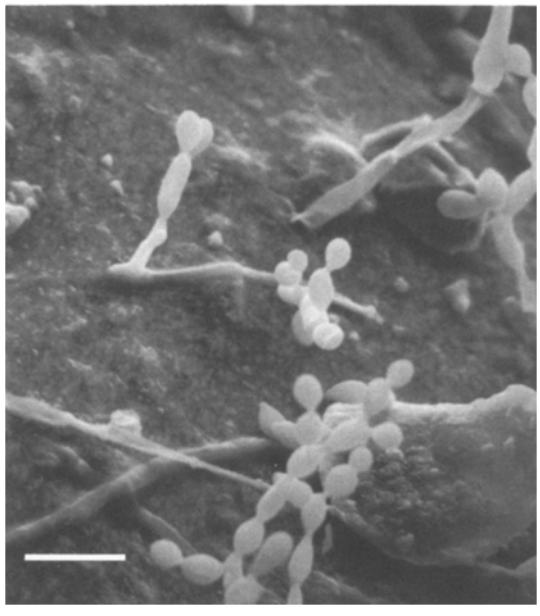

Fig. 2. SEM of painted metal surface of a register vane demonstrating conidiophores and conidia of *Cladosporium* (bar = 10 μm).

also *Epicoccum nigrum* were observed, but swab cultures of the areas yielded mostly confluent growth of *C. cladosporoides* or *C. herbarum*. At one site *Penicillium jenseni*(?) and the *Cladosporium* spp. were cultured from swab streaks of the metal surfaces at apparently equivalent densities. At a second site the *Cladosporium* spp. and *P. purpurogenum* were isolated from the swab streaks and from the indoor air. No recognizable *Penicillium* spp. were observed in the tape mounts from the colonized surfaces at these two sites. However, *Penicillium* spp. still were recovered by culture after 6 weeks of storage of the metal samples in plastic bags. At a third site conidiophores with mature conidia of both *C. cladosporioides* and *Penicillium* spp. were observed on tape slides and colonies of both genera were recovered in culture. Fungi were also recovered from swab samples of galvanized steel air ducts, but colonization of these non-painted surfaces was not demonstrated with tape mounts. Examination of representative painted metal surfaces from two sites with SEM showed that the hyphae of the *Cladosporium* spp. grew within the paint matrix and that short hyphal sections bearing conidiophores and chains of conidia protruded above the surface (Fig. 2). In three repeated experiments the *Cladosporium* spp. were recovered readily by culture from the lyophilized metal sections cut from the metal vanes. Light streaking of the metal section over the agar surface yielded confluent growth along the streak. Recovery of viable conidia from lyophilized suspensions in water was negligible.

DISCUSSION

We demonstrated the presence of viable *C. cladosporioides* and *C. herbarum* on painted metals associated with HVAC units by swab cultures. Acetate tape stripping and SEM showed that the fungi were present not in the form of dormant conidia originating from outside air, but as reproducing fungal colonies tightly adhered to the metal surfaces. Viable cladosporia were also recovered from the interiors of unpainted ducts, but colonization of these ducts was not observed. There was some suggestion that colonization was associated with paint deterioration and rust formation, but primary colonization of at least some surfaces seemed to be on surface films, probably comprised of condensed volatile organics. Amplification sites underlying the dispersal of fungi in indoor air are usually associated with a steady source of exogenous water [14], but there was no visual evidence of such water on the colonized metal surfaces and water condensation or dampness was not evident during collection of samples. The recovery of viable *Cladosporium* spp. colonies from the metal surfaces after lyophilization demonstrated the capacities of these fungi to withstand

variations in temperature, pressure and moisture beyond those suitable for growth.

At two colonization sites, monoverticillate *Penicillium* spp. were recovered in high densities from swab cultures, but they were not observed in tape mounts. These same *Penicillium* spp. were recovered from adjacent air samples generally in higher densities than the cladosporia. Possibly, the penicillia were growing on or parasitizing the cladosporia. Morey et al. [11] have indicated that air conditioning systems may act as reservoirs for species of these genera.

Morey [10] indicated that air sampling alone was insufficient for determining if a particular building was at risk for a sick-building-syndrome designation. This concept is supported further by our preliminary investigations which indicate that the presence of *Cladosporium* spp. on colonization sites may not be reflected by densities of airborne conidia. Pasanen et al. [12] recently demonstrated that the release of conidia of *Cladosporium* spp. from cultures required about twice the air velocity (1.0 ms^{-1}) of that for the release of conidia of *Penicillium* sp. or of *Aspergillus fumigatus*. Our investigations are of particular relevance to closed environmental systems such as NASA's Space Station Freedom and the proposed Lunar and Martian habitats. *Cladosporium* spp. are common constituents of the indoor and outdoor biota in southern parts of U.S.A. where most of NASA's fabrication, assembly and operational facilities are located. They are invariably present in prelaunch quarantine housings and they have been frequently isolated from nasal swabs of the crew, the interior surfaces, and air of the shuttle and space suits (NASA/JSC unpublished data). Species of *Cladosporium* and *Penicillium* are known to grow in orbiting spacecraft [2]. Because operational constraints are likely to preclude the possibility of maintaining a germ-free environment, *Cladosporium* spp. will probably find their way into future extra-terrestrial human habitats. The capacity of *Cladosporium* spp. on metal to withstand the lyophilization process without a protective protein supplementation also suggests that they may withstand the atmospheric and temperature fluctuations in the space station habitat. These fungi could pullulate in the fragile closed environment, and threaten the astronauts' health and the integrity of the spacecraft structures.

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