Soil Ecology of *Coccidioides immitis* at Amerindian Middens in California

GEORGE H. LACY AND FRANK E. SWATEK

Microbiology Department, California State University, Long Beach, California 90804

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Outbreaks of coccidioidomycosis and isolation of *Coccidioides immitis* have been reported from Amerindian middens. This study was undertaken to determine the most important ecological component(s) for the occurrence of *C. immitis* at archeological sites. Soils from 10 former Indian villages with no prior history of coccidioidal infection were collected and cultured. The physicochemical properties of the midden soils were compared with nonmidden soils and positive soils. The following theories for the sporadic distribution of the pathogen in the soil of the Lower Sonoran Life Zone were considered: (i) the *Larrea tridentata* (creosote bush) association, (ii) the preference for saline soils, (iii) isolation near rodent burrows, and (iv) animals as possible agents of dispersal. Results showed that a high percentage of the midden soils contained *C. immitis*, whereas none of the adjacent, nonmidden soils yielded the fungus. Physicochemical analyses revealed that the dark color and alkaline pH of the midden soils were due to past organic contamination. Repeated isolations were made from soils with low to moderate alkalinity. Alkalinity and sandy texture were consistent features of all soils in this study. However, the lack of any reports of nonsandy infested soils possibly indicates that the sandy texture and alkalinity may be factors in the distribution of this fungus. The organic content, soil parent material, and color were not important in the soil ecology. *L. tridentata* was not significant in the macroflora at the infested sites surveyed. Samples collected without reference to rodent burrows yielded a high percentage of recoveries. Animals, although not the major natural reservoir, cannot be ignored as possible factors in the ecology of *C. immitis*.

Our knowledge of the ecology of *Coccidioides immitis* is more advanced than for any other respiratory mycotic disease agent (1). However, as Huppert has pointed out, "A major gap in our knowledge of *C. immitis* is why the fungus should be so limited in its natural distribution" (10). Others have reported on the uneven, yet consistent, occurrence of the fungus in the soils of the Lower Sonoran Life Zone (15, 29). At one site, an area of approximately 2.5 m² has been positive from 1954 through this study, whereas the surrounding soil has yielded only rare positives.

The sporadic occurrence of the fungus in nature seems to contradict its laboratory physiology. It grows rapidly on all common media at temperatures of 20 to 30 C and is not exacting in its nutritional requirements. Growth occurs between pH 3.5 and 9.0 and on clay through sandy soils (13). The fungus is known to infect mammals, reptiles, fish, and amphibians (30) and thrives on parts of many desert plants (23).

Various theories have been advanced to explain the spotty distribution of *C. immitis* in the soils of endemic regions. Emmons (9) felt that the carcasses, sputa, urine, feces, and purulent materials of infected rodents were the sources of the pathogen in soil. Maddy (14) emphasized the presence of *Larrea tridentata* (creosote bush) at his isolation sites in Arizona. Egeberg and Ely (6) recovered the fungus from 13.6% of soil samples taken within 5 ft (15.24 m) of animal burrows, whereas only 3.4% of the samples collected further away were positive. Egeberg and his associates (7, 8) reported that high soil salinity was related to increased recovery of *C. immitis* and the suppression of antagonists. Swatek (27) noted that repeated isolations had been made from old Indian campsites and suggested a possible relationship between the fungus and the increased organic content of the soil on these sites.
The purpose of these studies was to define the most important ecological components for C. immitis infestation at former Amerindian habitation areas. Studies were undertaken to determine the relationship between the fungus and the midden soil (areas rich in charcoal, obsidian chips, and other evidence of domestic contamination) and the differences between midden and adjacent, nonmidden soil.

MATERIALS AND METHODS

Soil collection. Soils were collected aseptically from former Amerindian village sites having no known history of human infection in areas endemic for coccidioidomycosis. Villages in the widely separated Kern and Madera counties of California were chosen for this study, along with control midden soils from sites culturally positive for C. immitis from San Diego, Kern, Butte, and Merced counties (see Fig. 1.). Amerindian habitation sites may be delineated by various criteria. In this study they included: (i) soil darkened by domestic contamination near water-sources (some only seasonal) and, (ii) macroscopic artifacts of human occupation such as obsidian flakes or tools; bedrock mortars or metate stones; “house-floor” depressions of semisubterranean homes; soapstone, shell or glass beads; human remains and petroglyphs.

Determination of points for soil sampling within the middens was made by the use of grids or traverses. Collections of adjacent, nonmidden soils were made at the nearest area which resembled the midden in exposure, drainage and foliage.

Soil isolation. The technique of soil isolation employed was basically the double-pour, antibiotic-fortified, yeast extract agar method (28). Modifications included using 10 g of soil in 90 ml of sterile, distilled water in milk dilution bottles and agitating the 1-in-10 soil suspensions for 1 min and again at 20 and 40 min, and then leaving them undisturbed for 20 min.

The plates were incubated at ambient room temperature (23 to 27°C) in a humidified chamber for 3 to 5 weeks. The colonies were examined macro- and microscopically for similarities to C. immitis with consideration for the wide morphologic range possible (11). All fungi demonstrating arthroaleuriospores (20) or racquet cells, or both, were subcultured on Sabouraud dextrose agar. If, on examination of the subculture, it was felt there was still a resemblance to the pathogen, intraperitoneal inoculation of mice was carried out with an aqueous spore suspension. Only fungi which produced spherules and endospores in mice were reported as C. immitis.

Soil characterization. Selected midden and adjacent soil samples were compared physicochemically. Carefully mixed individual or composite (combined by equal portions) samples were passed through a no. 10 mesh screen. They were subjected to the following analytical scheme (manufacturers’ procedures were followed unless noted): (i) color was determined on dry and paste portions by using Munsell soil color charts (Munsell Color Company, Inc.); (ii) textural classification was made by use of stainless-steel, heat-sterilizable soil sieves; (iii) measurements of pH and Eh were made with a Beckman G pH-meter on 1:5 double distilled water, soil extracts, or soil pastes; (iv) electrical conductivities were determined with a model RC-16B2 Industrial Instruments, Inc., conductivity bridge on pastes and 1:5 extracts; (v) levels of principle ions were delimited with a Simplex soil testing kit (Edwards Laboratory), and (vi) organic and inorganic carbon and organic nitrogen determinations were made by wet combustion and micro-Kjeldahl techniques, respectively, on powdered (to pass 400 mesh), autoclaved soils (2, 12).

Soil comparisons. Comparisons of positive midden soils were made with analyses of other infested soils reported in the literature (8, 19), by personal communications and on soil samples received. The following persons provided either physicochemical data or samples of C. immitis-infested soils: J. L. Converse (Fort Detrick, Md.), D. H. Howard (University of California, Los Angeles), G. H. Kellogg (Butte Co. Public Health Department, Chico, Calif.), H. B. Levine and G. M. Scalarone (Naval Biomedical Research Laboratory, Oakland, Calif.), F. W. Ramdell (California State University, Chico), R. E. Reed (University of Arizona, Tucson), R. H. Sorensen (Veterans Administration Hospital, Fresno, Calif.), and H. A. Walch (California State University, San Diego).
RESULTS

Soil isolation. Ten sites of former Amerindian villages, with no known histories of coccidioidal infection, were chosen to represent the test middens. Two sites (4-Mad-117 and Fre-SFSC-1) associated with past infection, whose soils had never been studied mycologically, were included as additional controls. Table 1 summarizes the histories of infection and verification of prolonged human occupation at the midden sites surveyed. Results of the soil isolation of C. immitis are tabulated in Table 2. Most of the sites were included on archeological surveys. The Inyokern Cave was investigated by archeologists from the University of California at Los Angeles. The CaMad-169 through 186 sites were part of a survey of the Fresno River Valley by California State University, Long Beach. The Fre-SFSC-1 and 4-Mad-117 through 239 sites were part of similar surveys made by California State University, San Francisco near Lake Millerton and in the Chowchilla River Valley.

Soil characterization. The results of most of the physicochemical analyses of midden and adjacent, nonmidden soils are compiled in Table 3. The levels of principle ions in the 23 midden and adjacent soils may be summarized as follows: Mn²⁺, less than 1 ppm; K⁺, 15 to 20 ppm; Ca²⁺, less than 40 to 150 ppm; Mg²⁺, less than 2 to 6 ppm; Al³⁺, less than 3 ppm; Fe³⁺, less than 2 ppm; NH₄⁺, up to 2 ppm; NO₃⁻, less than 2 to greater than 25 ppm; SO₄²⁻, less than 1 to less than 3 ppm; PO₄³⁻, 0.5 to 5.0 ppm; Cl⁻, less than 20 ppm; and SO₄²⁻, less than 150 to 600 ppm.

Soil comparisons. Comparisons of positive midden soils with other C. immitis-infested soils in the literature, by personal communication and analyses, revealed that: (i) sandy-textured soils occurred in 98.0% of 51 samples (conflicting results were discovered for three of four soils studied by both us and Orr [19], and only the results of the mechanical analyses were used), (ii) alkaline soils occurred in 96.7% of 62 soils, (iii) organic carbon values for 12 soils ranged from 0.21 to 1.80% weight, (iv) organic nitrogen values for 21 soils ranged from 0.029 to 0.190% weight, (v) total organics for 47 soils ranged from 0.39 to 3.13% weight, and (vi) electrical conductivity values for 56 soils ranged from 37 to 27,000 × 10⁻⁴ mhos/cm at 25°C. C. immitis has been isolated from soils developed from diverse parent materials: granitic, volcanic, and sedimentary rocks (either stream or ocean derived) and alluvium or lacustrine depositions.

DISCUSSION

It is evident that a single factor does not determine the distribution of C. immitis in the soil of the Lower Sonoran Life Zone. Emmons has refuted his original hypothesis that rodents are the major reservoir of the pathogen in nature (1) and this is supported by the reports of Swatek et al. (29). Yet, Sorensen (24) has indicated that spherules and endospores protected by body fluids might survive long enough in the soil to allow mycelial growth. Maddy and Crecelius (16) reported the establishment of the fungus in soil with infected animal tissues. During the present study it was noticed that animal activity was increased on the midden sites (221 burrows were counted on eight 188-m² plots at six different middens compared to 146 on an equal number of adjacent plots). Animals, although not the major reservoir, cannot be ignored as possible factors in C. immitis dispersal.

Correlation of the distribution of C. immitis and the macroflora of the positive sites was unproductive. The sites ranged from sparsely vegetated deserts through oak woodlands with scattered pine (Fig. 2). Creosote bush was observed near only 1 positive site (IK-2) out of the 11 included in this survey. The repeated isolation of C. immitis from areas having vegetation markedly different than that of L. tridens-tata regions should broaden the search for C. immitis-macrofloral associations in nature. Riker (23), in her report on the growth of the pathogen on parts of desert plants, described the inhibition caused by creosote bush. She also noted that the parts of several plants, among them six Opuntia spp. (prickly pear cactus), supported abundant growth and sporulation of the fungus. Campins (5) and Mayorga (18) observed the same genus in endemic areas of Guatemala, Honduras, and Venezuela. The genus is also common in endemic regions of the United States and Mexico. It is possible that the fungus does form alliances with the macroflora, but they may be casual rather than distribution affecting.

Egeberg and Ely (6) published a report that incriminated the soil near rodent burrows as a source of C. immitis in nature. Midden samples taken without reference to animal burrows in this research yielded 9.5% positives (of 325 samples), which would indicate that other factors may also be important.

Elconin et al. (8) found a positive correlation between the recovery of C. immitis and high soil salinity. Repeated isolations (32 positive samples) from sites in this study revealed markedly
TABLE 1. Histories of infection and evidence of human occupation at the Amerindian archaeological sites included in this report

| Site and reference no. | History of coccidioidal infection | Dark soil (with charcoal and/or charred animal bones) | Artifacts | Housefloor depressions or petroglyphs | Human remains |
|------------------------|-----------------------------------|------------------------------------------------------|------------|--------------------------------------|---------------|
| Lakeside-1 San Diego Co. (31) | +** | + | - | + | - |
| Lakeside-2 San Diego Co. (29) | + | + | + | + | - |
| Santee San Diego Co. (29) | - | + | - | + | - |
| Inyokern Cave Kern Co. (21) | + | + | + | + | - |
| Inyokern-2 IK-2 Kern Co. | - | + | + | + | - |
| Inyokern-3 IK-3 Kern Co. | - | + | + | + | - |
| Rocky Point Kern Co. | - | - | + | + | - |
| Fre-SFSC-1 Fresno Co. | + | + | + | - | + |
| CaMad-171 Madera Co. | -< | + | + | + | + |
| CaMad-173 Madera Co. | -< | + | + | + | + |
| CaMad-177 Madera Co. | - | + | + | + | - |
| CaMad-179 Madera Co. | - | + | + | + | - |
| 4-Mad-117 Madera Co. | + | + | + | + | - |
| 4-Mad-118 Madera Co. | - | + | + | + | - |
| 4-Mad-136 Madera Co. | - | + | - | + | - |
| 4-Mad-239 Madera Co. | -* | + | + | + | + |
| Los Banos Merced Co. (21) | + | + | + | + | + |

* Other midden sites (not observed by authors): CaMad-167, 169, 183, 186, Madera Co., collected by cooperating archeologists; and Richardson Springs midden, Butte Co., collected by G. H. Kellog.

**+, Presence of infection and evidence of human occupation; -, absence of infection and evidence of human occupation.

* Human infection or suspicion of human infection was discovered after the site was sampled.
Table 2. Results of soil isolation of *C. immitis*.

| Site       | Classification* | Soils collected | Soils positive for *C. immitis* |
|------------|-----------------|-----------------|---------------------------------|
|            |                 | Mid-Aden | Mid-Aden |                     |
| Lakeside   |                 |          |          |                     |
| LS-1       | c               | 23       | 0        | 1                   | 0 |
| LS-2       | c               | 1        | 0        | 0                   | 0 |
| Santee     | c               | 1        | 0        | 1                   | 0 |
| Inyokern-1 | c               | 18       | 12       | 5                   | 0 |
| Inyokern-2 | a               | 22       | 6        | 3                   | 0 |
| Inyokern-3 | a               | 9        | 5        | 0                   | 0 |
| Rocky Point| a               | 8        | 5        | 0                   | 0 |
| Fre-SFSC-1 | c               | 21       | 5        | 0                   | 0 |
| CaMad-167  | b               | 1        | 0        | 0                   | 0 |
| CaMad-169  | b               | 4        | 0        | 1                   | 0 |
| CaMad-171  | a               | 39       | 4        | 5                   | 0 |
| Casad-173  | a               | 93       | 5        | 2                   | 0 |
| CaMad-177  | a               | 8        | 8        | 0                   | 0 |
| CaMad-179  | a               | 8        | 5        | 0                   | 0 |
| CaMad-183  | b               | 1        | 0        | 0                   | 0 |
| CaMad-186  | c               | 2        | 0        | 0                   | 0 |
| 4-Mad-117  | c               | 19       | 5        | 3                   | 0 |
| 4-Mad-118  | a               | 8        | 2        | 2                   | 0 |
| 4-Mad-136  | c               | 7        | 2        | 0                   | 0 |
| 4-Mad-239  | a               | 31       | 5        | 8                   | 0 |
| Los Banos  | c               | 1        | 0        | 0                   | 0 |
| Butte Co.  | c               | 1        | 0        | 1                   | 0 |

* Symbols: a, random middens collected by the authors; b, random middens collected by archeologists, and c, control middens.

The soils collected were from sites with less salinity (114 to 1,856 × 10⁻⁴ mhos/cm at 25°C) and may indicate that the high salinity might have been a local phenomenon, which, rather than limiting its distribution, represents a more halotolerant extension of the fungus physiology.

Swatek’s contention (27) that *C. immitis* infestation is enhanced in the soils of former Amerindian villages has borne out by this study. However, it appears that sandy texture and alkalinity were more important than the organic content of the soil in this relationship. The pathogen was recovered from 8.1% of 395 soils cultured. Considering just the midden soils, 9.8% were positive, or 8.7% after subtracting the control soils. Soils of 5 of the 10 random midden sites contained the pathogen. Additionally, 1 of 4 random sites, from which soils were collected by cooperating archeologists, was positive. Mention should also be made that the Santee site (29) was another positive midden with no prior history of *C. immitis* isolation. One of the two sites suspected of causing human infection, 4-Mad-117, yielded the fungus.

Historically, random soil samples from endemic areas have yielded between 2.0 and 3.4% positives for *C. immitis* (6, 9, 27). Considering these figures, the percentage of positives reported in this study would appear to be significant. The lack of any positives among the nonmidden soils supports this contention. The epidemiological impact of the association of *C. immitis* and midden soils will be presented elsewhere.

Darkening of the soil was a very stable character of the midden sites and was undoubtedly influenced by past human habitation. During cyclic periods of occupation, the soil was the final receptacle of charcoal, wood, thatch, domestic scraps, human wastes, and burials. Over as much as 1,500 years (at CaMad-173 and the Inyokern Cave), this amounted to a considerable localized increase in organic contamination. Soil color may be related to drainage, aeration, chemical content, and climate. In the midden soils, which were sandy, well drained, low in soluble iron and manganese, and situated in temperate, semiarid to arid regions, the color must be attributed directly to organic materials and charcoal.

The soil was alkaline at the middens surveyed. In general, except for IK-3 (which was in an alkaline area), the pH of the surrounding soil was lower. This was due to the accumulation of ash minerals and organic debris. Soil alkalinity is directly related to the intensity and duration of human occupation as revealed in a study of a Chowchilla River Valley site where a proportional increase among pH, artifact yield, and depth of the midden was found (17). Preliminary studies (by D. Rosenberg and J. Kelly, Department of Anthropology, California State University, Long Beach) indicated a similar pattern at CaMad-173.

Assessment of organic contamination at contemporary sites of human activity (since 1850) was not part of this study, but it was a contributing factor at two of the sites investigated. CaMad-173 had hydraulic gold mining and ranching activities until the present, and IK-2 had the foundations of a small building on it. Campers, sheepherders, and transients have been observed on some of the sites during this research.

All the positive soils studied exhibited an Eh range of 88 to 266 mV (uncorrected). The Eh-pH milieu occupied by *C. immitis* in nature appears to be naturally segregated when compared by the method of Baas-Becking (3) with adjacent, nonmidden and random Southern California soils (Fig. 3).

Midden soils tended to be higher in carbonates (0.05 to 1.55%) and phosphates (1.0 to 5.0...
**Table 3. Results of physicochemical analyses of midden and adjacent soils**

| Site                      | Parent material | Soil color (air dry) | Munsell notation | Texture (mechanical) | pH paste (1:5 extract) | Eh + mV (1:5 extract, uncorrected) | Electrical conductivities at 10⁻¹ mhos, at 25°C (paste, 1:5 extract) | Organics (wt %) | Carbonates (wt %) (HCl test) |
|---------------------------|-----------------|----------------------|-------------------|-----------------------|------------------------|-----------------------------------|---------------------------------------------------------------|-----------------|-----------------------------|
| Lakeside midden-1        | Granite         | Dark grayish brown   | 5YR-2/2           | Sand                  | 7.7                    | 218                               | 450                             | 0.19            | 1.80                        | 0.09             |
| Lakeside midden-2        | Granite         | Dark grayish brown   | 10YR-4/2          | Sand                  | 7.7                    | 108                               | 418                             | 0.08            | 1.05                        | +               |
| Santee midden            | Granite         | Very dark grayish brown | 10YR-3/2         | Sand                  | 7.4                    | 132                               | 223                             | 0.11            | 0.72                        | 0.05             |
| Inyokern (Cave) midden   | Sandstone       | Dark grayish brown   | 10YR-4/2          | Sand                  | 8.1                    | 266                               | 452                             | 0.04            | 0.57                        | 0.13             |
| Adjacent Inyokern (Cave) midden | Sandstone | Brown: dark brown | 10YR-4/3         | Sand                  | 7.4                    | 200                               | 400                             | 0.02            | 0.21                        | 0.02             |
| IK-2 midden              | Sandstone       | Brown                | 10YR-5/3          | Sand                  | 7.7                    | 198                               | 260                             | 0.02            | 0.45                        | -                |
| Adjacent IK-2 midden     | Sandstone       | Brown                | 10YR-5/3          | Sand                  | 7.4                    | 210                               | 398                             | 0.00            | 0.00                        | -                |
| IK-3 midden              | Sandstone       | Dark grayish brown   | 10YR-4/2          | Sand                  | 7.2                    | 214                               | 214                             | 0.00            | 0.00                        | -                |
| Adjacent IK-3 midden     | Sandstone       | Dark yellowish brown | 10YR-4/4          | Sand                  | 7.5                    | 187                               | 181                             | 0.00            | 0.00                        | -                |
| Sharktooth Hill Kern Co.* | Ocean deposits | Light brownish gray  | 2.5YR-6/2         | Sand                  | 6.6                    | 142                               | 1856                            | 0.00            | 0.00                        | -                |
| Same site                | Ocean deposits  | Pale olive           | 5YR-6/3           | Sand                  | 6.7                    | 148                               | 1560                            | 0.11            | 0.55                        | 0.01             |
| Fre-SFSC-1 midden        | Granite         | Dark grayish brown   | 10YR-4/2          | Sand                  | 7.5                    | 180                               | 317                             | 0.00            | 0.00                        | -                |
| Adjacent Fre-SFSC-1 midden | Granite       | Dark grayish brown   | 10YR-4/2          | Sand                  | 6.9                    | 175                               | 315                             | 0.00            | 0.00                        | -                |
| CaMad-171 midden         | Granite         | Grayish brown        | 10YR-5/2          | Sand or loamy sand    | 8.2                    | 194                               | 225                             | 0.04            | 0.57                        | 1.36             |
Table 3—Continued

| Site               | Parent material | Soil color (air dry) | Texture (mechanical) | pH paste (1:5 extract) | Eh (mV) (1:5 extract; uncorrected) | Conductivities at 10⁻⁴ mhos at 25 °C (paste, 1:5 extract) | Organics (% wt) | Carbonates (% wt) (HCl test) |
|--------------------|-----------------|----------------------|----------------------|------------------------|-------------------------------|-------------------------------------------------------------|----------------|-----------------------------|
| Same site          | Granite         | Dark grayish brown   | Sand                 | 8.4                    | 183                           | 114                                          | 0.10           | 1.60                        | 1.55                        |
| Adjacent CaMad-171 | Granite         | Brown                | Sand                 | 7.2                    | 185                           | 37                                           | 0.07           | 0.79                        | 0.02                        |
| midden             |                 | 10YR-4/2             |                      |                        |                               |                                              |                |                             |                             |
| CaMad-173 midden   | Granite         | Dark grayish brown   | Sand                 | 8.0                    | 203                           | 230                                          | 0.03           | 1.40                        | 0.88                        |
| Same site          | Granite         | Dark grayish brown   | Sand                 | 8.2                    | 180                           | 168                                          | 0.10           | 1.60                        | 1.55                        |
| CaMad-179 midden   | Granite         | Gravish brown        | Sand                 | 7.8                    | 178                           | 166                                          | 0.10           | 1.60                        | 1.55                        |
| Adjacent CaMad-179 | Granite         | Yellowish brown      | Sand                 | 7.6                    | 172                           | 248                                          | 0.03           | 1.40                        | 0.88                        |
| midden             |                 | 10YR-5/2             |                      |                        |                               |                                              |                |                             |                             |
| 4-Mad-117 midden   | Granite         | Dark grayish brown   | Sand                 | 8.1                    | 148                           | 170                                          | 0.05           | 1.53                        | 0.74                        |
| Adjacent 4-Mad-117 | Granite         | Dark yellowish brown | Sand                 | 6.4                    | 194                           | 163                                          | 0.14           | 1.51                        | 0.02                        |
| Los Banos midden   | Alluvium        | Gravish brown        | Sand                 | 8.6                    | 182                           | 535                                          | 0.08           | 0.83                        | 0.30                        |

*This paleontological site was positive for *C. immitis* (29); however, the samples included here did not yield the pathogen.

ppm) than adjacent soils (0.02% and 0.5 to 1.8 ppm, respectively). Except for sites liable to seasonal flooding (Fre-SFSC-1, IK-2, and IK-3), all middens demonstrated strong to violent effervescence when tested with 10% hydrochloric acid. Bone and shell, rich in these ions, were part of the aboriginal contamination of the middens. These components may have ecological significance as buffering agents.

Comparisons of infested soils revealed physicochemical characters similar to those reported by Cameron in studies of California desert soils (4). Possibly, ecologically limiting factors for *C. immitis* may include soil pH and texture, whereas nonlimiting factors would include color, organic content, salinity, and soil parent materials. Stotzky (25, 26) reported that a positive correlation existed between the presence of clay minerals and the isolation of *Histoplasma capsulatum* from soil, but no conclusions could be formed concerning *C. immitis*. Analyses of soils collected for this study revealed that some contained montmorillonite, but the correlation with the presence of the pathogen was not as good as obtained with *H. capsulatum* (Stotzky, personal communication).

It is evident that *C. immitis* is a physiologically versatile organism, yet the contradiction remains that is has a spotty distribution in nature to the limit of present soil isolation.
techniques. The limiting factor may be the competitive saprophytic ability of the fungus. Observations of its behavior in the mixed cultures of soil isolation plates and comments of other workers support the hypothesis that C. immitis may be a poor competitor for nutrients and biological space. Another consideration is that the consistent soil physicochemical characters determined in this study may well be those most favorable for control of its microbial competitors. Manipulation of these conditions should be considered for the control of the saprophytic phase of C. immitis.

The results of this research indicated that C. immitis is strongly associated with Amerindian middens in California. Comparisons of infested soils in the literature and in the laboratory revealed that the chief factors for this association were the presence of alkaline and sandy soils. At the midden sites surveyed, the soil alkalinity and color were due to past accumulation of domestic contaminants.
FIG. 3. $E_h$ (uncorrected) and pH comparisons of 30 soils from Amerindian archeological sites and non-midden areas culturally positive for C. immitis (○), seven adjacent non-midden soils not containing the pathogen (△), and 19 random southern California soils not processed culturally (O). (Random soils courtesy of M. R. Sneller, California State University, Long Beach.)

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