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Occurrence of *Pasteuria*-like Organisms on Selected Plant-Parasitic Nematodes of Pineapple in the Hawaiian Islands[1]

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Abstract: Soils from 320 sites representing diverse undisturbed habitats from five Hawaiian Islands were assessed for occurrence of *Pasteuria*-like organisms. Mean annual rainfall at sites ranged from 125–350 cm, elevation from 69–2,286 m, and annual mean temperature from 12–24 C. Seven different natural communities were represented: wet lowland, mesic lowland, wet montane, mesic montane, dry montane, mesic subalpine, and dry alpine. *Pasteuria* spp. in a soil sample was detected by baiting with infective stages of *Helicotylenchus dihystera, Meloidogyne javanica, Pratylenchus brachyurus,* and *Rotylenchulus reniformis,* followed by cultivation of the nematodes on pineapple plants for 10–11 months. All nematode baits except *R. reniformis* were readily recovered from the soil samples. A sample was considered *Pasteuria*-positive if at least 5% of the nematode specimens showed endospore attachment. Thirteen percent of all samples were positive for *Pasteuria*-like organisms. The frequencies of association between *Pasteuria* spp. and *Meloidogyne, Helicotylenchus,* or *Pratylenchus* species were 52%, 24%, and 24%, respectively. Positive samples were more prevalent on the older islands of Kauai and Oahu (75%), in lowland communities (61%), and in areas with introduced vegetation (60%). More than 27% of the positive samples were associated with plant species in a few selected families that included Meliaceae and Myrtaceae. Occurrence of *Pasteuria* spp. seemed to be positively associated with mean annual rainfall or temperature, but negatively associated with elevation.

Key words: bacterium, biological control, Hawaiian Islands, *Helicotylenchus, Meloidogyne,* microbial ecology, native vegetation, natural community, *Pasteuria,* pineapple, plant-parasitic nematode, *Pratylenchus, Rotylenchulus,* tropics.

Species of the genus *Pasteuria* (Thorne) Sayre & Starr are obligate endoparasites and pathogens of plant-parasitic nematodes (7,9,18,26,28,29). These parasites, which are among the most promising biological control agents (4,27,34), have been recorded in many countries worldwide (27,37). However, almost all have been found incidentally in cultivated soils during routine sampling or diagnostics. Only a few deliberate surveys of the parasite have been reported (12,30,35,41). Surveys of the parasites in citrus groves in Florida (41) and pineapple fields in Hawaii (M. Ko, per. obs.) have yielded only one isolate, or none at all. In contrast, the *Pasteuria* spp. was found in every turfgrass field sampled in southern Florida (12). In similar surveys in South Africa, *Pasteuria* spp. occurred in 34% of 74 sugarcane fields sampled (30). The bacteria were found to be widespread when sampled in South Australian vineyards (35). In the latter two cases, the incidence of *Pasteuria* was much greater in older fields (i.e., >10 years of production in Australian vineyards) than in young ones. This provides some evidence that the parasites might be present initially in new and previously undisturbed fields at a low level but require time to build up to a detectable level, following an increase in population densities of the nematode host. Thus, the chance of detecting *Pasteuria* spp. in a soil sample would be similarly increased if nematode baits were incubated in the soil for a prolonged period with a plant host. Such strategy may be exploited to examine the distribution of *Pasteuria* spp. in undisturbed natural areas, where population density of individual nematode species is likely to be low (21).

The study of natural distributions of *Pasteuria* spp. may lead to identifying the

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ecological determinants necessary for establishment, multiplication, and spread of *Pasteuria* spp. It may also uncover new isolates of diverse genetic bases to supplement those currently available. The latter aspect is particularly important because biological control of plant-parasitic nematodes with *Pasteuria* spp. is unlikely to succeed unless populations of *Pasteuria* spp. with broad host specificity can be deployed (3,10,34). Hawaiian Islands, with its diverse ecological habitats within a short distance and hence its richness in biodiversity (5,36), are likely places for uncovering new strains of the bacteria. Local *Pasteuria* isolates, if found, could serve as non-exotic biological control agents to manage plant-parasitic nematodes, which are serious pests in pineapple and other crop production in Hawaii (6).

The objectives of this study were to assess the occurrence of *Pasteuria* spp. in undisturbed areas in the Hawaiian Islands, and to relate their distribution to environmental factors such as vegetation, elevation, temperature, and rainfall.

Materials and Methods

Collection of soil samples: Stratified random soil sampling was used to sample diverse undisturbed sites from a range of elevation, rainfall, temperature, and native vegetation communities (Table 1). A total of 320 sites from 17 native trails or regions on five major Hawaiian Islands (Kauai, Oahu, Maui, Molokai, and Hawaii) were sampled during July and August 1991. At each site, 10 random samples (each consisting of 2 cores) were taken over an area of approximately 5 m^2 to give about 1,000 cm^3 of soil. Each core, including soil and duff, was collected 15 cm deep with a standard soil sampling tube (2-cm-d Oakfield tube). The samples were placed in a plastic bag and mixed to produce one composite sample per site. Site location and associated vegetation were recorded and designated as endemic, indigenous, or naturalized according to Wagner (40). Annual temperature and rainfall were estimated from the nearest weather stations (11,20); elevation and biogeoclimatic zones were derived from either topographic (38) or vegetation (25) maps. Nematodes, in an aliquot of 200 cm^3 soil, were extracted by elutriation, fixed in formalin, dehydrated, and mounted in glycerol for species identification to characterize the native nematode fauna (2). Additional aliquots of 25 cm^3 and 250 cm^3 soil were bioassayed for the presence of *Pasteuria* spp. with nematode baits as described in the following section.

Nematode bait bioassays: Helicotylenchus dihystera (Cobb) Sher, *Meloidogyne javanica* (Treub) Chitwood, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, and *Rotylenchulus reniformis* Linford & Oliveira were chosen as baits because of their economic importance and wide distribution in the Hawaiian Islands (6). Original populations of these nematodes were collected from pineapple fields on the island of Oahu. The nematodes were cultured in the greenhouse for 3–5 months before being used as baits in these experiments. *Helicotylenchus dihystera* and *P. brachyurus* were reared on pineapple (*Ananas comosus* (L.) Merr. cv. Smooth Cayenne); *M. javanica* and *R. reniformis* were cultured on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) or cowpea (*Vigna unguiculata* L. cv. Purple Hull). Juveniles and adults of *Helicotylenchus dihystera* and *P. brachyurus* were obtained from infested soil in these cultures by wet sieving and centrifugal flotation (15). Eggs of *M. javanica* and *R. reniformis* were harvested from tomato or cowpea roots with sodium hypochlorite (14) and placed on a 38-μm pore sieve (layered with a double sheet of tissue paper) in a shallow dish of water at 25–28 C for 3–5 days. Juveniles that had hatched and migrated through the sieve were collected and examined to ensure that they were free of *Pasteuria* before being used as baits. For the bioassay, pineapple plants (seedlings) free of nematodes and *Pasteuria* were obtained using a modified stem segment technique (16): Stem segments (5–10 cm) of ratooning pineap-

TABLE 1. Location, climate, elevation, and vegetation of sampling sites for *Pasteuria* spp. in the Hawaiian Islands during Summer 1991.

Island (no. samples)	Trail	Location	Rainfall[a] cm	Elevation[b] m	Temp.[c] C	Natural community[d]	Vegetation[d]
Oahu	Puu Ohia	Tantalus	300	349	22.7	Wet lowland	Shrubs and closed forest
(71)	Manoa Cliff	Tantalus	300	427	22.7	Wet lowland	Shrubs and closed forest
	Moleka	Tantalus	300	244	22.7	Wet lowland	Open forest and shrubs
	Aiea Loop	Aiea Heights	350	366	22.7	Wet lowland	Closed forest
	Waiahole	Waiahole	250	69	23.7	Wet lowland	Shrubs and closed forest
	Opaeuka Stream	Opaeuka	125	183	21.7	Mesic lowland	Shrubs and closed forest
	Wahiawa	Wahiawa	125	305	22.7	Mesic lowland	Shrubs and closed forest
Kauai	Pihea	Napali-Kona Forest Reserve	175	1,067	15.0	Mesic montane	Mixed open forest
(22)	Alakai	Napali-Kona Forest Reserve	200	1,158	15.0	Mesic montane	Mixed closed forest
Maui	Halemauu	Haleakala National Park	125	2,286	12.4	Mesic subalpine	Upland open shrubland
(65)	Hosmer Grove	Haleakala National Park	125	2,085	12.4	Mesic subalpine	Upland open shrubland
	Maile	Waikamoi Preserve	125	1,372	15.7	Mesic montane	Open forest and shrubland
Molokai	Pepeopae	Kamakou Preserve	250	1,219	21.9	Wet montane	Closed forest
(37)							
Hawaii	Manua Loa	Volcano National Park	70	2,439	7.9	Dry alpine	Mainly upland open shrubland
(56)	Kipuka Ki	Volcano National Park	113	1,295	16.7	Dry montane	Open forest and shrubs
	Olaa Forest	Olaa Forest Preserve	318	1,097	16.7	Wet montane	Open forest
	Kilauea Crater	Volcano National Park	113	1,158	16.7	Dry montane	Open forest and shrubs

[a] From reference 11.
[b] From reference 38.
[c] From reference 20, 42.
[d] From reference 25: Wet = >250 cm, mesic = 125–250 cm, dry = <125 cm rainfall/year; lowland = <1,000, montane = 1,000–2,000, subalpine = 2,000–2,800 alpine = >2,800 m in altitude; closed forest = >60% trees, open forest = 25–60% trees, woodland = <25% trees-shrubs-grasses, parkland = forest patches, shrubland = woody shrubs, grassland = grasses-sedges-rushes.

ple plant were surface-sterilized for 10–15 minutes in a 1:2:7 mixture of 95% alcohol, 5.25% NaOCl, and distilled water, and then planted in sterile coarse sand in 15-cm-d clay pots (four segments/pot); sprouting pineapple suckers (5–7 cm long) with roots were selected for use after 2.5 to 4 months of growth in a 25–36 C greenhouse.

The incidence of *Pasteuria* spp. was assessed within a week of sampling using the following two methods: i) Approximately 2,000 vermiform stages each of *H. dihystera, M. javanica, P. brachyurus,* and *R. reniformis* mixed in 2 ml of water were added to petri dishes containing 25-cm^3 aliquot of soil. The dishes were incubated at 30 C for 48 hours (32) before the nematodes were extracted by shaking soil in water, decanting through a 38-μm sieve, and centrifuging the residue on the sieve in sugar solution (484 g/liter). The number of nematodes with endospores attached was counted in a sample containing 225–300 nematodes (approximately 75 of each nematode bait); ii) another aliquot of soil (250 cm^3) from each sample was placed in 7.5-cm-d clay pot and baited with a combination of 2,000 infective stages each of the same nematodes. Rooted pineapple suckers were transplanted to the pots at the same time. Pineapple suckers growing in steam-sterilized soil (Wahiawa silty clay) contained in similar pots were used as *Pasteuria*-negative controls (1 control for every 15 soil samples). After a growth period of 10–11 months in a 25–36 C greenhouse, plants were topped and roots gently shaken to remove the bulk soil without loosening the rhizosphere soil (soil still adhering to roots). Roots from each sample were placed on a 150-μm-pore sieve nested over a 20-μm-pore screen, and nematodes were dislodged from rhizosphere and rhizoplane with a pressurized water-jet spray. Nematodes caught on the bottom screen were separated from debris with centrifugal flotation (15) and then examined for *Pasteuria* attachment with the aid of an inverted microscope (×200). A sample was considered *Pasteuria*-positive if a random sample of 100 nematodes had more than five individuals whose cuticles were laden with at least five *Pasteuria*-endospores. Such a stringent criterion was set to minimize false positives, where an endospore-like protuberance on a nematode body might be merely an aberrant growth (19). However, during the course of the experiment, only one sample fell into the 1–4 endospores attachment category among the ones containing nematodes with endospore attachments.

Experimental design and statistical analysis: Pots for the bioassay were arranged randomly on a greenhouse bench. Of the 320 samples, only 251 provided data that could be analyzed. Data with nominal (categorical) or ordinal variables were subjected to analysis by Chi-square test, and data with interval variables were subjected to analysis by ANOVA. Values were assigned to the ordinal variables in the following manner: i) Vegetation: endemic = 1, indigenous = 2, naturalized = 3; ii) age of the islands (from oldest to youngest (5)): Kauai = 5, Oahu = 4, Molokai = 3, Maui = 2, Hawaii = 1; and iii) natural community zones (25): wet lowland = 1, mesic lowland = 2, wet montane = 3, mesic montane = 4, dry montane = 5, mesic subalpine = 6, and dry alpine = 7. Cramer's V for nominal variables, Goodman and Kruskal's Gamma, and Somers' d for ordinal variables were then calculated to measure the strength of the association based on the χ^2 or concordant and discordant pairs (22). All analyses were done with the aid of $SPSS^X$ (31).

Results

Distribution of Pasteuria spp. on nematode baits: Bioassay of incubating nematode baits directly in soil samples for 48 hours (Method 1) did not yield one nematode with endospores attached. Therefore, assessment of *Pasteuria* incidence was based solely on the bioassay with pineapple plants (Method 2). After 10–11 months,

only 251 out of the 320 samples were recoverable because of inadequate soil samples or death of some plants during the lengthy bioassay period. The nematode baits *M. javanica, P. brachyurus,* and(or) *H. dihystera* were recovered from 94% of the samples and all of the *Pasteuria*-negative controls. *Rotylenchulus reniformis* was recovered from only 0.25% of the samples and 0.5% of the controls. Nematode baits were absent in 5.6% of the samples. Among the samples and controls where the nematode baits could be recovered, *Meloidogyne* was generally the dominant nematode species (60.5%), followed by *Helicotylenchus* (25.1%) and *Pratylenchus* (14.4%). Of 251 samples, 33 (13%) met the *Pasteuria*-positive criterion. Only one sample failed the criterion test but had three *H. dihystera* females with two endospores attached. No endospore attachment was observed on any of the nematodes in the negative controls. Among the positive samples, 51.5% occurred with *M. javanica,* 24.2% with *P. brachyurus,* and 24.2% with *H. dihystera.* Endospore attachment generally was limited to one species of nematode per sample; in only two samples was attachment observed on more than one species of nematode. Endospore attachment on the nematode appeared as a raised refractile circular protuberance on the cuticle. Regardless of bait species, endospores adhered to the host along the entire length of the nematode cuticle, although more spores adhered to the cephalic region (Fig. 1).

The nematode fauna observed at test sites included plant-parasitic nematodes *Xiphinema* spp., *Helicotylenchus* spp., *Criconema* spp., *Paratylenchus* spp., and *Prismatolaimus* spp., none of which were observed to be parasitized by *Pasteuria* spp. Other nematodes in many orders, including Mononchida, Tylenchida, Cephalobida, and Dorylaimida, were also found but none were noted to have *Pasteuria* endospores attached.

Distribution of samples and Pasteuria-positive samples with respect to island location: Most *Pasteuria*-positive samples were found on Oahu and the smallest number on the island of Hawaii (Fig. 2). The older islands of Kauai and Oahu yielded 37% of all samples but 75% of the *Pasteuria*-positive samples (Fig. 3A). On the other hand, the younger islands of Maui and Ha-

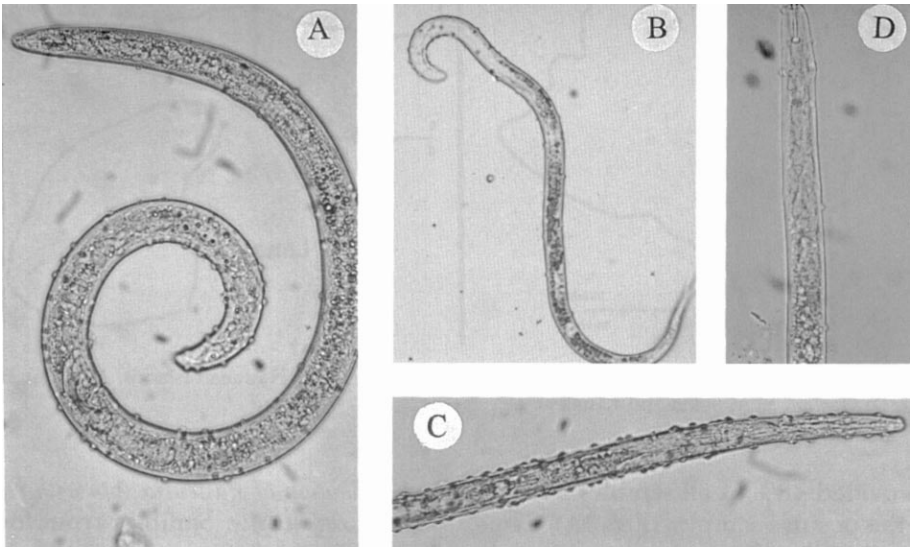

FIG. 1. Appearances of plant-parasitic nematodes infected with *Pasteuria* spp. A) A female of *Helicotylenchus* spp. B) A second-stage juvenile of *Meloidogyne javanica.* C) An endospore-laden juvenile of *M. javanica.* D) A female of *Pratylenchus* spp.

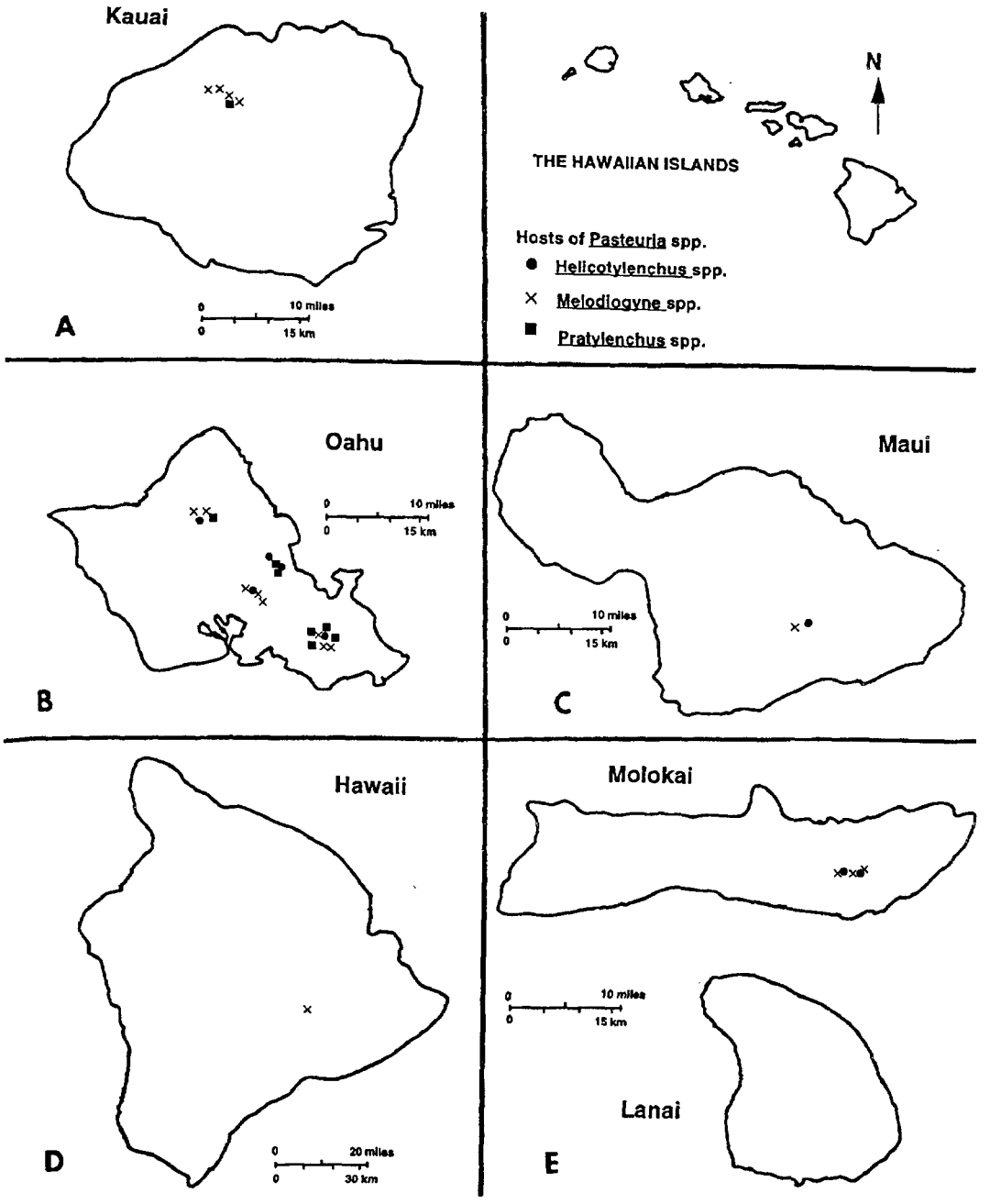

FIG. 2. Distribution of naturally occurring *Pasteuria* spp. in the major Hawaiian Islands. A) Kauai. B) Oahu. C) Maui. D) Hawaii. E) Molokai and Lanai.

waii provided 48% of all samples but only 9% of the positive samples (Fig. 3A). Thus, the distribution of *Pasteuria* relative to island age was not an independent event (χ^2: $P < 0.001$).

Distribution of Pasteuria spp. with respect to natural community: Samples from lowlands were 28.3% of all samples collected; yet more than 60% of the positive samples with *Pasteuria* were from these areas (Fig.

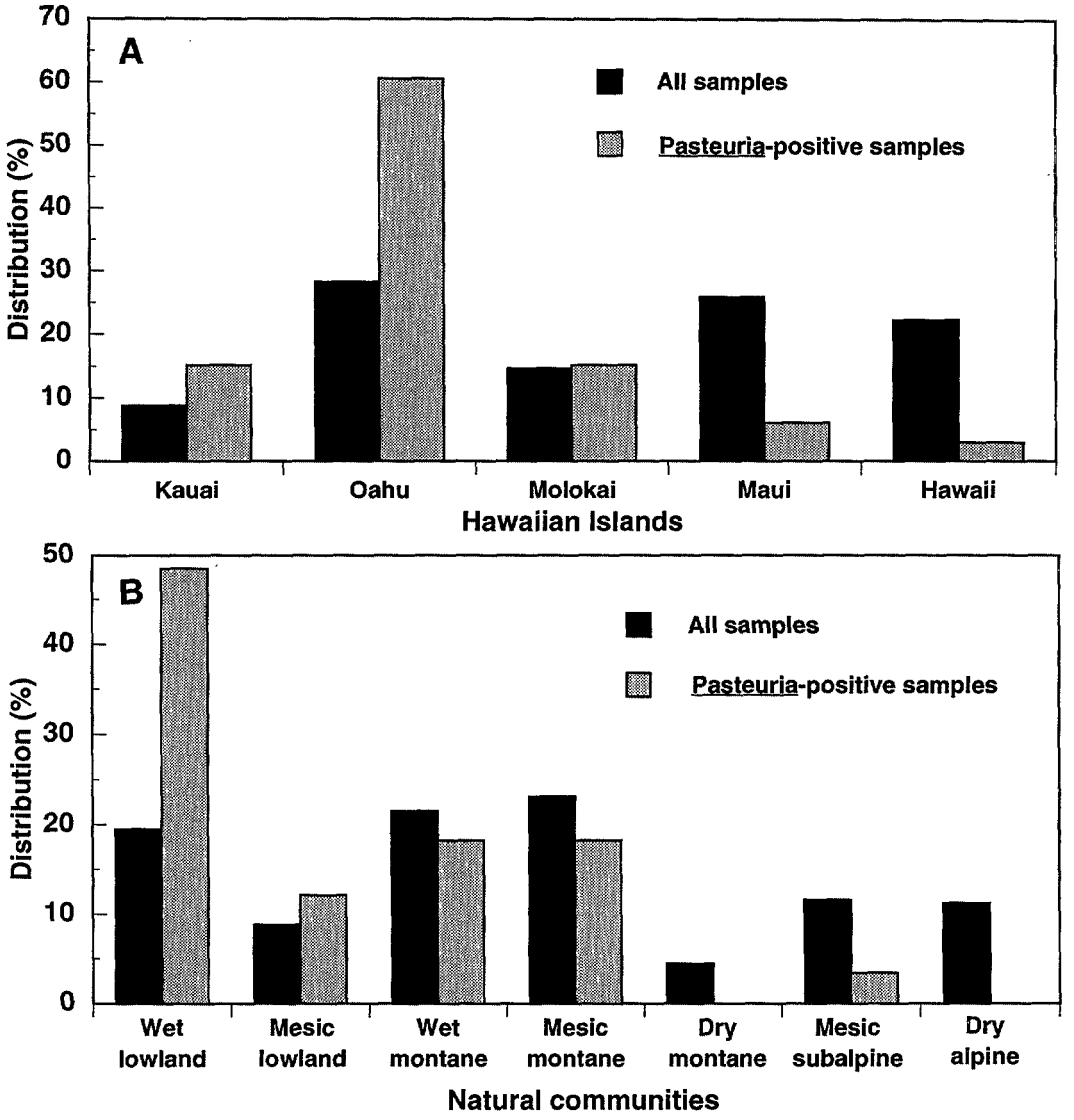

FIG. 3. Distribution of *Pasteuria* spp. with respect to island location (A) and natural community zones (B). All samples: distribution (%) = no. of samples (regardless of positive or negative for *Pasteuria*) taken from a specific location or zone/no. of samples (regardless of positive or negative for *Pasteuria*) taken from all locations or zones × 100%. *Pasteuria*-positive samples: distribution (%) = no. of samples positive for *Pasteuria* found in a specific location or zone/total no. of samples positive for *Pasteuria* in all locations or zones × 100%.

3B). In contrast, 23% of the samples were from subalpine and alpine regions but only 3% of the *Pasteuria*-positive samples came from these regions. The remainder of the samples (49%) were collected from the montane zones, representing 36% of the *Pasteuria*-positive samples (Fig. 3B). Thus, the association of *Pasteuria* spp. with a specific natural community was also not random (χ^2: $P < 0.001$).

Distribution of Pasteuria spp. with respect to mean annual temperature, rainfall, and elevation: Pasteuria spp. occurred more often in moist–wet rather than dry areas. About 50% of all samples were collected from areas with more than 150 cm annual rainfall, and these samples contained more than 82% of the *Pasteuria*-positive samples. The remaining 50% were taken from areas with less than 150 cm annual rainfall, and

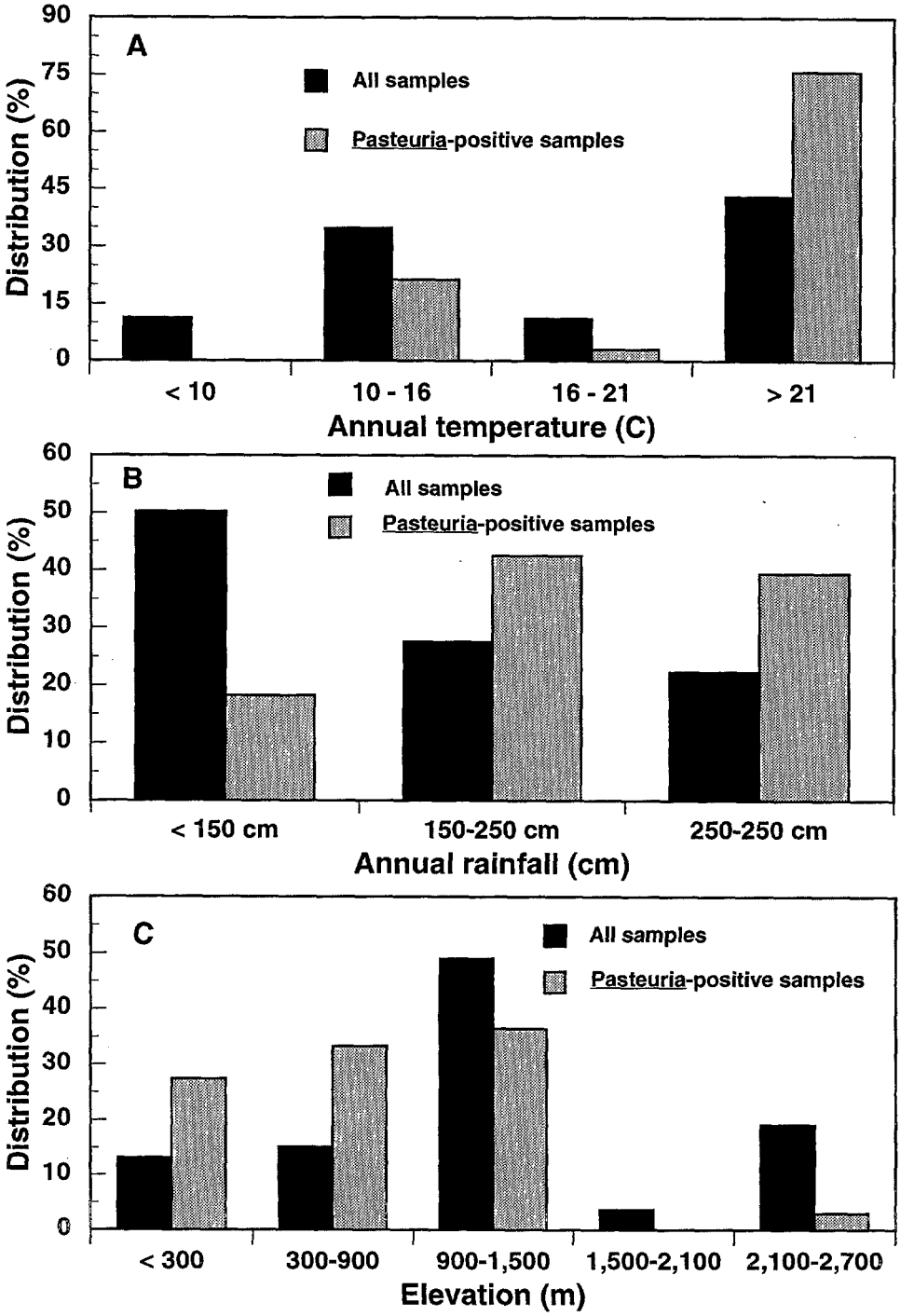

FIG. 4. Distribution of *Pasteuria* spp. with respect to (A) mean annual rainfall, (B) mean annual temperature, and (C) elevation. All samples: distribution (%) = no. of samples (regardless of positive or negative for *Pasteuria*) taken from an area with a specific rainfall, temperature, or elevation/total no. of samples (regardless of positive or negative for *Pasteuria*) taken from all areas × 100%. *Pasteuria*-positive samples: distribution (%) = no. of samples positive for *Pasteuria* from an area with specific rainfall, temperature, or temperature/total no. of samples positive for *Pasteuria* from all areas × 100%.

TABLE 2. Geoclimatic characteristics of 33 *Pasteuria*-positive sampling sites.

Geoclimatic parameter	Statistics			
	Mean ± SD	Median	Range at sampling sites	Statewide range[a]
Annual rainfall (cm)	244.5 ± 74.2	250	125–350	<25 to >1,000
Annual temperature (C)	20.6 ± 3.6	21.9	12.4–23.7	<6.1 to 23.9
Elevation (m)	666.8 ± 538.2	427	69–2286	<0 to >4,200

[a] From reference 42.

these samples contained only 18% of the *Pasteuria*-positive samples (Fig. 4B).

Pasteuria spp. was not found in areas with temperatures lower than 10 C (mean annual temperature), which comprised 11% of all samples; most positive samples (76%) were found in areas where mean annual temperature was higher than 21 C, which constituted 43% of all samples (Fig. 4A). The intermediate temperature zone of 10–21 C comprised 45% of all samples but only 24% of the positive samples (Fig. 4A).

Pasteuria spp. was more prevalent below 900 m than at higher elevations (Fig. 4C). The former had 61% of positive samples with only a 28% share of all samples. The elevation zones higher than 1,500 m contained less than 3% of the positive samples with a 23% share of all samples. Again, the association of *Pasteuria* incidence with temperature, rainfall, or elevation was not random (χ^2: $P < 0.001$).

The geoclimatic characteristics of the 33 positive sites are averaged in Table 2. The median annual rainfall of 250 cm at the positive sites is considered relatively moderate in a state with a range of less than 25 to more than 1,000 cm annually; and the median elevation of 427 m is considered relatively low in a state where elevation ranged from below sea-level to 4,200 m (at the summit area of Mauna Kea). Maximum rainfall occurred between 600 and 1,800 m in elevation (42). The median temperature of 22 C at the positive sites is near the upper end of the annual mean range of 6–24 C throughout the state (Table 2).

Distribution of Pasteuria spp. with respect to vegetation: Endemic plant species constituted more than 75% of the vegetation associated with the sampling sites on Kauai, Molokai, Maui, or Hawaii (Table 3). Nearly 2.5 times as many introduced (indigenous and naturalized) species as endemic species were found in sampling sites on the most populous island, Oahu. On Oahu, endemic species were associated (not exclusively) with 29% of all sampling sites and 25% of the positive sites; introduced species were associated with 71% of all sampling sites and 75% of the positive sites (Table 4). The association of *Pasteuria* incidence and origin of vegetation on Oahu was a random (independent) event (χ^2: $P < 0.001$). However, when sampling sites from all islands were considered, introduced species were associated with 37% of all sampling sites but 59% of the positive sites; endemic species shared 63% of the sampling sites but 41% of the positive sites (Table 4). The association of *Pasteuria* incidence and vegetation in this case is not random (χ^2: $P < 0.001$).

Table 5 lists some of the botanical families most often encountered at the positive sampling sites. Although 27% of the plants

TABLE 3. Origins of observed vegetation associated with 251 sites sampled for *Pasteuria* spp. in the Hawaiian Islands.

Original source[a]	Distribution (%)				
	Kauai	Oahu	Molokai	Maui	Hawaii
Endemic	78.9	28.8	75.0	75.0	78.4
Indigenous	10.5	22.7	8.3	12.5	17.6
Naturalized	10.5	48.5	16.7	12.5	3.9

[a] Endemic = native plants before the arrival of the Polynesians; indigenous = plants introduced by the Polynesians; naturalized = plants introduced after the European discovery of the Hawaiian Islands (40).

TABLE 4. Distribution of *Pasteuria* spp. with respect to origins of associated vegetation on Oahu and all the islands.

Plant origin[a]	Distribution on Oahu (%)		Distribution on all islands (%)	
	All samples[b]	*Pasteuria*-positive samples[c]	All samples[b]	*Pasteuria*-positive samples[c]
Endemic	28.8	25	62.7	40.6
Indigenous	22.7	10	15.8	18.7
Naturalized	48.5	65	21.5	40.7

[a] Endemoc = native plants before the arrival of the Polynesians; indigenous = plants introduced by the Polynesians; naturalized = plants introduced after the European discovery of the Hawaiian Islands (40).
[b] Distribution for "all samples" (%) = no. of endemic, indigenous, or naturalized plants at all sampling sites/total no. of plants at all sampling sites × 100%.
[c] Distribution for "*Pasteuria*-positive samples" (%) = no. of endemic, indigenous, or naturalized plants at *Pasteuria*-positive sites/total no. of plants at *Pasteuria*-positive sites × 100%.

belonging to these botanical families were encountered in all sampling sites, 2.4 times as many of them (64%) were encountered at the positive sites. *Pasteuria* incidence therefore seemed to be preferably associated with member species of these families (χ^2: $P < 0.001$). Plants in Myrtaceae (e.g., *Eucalytus* spp.) and Fabaceae (e.g., *Acacia koa*) were among the most frequently observed at the positive sampling sites. Both endemic and introduced species were observed within the families. Moss, fern, and litter beds were also included in the sampling sites, but *Pasteuria* spp. was associated with only a few of them, constituting an insignificant share of the positive samples.

TABLE 5. Frequency of occurrence of selected botanical families associated with *Pasteuria*-positive soil samples.

Botanical family	Distribution (%)	
	All samples[a]	*Pasteuria*-positive samples[b]
Myrtaceae	10.4	21.2
Fabaceae	10.0	15.2
Malvaceae	0.8	6.1
Meliaceae	1.2	6.0
Anacardiaceae	0.4	3.0
Apocynaceae	0.4	3.0
Urticaceae	0.4	3.0
Cyperaceae	1.2	3.0
Liliaceae	2.0	3.0

[a] Distribution for "all samples" (%) = no. of plants belonging to a specific botanical family at all sampling sites/total no.of plants at all sampling sites × 100%.
[b] Distribution for "*Pasteuria*-positive samples" (%) = no. of plants belonging to a specific botanical family at *Pasteuria*-positive sites/total no. of plants at *Pasteuria*-positive sites × 100%.

Strength of association of Pasteuria incidence and the selected biogeoclimatic parameters: Table 6 lists some statistical coefficients relating the occurrence of *Pasteuria* to biogeoclimatic factors. According to Cramer's V, the association of *Pasteuria* incidence and various geoclimatic factors was rather weak but equal in magnitude. Somers' d and Gamma indicate a positive association between *Pasteuria* occurrence and annual rainfall, temperature, origin of vegetation, or age of the islands; the coefficients also reveal a negative association between *Pasteuria* incidence and natural community zones or elevation at the sampling sites. Somers' d values calculated using *Pasteuria* incidence as an independent variable were higher than the values calculated using *Pasteuria* incidence as a dependent valuable (Table 6). This may mean that a *Pasteuria*-positive sampling site was likely to be associated with a naturalized vegetation in the lowlands on Kauai or Oahu, where average conditions were warm and moderately wet. However, a site with such conditions might have less likelihood of being *Pasteuria*-positive.

DISCUSSION

A *Pasteuria*-like organism was first discovered by Cobb in 1904 in Hawaii on a free-living nematode *Dorylaimus bulbiferous* (8). Despite reports of presence worldwide

TABLE 6. Degree of association of *Pasteuria* spp. with island location, origin of associated vegetation, natural community, rainfall, temperature, and elevation of sampling sites.

	Degree of association				
		Somers' d[b]			
Biogeoclimatic parameter	Cramer's V[a]	Symmetric	Dependent[c]	Independent[c]	Gamma[b]
Island location[d] (age of the islands)	0.333	0.232	0.15	0.509	0.637
Origin of vegetation[e] (endemic to naturalized)	0.203	0.177	0.128	0.285	0.454
Natural community[f] (wet-low to dry-high)	0.320	−0.215	−0.137	−0.497	−0.600
Annual rainfall (cm)	0.327	0.206	0.141	0.384	0.537
Annual temperature (C)	0.348	0.208	0.132	0.494	0.625
Elevation (m)	0.376	−0.224	−0.149	−0.605	−0.605

[a] On a scale of 0 to 1: 0 = no association, 1 = highest association (22).
[b] On a scale of −1 to +1: 0 = no relationship, −1 = perfect negative relationship, +1 = perfect positive relationship (22).
[c] Dependent = *Pasteuria* incidence as a dependent variable; independent = *Pasteuria* incidence as an independent variable (22).
[d] Age of island: Kauai = 5, Oahu = 4, Molokai = 3, Maui = 2, Hawaii = 1 (5).
[e] Ordinal values assigned to origin of vegetation: endemic = 1, indigenous = 2, naturalized = 3.
[f] Ordinal values assigned to natural plant community: Wet lowland = 1, mesic lowland = 2, wet montane = 3, mesic montane = 4, dry montane = 5, mesic subalpine = 6, dry alpine = 7 (25).

since then, it has not been further reported in Hawaii on any nematode until this survey. Although we used endospore attachment as the only criterion in our bioassay and proof of bacterial endospore germination, penetration, and completion of life-cycle on a nematode host have yet to be undertaken, the characteristic refractile protuberance on the nematode cuticles indicate the bacteria's wide distribution in the lowland, warm-temperature, and moist areas of the Hawaiian Islands. The use of attachment of endospores to the nematode cuticles, despite the limitation that attached endospores might not germinate and penetrate (33), is the simplest means available to detect the presence of *Pasteuria*. It has been used to study host range (13,34) and the population dynamics of root-knot nematode-*Pasteuria* interactions under field conditions in several laboratories (18,23,24,39).

Initially in our study, bioassays of incubating nematode baits with aliquot of soil samples for 48 hours as described by Sterling (32) yielded no *Pasteuria* endospore-attached nematodes. Thus, the lengthy but low-maintenance pineapple plant bioassay was devised, with nematodes as bait for the bacteria. The inoculum potential of *Pasteuria* spp. endospores probably was low in the field as population density of a suitable nematode host(s) in natural areas was also low (21); consequently, a lengthy incubation period of the soil with nematode baits on a plant host was necessary to increase the chance of detection. The warm greenhouse temperature (which, during the summer months, reached as high as 36 C) also favored the multiplication of the bacteria on the nematode host (32). The absence of endospore attachment to any nematodes in the *Pasteuria*-negative controls eliminates the possibilities that the observations were contaminations from the greenhouse.

Pasteuria spp. was found in all nematode-bait types except one (*R. reniformis*). Isolates from *Meloidogyne* spp. were the most prevalent, in agreement with the fact that *Pasteuria* spp. on *Meloidogyne* spp. is the most widely distributed (27). The near absence of reniform nematode at the end of the bioassay indicates that either it was a weak competitor with the other bait nematodes or growth conditions in the greenhouse were not conducive to its multiplication during the incubation period. *M. javanica* has been shown to displace *R. reniformis* in pineapple fields as production

practices have changed (6). The possibility that *R. reniformis* was completely eliminated by *Pasteuria* spp. or other biological control agents in the samples were unlikely, since no biological agent of nematodes, including *Pasteuria* spp., has been shown to completely exterminate its hosts. In any case, the near absence of reniform nematodes in the controls also indicates factor(s) other than *Pasteuria* was the culprit. Therefore, the existence of an isolate of *Pasteuria* parasitic on *R. reniformis* in Hawaii is still not known, although one with such host preference for closely related nematode genera has been reported (27). Separating the nematode baits for the bioassay may be necessary in similar future studies. The identity, taxonomic, morphological, and physiological status of these Hawaiian isolates of *Pasteuria* spp., and their potential for biological control of nematodes, were not evaluated, but accession numbers were given to each isolate for future reference.

Pasteuria-positive sites were found mostly on the islands of Oahu and Kauai (oldest members of the island chain), and only a few were found in the islands of Hawaii or Maui (youngest members of the island chain (5)), with numbers in Molokai being intermediate. Plant-parasitic nematode species diversity in native vegetation was also greatest on the oldest island of Kauai (15 species) and lowest on the youngest island of Hawaii (five species) (2), an indication that distribution of *Pasteuria* spp. may be related to the distribution of their natural hosts. However, it is not certain from this study whether the distribution in the islands can be attributed to the age of the islands rather than to the biogeoclimatic zones, since all sampling sites involved only lowland communities in the older islands and subalpine or alpine communities in the younger islands. It is clear, however, that the distribution of various *Pasteuria* strains was stratified by moisture, temperature, and elevation. *Pasteuria* isolation was positively correlated with mean annual temperature and moisture, but negatively associated with elevation. It was also moderately associated with introduced vegetation and lowland communities. Again, this may be related to the distribution of natural nematode hosts, which also is dependent on the distribution of specific plants. Temperature and rainfall may be the two primary factors determining the distribution of *Pasteuria,* since elevation exerts effects indirectly through its influence on these two factors in Hawaii (42). Temperature and rainfall not only affect vegetation and soil, but also may have direct effects on *Pasteuria* spp. Temperature influences *P. penetrans* parasitism of *M. javanica* (32) and is implicated in eliciting the resurgence of *Pasteuria* spp. in soil solarization experiments to suppress plant-parasitic nematodes (17). Rainfall or percolating water may facilitate the dispersal of the bacterial endospores (23). The observation that *P. penetrans* was more frequently found in sand and loamy sand than in other soil types in sugarcane fields (30) may in part be explained by better water percolation in the former soils. Other factors, such as soil texture, salinity, pH, organic content, and native nematode fauna, may also influence distribution patterns and should be evaluated in future studies.

Of the 33 positive sites, no natural nematode host was detected for the *Pasteuria* spp., raising serious questions about its ecological role in natural Hawaiian plant communities. Sampling was conducted during the relatively dry and host months of the summer, when nematode population densities were likely to be at their lowest in the Hawaiian Islands (43). Therefore, natural nematode hosts may have been present but their numbers may have been depressed by the parasites as well as by the season, thereby escaping detection. Alternatively, the natural nematode hosts for *Pasteuria* at the sampling sites may not belong to the same genera as the bait nematode species. The bait nematode species may have selected only those isolates of *Pasteuria* that could use them as alternative hosts. Other *Pasteuria* isolates could be present at the sampling sites but

not be detected by our bioassay. In any case, the isolates detected were initially present at low density and thus required a lengthy incubation period for detection. A particular population of *P. penetrans* consists of a heterogeneous group of isolates (10,28,34). Further studies are required to ascertain the ecological distribution of *Pasteuria* spp. in the natural environment and the role played there. Such studies will not only add more genetic diversity of the bacteria to the current collection of available *Pasteuria* isolates but also clarify the climatic, edaphic, and biological factors affecting their distribution. This information is important to the successful introduction and establishment of a *Pasteuria* spp. and to the management of resident species to control plant-parasitic nematodes.

The origin of *Pasteuria* spp. in the Hawaiian Islands is unclear. The restricted distribution of the bacteria in older islands indicates an ancient presence. On the other hand, the strong association of the bacteria with the most populous island and with introduced vegetation indicates historical presence. The Hawaiian isolates may have been inadvertently introduced during the immigration of Polynesians and Europeans, probably in soil introduced with exotic plants, farm implements, or ships' ballast, as speculated by Akhurst and Bedding (1) for nematodes introduced into Australia. Another possibility is that distinct indigenous isolates also exist, as indicated by the existence of isolates found in more remote parts of the islands among endemic plants. Future characterization of these isolates with DNA technology may resolve this issue.

Literature Cited

1. Akhurst, R. J., and R. A. Bedding. 1986. Natural occurrence of insect pathogenic nematodes (Steinernematidae and Heterorhabditidae) in soil in Australia. Journal of the Australian Entomological Society 25:241–144.

2. Bernard, E. C., and D. P. Schmitt. 1992. Nematodes of Hawaiian native plant communities. Journal of Nematology 24:582 (Abstr.).

3. Bird, A. F., and P. G. Brisbane. 1988. The influence of *Pasteuria penetrans* in field soils on the reproduction of root-knot nematodes. Revue de Nématologie 11:75–81.

4. Brown, S. M., J. L. Kepner, and G. C. Smart. 1985. Increased crop yields following application of *Bacillus penetrans* to field plots infested with *Meloidogyne incognita*. Soil Biology and Biochemistry 17:483–486.

5. Carlquist, S. 1980. Hawaii, a natural history: geology, climate, native flora, and fauna above the shoreline. 2nd ed. Lawai, HI: Pacific Tropic Botanic Garden.

6. Caswell, E. P., J. L. Sarah, and W. J. Apt. 1989. Nematode parasites of pineapple. Pp. 519–537 *in* M. Luc, R. A. Sikora, and J. Bridge, eds. Plant-parasitic nematodes in subtropical and tropical agriculture. Wallingford, UK: CAB International.

7. Ciancio, A., R. Mankau, and M. Mundo-Ocampo. 1992. Parasitism of *Helicotylenchus lobus* by *Pasteuria penetrans* in naturally infested soil. Journal of Nematology 24:29–35.

8. Cobb, N. A. 1906. Fungus maladies of sugarcane, with notes on associated insects and nematodes. Hawaii Sugar Plant Association Experiment Station, Division of Pathology Physiology Bulletin 5:163.

9. Davies, K. G., C. A. Flynn, V. Laird, and B. R. Kerry. 1990. The life-cycle, population dynamics, and host specificity of a parasite of *Heterodera avenae*, similar to *Pasteuria penetrans*. Revue de Nématologie 13:303–309.

10. De R. Channer, A. G., and S. R. Gowan. 1992. Selection for increased host resistance and increased pathogen specificity in the *Meloidogyne–Pasteuria penetrans* interaction. Fundamental and Applied Nematology 15:331–339.

11. Giambelluca, T. W., M. A. Nullet, and T. A. Schroeder. 1986. Rainfall atlas of Hawaii. Report 76. Honolulu, HI: Division of Water and Land Development, Hawaii Department of Land and Natural Resources.

12. Giblin-Davis, R. M., L. L. McDaniel, and F. G. Bilz. 1990. Isolates of the *Pasteuria penetrans* group from phytoparasitic nematodes in Bermudagrass turf. Supplement to the Journal of Nematology 22: 750–762.

13. Hewlett, T. E., and D. W. Dickson. 1993. A centrifugation method for attaching endospores of *Pasteuria* spp. to nematodes. Supplement to the Journal of Nematology 25:785–788.

14. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025–1028.

15. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

16. Kotalawala, J. 1971. Mass production of pineapple planting material. Tropical Agriculture 127: 199–201.

17. Lamberti, F., and N. Greco. 1990. Effectiveness of soil solarization for control of plant parasitic nematodes. Pp. 167–172 *in* J. E. Devay, J. J. Stapleton, and C. L. Elmore, eds. Soil solarization: Proceed-

ings of the First International Conference of Soil Solarization. FAO Publication no. 109. Amman, Jordan.

18. Mankau, R. 1975. *Bacillus penetrans* n. comb. causing a virulent disease of plant-parasitic nematodes. Journal of Invertebrate Pathology 26:333–339.

19. McClure, M. A. 1989. Neoplastic growths in preparasitic juveniles of *Meloidogyne incognita*. Journal of Nematology 21:427–430.

20. National Climatic Data Center. 1991. Climatological Data, Hawaii and Pacific 87(13):1–36.

21. Norton, D. C. 1978. Ecology of plant-parasitic nematodes. New York, NY: John Wiley.

22. Norusis, M. J. 1988. The SPSS guide to data analysis for SPSS^X. Chicago, IL: SPSS.

23. Oostendorp, M., D. W. Dickson, and D. J. Mitchell. 1990. Host range and ecology of isolates of *Pasteuria* spp. from the southeastern United States. Journal of Nematology 22:525–531.

24. Oostendorp, M., D. W. Dickson, and D. J. Mitchell. 1991. Population development of *Pasteuria penetrans* on *Meloidogyne arenaria*. Journal of Nematology 23:58–64.

25. Ripperton, J., and E. Hosaka. 1942. Vegetation zones of Hawaii. Hawaii Agricultural Experiment Station Bulletin 89:1–60.

26. Sayre, R. M., and M. P. Starr. 1985. *Pasteuria penetrans* (ex Thorne 1940) nom. rev., comb. n., sp. n., a mycelial and endospore-forming bacterium parasitic in plant-parasitic nematodes. Proceedings of the Helminthological Society of Washington 52:149–165.

27. Sayre, R. M., and M. P. Starr. 1988. Bacterial diseases and antagonisms of nematodes. Pp. 69–101 *in* G. O. Poinar, Jr. and H.-B. Jansson, eds. Diseases of nematodes, vol. 1. Boca Raton, FL: CRC Press.

28. Sayre, R. M., M. P. Starr, A. M. Golden, W. P. Wergin, and B. Y. Endo. 1988. Comparison of *Pasteuria penetrans* from *Meloidogyne incognita* with a related mycelial and endospore-forming bacterial parasite from *Pratylenchus brachyurus*. Proceedings of the Helminthological Society of Washington 55:28–49.

29. Sayre, R. M., W. P. Wergin, J. M. Schmidt, and M. P. Starr. 1991. *Pasteuria nishizawae* sp. nov., a mycelial and endospore-forming bacterium parasite on cyst nematodes of genera *Heterodera* and *Globodera*. Research in Microbiology 142:551–564.

30. Spaull, V. W. 1984. Observations on *Bacillus penetrans* infecting *Meloidogyne* in sugarcane fields in South Africa. Revue de Nématologie 7:277–282.

31. SPSS Inc. 1988. SPSS-X™ User's Guide, 3rd ed. Chicago, IL: SPSS.

32. Stirling, G. R. 1981. Effects of temperature on infection of *Meloidogyne javanica* by *Bacillus penetrans*. Nematologica 27:458–462.

33. Stirling, G. R. 1984. Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. Phytopathology 74:55–60.

34. Stirling, G. R. 1991. Biological control of plant-parasitic nematodes. Wallingford, UK: CAB International.

35. Stirling, G. R., and A. M. White. 1982. Distribution of a parasite of root-knot nematodes in South Australian vineyards. Plant Disease 66:52–53.

36. Stone, C. P., and D. B. Stone, editors. 1989. Conservation biology in Hawaii. Honolulu, HI: University of Hawaii Press.

37. Sturhan, D. 1988. New host and geographic records of nematode-parasitic bacteria of the *Pasteuria penetrans* group. Nematologica 34:350–356.

38. U.S. Geological Survey. Hawaii 7.5-minute Quadrangles [map]. 122 sheets. 1:24,000. 7.5-minutes Series (Topographic). Denver, CO: USGS. 1980–1982.

39. Verdejo-Lucas, S. 1992. Seasonal population fluctuation of *Meloidogyne* spp. and the *Pasteuria penetrans* group in kiwi orchards. Plant Disease 76:1275–1279.

40. Wagner, W. L. 1990. Manual of the flowering plants of Hawaii. vol 2. Honolulu, HI: University of Hawaii Press.

41. Walter, D. E., and D. T. Kaplan. 1990. Antagonists of plant-parasitic nematodes in Florida citrus. Journal of Nematology 22:567–573.

42. Warwick, A. R., and J. A. Bier, eds. 1993. Atlas of Hawaii. Honolulu, HI: University of Hawaii Press.

43. Zhang, F. R., and D. P. Schmitt. 1995. Spatial-temporal patterns of *Meloidogyne konaensis* on coffee in Hawaii. Journal of Nematology 27:109–113.