Comparison of Populations of *Heteropsylla cubana* Crawford (Homoptera: Psyllidae) and its Parasitoids on Seven Accessions of *Leucaena* (Leguminose: Mimosoideae) in Hawaii.[1]

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ABSTRACT. Seven accessions of *Leucaena* Benth. supported significantly different population densities of *Heteropsylla cubana* Crawford. The highest populations occurred on the *L. leucocephala* (Lamb.) De Wit accessions K8, K527, and K784, the *L. diversifolia* (Schlecht.) Benth. accession K156, and the *L. leucocephala* X *L. diversifolia* accession K743. The lowest psyllid densities occurred on the *L. pallida* Britton and Rose accession K376. Accessions K8, K527, and K636 were the most severely damaged. Accessions K743 and K156 were often slightly damaged and only occasionally moderately damaged. Only slight damage was observed on accessions K784 and K376.

Parasitism of *H. cubana* by *Psyllaephagus yaseeni* Noyes was low (1.9%), probably due to hyperparasitization levels averaging 39.6%.

The accidental introduction of the leucaena psyllid, *Heteropsylla cubana* Crawford, into Hawaii in 1984 and its subsequent spread throughout the tropical Pacific has resulted in major economic damage to *Leucaena leucocephala* (Lamb.) De Wit (Mitchell & Waterhouse 1986, Waterhouse & Norris 1987). The leucaena psyllid was first detected in Hawaii on Oahu Island in April 1984 (Nakahara and Lai 1984) attacking koa-haole *(Leucaena leucocephala)* and monkeypod (*Samanea samen* (Jacq.) Merr.). By June 1984 the psyllid had spread to all of the major Hawaiian Islands (Kauai, Molokai, Maui, Lanai and Hawaii) (Nakahara & Lai 1984, Mitchell & Waterhouse 1986). Severe damage to several cultivars of *Leucaena* Benth. in Hawaii was observed by August 1984 (Sorensson and Brewbaker 1984).

In 1984, the Hawaii Department of Agriculture initiated a search for biological control agents of the psyllid in the West Indies (Nakahara & Funasaki 1986). An endoparasitoid, *Psyllaephagus yaseeni* Noyes (Hymenoptera: Encyrtidae) (Noyes 1990) from Tobago was released in Hawaii in 1987 (Nagamine et al. 1991). In 1988, three hyperparasitoids, *Syrphophagus aphidivorous* (Ashmead) (Hymenoptera: Encyrtidae), *Syrphophagus* sp. (Hymenoptera: Encyrtidae), and *Pachyneuron siphonophorae* (Ashmead) (Hymenoptera: Pteromalidae) were reared from *H. cubana* (Beardsley and Uchida 1991).

[1]Journal Series No. 3682 of the Hawaii Institute of Tropical Agriculture and Human Resources. Research supported in part by U.S. Department of Agriculture under CSRS Special Grant No. 87-CRSR-2-3134, Managed by the Pacific Basin Advisory Group (PBAG).
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The spread of the leucaena psyllid throughout the South Pacific has brought attention to several tolerant or resistant species and strains of *Leucaena* as potential substitutes, or sources of crossing stock for genetically controlled resistance (Othman & Prine 1984, Bray 1987, Brewbaker 1987, Sorensson & Brewbaker 1987). Field observations in Hawaii indicated that one species, *L. diversifolia* (Schlecht.) Benth., was moderately tolerant of the psyllid (Sorensson & Brewbaker 1987). In addition, observations of tolerance to the psyllid were reported for other species of *Leucaena;* specifically *L. esculenta* (Moc. & Sesse) Benth., *L. collinsii* Britton and Rose, *L. greggii* S. Watson, *L. pallida* Britton & Rose, and *L. retusa* Benth. (Sorensson & Brewbaker 1987). The Nitrogen Fixing Tree Association (NFTA) set up the International Leucaena Psyllid Trial (LPT) Network in 1987, with trials established in Hawaii and other localities throughout the tropics, to test the stability of resistance in *Leucaena* over time at different geographical localities (Glover 1988, Wheeler 1988).

The purposes of our study were: to determine whether there were significant differences in psyllid populations supported by the different accessions of *Leucaena* used in the Leucaena Psyllid Trials; to evaluate a damage rating system (modified after Wheeler 1988) which was designed to detect differences in the response of accessions of *Leucaena* to feeding damage; and to examine the interrelationships among psyllids, parasitoids, and hyperparasitoids.

MATERIALS AND METHODS

The study site for trial LPT-86-2 of Brewbaker and Wheeler (1988) was located at the Waimanalo Research Station, Oahu Island in Hawaii. Seven accessions of *Leucaena* (Table 1) were planted within each of three rectangular blocks (2 × 35 m) in a random block design. For each accession, 40 plants were planted in two rows (20 plants per row) with each row 5 m long and 1 m apart. Plants were spaced 0.25 m apart within each row.

Five 20 cm lateral leaf shoots were randomly collected once a week in plastic bags and stored in an ice cooler, from each accession of *Leucaena* in each of the three blocks. The accessions K784, K743, K156, and K376 were sampled from August 9 to September 6, 1988. Accessions K527, K8, and K636 were sampled from August 9 until October 25, 1988.

TABLE 1. Accession numbers, species names, and natural distribution of *Leucaena*.

Accession Nos.	Leucaena species	Distribution
K8, K527, K636	*L. leucocephala* (Lamb.) De Wit	Yucatan Peninsula, Latin America[1]
K156, K784	*L. diversifolia* (Schlecht.) Benth.	Honduras to Mexico[2]
K743	*L. leucocephala* X *L. diversifolia*	Artificially hybridized
K376	*L. pallida* Britton and Rose	Central and Western Mexico[2]

[1]Lowland species.
[2]Highland species.

TABLE 2. Comparison of mean (SEM) number per leaf shoot of immatures of the leucaena psyllid, *Heteropsylla cubana*, collected weekly on seven accessions of *Leucaena*.

ACC.	AUG 9	AUG 16	AUG 23	AUG 30	SEP 6	SEP 13
K527	1769.2(1592.5)ab	82.9(45.1)a	501.9(115.0)a	1059.9(928.2)a	401.2(386.9)a	1214.4(409.0)a
K8	2708.6(462.2)a	98.6(101.7)a	285.0(244.6)ab	830.7(458.9)ab	741.9(742.0)a	415.8(490.8)b
K743	1333.9(1312.4)ab	438.4(351.8)a	130.1(197.2)ab	330.8(278.5)ab	118.9(99.7)a	-
K156	586.7((431.4)ab	1124.6(1189.9)a	28.1(24.7)b	135.3(94.7)ab	520.1(477.1)a	-
K636	1078.5(780.8)ab	135.2(76.7)a	81.5(113.9)b	105.1(54.0)ab	161.7(13.3)a	228.7(208.3)b
K784	68.6(49.0)b	106.7(84.7)a	4.5(3.5)b	2.1(2.5)b	34.9(55.4)a	-
K376	15.1(21.4)b	2.3(1.6)a	4.0(5.6)b	36.7(47.9)ab	5.8(3.6)a	-

ACC.	SEP 19	SEP 27	OCT 4	OCT 11	OCT 18	OCT 25
K527	1583.9(1479.1)a	224.1(140.9)a	1175.1(465.9)a	494.7(157.8)a	425.8(599.6)a	1055.1(854.5)a
K8	586.3(388.6)a	208.7(164.5)a	1012.7(730.7)a	390.2(211.6)a	18.3(25.4)a	800.9(97.9)a
K743	-	-	-	-	-	-
K156	-	-	-	-	-	-
K636	276.8(267.0)a	86.0(84.8)a	141.7(72.2)a	144.8(73.1)a	24.7(34.9)a	78.9(131.9)a
K784	-	-	-	-	-	-
K376	-	-	-	-	-	-

Means followed by the same letter within a column are not significantly different ($P<0.05$; Tukey's HSD test).

The plastic bags containing the sample shoots were brought to the laboratory and flooded with water. The contents were decanted into a stack of metal sieves with stainless steel mesh, and were washed with a strong stream of water to separate psyllid adults and nymphs from leaf terminals. The sieves were stacked according to increasing mesh sizes (in Tyler equivalents of 80, 60, 40, 30, and 20 meshes per square inch) to separate the I, II, III, IV instars, and V instar and adults, respectively. Each stage was transferred to petri dishes with 70% ethanol, and counted with the aid of a dissecting microscope. An estimate of observable damage to the leaf shoots was made on the leaf directly below the leaf which supported the greatest number of nymphs. These were chosen because the leaves above did not show obvious signs of damage. Lower leaves, although often damaged, were mostly too old to be infested with psyllids. Damage was rated in the following manner: 1 = no damage; 2 = curling of pinnules; 3 = curling and yellowing of pinnules; 4 = up to 25% loss of pinnules; 5 = up to 50% loss of pinnules; 6 = up to 75% loss of pinnules; 7 = up to 100% loss of pinnules.

The three accessions K636, K527 and K8 were sampled for parasitoids and hyperparasitoids from August 23 to September 6, 1988. The pinnules of the leaf shoots with parasitized psyllid mummies were removed from the two lowest mature leaves of the sample shoot and held in 7 dram plastic vials for 3 weeks to allow the adult parasitoids and hyperparasitoids to emerge. The adults were identified and counted.

The data were analyzed with ANOVA, and means were compared using Tukey's HSD test (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

Psyllid Population. There were significant differences ($P<0.05$) in the mean number of psyllids among seven accessions of *Leucaena*, for four cf twelve sample dates (Table 2). The highest psyllid populations were found on *L. leucocephala* (K527, K8, and K636), *L. diversifolia* (K156), and the hybrid, *L. leucocephala* X *L. diversifolia* (K743). The lowest psyllid populations occurred on *L. pallida* (K376). On August 9, K8 supported significantly more psyllids (2708.6) than K784 (68.6) and K376 (15.1). On August 23, significantly more psyllids were found on K527 (501.9) than on K156 (28.1), K636 (81.0), K784 (4.5), and K376 (4.0). Significantly higher densities of psyllids occurred on accession K527 (1059.9) than K784 (2.1) on August 30, and on September 13 density on K527 was higher (1214.4) than on K8 (415.8) and K636 (228.7). Ironically, collections made on August 16, September 6, 19, and 27, and October 4, 11, 18, and 25, showed no significant differences among the means.

Defoliation of *Leucaena* occurred when psyllid populations reached high densities. This defoliation caused a drop in the psyllid densities on the affected trees at a time when other trees of the same accession were not yet affected, resulting in high variation of the data. Therefore, differences in psyllid densities among accessions were not always significant.

TABLE 3. Comparison of mean (SEM) damage ratings for seven accessions of *Leucaena*.

ACC.	AUG 9	AUG 16	AUG 23	AUG 30	SEP 6	SEP 13
K527	3.0(0.8)ab	4.2(1.5)a	2.5(1.2)a	3.1(2.3)ab	2.5(0.6)a	2.4(1.2)a
K8	4.0(0.7)a	6.1(1.6)a	3.0(1.7)a	3.9(1.6)a	2.0(1.6)ab	2.2(1.2)a
K743	1.8(0.7)bc	2.3(1.6)bc	1.3(0.5)b	1.5(0.6)bc	-	-
K156	1.1(0.3)c	1.5(0.7)c	1.0(0)b	1.8(1.6)bc	-	-
K636	2.7(0.9)ab	3.7(1.6)ab	2.5(2.0)ab	3.2(1.3)a	1.7(0.8)a	2.1(1.5)a
K784	1.0(0)c	1.0(0)c	1.0(0)b	1.0(0)c	-	-
K376	1.0(0)c	1.0(0)c	1.0(0)b	1.0(0)c	-	-

ACC.	SEP 19	SEP 27	OCT 4	OCT 11	OCT 18	OCT 25
K527	3.3(1.7)a	3.5(1.6)a	3.9(1.9)a	3.7(2.5)ab	5.5(1.9)a	3.1(1.9)a
K8	2.5(1.7)a	1.9(1.7)a	3.7(1.6)a	5.1(1.3)a	5.3(1.0)a	2.5(1.9)a
K743	-	-	-	-	-	-
K156	-	-	-	-	-	-
K636	2.1(1.5)a	1.7(1.3)a	2.7(1.9)a	2.3(1.5)b	3.0(1.9)b	1.9(1.5)b
K784	-	-	-	-	-	-
K376	-	-	-	-	-	-

Means followed by the same letter within a column are not significantly different ($P<0.05$; Tukey's HSD test).

TABLE 4. Relationships between the number of psyllids and damage ratings for seven accessions of *Leucaena*.

Damage Ratings	x̄ (SEM) number of psyllids per shoot						
	K527	K8	K636	K743	K156	K784	K376
1	117.2(33.3)b	252.0(44.9)b	40.3(13.7)b	64.7(21.8)	253.5(70.7)	42.3(10.9)	13.9(7.5)
2	983.4(147.7)ab	413.9(86.3)b	488.3(83.7)ab	720.9(120.8)	1446.0(40.3)	-	-
3	1517.0(246.3)a	1333.4(195.2)a	540.0(164.7)a	2176.0(1000.4)	714.5(292.3)	-	-
4	961.8(385.1)ab	1392.6(493.8)a	440.6(383.5)ab	-	-	-	-
5	273.0(127.9)b	1012.8(559.2)a	-	-	273.0(127.9)	1012.8(559.2)	384.3(251.5)
6	594.5(173.2)ab	303.9(97.0)b	187.1(82.3)ab	617.0(377.0)	633.0( )	-	-
7	267.4(126.0)b	191.3(36.7)b	93.9(41.8)ab	-	-	-	-

Means followed by the same letter within a column are not significantly different ($P<0.05$; Tukey's HSD test).

Damage Ratings. In Table 3, the highest psyllid damage ratings were obtained on accessions K527, K8, and K636; the lowest on K784 and K376, and intermediate on K743 and K156.

Among the *L. leucocephala* accessions (K527, K8, and K636), K636 had significantly lower ratings than K527 and K8 (on October 11, 18, and 25). Damage on K8 was significantly higher than damage observed on accessions K743, K156, K784, and K376 for each sample taken on August 9, 16, 23, and 30. K527 was significantly different from K743 on August 16 and 23, and K636 was different from K156 on August 9, 16, and 30. *L. diversifolia* and *L. pallida* (K784 and K376, respectively) had the lowest damage levels, but there was no significant difference between *L. leucocephala* × *L. diversifolia* (K743) and *L. diversifolia* (K156).

Psyllid Density vs Damage. There were significant differences in psyllid densities for each damage rating value for accessions K527, K8, and K636 of *Leucaena leucocephala* (Table 4). Determination of differences for K743, K156, K784, and K376 was not possible due to the lack of data for higher damage levels. The numbers of psyllids on leaves with a damage rating of 3 for K527 was significantly higher than for psyllid damage ratings of 1, 5, and 7. On K8, the numbers of psyllids on leaves with damage ratings of 3, 4, and 5 were not significantly different from each other, but were significantly different from those on leaves with ratings of 1, 2, 6, and 7. For K636, leaves with a damage rating of 3 had significantly more psyllids than leaves with less damage (damage rating = 1). These results suggest that accession K636 was less tolerant of psyllid feeding than K8 and K527 and that K636 supported lower psyllid population levels than K8 and K527, but suffered a similiar level of damage.

In general, the highest psyllid densities were associated with the midrange damage ratings (3, 4, and 5) and the lowest psyllid densities were associated with the lowest (1 and 2) and highest (6 and 7) damage ratings. The low psyllid densities at the high damage ratings were due to the loss of leaf surface area caused by defoliation. Thus, psyllids which had previously occupied the defoliated parts of the leaves were probably either eliminated from the plant, migrated onto less damaged and more nutritious shoots, or starved. Thus, damage ratings and psyllid population levels were not significantly correlated over the entire range of damage ratings. Rather, as damage increased, psyllid density increased until defoliation occurred, which was followed by a decline in psyllid density.

Parasitoids. *P. yaseeni*, the only primary parasitoid reared from psyllid mummies, was relatively uncommon. Based on the total number of fifth instar psyllid nymphs sampled (N=19,711) for accessions K636, K8, and K527, parasitization by that species was 1.91%. This low level of parasitism may have been due to the high level of hyperparasitism. Three hyperparasitoids, *S. aphidivorous*, *S.* sp., and *P. siphonophorae*, were reared from *P. yaseeni* (Beardsley and Uchida 1991). A mean of 39.6% of *P. yaseeni* were hyperparasitized by these three wasps. The dominant and most important hyperparasitoid was *S. aphidivorous*. *S.* sp. and *P. siphonophorae* also contributed to the suppression of the primary psyllid parasitoid, but apparently played a relatively minor role.

An average of 1.42% of fifth instar psyllid nymphs (N=12,293) were parasitized on K527 (Table 5). *S. aphidivorous, S.* sp., and *P. siphonophorae* were present in 90, 20, and 10% of the samples. The mean percent hyperparasitism by these species were 43.2, 11.7, and 3.9%, respectively.

TABLE 5. Numbers of *Heteropsylla cubana* Crawford parasitized and hyperparasitized individuals on K527 (*Leucaena leucocephala* (Lamb.) De Wit). Numbers in parenthesis indicate %.

		No.(%)			
		Parasite	Hyperparasites		
COLL. DATE	N	P	A	S	P
Aug. 23	227	9(3.96)	0(0)	0(0)	0(0)
30	2,683	10(0.37)	3(30.0)	1(10.8)	0(0)
Sep. 6	242	25(10.33)	12(48.0)	0(0)	0(0)
13	1,607	5(0.31)	2(40.0)	0(0)	0(0)
19	3,298	8(0.24)	3(37.5)	1(12.5)	0(0)
27	606	29(4.79)	12(41.4)	0(0)	0(0)
Oct. 4	1,079	13(1.20)	6(46.2)	0(0)	0(0)
11	1,429	51(3.57)	22(43.1)	0(0)	2(3.9)
18	821	18(2.19)	10(55.6)	0(0)	0(0)
25	301	6(1.99)	2(50.0)	0(0)	0(0)
	$\bar{X}$%=	(1.42)	(43.5)	(11.7)	(3.9)

N = number of fifth instar psyllid nymphs, P = *Psyllaephagus yaseeni* Noyes, A = *Syrphophagus aphidivorous* (Ashmead), S = *Syrphophagus* sp., P = *Pachyneuron siphonophorae* (Ashmead).

A mean parasitism of 2.18% of fifth instar psyllid nymphs (N=5,363) was observed on K8 (Table 6). On this accession, *S. aphidivorous, S.* sp., and *P. siphonophorae* were present in 80, 30, and 0% of the samples, and hyperparasitized 54.3, 6.8, and 0.0% of *P. yaseeni*, respectively.

A mean parasitism of 4.14% of fifth instar psyllid nymphs (N=2,055) was observed on K636 (Table 7). *S. aphidivorous, S.* sp., and *P. siphonophorae* occurred in 70, 1, 0% of the samples and hyperparasitized a mean of 19.2, 5.0, and 0% of *P. yaseeni*, respectively.

CONCLUSIONS

The high variation in the mean numbers of psyllids among the seven accessions of *Leucaena* occurred because all of the leaves sampled on one day were not in the same state of damage. The presence of defoliated leaves on some sample shoots had a negative affect on the number of psyllids present, because defoliated leaves with less surface area supported fewer

TABLE 6. Numbers of *Heteropsylla cubana* Crawford parasitized and hyperparasitized individuals on K8 *(Leucaena leucocephala* (Lamb.) De Wit). Numbers in parenthesis indicate %.

COLL. DATE	N	No.(%) Parasite P	Hyperparasites A	S	P
Aug. 23	217	12(5.53)	8(66.7)	0(0)	0(0)
30	616	15(2.44)	11(73.3)	0(0)	0(0)
Sep. 6	232	38(16.38)	18(47.4)	1(2.6)	0(0)
13	672	1(0.15)	0(0)	0(0)	0(0)
19	747	9(1.20)	2(22.2)	1(11.1)	0(0)
27	601	15(2.50)	3(20.0)	1(6.7)	0(0)
Oct. 4	868	6(0.69)	0(0)	0(0)	0(0)
11	1,168	14(1.20)	3(21.4)	0(0)	0(0)
18	13	6(46.15)	5(83.3)	0(0)	0(0)
25	229	1(0.44)	1(100.0)	0(0)	0(0)
	$\bar{x}$% =	(2.18)	(54.3)	(6.8)	(0)

N = number of fifth instar psyllid nymphs, P = *Psyllaephagus yaseeni* Noyes, A = *Syrphophagus aphidivorous* (Ashmead), S = *Syrphophagus* sp., P = *Pachyneuron siphonophorae* (Ashmead).

TABLE 7. Numbers of *Heteropsylla cubana* Crawford parasitized and hyperparasitized individuals on K636 *(Leucaena leucocephala* (Lamb.) De Wit). Numbers in parenthesis indicate %.

COLL. DATE	N	No.(%) Parasite P	Hyperparasites A	S	P
Aug. 23	8	4(50.00)	0(0)	2(50.0)	0(0)
30	102	4(3.92)	1(25.0)	0(0)	0(0)
Sep. 6	178	30(16.85)	7(23.3)	0(0)	0(0)
13	303	9(2.97)	1(11.1)	0(0)	0(0)
19	371	14(3.77)	2(14.3)	0(0)	0(0)
27	124	2(1.61)	0(0)	0(0)	0(0)
Oct. 4	189	11(5.82)	2(18.2)	0(0)	0(0)
11	714	7(0.98)	2(0.28)	0(0)	0(0)
18	16	2(12.50)	0(0)	0(0)	0(0)
25	50	2(4.00)	2(100.0)	0(0)	0(0)
	$\bar{x}$% =	(4.14)	(27.5)	(50.0)	(0)

N = number of fifth instar psyllid nymphs, P = *Psyllaephagus yaseeni* Noyes, A = *Syrphophagus aphidivorous* (Ashmead), S = *Syrphophagus* sp., P = *Pachyneuron siphonophorae* (Ashmead).

psyllids than intact leaves. A modification of the sample method, such as increasing the number of samples, is needed to reduce this variation.

Based on damage ratings, the seven accessions of *Leucaena* can be separated into three groups. The accessions in the group with the highest overall damage ratings were K527, K8, and K636; the group that was intermediate consisted of K743 and K156, and the group with the lowest damage ratings included K784 and K376. Except for K784 and K376, which were consistently rated at 1, the other accessions fluctuated in their mean damage ratings from week to week, resulting in small to large variance. In order to reduce the amount of variance, the damage rating system needs further modification.

Psyllid densities and damage ratings were not significantly correlated because psyllid densities did not increase continuously with damage. Psyllid densities reached a maximum at the midrange of damage ratings, and declined at higher ratings. Thus, it can be stated that damage ratings are reliable indicators of differences in psyllid damage, but are not good indicators of psyllid abundance.

The primary parasitoid, *P. yaseeni*, reared from the fifth instar psyllid nymphs, was relatively uncommon. This may have been due to the high level of hyperparasitism by *S. aphidovorous*, and to a lesser degree by *S.* sp. and *P. siphonophorae.*

ACKNOWLEDGEMENTS

We thank J.L. Brewbaker and C.T. Sorensson for providing advice throughout the project, and the former for comments on the manuscript. We are also grateful to four anonymous reviewers for their helpful suggestions.

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