Host shift capability of a specialist seed predator of an invasive plant: roles of competition, population genetics and plant chemistry

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Abstract *Acanthoscelides macrophthalmus* is a seed predator that has become widely distributed along with its native host, *Leucaena leucocephala* (Mimosoideae), which is a neotropical leguminous tree and one of the most invasive plants worldwide. Previous studies revealed that *A. macrophthalmus* is able to host-shift to several mimosoid species. Here, we aim to test the host-shift potential to other mimosoid and non-mimosoid plants and possible roles of interspecific competition, genetic background, and plant chemistry in host-shift. First, we found that *A. macrophthalmus* predator completed development on two new hosts: pigeon pea *Cajanus cajan* and *Cajanus scarabaeoides* (Faboideae), by rearing from seeds collected in South/Southeast Asia and Hawaii. In contrast, in most regions, both *Cajanus* species were infested only by other beetle species. Second, we performed no-choice tests using 11 leguminous plants, covering all three subfamilies as potential hosts, including the two new hosts. A Taiwanese *A. macrophthalmus* population reared in the laboratory on *Leucaena* did not deposit eggs on any of the seeds of each tested species. To compare host-shift responses between populations, we also used a Hawaiian *A. macrophthalmus* population that had completed its development on freshly collected *Leucaena* seeds from the field. This population deposited eggs onto and hatching larvae burrowed into *C. cajan* seeds, although none developed beyond the larval stage. Third, the surface chemical composition of seed-pods of *L. leucocephala* and the two *Cajanus* species was dissimilar, although that of seeds was highly similar. Finally, all of the host-shifting *A. macrophthalmus* populations shared the same haplotypic group.

Key words *Bruchidae · Biocontrol agent · Phytophagous insect · Ecological fitting · Host range · Hydrocarbon*

Introduction

Choice of host plants by herbivores shapes plant assemblages and their herbivores. Recent studies show that even specialist (monophagous and oligophagous) herbivorous insects are flexible in their choice of host plants (Messina et al. 2009; Tuda et al. 2009). These findings of host plant flexibility support the ecological fitting hypothesis (Janzen 1985), or phenotypic plasticity, an additional mechanism for coevolution (Ehrlich and Raven 1964), in which evolution of either plant or herbivore species is not a prerequisite (Agosta 2006). Ecological fitting without evolutionary change can promote the expansion of the host range for specialist species (Amarillo-Suarez and Fox 2006; Agosta et al. 2010), which often are considered an evolutionary dead end (Moran 1988). This hypothesis may explain how generalists evolve from specialist lineages (Nosil and Mooers 2005; Kergoat et al. 2007; Kato et al. 2010; Forister et al. 2012).

Ecological and genetic mechanisms behind host shift are yet to be understood (Janz 2011). Examples of factors promoting host-shift are competition among herbivores, phylogenetic closeness of potential hosts, and herbivore's genetic background. Interspecific competition is reduced by host shifts (Feder et al. 1995). Specialist herbivorous insects tend to host shift to plants that are phylogenetically close to their original hosts (Janz and Nylin 1998; Bertheau et al. 2010; Benda et al. 2012). Some genotypes are more adept than others at using new hosts (Tilmon et al. 1998).

Female acceptance of hosts for oviposition is the first important step in the process of host shifts (Jermy 1984), especially for insects in which larvae are more or less sedentary. Both physical and chemical composition of the substrate elicits female oviposition response (Hora and Roessingh 1999; Sulehrie et al. 2003; Gouinguene et al. 2005; Nazzi et al. 2008; Sambaraju and Phillips 2008; Hudaib et al. 2010; Nawrot et al. 2010), and plays an important role in determining the diet breadth and host shift potential of phytophagous insects (Jaenike 1990). Epicuticular waxes of plants, such as alkanes, stimulate oviposition of numerous insect species in the Coleoptera and Lepidoptera (Guerra et al. 1994; Eigenbrode and Espelie 1995; Li and Ishikawa 2006; Nawrot et al. 2010).

Acanthoscelides macrophthalmus (Schaeffer) (Chrysomelidae: Bruchinae) is a seed predator native to Central America (Kingsolver 2004) that has become widely distributed along with its native host, *Leucaena leucocephala* (Lam.) de Wit (Fabaceae: Mimosoideae), a neotropical tree and one of the most invasive plants throughout the tropics and subtropics (Lowe et al. 2000). *Acanthoscelides macrophthalmus* is established in Australia (Raghu et al. 2005), Cyprus (Vassiliou and Papadoulis 2008), Taiwan (Wu et al. 2007; Tuda et al. 2009), southern Vietnam (Kergoat et al. 2005), China (Yunnan), India, Thailand, Japan (Tuda et al. 2009), China (Hong Kong and Hainan), northern Vietnam, and Myanmar (Tuda et al., unpublished data). This insect is oligophagous, feeding only on species belonging to a single genus *Leucaena* in its native range (Johnson 1979; Hetz and Johnson 1988; Hughes and Johnson 1996; summarized in Tuda et al. 2009). Recently, however, the seed predator was observed attacking *Falcataria moluccana* (Miq.) Barneby & Grimes (Mimosoideae), an introduced tree in Taiwan, and potential alternate host (Tuda et al. 2009).

Bruchine seed predators are used as biocontrol agents of weedy plants (Tuda 2007). *Acanthoscelides macrophthalmus* has the potential to limit the invasiveness of *L. leucocephala* in dry areas where leucaena psyllids are not abundant (Raghu et al. 2005) and was released in South Africa and other African countries to control *Leucaena* seed dispersal (Olckers 2004). The life history of *A. macrophthalmus* has been studied by Shoba and Olckers (2010), Effowe et al. (2010) and Wu et al. (2012). There have been no reports of host-shifting in these countries and experimental host-shift tests within the plant subfamily (Mimosoideae) have indicated acceptance and development into adults on some species (Shoba and Olckers 2010; Olckers 2011). There is, however, an example of a bruchine beetle (i.e., *Bruchidius villosus* (F.)) host-shifting to a non-target introduced plant congeneric to the target (*Cytisus*) (Syrett 1999).

Leucaena leucocephala is a fast-growing nitrogen-fixing tree. However, this multipurpose beneficial tree is, a “conflict-of-interest” plant (Neser 1994) because it excludes plants growing nearby via allelopathy caused by the alkaloid mimosine or leucenol (Mascre 1937; Adams et al. 1945), produced and stored especially in young leaves and mature seeds (Williams and Hoagland 2007). The extract of *L. leucocephala* also has insecticidal properties (Cavalcante et al. 2006). Seed production of *L. leucocephala* is heavy and prolific, with 2–4 seed crops

produced annually (Raghu et al. 2005). Seed dispersal by rodents and birds or through cattle manure, following seed release as pods dehiscence, promotes the plants spread throughout adjacent areas (Smith 1985). *Leucaena leucocephala* has become an aggressive invader in disturbed areas where it was introduced (Walton 2003) and is recognized as one of the worst invasive alien species in the world (Lowe et al. 2000). This plant also promotes the invasion by more aggressive alien plants (Yoshida and Oka 2004).

Seed pods of legumes had been surveyed for possible bruchine infestation (Tuda et al. 2005, 2009) and *A. macrophthalmus* was found to use not only its original host *L. leucocephala* and a new host *F. moluccana* (Tuda et al. 2009) but also *Cajanus cajan* (L.) Millsp. and *Cajanus scarabaeoides* (L.) Thouars (Fabaceae: Faboideae). Pigeon pea, *C. cajan*, an erect shrub, is an important crop that is cultivated as an annual or semi-perennial in semi-arid tropical and subtropical farming systems as food, animal feed, and firewood (Shanower et al. 1999). Although approximately 90% of the world's production of *C. cajan* is in Asia, this legume also is an important crop in Africa and Latin America. In Hawaii, pigeon peas had been imported and cultivated especially by immigrants from the Philippines. *Cajanus scarabaeoides* (L.) Thouars, a wild herb, is widely distributed but most abundant on the Indian subcontinent and Australia (Saxena et al. 1990).

Here, we tested the host-shift potential of *A. macrophthalmus* to phylogenetically distant but confamilial plants. First, by collecting leguminous seeds and rearing larvae, if any, from the seeds in Asia and Hawaii, we investigated host shift events by *A. macrophthalmus*, along with presence of other seed predator insects. Second, under a no-choice laboratory scenario, 11 leguminous plants were tested, covering all three subfamilies for the first time, as potential new hosts of the seed predator. Two geographic insect populations were used to compare population responses to non-host plants. Third, to test if similarity of plant surface chemicals promoted host-shifts (Parr et al. 1998), surface chemical components were analyzed of seed-pods and seeds of the original, new host, and non-host species. Finally, to test if genetic makeup of the seed predator promoted host-shifts, a mitochondrial region was sequenced of host-shifting and non-host-shifting *A. macrophthalmus* populations.

Materials and methods

The insect and its native host plant

Two geographic populations of *Acanthoscelides macrophthalmus* were used for non-choice host shift experiment. One was a mixed population collected in Pingtung County (22.01 N, 120.75 E) and on Hsinchu-Miaoli County border (24.68 N, 120.96 E), Taiwan, in April 2005 (Wu et al. 2013), and the other was a population collected in Hawaii Kai, Oahu, Hawaii, USA (21.31 N, 157.70 W) in February 2011. *Acanthoscelides macrophthalmus* was first found in Taiwan, Thailand and in Hawaii (on Oahu) in 1995, 1998 (Tuda et al. 2009) and in 1990 (Samuelson 1991), respectively. There have been no attempts of deliberate introduction of *A. macrophthalmus* for biocontrol in these countries.

Leucaena leucocephala, a native host plant of *A. macrophthalmus*, has been deliberately introduced to tropical/subtropical regions. Introductions into Asia and a Hawaiian island were as early as the 13-14th century and the 19th century, respectively (Table 1).

Field collection of seeds and rearing

Cajanus cajan and *C. scarabaeoides* seeds were collected from various regions from 1995–2011 and reared for possible emergence of the seed predator. *Cajanus cajan* was collected on Oahu Island, Hawaii, USA (21.39 N, 157.98 W), and Batangas, Luzon Island, the Philippines (13.74 N, 121.09 E), and *C. scarabaeoides* was collected in Pingtung County, Taiwan (21.95 N, 120.74 E), Chiang Mai (18.50 N, 98.39 E; 18.41 N, 98.37 E; 18.44 N, 98.68 E), Mae Hong Son (19.29 N, 98.46 E), Lamphun (18.45 N, 99.20 E) and Lampang (17.61 N, 99.23 E) Provinces, Thailand, and in Yunnan Province, China (25.42 N, 101.47 E). The collected seeds from each legume population were kept in a paper bag under semi-natural conditions (Oahu, 17–28°C; Batangas, 24–32°C; Yunnan, 8–23°C; Taiwan, 11–28°C; Chiang Mai, 13–31°C) in the country where they were collected. The bags were checked every week for the first 2 months and then every month for 4 additional months.

No-choice host shift experiment

Eleven leguminous plants, including the two *Cajanus* species, were evaluated as potential new hosts of *A. macrophthalmus* (Table 1). The additional nine species were selected based on phylogenetic closeness to the target, similarity of pod morphology to *Leucaena*, or dominant abundance in Taiwan. Ten to 15 pairs of adults emerging within 24 h that had been incubated with *Leucaena* seeds were paired and put into a 50 ml plastic tube with 30 seeds of one of the legume species. The seeds were replaced with 30 new seeds every day until all of the female *A. macrophthalmus* died (the sex was determined according to the keys in Wu et al. 2007). This no-choice test for each plant species was replicated three times, and we used the Taiwanese population of *A. macrophthalmus* for these tests. This experiment was conducted at $27 \pm 1^\circ\text{C}$, $60 \pm 5\%$ relative humidity and with 12L:12D photo cycle. These tests were performed in 2007, except for the two *Cajanus* species, which were completed in 2011.

The Hawaiian *A. macrophthalmus* population was also used for an additional test. Five pairs of adults emerging within 7 days from field-collected *Leucaena* seeds were put in a 5.2-cm-diameter Petri dish (30 ml) containing 10 seeds of *C. cajan*. The numbers of hatched and unhatched eggs were counted after all adults had died. This experiment was completed at $20 \pm 2^\circ\text{C}$, $45 \pm 5\%$ relative humidity and with 14L:10D. This test was performed in 2011.

Chemical analysis of plant surface

Surface chemicals (mostly hydrocarbons) were extracted from seed coats of *L. leucocephala*, *C. cajan*, and *C. scarabaeoides*, and from seed-pods (pericarp) of *L. leucocephala* and *C. scarabaeoides*, by dipping them in ethyl acetate at room temperature. The crude extract was concentrated by purging with nitrogen and resuspended in hexane/ ethyl acetate (10:1). The sample extract was transferred to the column (7 mm i.d.) with 0.7 g of silica gel (63-210 μm i.d.) and eluted with 5 ml of hexane : ethyl acetate (10:1). The eluates were reduced to small volumes by purging with nitrogen.

Gas chromatography-mass spectrometry (GC–MS) was performed for analysis of chemicals on a coupled GC–MS system (GC17A-QP5050A, Shimadzu Inc., Kyoto, Japan). The gas chromatograph was equipped with a fused silica

capillary column (30 m length, 0.25 mm i.d.) coated with 0.25 mm of crosslinked poly-dimethylsiloxane (SPB-1, Supelco, Inc., USA). Plant sample extract (1 μ l) was injected with a splitless injector at 280°C (carrier). The gas chromatography oven temperature was maintained at 100°C for 3 min, then increased at a rate of 10°C min⁻¹ to 30°C, and held for 17 min, making a total time of 40 min. The interface with the MS was set at 280°C and MS performed in full scan mode using 70 eV EI⁺ and scanned from 50 to 400 m/z (carrier: helium; flow rate: 0.9 ml min⁻¹). Chemical components were identified by comparing peak retention times with those of authentic standards, and by a GC–MS analysis of representative samples. Chemical compositions were measured based on peak areas. Each plant sample extract was analyzed twice. Multivariate correlations of chemical composition between the original and new host plants or non-host plants were estimated with JMP8.0.

DNA analysis

Crude DNA was extracted from a sample of the 10 beetles (60 total) from the Yunnan, Oahu, Lampang, and Lamphun populations and the Taiwanese laboratory population, all from *L. leucocephala* and Nantou population from *F. moluccana* (23.75 N, 120.86 E; Tuda et al. 2009) using a DNeasy Tissue Kit (Qiagen, Tokyo, Japan). Amplification of a 522-bp mitochondrial *COI* region was performed by polymerase chain reaction (PCR) with the primers COI2-1 (Tuda et al. 1995, 2004) and COI2-2 (Tuda et al. 2004) and sequencing followed. For the PCR and sequencing, the protocol previously described in Tuda et al. (2004, 2006) was followed. To estimate a statistical parsimony haplotype network (Templeton et al. 1992), we used TCS 1.21 (Clement et al. 2000) with the connection limit set at 95%.

Results

Field collection of seeds and beetle emergence

Acanthoscelides macrophthalmus adults emerged from *C. cajan* seeds collected in an urban garden center on Oahu (Hawaii) and from two populations of *C.*

scarabaeoides seeds collected from bare ground along roadsides with little vegetation in Yunnan (China) at seed predation rates 0.0070-0.0168 (Table 2). No *A. macrophthalmus* emerged from *C. scarabaeoides* seeds collected in other Asian countries or from *C. cajan* seeds collected in an agricultural experimental field surrounded by a relatively natural environment in Oahu (Table 2). Instead, several species of *Callosobruchus* beetles (Chrysomelidae: Bruchinae) and apionid weevils (Curculionoidea) emerged at seed predation rates 0.0044-0.0479 (Table 2). These *Callosobruchus* species all belonged to the *chinensis* phylogenetic group that is highly associated with Cajaninae, including *Cajanus* (Tuda et al. 2005, 2006).

No-choice host shift experiment

In the no-choice test on *Cajanus* species, the females of the Taiwanese laboratory population of *A. macrophthalmus* did not deposit eggs on seeds of either species, while depositing 26.4 ± 13.8 (mean $\pm$ SE) eggs on *L. leucocephala* seeds. In contrast, three hatched eggs of the Hawaiian beetle population were confirmed on the seeds of *C. cajan*. However, none of the early instar larvae from the hatched eggs developed into late instar larvae in the seeds. For the other nine leguminous species tested, the Taiwanese *A. macrophthalmus* did not deposit eggs on any of the seeds.

Chemical analysis of plant surface

Chemical compositions of the seed surface for the original (*L. leucocephala*) and novel hosts (*C. cajan* and *C. scarabaeoides*) were similar (Table 3a). For the seed-pod surface, however, chemical compositions of the original (*L. leucocephala*), novel hosts (*C. scarabaeoides*), or non-hosts were not similar (Table 3b). A sesquiterpene caryophyllene was confirmed from the seed-pod of *C. scarabaeoides*. This volatile compound was reported from *C. cajan* leaves (Hartlieb and Rembold 1996; Ogunbinu et al. 2009) and from *L. leucocephala* (Finlay-Doney and Walter 2005).

DNA analysis

In total, 5 haplotypes were identified and separated into two major groups (Fig. 1). All individuals from the Yunnan, Oahu (both host-shifted to *Cajanus* in the field), Nantou and Taiwanese laboratory populations (host-shifted to *Falcataria*, in the field and in the laboratory, respectively, Tuda et al. 2009) belonged to the same haplotypic group (Fig. 1). In contrast, individuals from Lampang and Lamphun (not host-shifted) formed another haplotypic group, except for four individuals from Lamphun that belonged to the former (the majority host-shifted) haplotypic group (Fig. 1).

Discussion

Our experiments showed that although a shift to a new host by a specialist, or an oligophagous herbivorous insect, is difficult, development under natural conditions may allow a shift to a new plant host that is phylogenetically distant from the original host. Host shift by introduced or invading herbivores has been observed in Bruchinae, but the shift is usually within a related plant group (within plant tribes, Syrett 1999; Tuda et al. 2004, 2005; between plant tribes but still within plant subfamily, in *A. macrophthalmus*, Tuda et al. 2009; from *Erythrina* to *C. cajan* in *Specularius*, Southgate and McFarlane 1979). This is also true for native specialist insects that encountered introduced plants (Bertheau et al. 2010). However, host shifts can be to more distantly related plants such as from *Bauhinia reticulata* DC. (Caesalpinioideae) to *Arachis* (Fabaceae) by *Caryedon* (Sembene 2006) and from *Acacia* (Mimosoideae) to *Parkinsonia* (Caesalpinioideae) by *Stator* (Johnson and Siemens 1991).

The results of our field collections and rearing studies to detect seed predators suggest that frequent encounters with plants without encountering competitors may facilitate a host shift (Table 2), supporting the effect of competitors in host shifts (Janzen 1973; Denno et al. 1995; Feder et al. 1995). The longer an original host (see '*Leucaena* introduction' in Table 2) or new potential host (see '*status*' in Table 2) has been present in a particular area (results of no-choice experiments and field observation, Table 2), the lower the host shift probability. This is probably because the number of phytophagous insects associated with each plant increase through time (Strong 1977) and serve as

competitors against recently introduced insects. For the laboratory population kept for several years only on the original host, *A. macrophthalmus* population may have lost preference for low-rank hosts.

Possible cues for accepting *Cajanus* as a new host in the natural habitat are currently unknown. Pod morphology and size are not similar between *Leucaena* and *Cajanus*; smooth, flat, elongate pods for the former and hairy, rotund, short pods for the latter. Seed morphology also is different; flat for the former and spherical for the latter. In terms of the oviposition site for *A. macrophthalmus*, there may be some common features between the two genera. *Acanthoscelides macrophthalmus* deposits eggs on *Leucaena* pods and seeds that are both on the plant and on the ground and tend to prefer those on the ground, although egg mortality does not differ between the two microhabitats (Egli and Olckers 2012). *Cajanus scarabaeoides* is a non-climbing herb and *C. cajan* is a shrub, both of which have pods closer to the ground than *Leucaena* pods on the tree.

The absence of a toxic chemical also may be a factor for *Cajanus* acceptance: For example, L-canavanine is absent in both *Leucaena* and *Cajanus* species (Bell et al. 1978). In contrast, chemical cues for oviposition are unlikely to be the factor promoting these host-shifts. Although chemical components between the original and new host plants were similar for seed surfaces (Table 3a), those for seed-pod surfaces were dissimilar between the original and new hosts (Table 3b). Because the seed-pods of both *Cajanus* species do not dehisce until they are mature and dry (Smartt 1990; M. Tuda, personal observation), the seed predator probably had little chance to assess seed surface directly before we collected seed-pods that had not dehisced yet. We found caryophyllene, a volatile terpene, from the seed-pods of *C. scarabaeoides*, the new host plant, but not from *L. leucocephala* (Table 3b). There is, however, evidence that *L. leucocephala* emits caryophyllene, the amount of which depends on the environment (Finlay-Doney and Walter 2005). This compound also is found from *C. cajan* (Hartlieb and Rembold 1996; Ogunbinu et al. 2009). Therefore, the chemical similarity among the original and new hosts may be higher in nature than was identified in our chemical analysis. Note, however, caryophyllene alone may not be attractive for phytophagous insects. For example, in another study caryophyllene did not attract the *Helicoverpa* moths but its mixture with other sesquiterpenes acted as an oviposition stimulant (Hartlieb and Rembold 1996). Likewise, caryophyllene

alone did not influence the behavior of *leucaena* psyllids (Finlay-Doney and Walter 2005).

Furthermore, the genetic makeup of the herbivorous insect also can be another factor facilitating host shifts (Wassermann 1986; Bernays and Chapman 1994). The populations that host-shifted belonged to the same haplotypic group (Yunnanese, Hawaiian (this study), Nantou and Taiwanese laboratory (Tuda et al. 2009) populations) (Fig. 1). Contribution of the genome of herbivore populations to host-shift tendency is likely at least in the field. Host-shift tendency, however, may be a consequence of other life history traits, such as high fecundity that often leads to indiscriminate oviposition and is likely influenced maternally. Note that the plant species to which herbivores host-shift probably depends on other factors, such as the composition of local species assemblages (Mitter et al. 1991), or more specifically, distance, apparency [abundance (Bernays and Chapman 1994; Yotoko et al. 2005) and history (Strong 1977)], as well as phenological matching with each species. Further work should clarify the relative contributions of genetics and other ecological and environmental factors that influence host shifts.

In conclusion, our study illustrated rare host-shift occurrences of an oligophagous seed predator that had been considered host-specific. This indicates the lability or plasticity of host range even in so-called specialist herbivores. This case study demonstrated that specialists can host shift to phylogenetically distant plants, which is a counter example of phylogenetically constrained pattern of host shifts (Mitter et al. 1991). Therefore, specialists are not evolutionary dead ends and ecological fitting or plasticity may rescue them as demonstrated in this study, which may eventually fuel evolution towards generalists and host races.

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Table 1 Leguminous plants used as potential new hosts of *Acanthoscelides macrophthalmus*, the seed predator of *Leucaena*, in no-choice tests.

Fabaceae: Mimosoideae

Acacia confusa Merr.

Acacia farnesiana (L.) Willd.

Albizia lebbbeck (L.) Benth.

Fabaceae: Caesalpinioideae

Bauhinia variegata L.

Senna tora (L.) Roxb.

Peltophorum pterocarpum (DC.) K. Heyne

Fabaceae: Faboideae

Abrus precatorius L.

Cajanus cajan (L.) Millsp.

Cajanus scarabaeoides (L.) Thouars

Clitoria ternatea L.

Crotalaria similis Hemsl.

Table 2 *Cajanus* species collected as potential alternative hosts of *Acanthoscelides macrophthalmus* and emerging seed predator beetles.

Host legume (<i>Cajanus</i>)	Site	Time	Stat ¹	<i>Leucaena</i> introduction (year)	Emerging beetles ²	Number of emerging adults	Number of seeds	Seed predation rate
<i>Cajanus cajan</i>	Oahu Is. 1, Hawaii, USA	Feb., 2003	I	1860s- early 1880s*	<i>Acanthoscelides macrophthalmus</i>	2	285	0.00702
<i>Cajanus cajan</i>	Oahu Is. 2, Hawaii, USA	Feb., 2003	I	1860s- early 1880s	<i>Callosobruchus pulcher</i> ^{§§}	4	906	0.00442
<i>Cajanus cajan</i>	Luzon Is., Philippin es	Oct., 1998	I	prior to 1815 [#]	<i>Callosobruchus pulcher</i> ^{§§}	NA ³	NA	NA
<i>Cajanus scarabaeoides</i>	Yunnan 1, China	Oct., 2004	U	after 1961 [§]	<i>Acanthoscelides macrophthalmus</i>	7	416	0.0168
<i>Cajanus scarabaeoides</i>	Yunnan 2, China	Nov., 2004	U	after 1961	<i>Acanthoscelides macrophthalmus</i>	5	452	0.0111
<i>Cajanus scarabaeoides</i>	Pingtung, Taiwan	Nov., 1995	N	1645**	<i>Callosobruchus theobromae</i> ^{§§}	4	107	0.0374
<i>Cajanus scarabaeoides</i>	Lampang , Thailand	Nov., 1998	U	1238– 1378 ^{##}	<i>Callosobruchus theobromae</i> ^{§§}	21	116 9	0.0180
<i>Cajanus scarabaeoides</i>	Chiang Mai 1, Thailand	Nov., 2002	U	1238– 1378	<i>Callosobruchus theobromae</i> , <i>C. chinensis</i> , <i>C. nigripennis</i>	15	115 0	0.0130
<i>Cajanus scarabaeoides</i>	Chiang Mai 2, Thailand	Nov., 2002	U	1238– 1378	<i>Callosobruchus dolichosi</i>	8	510	0.0157
<i>Cajanus scarabaeoides</i>	Chiang Mai 3, Thailand	Dec., 2003	U	1238– 1378	<i>Callosobruchus theobromae</i>	12	107 8	0.0111
<i>Cajanus scarabaeoides</i>	Pai, Thailand	Dec., 2003	U	1238– 1378	<i>Callosobruchus nigripennis</i>	10	449	0.0223
<i>Cajanus scarabaeoides</i>	Lamphun , Thailand	Dec., 2003	U	1238– 1378	<i>Callosobruchus theobromae</i> , <i>C. nigripennis</i>	10	252	0.0397
<i>Cajanus scarabaeoides</i>	Chiang Mai 3, Thailand	Dec., 2005	U	1238– 1378	<i>Callosobruchus theobromae</i> , <i>C. dolichosi</i> , <i>C.</i>	103 (Cal loso	215 0	0.0479

<i>chinensis</i> , <i>C.</i>	bruc
<i>nigripennis</i> , <i>C.</i>	hus)
<i>imitator</i> ,	, 1
Apionidae	(Api
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¹Status of the *Cajanus* species in the countries where seeds were collected is indicated by I: introduced; N: native; U: unknown (International Legume Database & Information Service, www.ildis.org)

² *: Little Jr. and Skolmen (1989), # Merrill (1921–1926), §: Yang (2000), **: Wu *et al.* (2003), ##: Tuda *et al.* (2009), §§: Tuda *et al.* (2005)

³Not available

Table 3 Chemical composition (%) of (a) seed surface and (b) seed-pod surface of *Leucaena leucocephala* (the original host plant of *Acanthoscelides macrophthalmus*), *Cajanus cajan* and *Cajanus scarabaeoides* (new host plants), and *Albizia lebbeck* and *Senna tora* (non-host plants). -: not detected, NS: $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$

(a)						
Seed surface	Retenti on time (min)	<i>Leucaena leucoceph ala</i>	<i>Cajanus cajan</i>	<i>Cajanus scarabaeoi des</i>		
Squalene	23.8	85.3	74.5	42.2		
Nonacosane	24.5	5.3	8.1	10.1		
Hentriacontane	26.6	4.6	3.0	-		
Heptacosane	22.8	-	-	22.6		
a	25.6	4.8	-	-		
1-Hexacosanol	24.3	-	8.8	-		
b	26.3	-	5.6	-		
c	30.2	-	-	10.1		
d	31.4	-	-	7.4		
e	32.8	-	-	7.5		
Correlation with <i>L. leucocephala</i>		1.000***	0.987***	0.831**		
(b)						
Seed-pod surface	Retenti on time (min)	<i>Leucaena leucoceph ala</i>	<i>Cajanus cajan</i>	<i>Cajanus scarabaeoi des</i>	<i>Albizia lebbeck</i>	<i>Senna tora</i>
Nonacosane	24.5	-	17.1	44.4	78.7	79.6
Hentriacontane	26.6	27.5	46.4	17.4	6.6	14.9
Lupenone	30.2	-	-	1.8	5.6	-
Caryophyllene	11.9- 12.9	-	-	32.2	-	-
Trtriacontane	29.2	52.6	-	-	-	-
Triacontane	25.4	-	-	-	-	1.8
Heptacosane	22.8	-	-	1.6	-	-
f	29.4&2 9.8	1.1	36.5	2.6	3.5	2.8
g	31.4	18.8	-	-	-	-
Lupenol	30.5	-	-	-	5.6	-
h	30.5- 30.8	-	-	-	-	1.0
Correlation with <i>L. leucocephala</i>		1.000***	0.161 ^{NS}	-0.128 ^{NS}	-0.159 ^{NS}	-0.101 ^{NS}

Figure legend

Fig. 1 Statistical parsimony network of *COI* sequences of geographic populations of the seed predator, *Acanthoscelides macrophthalmus*. The size of the pie graph is proportional to the number of individuals. Small blank circles indicate hypothetical haplotypes.

Fig.1

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