LACK OF ASSOCIATIONAL EFFECTS BETWEEN TWO HOSTS OF AN INVASIVE HERBIVORE: *OPUNTIA* SPP. AND *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE)

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Abstract

Associational susceptibility has been predicted to arise when a target plant's neighbor is more palatable to an herbivore than the target plant itself, resulting in attraction and spill-over of the herbivore onto the target plant. This prediction was tested on two hosts of the invasive pyralid moth *Cactoblastis cactorum* Berg. A combination of observational surveys and experimental plantings were used to test for differences in herbivore damage and plant size on isolated *Opuntia humifusa* (Raf.) Raf. plants, *O. humifusa* paired with *O. stricta* (Haw.) Haw., and *O. humifusa* in conspecific pairs. Based on general patterns of associational effects and specific *C. cactorum* behavior, it was predicted that *O. humifusa* would have higher levels of *C. cactorum* damage when growing near *O. stricta*, as compared with isolated plants or those growing near conspecifics. However, little support for associational resistance or susceptibility was found. Surveys revealed that isolated *O. humifusa* plants had on average more cladodes than isolated *O. stricta* plants or paired plants. Additionally, *O. humifusa* paired with *O. stricta* had more *C. cactorum* larvae and old damage than pairs of *O. humifusa*, but did not have more than isolated *O. humifusa* plants. Plants from the site used for the experimental plantings had little to no *C. cactorum* damage, regardless of plant neighbors, and revealed no associational effects for native herbivores. The occurrence of associational susceptibility driven by a spillover mechanism may require a higher herbivore density and a sparser distribution of the herbivore's preferred host than was present at the sites use here.

Key Words: biological invasions, herbivory, associational resistance, Cactaceae

Resumen

Se ha previsto que la susceptibilidad asociativa surge cuando una planta vecina es más aceptable para un herbívoro que la planta clave, lo que resulta en la atracción y el desbordamiento de los herbívoros sobre la planta clave. Pusimos a prueba esta predicción en 2 hospederos de la polilla invasora Pyralidae, *Cactoblastis cactorum* Berg. Se utilizó una combinación de estudios de observacion y plantaciones experimentales para probar las diferencias en el daño de herbívoros y el crecimiento de cladodios en plantas aisladas de *Opuntia humifusa* (Raf.) Raf., plantas de *O. humifusa* emparejadas con plantas de *O. stricta* (Haw.) Haw., y en plantas de *O. humifusa* en pares conespecificas. Basado en el patron general de los efectos asociativos y del comportamiento específico de *C. cactorum*, predijimos que *O. humifusa* tendría un mayor nivel de daño causado por *C. cactorum* cuando crecen cerca de *O. stricta*, en comparación con las plantas aisladas de *O. humifusa* o con las plantas *O. humifusa* creciendo cerca de otras plantas conespecíficas. Sin embargo, hemos encontrado muy poca evidencia de la resistencia o de la susceptibilidad asociativa. El sondeo revelo que las plantas de *O. humifusa* en parejas interespecíficas tenían más larvas de *C. cactorum* y daño viejo que las plantas en parejas conespecificas, pero no tenían más que las plantas aisladas de *O. humifusa* plantas, lo que significa este resultado podría ser impulsado por la densidad de plantas y no con la diversidad de las plantas hospederas. Las plantas en el sitio utilizado para las plantaciones experimentales tenían poco o ningún daño causado por *C. cactorum*, independientemente de las plantas vecinas, y no mostró efectos asociados de los herbívoros nativos. La ocurrencia de susceptibilidad asociativa impulsado por un mecanismo de desbordamiento puede requerir una mayor densidad de herbívoros y una distribución más escasa del hospedero preferido del herbívoro del que estaba presente en nuestros sitios.

Palabras Clave: *Opuntia*, invasiones biológicas, herbivoría, resistencia por asociación, Cactaceae

The risk of herbivory for a plant is influenced by the diversity and spatial distribution of its surrounding plant community. A host plant may gain protection from its herbivores when found in a diverse, versus simple, community or when growing in close association with non-host or less palatable species. This phenomenon is termed 'associational resistance' (hereafter AR) and was first used to describe reduced herbivory on crops grown in polycultures versus those grown in monocultures (Root 1973; Tahvanai & Root 1972). AR was later extended to include other types of 'damage' (i.e., reduced predation or parasitism), as can arise when a target host/prey is associated with a more resistant or less preferred host/prey (Hjalten & Price 1997; LoGiudice et al. 2003; Ostfeld & Keesing 2000; Raffel et al. 2008; Stenberg et al. 2007; Stiling et al. 2003; Wahl & Hay 1995), even if that host is a conspecific (Hjalten & Price 1997). Conversely, if a target host experiences increased 'damage' when associated with a less resistant or more preferred host, it is termed 'associational susceptibility' (hereafter AS) (Brown & Ewel 1987; Thomas 1986; Wahl & Hay 1995; White & Whitham 2000). Multiple mechanisms and hypotheses have been proposed to explain AR and AS, including the resource concentration hypothesis (Root 1973), enemies hypothesis (Root 1973), plant defense guilds (Atsatt & Odowd 1976; Tahvanainen & Root 1972), plant 'eavesdropping' (Himanen et al. 2010; Karban 2001, 2007), shared natural enemies (or apparent competition) (Holt & Lawton 1994), and biotic or abiotic habitat modification (Barbosa et al. 2009; White & Andow 2006); these mechanisms and hypotheses have been reviewed by Russell (1989); Andow (1991); Agrawal et al. (2006); Barbosa et al. (2009) and Letourneau et al. (2011).

Many of the mechanisms listed above could, in theory, lead to either AR or AS. For example, a plant neighbor that serves as an attractant could reduce damage on the target plant by drawing away shared herbivores (AR). This is the conceptual basis for trap cropping in agricultural systems that involves mixing attractive, but less economically valuable, plant species with crops in order to lure herbivores away from the crops (Hokkanen 1991). However, if an herbivore species increases in abundance due to feeding on the attractive neighbor(s), spillover onto the target plant could occur, thereby increasing damage (AS).

In general, AR is more likely when a neighbor is less palatable relative to the target plant (Barbosa et al. 2009; Hjalten & Price 1997; Pfister & Hay 1988), whereas AS is more likely when a neighbor is more palatable (Brown & Ewel 1987; White & Whitham 2000). For example, White and Whitman (2000) found significantly higher fall cankerworm (*Alsophila pometaria* Harris; Lepdioptera: Geometridae) densities and percent-age defoliation on potted cottonwoods (*Populus* spp.; Malpighiales: Salicaceae) placed under the worm's preferred host, box elder (*Acer negundo* L.; Sapindales: Sapindaceae), than on isolated cottonwoods or cottonwoods placed under conspecifics. Agrawal (2006) refined this prediction for specialist herbivores, by observing that for specialists, AS should become more likely as plant neighbors offer increasingly essential resources. However, actual occurrence of AR or AS will clearly be linked to other factors including herbivore density and motility (Brown & Ewel 1987; Russell et al. 2007; White & Whitham 2000), presence of other herbivores and natural enemies (Hamback et al. 2006; Stiling et al. 2003; White & Andow 2006) and distance between target and neighbor plants (Russell et al. 2007; Stiling et al. 2004; Stiling & Moon 2001; White & Whitham 2000).

The primary objective of this study was to determine whether *Opuntia humifusa* (Raf.) Raf. (Caryophyllales: Cactaceae), a host of the invasive cactus moth, *Cactoblastis cactorum* Berg, experiences associational effects when growing near to *O. stricta* (Haw.) Haw.*, a second host of *C. cactorum*. In a field study, *O. stricta* was found to have more *C. cactorum* eggs and damage than *O. humifusa* (Baker & Stiling 2009); however, laboratory host choice experiments found no difference in oviposition or larval preference between the 2 species (Johnson and Stiling 1996). Evidence suggests that *C. cactorum* females normally disperse very little and tend to oviposit very near to their emergence site, leading to a clumped distribution of eggsticks (Dodd 1940; Myers et al. 1981; Robertson 1987b; Zimmermann et al. 2004). For insect herbivores, AS has been more commonly observed than AR (Barbosa et al. 2009), and is more likely when a neighbor is preferred over a target plant; this, coupled with specific behavior of *C. cactorum* females, suggests that spillover from *O. stricta* is likely to lead to AS for *O. humifusa* growing in close proximity. The prediction was therefore made that *O. humifusa* would have higher levels of *C. cactorum* damage when growing near *O. stricta*, as compared with damage levels when growing near conspecifics or isolated. The secondary objectives were to examine the co-occurrence of *C. cactorum* and native *Opuntia*-feeding insects and to determine if differences in damage levels or abundance of these insects could be attributed to associational effects of the 2 *Opuntia* species. Four native herbivores were examined: *Chelinidea vittiger aequoris* McAtee (Hemiptera: Coreidae), *Dactylopius confusus* Cockerell (Coleoptera: Dactylopiidae), *Diaspis echinocacti* Bouche (Hemiptera: Diaspididae)*, and *Gerstaeckeria hubbardi* LeConte (Coleoptera: Curculionidae) (Table 1). Preferences for *O. humifusa* versus *O. stricta* have not been documented for any of these species, therefore specific predictions concerning the occurrence of AR or AS were

TABLE 1. The four native *Opuntia* herbivores examined in the study.

Insect species	Order:Family	Common Name	Description of feeding/damage
Chelinidea vittiger aequoris McAtee	Hemiptera: Coridae	Cactus bug	Mobile sap sucker; leaves pale half or full circles on cladode surface; severe damage can lead to desiccation of cladode[a,b]
Dactylopius confusus Cockerell	Hemiptera: Dactylopiidae	Cochineal	Sessile sap sucker; exudes fluffy white substance on surface of cladodes[b]
Diaspis echinocacti Bouché	Hemiptera: Diaspididae	Cactus scale	Sessile sap sucker; circular, slightly convex, whitish to tan scale; severe damage causes chlorotic patches and desiccation of cladode[c]
Gerstaeckeria hubbardi LeConte	Coleoptera: Curculionidae	Cactus weevil	Internal feeder; leaves light brown circular cell; cladode appears pierced by tiny "bullet hole"[b,d]

[a]Hamlin (1924)
[b]Mann (1969)
[c]Oetting (1984)
[d]Woodruff (2009)

not made. In order to address the 2 objectives, observational surveys and experimental plantings were carried out.

MATERIALS AND METHODS

Study System

Cactoblastis cactorum Berg (Lepidoptera: Pyralidae), is well known for its role in the biological control of *Opuntia* species, including the highly successful campaign in Australia during the 1920s and 1930s, the less successful campaign begun in the late 1930s in South Africa, and the campaign against native cacti in the Caribbean begun in the 1950s (Bennett & Simmonds 1966; Dodd 1940; Pettey 1948; Zimmermann & Moran 1991). However, since its discovery in the Florida Keys in 1989, the moth has become a potential threat to North American opuntioid biodiversity (Bennett & Habeck 1995; Stiling 2002; Zimmermann et al. 2000). Presently, *C. cactorum* is established in the southeastern United States as far north as Bull Island, South Carolina and as far west as southeastern Louisiana (Simonsen et al. 2008; USDA-APHIS-PPQ 2009). Female moths lay about 40-100 eggs one on top of the other to form an eggstick that is attached to the tip of a cactus spine or directly to a cladode. The larvae feed internally and gregariously on a number of opuntioid species. Larval damage results in hollowed out cladodes and can lead to secondary infections and in some cases, the death of entire plants (Zimmermann et al. 2004). After completing 6 instars, larvae pupate in the soil or in hollowed out cladodes. In central Florida, there are 3 non-overlapping flight periods per yr: a spring flight beginning in mid- Feb, a summer flight beginning in early Jun, and a fall flight beginning in late Aug (Hight & Carpenter 2009).

Four native *Opuntia*-feeding insect species were included in damage surveys: *C.v. aequoris*, *D. confusus*, *D. echinocacti,* and *G. hubbardi*. *Diaspis echinocacti* feeds on a variety of cactus species and the remaining 3 species are specialists on opuntioid cacti (Hunter et al. 1912, Mann 1969). *Dactylopius confusus* and *D. echinocacti* are primarily sessile and live adult stages are therefore easily quantified. *Chelinidea vittiger aequoris* nymphs and adults are motile and *G. hubbardi* larvae feed internally, making accurate, direct counts of these 2 species more difficult. However, both leave characteristic signs of feeding (Table 1) that allow for quantification of damage even if insects are not directly observed.

Two *Opuntia* species native to the southeastern United States were used in this study, *O. stricta* and *O. humifusa*. Both are common hosts of *C. cactorum*, although *O. stricta* is attacked more often than *O. humifusa* (Jezorek et al. 2012; Baker & Stiling 2009). They exhibit slightly different

morphologies; *O. stricta* has a more erect growth form and more spines and *O. humifusa* has a low, spreading growth form and fewer spines (Baker & Stiling 2009). However, both species can range in size from a few to several hundred cladodes.

Observational surveys were conducted at Honeymoon Island State Park (Pinellas County, Florida; hereafter referred to as HI) and experimental plantings were conducted at Fort deSoto Park (Pinellas County, Florida; hereafter referred to as FDS). Both sites are part of Florida's west-central barrier island chain and have similar plant and herbivore communities (P. D. S. personal observation). *O. stricta* and *O. humifusa* are common in the dune and coastal scrub areas and *C. cactorum* is well-established (Jezorek et al. 2012).

Data Collection and Analysis

Observational Surveys. In Sep 2006, 190 *Opuntia* plants were marked at HI as follows: Group 1 - isolated *O. humifusa* (n = 50), Group 2 - isolated *O. stricta* (n = 52), Group 3 - interspecific pairs (n = 44 pairs). Plants in "isolated" groups were > 5m from any other *Opuntia* individual and plants in "paired" groups were ≤ 1m apart, but > 5m from any other *Opuntia* individuals. Distances were measured between the 2 closest cladodes of the 2 plants in question at the time of marking. In order to meet these distance criteria, plants could not be chosen at random. When marking plants, distance to other *Opuntia* individuals was considered, but other factors, such as plant size or pre-existing herbivore damage were not. All plants were surveyed in Sep 2006 and Dec 2006. During each survey, the following variables were recorded: plant size (measured as the number of live, non-woody cladodes), proportion of cladodes with *C. cactorum* larvae, proportion of cladodes with old *C. cactorum* damage, number of *C. cactorum* eggsticks per cladode, and proportion of cladodes with *D. confusus*, *D. echinocacti* (live insects present), *C. v. aequoris*, and *G. hubbardi* (feeding marks present). Old damage from *C. cactorum* is indistinguishable from that of *Melitara prodenialis* Walker (Lepidoptera: Pyralidae), the native cactus borer, however the larvae and eggsticks of the 2 moths are easy to tell apart. From 2003-2005, Baker & Stiling (2009) found no *M. prodenialis* at HI or FDS. Similarly, no *M. prodenialis* specimens were found from 2005-2011 while conducting this study and others at HI and FDS (Jezorek et al. 2011, 2012). Therefore, the assumption was made that old damage was solely due to *C. cactorum*.

All analyses were conducted using IBM SPSS Statistics 19. Data were highly non-normal so to test for differences in herbivore damage between *O. stricta* and *O. humifusa*, a Mann-Whitney test was performed with plant species as the independent variable. To check for associational effects on herbivore damage, a non-parametric ANOVA by ranks was used to compare plant groups. Where appropriate, Dunn's test for multiple contrasts was used for pair wise comparisons. Spearman's rank correlation was used to test for relationships between *C. cactorum* larvae and native herbivores and was performed separately for *O. humifusa* and *O. stricta*, as well as for plant groupings.

The design described above allows for testing of *O. humifusa* versus *O. stricta* for cladode number and herbivore damage levels. However, because conspecifc pairs were not marked, it does not allow differentiation between associational effects due to density of *Opuntia* versus those due to species of *Opuntia*. For example, if *O. humifusa* in interspecific pairs were found to have significantly more *C. cactorum* larvae than isolated *O. humifusa* plants, the effect could be due to having any *Opuntia* neighbor (density effect), or to specifically having an *O. stricta* neighbor (species effect). To address this, a second set of surveys were conducted at HI in 2009 with 100 *Opuntia* plants marked as follows: Group 1 - isolated *O. humifusa* (n = 20), Group 2 - conspecific pairs of *O. humifusa* (n = 20 pairs), and Group 3 - interspecific pairs (n = 20 pairs). Distances for "isolated" and "paired" groups were as described for 2006 surveys. All plants were surveyed in May, Jul, and Nov of 2009 with the same variables as in 2006 recorded, plus maximum height. Data were again highly non-normal, so similar methods were used for analysis. All variables except height and size were averaged over the 3 mo. As this survey encompassed the latter part of the *Opuntia* growth season, the change in height and the proportional change in number of live cladodes from May to Nov was used instead of the average. A non-parametric ANOVA by ranks was used to compare *O. humifusa* plants with plant group as the independent variable. Spearman's rank correlation was performed as previously described.

Experimental Plantings. Analysis of data from the observational surveys resulted in a "snapshot" assessment of associational effects. However, the time since establishment in a plant grouping was unknown for each plant and initial damage levels were not controlled for, so changes over time were not of primary interest for the observational surveys. In order to control for these factors *Opuntia* plants raised in a greenhouse at the University of South Florida Botanical Garden were used in an outplanting experiment. Undamaged cladodes from FDS were collected in spring of 2007 and a single cladode was planted in a 6-in (15 cm) pot using a mix of sand and potting soil. Plants were fertilized twice a year, and watered as needed. In Apr

of 2009 the experimental (greenhouse raised) plants were used to create 2 plant groupings at FDS. At the time of planting, the experimental *O. stricta* plants had fewer cladodes than the experimental *O. humifusa*. In order to keep the number of experimental cladodes similar for all groupings, different numbers of plants were used such that the total number of experimental cladodes per grouping ranged from 5-9. Group 1 was interspecific, consisting of 2 experimental *O. stricta* planted ≤0.5m from one naturally occurring *O. humifusa* (*n* = 18 groupings), and Group 2 was conspecific, consisting of 1 experimental *O. humifusa* planted ≤0.5 m from 1 naturally occurring *O. humifusa* (*n* = 16 pairs).; the use of 2 *O. stricta* per grouping for Group 1 kept the number of experimental cladodes within a smaller range. Plants were also marked for an isolated grouping, Group 3, which consisted of 15 naturally occurring isolated *O. humifusa* with distance criteria as described for observational surveys. Experimental plants were initially free of damage from *C. cactorum*, *D. confusus*, *C. v. aequoris*, and *G. hubbardi*, but because of an outbreak in the greenhouse, all experimental plants had *D. echinocacti*. Naturally occurring plants were initially free of *C. cactorum* larvae and had little or no old *C. cactorum* damage (maximum = 2 cladodes with old damage); initial damage levels of the 4 native herbivores varied as finding plants free of all types of native herbivores proved impossible. All plants were surveyed monthly from Apr 2009 to Nov 2009, and the same variables as in the 2009 observational survey were recorded. Although changes over time were of inherent interest, data could not be transformed to meet assumptions of parametric repeated measures techniques. Instead, the difference in height, the proportional difference in cladode number, and the difference in each herbivore-related variable between the Apr and Nov surveys was used. A non-parametric ANOVA by ranks was used to compare *O. humifusa* plants with plant group as the independent variable.

RESULTS

Mann-Whitney tests of all *O. stricta* versus all *O. humifusa* from the 2006 survey showed that *O. stricta* plants had significantly higher proportions of cladodes with larvae and old damage, and more eggsticks per cladode. *Opuntia humifusa* had higher proportions of cladodes with *C. v. aequoris, D. confusus*, and *G. hubbardi,* and were significantly larger, (as measured by number of live cladodes, Table 2). ANOVA by ranks for plant groups were significant for all variables except proportion of cladodes with *D. echinocacti*. However, pairwise comparisons revealed that this was driven almost entirely by interspecific differences. Isolated and paired *O. stricta* groups were not significantly different for any variables. The sole intraspecific differences between isolated and paired *O. humifusa* groups were for size (*P* = 0.005) and proportion of cladodes with *G. hubbardi* (*P* < 0.0001). For both of these variables, the isolated *O. humifusa* group had the highest value of the 4 groups and was significantly different than all other groups (Fig. 1).

No significant relationships between *C. cactorum* larvae and native insects were found in 2006.

The 2009 observational data were used to test for differences between isolated *O. humifusa* plants and those in conspecifc and interspecific pairs. Plants that were in interspecific pairs had the greatest proportion of cladodes with *C. cactorum* larvae and old damage (Figs. 2 and 3). The difference between groups was significant for old damage (*P* = 0.035), but was marginal for larvae (*P* = 0.080). Pairwise comparisons showed that *O. humifusa* plants from interspecific pairs had significantly more old damage than *O. humifusa* in

TABLE 2. RESULTS FROM A MANN-WHITNEY TEST FOR DIFFERENCES BETWEEN *OPUNTIA HUMIFUSA* (*N* = 94) AND *O. STRICTA* (*N* = 96). PLANTS WERE SURVEYED IN SEP AND DEC OF 2006 AND VALUES FOR ALL VARIABLES WERE AVERAGED ACROSS THE TWO SURVEYS BEFORE ANALYSIS.

Variable	*O. humifusa* rank	*O. stricta* rank	Standardized test statistic[a]	*P*
Total cladodes	104.27	86.91	-2.176	0.030
C. cactorum larvae[b]	82.45	108.28	4.54	0.000
C. cactorum old damage[c]	74.80	115.79	5.62	0.000
Eggsticks/cladode	92.50	98.44	2.46	0.014
D. confusus[b]	107.63	83.62	-5.04	0.000
G. hubarddi[c]	121.26	70.28	-7.19	0.000
C.v. aequoris[c]	121.30	70.23	-6.56	0.000
D. echinocacti[b]	97.85	93.20	-0.85	0.394

[a]Negative values of the standardized test statistic indicate that the variable was greater for *O. humifusa* than for *O. stricta*; positive values indicate it was lower for *O. humifusa* than for *O. stricta*.

[b]Recorded as the proportion of cladodes with the herbivore present.

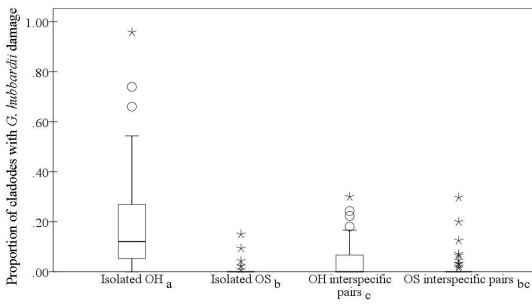[c]Recorded as the proportion of cladodes with characteristic damage.

Fig. 1. Proportion of cladodes with damage from the weevil *Gerstaeckeria hubbardi* for the 3 plant groups surveyed at HI in 2006. OH = *Opuntia humifusa*, OS = *Opuntia stricta*. Boxes indicate interquartile ranges, bold lines indicate medians, whiskers indicate inner fences, points indicate outliers, and asterisks indicate extreme values. Different lower case letters to the right of x-axis labels represent significant pairwise comparisons according to Dunn's test.

Fig. 3. Proportion of cladodes with old *Cactoblastis cactorum* damage from the 3 plant groups surveyed at Honeymoon Island State Park (= HI) in 2009. Boxes indicate interquartile ranges, bold lines indicate medians, whiskers indicate inner fences, points indicate outliers, and asterisks indicate extreme values. Different lower case letters to the right of x-axis labels represent significant pairwise comparisons according to Dunn's test.

conspecific pairs (adjusted P = 0.030), but neither group was significantly different than isolated *O. humifusa* (Fig. 3). Isolated *Opuntia humifusa* plants added significantly more height than *O. humifusa* in interspecifc or conspecific pairs (adjusted P = 0.004 for both pair wise comparisons). No other variables were significantly different among the 3 plant groups. The proportion of cladodes with *C. cactorum* larvae and *D. confusus* was negatively related on *O. humifusa* (r_s = -0.232, P = 0.038); *D. confusus* was not found on *O. stricta* plants in the 2009 survey. No other significant relationships between *C. cactorum* larvae and native insects were found.

Cactoblastis cactorum damage levels were extremely low at FDS, where the experimental plantings took place. Over the 7 mo, only 1 plant had larvae and only 1 eggstick was found. Eight plants showed old damage, but half of these were plants with 1-2 cladodes of pre-existing old damage. Therefore, measures of *C. cactorum* larvae, old damage, and eggsticks were not included in the analysis. Results from the ANOVA by ranks indicated significant differences between plant groups for change in height, proportional change in cladodes, and change in *D. echinocacti* damage (P = 0.016, 0.023, and 0.004, respectively). Further examination revealed that results for the latter 2 variables were driven solely by differences between experimentally planted *O. humifusa* and naturally occurring *O. humifusa*. The experimentally planted *O. humifusa* had a significantly higher proportional change in cladodes when compared with *O. humifusa* in conspecific and interspecific pairs and naturally occurring isolated *O. humifusa* (adjusted P = 0.000 for all 3 pairwise comparisons). Experimental *O. humifusa* also had a significantly larger change in the proportion of cladodes with *D. echinocati* as compared with all 3 groups of naturally occurring plants (adjusted P = 0.000 for all 3 pairwise comparisons). Experimental *O. humifusa* had *D. echinocacti* colonies present at the time of planting and the large decrease in cladodes with *D. echinocacti* reflects the insects' tendency to reach much higher densities in greenhouse or plantation settings than in naturally occurring *Opuntia* populations (Hunter et al. 1912; H. Jezorek personal observation).
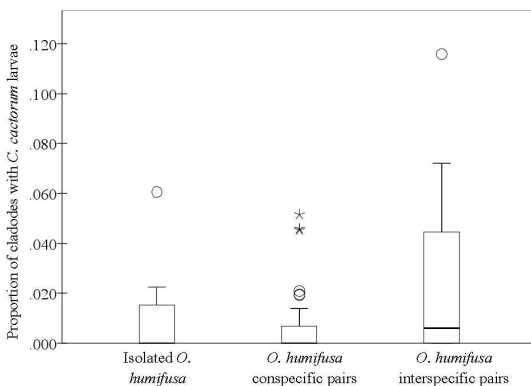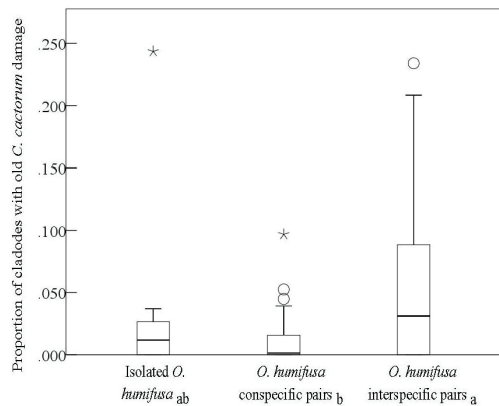

Fig. 2. Proportion of cladodes with *Cactoblastis cactorum* larvae from the 3 plant groups surveyed at HI in 2009. Boxes indicate interquartile ranges, bold lines indicate medians, whiskers indicate inner fences, points indicate outliers, and asterisks indicate extreme values.

DISCUSSION

The findings here are in agreement with previous reports of *O. stricta* being preferred by *C. cactorum* over *O. humifusa* (Baker & Stiling 2009; Jezorek et al. 2012); *O. stricta* plants had a higher proportion of damaged cladodes and cladodes with larvae present. In cases where a neighboring species is preferred by an herbivore, the expectation is of AS for the target species, provided that target species is an acceptable host (Brown & Ewel 1987; White & Whitham 2000, Barbosa et al. 2009). Contrary to this, little evidence was found of AS to *C. cactorum* for *O. humifusa* paired with *O. stricta*. The 2006 survey revealed no differences in *C. cactorum*-related variables between conspecific *Opuntia* in different plant groups. The 2009 survey produced only partial support for the prediction of AS in that *O. humifusa* plants paired with *O. stricta* had higher proportions of cladodes with *C. cactorum* larvae and old damage than did isolated *O. humifusa* plants or those paired with conspecifics. Although the result for larvae was marginal, it merits mention because observation of *C. cactorum* larvae is infrequent compared with observation of old damage. This results in lower power to detect significant differences in the proportion of cladodes with larvae. The difference for old damage between plants in interspecifc and conspecifc pairs is of particular interest because the comparison between these 2 groups controls for density of host plants, making it more likely that the observed difference was due to diversity of hosts (that is, to the specific effect of *O. stricta*). However, a significant difference between interspecific pairs and isolated plants, which would have strengthened the support for AS, was not observed.

Opuntia humifusa was found to have significantly more cladodes with the native insects *C.v. aequoris, D. confusus,* and *G. hubbardi* than *O. stricta*. Although the reason for *C. cactorum*'s preference for *O. stricta* over *O. humifusa* is unknown, native herbivores may play a role. Plants that have actively feeding herbivores, or eggs of herbivores, on them can produce feeding- or oviposition-induced surface compounds and volatiles that serve to repel gravid females from oviposition (Blaakmeer et al. 1994; Harmon et al. 2003; Hilker & Meiners 2011). *Cactoblastis cactorum* females are known to respond to slight variations in the CO_2 concentration at the boundary layer of plants, a behavior hypothesized to enable them to choose more actively growing, healthier plants for their offspring (Stange 1997; Stange et al. 1995). It is also possible that they respond to induced surface compounds or volatiles to avoid *Opuntia* individuals that are relatively more damaged and seek out individuals that are relatively less damaged. Given that 3 of the native insects examined were found in higher levels on *O. humifusa* than

O. stricta, such behavior in this system would result in *C. cactorum* females choosing an *O. stricta* individual more often than an *O. humifusa* individual. Avoidance of damaged plants could also explain the negative relationship between *C. cactorum* and *D. confusus* found for *O. humifusa* during the 2009 survey, although controlled experiments would be required to determine which insect is responsible for deterring the other.

If, based on our findings, one assumes *G. hubbardi* prefers *O. humifusa* over *O. stricta*, the expectation would be of AR for *O. humifusa* when paired with *O. stricta* (Barbosa et al. 2009). Indeed, the 2006 data showed that isolated *O. humifusa* plants had a higher proportion of cladodes with *G. hubbardi* damage than did *O. humifusa* in interspecific pairs. In theory, this could result from *O. stricta* acting as a repellent/masking plant (Atsatt & Odowd 1976) by interfering with the ability of *G. hubbardi* to locate its preferred host, or from spillover from *O. humifusa* onto *O. stricta* (Agrawal et al. 2006; Barbosa et al. 2009). If the mechanism at work were repellency/masking, one would not necessarily expect a difference between isolated *O. stricta* and those in interspecific pairs. However, if spillover were occurring, one would expect lower levels of *G. hubbardi* on isolated *O. stricta* than on *O. stricta* in interspecific pairs, as it would be receiving AS from being paired with *O. humifusa*. The actual results cannot distinguish the mechanism for 3 reasons. First, although *O. stricta* in pairs did have higher *G. hubbardi* damage than isolated *O. stricta*, the difference was not significant. Second, conspecific pairs of *O. humifusa* were not marked in 2006, so a density effect, whereby *G. hubbardi* damage is diluted or spread out when host plants are more densely arrayed, cannot be ruled out. Third, no evidence for associational effects of *G. hubbardi* from the 2009 survey or from the experimental plantings was found.

There are several possible explanations for the lack of evidence for AS/AR, one of which concerns the distance criterion for determining plant groupings. Russell et al. (2007) found that egg loads of *Rhinocyllus conicus* Frolich on the native thistle *Cirsium undulatum* (Nutt.) Spreg. decreased significantly as distance from *Carduus nutans* L., the invasive musk thistle, increased. Similarly, White and Whitham (2002) found that both cankerworm densities and defoliation rates on cottonwoods decreased as a function of distance from box elder, the worm's preferred host. Distances in this study were based on the behavior of *C. cactorum*, as documenting AS to this herbivore was the primary objective. Larvae are able to disperse very short distances, but only do so when no cladodes remain on their original host (Dodd 1940). Females are reluctant to fly, and several studies have shown that previously attacked plants tend to have higher densities of

eggsticks than unattacked plants (Dodd 1940; Myers et al. 1981; Robertson 1987a), suggesting that first oviposition often takes place near the emergence site. Females will disperse over larger distances, but only as hosts become sparser. Dodd (1940) reported the longest known dispersal of a female as 24 km, while a recent flight mill experiment found the maximum total distance flown by females was 22 km (Sarvary et al. 2008). It was thought that plants > 5 m from another *Opuntia* would be sufficiently isolated, but it is possible that associational effects may be more readily detected by using a larger distance between isolated and paired plants. Stiling et al. (2004) found no significant difference in *C. cactorum* attack rates for *O. corallicola* plants ≤ 5m from an *O. stricta* and those > 20 m from an *O. stricta*. However, they found that *O. corallicola* plants that were ≥ 500 m from an *O. stricta* suffered no attacks by *C. cactorum*. Perhaps the distance needed for a host plant to be truly isolated in terms of normal *C. cactorum* dispersal is more on the order of tens or hundreds of meters. If so, the strength of associational effects could vary spatially, appearing weak or undetectable at sites where few host plants meet such a large distance criterion, and stronger at sites with widely separated host plants.

The occurrence of a spillover effect depends on the herbivore in question reaching a high enough density to actually begin using the less preferred host. For example, White & Whitham (2000) note that AS of cottonwoods, because of spillover from the attractant "sink" plant, box elder, was only seen in areas with high cankerworm densities. In areas with low densities, cankerworm completed their full life cycle on box elder. Although *C. cactorum* and native insect damage was present on both *Opuntia* species, the density of these herbivores during this study may have been too low to reveal significant correlations between species or to result in spillover onto their less preferred hosts. In the future, if the density of any of the herbivores increases, or the density of one of their host species decreases, associational effects could be more readily documented. A recent study (Jezorek et al. 2012) showed that mortality of *O. stricta* is higher than that of *O. humifusa*, and that *O. humifusa* plants are more likely to survive a *C. cactorum* attack. If *O. stricta* plants are dying faster than they are being recruited, they may become scarce enough to consistently suffer complete "defoliation" by *C. cactorum* within a single generation, a phenomenon that is currently rare. If this were the case, *O. humifusa* plants close to *O. stricta* could end up as the most likely oviposition sites for the next generation of moths.

The phenomenon of AR and AS for mixtures of host plants with shared herbivores has been fairly well-documented. Despite this, only weak support was found for the prediction of AS to *C.* *cactorum* for *O. humifusa* plants near *O. stricta* and no evidence for AR from or AS to 4 native opuntioid-feeding insects. The lack of support can be attributed in part to the factors of host plant and herbivore density. Studies performed at locations with relatively high *C. cactorum* densities would provide valuable information about how associational effects change with varying host and herbivore densities.

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