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Agronomy of strip intercropping broccoli with alyssum for biological control of aphids

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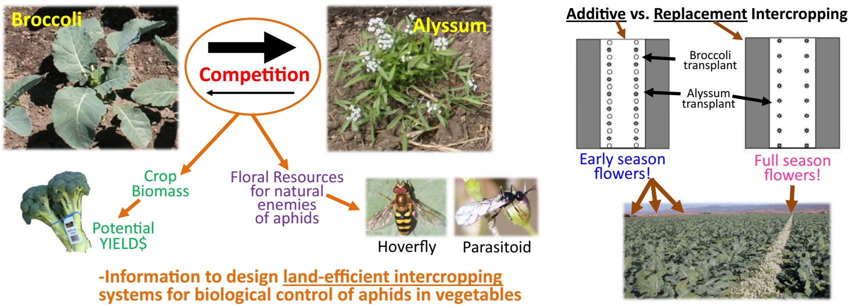
HIGHLIGHTS

- Broccoli was much more competitive than alyssum and produced more shoot dry matter.
- Alyssum flower counts increased linearly with alyssum plant size.
- Alyssum transplants produced more flowers per transplant on beds without broccoli.
- Bed sections with only alyssum are recommended for all-season floral resources.
- Additive intercropping is recommended to efficiently provide early-season flowers.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic broccoli growers in California typically control aphids by intercropping broccoli with strips of alyssum (*Lobularia maritima* (L.) Desv.) which attracts hoverflies (Diptera: Syrphidae) that are important predators of aphids. A three year study with transplanted organic broccoli in Salinas, California evaluated agronomic aspects of broccoli monoculture (B100) and broccoli-alyssum strip intercropping on beds in replacement intercropping treatments where alyssum transplants replaced 4 or 8% of the broccoli transplants, and an additive intercropping treatment (B100 + A100) where alyssum transplants were interspersed between broccoli without displacing it. The replacement patterns included alyssum planted on both lines of a bed (A100), beds with 50% broccoli and 50% alyssum transplants in different lines (B50A50D), and beds with 50% broccoli and 50% alyssum alternating in the same lines (B50A50S). To evaluate competition, shoot dry matter (DM) of alyssum and broccoli was measured at 36–43 days (harvest 1) and 59–66 days (harvest 2) after transplanting, and alyssum flowering was assessed at both harvests. The treatments performed consistently across years. The number of flowering alyssum shoots was highly correlated with alyssum DM. Per alyssum transplant, alyssum DM was highest in A100 and B50A50D at harvest 1, and by harvest 2 (3–7 days before broccoli maturity) was in order of A100 > B50A50D > B50A50S = B100 + A100. Broccoli was much more competitive than alyssum and by harvest 2 produced larger broccoli shoots per transplant in B50A50S (122 g) and B50S50D (96 g) than the more ideally sized shoots (73 g) in B100 and B100 + A100. The A100 pattern may be the most efficient replacement intercropping strategy to provide hoverflies and parasitoids with floral resources through the whole season, however, additive intercropping may also be useful to augment floral resources early in the season without displacing broccoli. These results can help growers reduce the cost of alyssum intercropping in high-density broccoli systems (>100,000 transplants per ha). The practical management implications and future research needs to further improve the efficiency of these systems are discussed.

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1. Introduction

After lettuce (*Lactuca sativa* L.), broccoli (*Brassica oleracea* L. var. *italica* Plenck) is the most economically important vegetable grown in the Salinas Valley (Monterey County) on the central coast of California, with an annual production value of more than U.S. $400 million harvested from 26,538 ha (Monterey County Agricultural Commissioner, 2013). While broccoli and other crucifer vegetables are widely known for their anticancer health benefits to humans (Verhoeven et al., 1997), growing broccoli in rotation with lettuce and strawberry (*Fragaria* × *ananassa* Duch.) may also improve soil health by suppressing soil-borne diseases (Hao et al., 2003; Subbarao et al., 2007). With the dramatic growth of the organic industry in the Salinas Valley from a production value of approximately $11 million in 1994 to $274 million in 2014 (Monterey County Agricultural Commissioners, 1999, 2014), there has been increased research on intercropping vegetables with insectary plants to enhance biological control of aphids (Brennan, 2013; Chaney, 1998; Gillespie et al., 2011; Smith and Chaney, 2007). However, these previous studies were all focused on lettuce. The only known intercropping research to control aphids in other vegetables in Salinas Valley was with living mulch cover crops in broccoli that reduced aphid infestations (Costello, 1994; Costello and Altieri, 1995); however, this practice has not been adopted here.

Insectary plants attract beneficial insects into fields and provide floral resources (pollen and nectar) that these insects need to survive and reproduce, and which contributes to biological control of pest insects (Parolin et al., 2012). This is a form of conservation biological control that can make highly disturbed agroecosystems more hospitable environments for natural enemies of agricultural pests (Jonsson et al., 2008; Landis et al., 2000). Alyssum (*Lobularia maritima* (L.) Desv.) is a frequently studied plant for biological control in many agroecosystems (Araj and Wratten, 2015; Brennan, 2013; Fiedler et al., 2008; Gontijo et al., 2013) and is a popular insectary plant in California because it flowers quickly, attracts several beneficial insect species and few pests, and is not overly aggressive or likely to become a weed (Chaney, 1998). Hoverflies (Diptera: Syrphidae) are a common beneficial insect in California organic vegetable production (Bugg et al., 2008) and alyssum pollen is an important food for adult hoverflies in these systems (Hogg et al., 2011).

The cabbage aphid (*Brevicoryne brassicae* L.) is the primary insect pest of broccoli in Monterey County and its most common natural enemies here are a parasitoid wasp (*Diaeretiella rapae*, McIntosh) and aphidophagous hoverflies (Nieto et al., 2006). The most common approach that growers here use to control cabbage aphid in organic broccoli is to interplant broccoli with alyssum. Perennial hedgerows on field edges are also used on some organic farms in California to provide floral resources for a diversity of natural enemies of aphids and other pest insects (Brennan, 2015; Earnshaw, 2004; Gareau et al., 2013; Morandin et al., 2011). To increase the adoption and efficiency of vegetable-insectary intercropping for biological control of pest insects, farmers need basic agronomic information on growth characteristics of insectary plants that will maximize their flower production per unit of land area. This is especially true in regions like the Salinas Valley where high agricultural land rents ($3700–7400 per ha) limit the land area that farmers can allocate to insectary plants. Historically, 'replacement intercropping', whereby vegetable plants were replaced (i.e., displaced) by insectary plants in strips or scattered through the field, was the most common approach used; alyssum intercrops typically replace 5% of the broccoli in organic production systems (Tourte et al., 2004). However, research with lettuce found that additive intercropping, whereby insectary plants are inserted between lettuce plants without displacing them, was a far more land-efficient intercropping approach than replacement intercropping (Brennan, 2013) and effective for aphid control (Brennan, 2014).

A three year study was conducted in transplanted organic broccoli that was strip intercropped with 'replacement' and 'additive' arrangements of alyssum from July to September. The objectives were 1) to determine the relationship between alyssum shoot biomass and flower production in broccoli, 2) to evaluate competition between the intercropped plants by measuring their shoot biomass, and 3) to identify the most land-efficient intercropping strategies to maximize alyssum flower production in high density broccoli production (i.e., >100,000 plants per ha).

2. Methods

2.1. Site description, field preparation, and soil amendments

The experiment occurred at the USDA-ARS organic research farm in Salinas, CA (lat. 36.622658, long. −121.549172, elevation 37 m), where the soil is a Chualar loamy sand (fine-loamy, mixed, superactive, thermic Typic Argixerol). The site has been certified organic since 1999, and inputs described were allowable under the USDA National Organic Program. The experiment occurred in a 48 by 15 m area on the east side of a 0.9 ha field that has been in a long-term, commercial-scale trial (Brennan and Boyd, 2012b) with an annual rotation of romaine lettuce (May to June), broccoli (July to October), and winter cover crops (October to March), since 2003. Management details of the cover crops and lettuce that preceded the broccoli each year are in Brennan (2013). During the 23–33 d period between the harvest of the lettuce and transplanting of broccoli, the following field preparation occurred: (1) the lettuce residue was incorporated into the soil with standard tillage equipment as needed to promote decomposition, and peaked beds (101.6 cm wide) were formed, (2) urban yard-waste compost (C:N ≈ 22) was broadcast at approximately 7.6 Mg per ha (oven-dry basis) onto the beds and incorporated with a rolling cultivator, (3) pelleted organic fertilizer of chicken manure and feather meal (8N-1P-1K) was injected into the beds at rates of 133, 125, and 141 kg N per ha with a fertilizer applicator in two bands 27 cm apart, and approximately 15 cm deep on 13 July, 26 June, and 2 July, for 2007, 2008 and 2009, respectively, and (4) the peaked beds were then shaped with a bed harrow to produce a flat planting area on the bed top that was approximately 50 cm wide and 15 cm above the furrow bottoms (Fig. 1). These field preparation procedures are typical for commercial-scale, organic broccoli production in this region.

2.2. Experimental design and intercropping arrangement

The experimental design was a randomized complete block with 4 blocks of five treatments of interest including broccoli monoculture (B100) and four strip intercropping treatments. Each block was 10.2 m wide (10 beds) and 15 m long. The experimental unit for each treatment was a single bed with two transplant rows. In addition to the five treatments described here, each block contained five additional broccoli-alyssum intercropping treatments that were not of interest and were excluded from the analysis. As in similar research with lettuce (Brennan, 2013), the furrow between adjacent beds was considered an adequate buffer area to prevent competition between adjacent treatments. This assumption that plants on adjacent beds did not affect each other is reasonable up to the first harvest where the leaves from adjacent beds were not overlapping. Moreover, if there was any competition between plants on adjacent beds thereafter, this would not likely

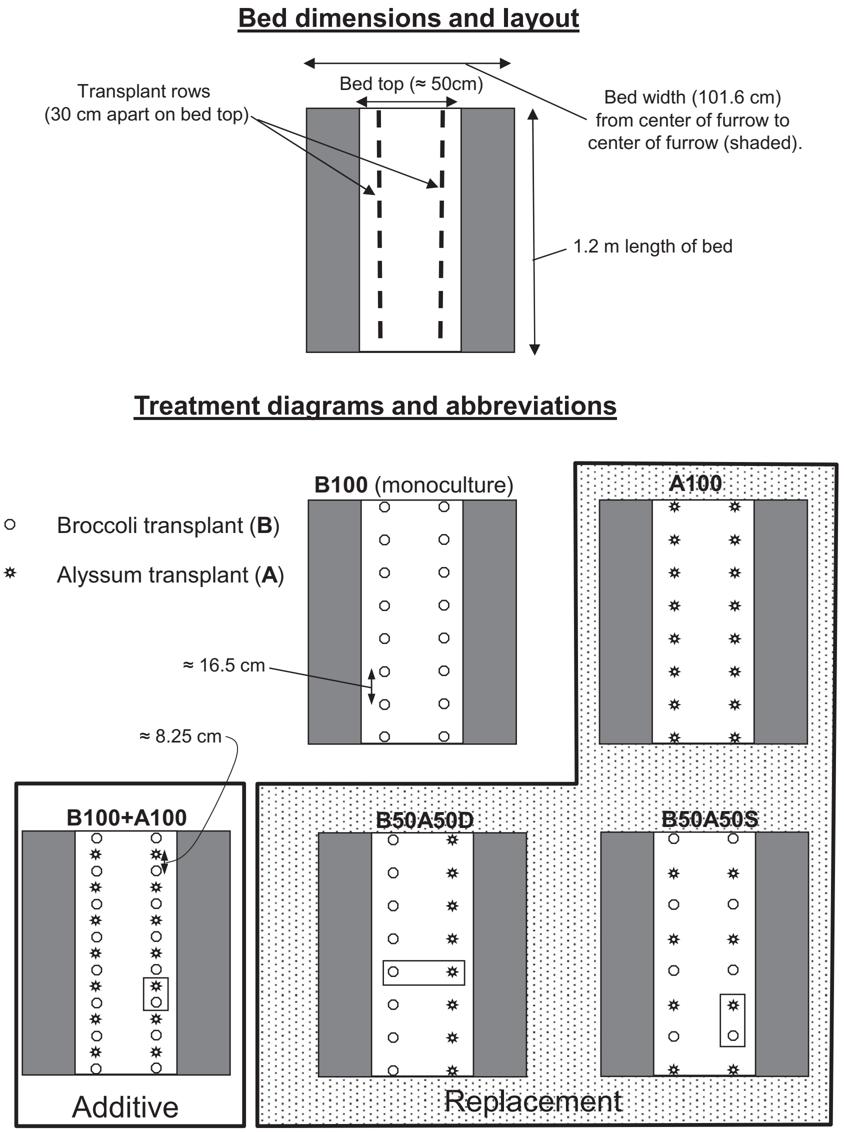

Fig. 1. Diagram illustrating the raised bed dimensions and layout of monoculture broccoli (B100) and 4 broccoli-alyssum strip intercropping treatments evaluated in Salinas, CA, during 2007, 2008 and 2009. Broccoli transplants contained one plant per transplant plug, but the alyssum transplants contained an average of 10–17 plants per plug. To illustrate the repeating pattern for each treatment, 8 plants at the standard broccoli spacing are shown within the 1.2 m length of bed; additional details on transplant density per ha are in Table 1. A square alignment of transplants in the two rows is shown, however, transplant alignment was not always square due to slippage of the press wheels of the transplanter that control the within row spacing for each row separately. The rectangles around pairs of alyssum and lettuce transplants in the intercropped treatments indicate the adjacent pair of plants that were harvested for above ground dry matter measurement on beds with alyssum and broccoli.

have occurred until near the end of the season and would presumably have a minimal overall effect on the results given that plants from both sides of all beds were harvested, and that the order of treatments within block were randomized each of the three years. It should be highlighted that in a typical commercial field, any of the treatments with alyssum could theoretically be positioned on the outside bed of a field, or inserted further into the field with broccoli on both sides. Thus, the experimental approach was considered a robust and valid way to evaluate various intercropping treatments.

Intercropping was evaluated in various patterns on two rows per bed to simulate strip intercropping as practiced in commercial organic broccoli fields in the region. The intercropping treatments included 3 ‘replacement’ treatments (A100, B50A50D, B50A50S) with the same total transplant density (118,788 transplants per ha) as B100, and an ‘additive’ treatment (B100 + A100) with the broccoli density of B100 plus additional alyssum transplants at 9697 transplants per ha, respectively (Fig. 1, Table 1). The treatment abbreviations refer to the percentage of broccoli (B) and alyssum (A) on the intercropped beds alone. The percentage of broccoli plants displaced by alyssum per ha in the replacement treatments ranged from 4% in B50A50 to 8% in A100 (Table 1). In this scenario, I assumed each intercropping treatment would occupy 8 evenly spaced beds in a 1 ha field containing 98 beds. For example, all 98 beds would be in broccoli in B100, whereas A100 would have 90 beds of broccoli and eight beds of 100% alyssum (i.e., one alyssum bed followed by 11 broccoli beds with the first alyssum bed at bed seven). The ‘D’ and ‘S’ of the B50A50 treatments indicate if the 50% broccoli and 50% alyssum ratio occurred on the same row (S) or different row (D) of the bed. Alyssum did not replace broccoli in the additive treatment, but instead was inserted between broccoli plants within the row. Therefore, the additive treatment abbreviation, B100 + A100, refers to the B100 plus (+) the percentage of alyssum in A100.

Table 1
Transplant density of broccoli and alyssum, and broccoli area displaced by alyssum in monoculture broccoli (B100) and 4 intercropping treatments evaluated over three years in Salinas, CA.

Treatment[b]	Transplant density[a]			Broccoli displaced (%)[c]
	Broccoli	Alyssum	Total	
	(Transplants per ha)			
B100	118,788	0	118,788	–
A100	109,091	9697	118,788	8
B50A50D	113,940	4849	118,788	4
B50A100S	113,940	4849	118,788	4
B100 + A100	118,788	9697	128,485	0

[a] Density assuming that the treatments were applied to eight beds in a 1-ha field containing 98, 100 m long beds (9800 m of total bed length) that were 101.6 cm wide. Due to rounding, totals may not equal the sum of broccoli plus alyssum.

[b] Treatment codes indicate the percentage of broccoli (B) and alyssum (A) in monocropped broccoli (B100), replacement intercropping treatments (A100, B50A50D, B50A50S), and an additive intercropping treatment (B100 + A100). Beds contained two rows, and the 'D' and 'S' of the B50A50 treatments indicate if the 50% broccoli and 50% alyssum ratio occurred on the same row (S) or different row (D) of the bed. B100 + A100 had one alyssum transplant within the row between each broccoli transplant.

[c] Percentage of broccoli transplants per ha that were displaced by alyssum transplants in the intercropping treatment compared with the B100 treatment.

2.3. Transplanting procedures

Transplants of 'Patron' broccoli (Sakata Seed America, Morgan Hill, CA, U.S.A.) and alyssum ('Sweet Alyssum', Kamprath Seed Inc. Manteca, CA, U.S.A.) were produced in a commercial greenhouse in 2.5 square by 5 cm deep cells in plastic trays for transplanting approximately 30–40 d later. Broccoli transplants contained one plant per cell, whereas alyssum transplants had an average of 17, 18, and 10 plants per cell in 2007, 2008 and 2009, respectively. In this paper, 'transplant' refers to a single broccoli plant grown in one cell or a group of 10–18 alyssum plants grown in one cell. Alyssum transplants for insectary plantings in this region typically contain multiple plants per transplant plug because seed singulation is not possible with small raw seed that is used for the automated system for seeding transplant trays; furthermore, raw alyssum seed is relatively inexpensive (approximately $30–55 per kg) and transplant plugs with multiple plants are easier to pull from the transplant tray (with less plant damage) and load by hand in a mechanical transplanter. A hand-loaded cell-type carrousel transplanter was used to transplant the broccoli in two rows (Fig. 1) at a spacing of 16.5 cm between transplants within a row on July 19, 17 and 24 in 2007, 2008, and 2009 respectively. After transplanting the broccoli on all beds, the 4 intercropping treatments with alyssum at various densities and arrangements (Fig. 1) were created by hand with a trowel as needed by replacing broccoli transplants with alyssum in the replacement treatments, or adding alyssum between broccoli in the additive treatment.

2.4. Post-transplanting management and climate

Sprinkle irrigation was applied immediately after transplanting but drip irrigation with a single drip tape line at the bed center was used as the primary irrigation method for the first 40 d after transplanting (DAT), after which a combination of drip and sprinkle irrigation were used. The drip tape was buried approximately 5 cm below the bed surface in 2007, but was on the soil surface the other years. Irrigation scheduling was based on daily evapotranspiration from the California Irrigation Management Information System (Station 89), and soil moisture sensors at 20 and 46 cm depth. No precipitation occurred during the trial and total irrigation was 332, 269, and 293 mm in 2007, 2008 and 2009, respectively. The climatic conditions were similar across years with average daily air temperatures of 14–17 °C, and average daily soil temperature of 22–24 °C. Liquid, fish-based fertilizers (6N-2P-0K, 5N-1P-1K) were applied through the drip tape at 15–43 DAT to bring the total rate of N applied (preplant + fertigation) to between 163 and 168 kg N per ha each year. Weeds were controlled with a tractor mounted cultivator once at 11–15 DAT, and hand-hoeing once at 18–25 DAT.

2.5. Plant sampling and alyssum flowering analysis

Above ground shoot dry matter (DM) was determined for broccoli and alyssum at 24–31 August and 16–28 September. These harvests were at 36–37, and 59 DAT in 2007, 42–43 and 61 DAT in 2008, and 39 and 66 DAT in 2009. The second harvest occurred 3–7 d before the broccoli in the remainder of the field was harvested by a commercial crew. For B100 and A100, the DM harvests included one transplant from both rows of each bed. A similar procedure was used to determine broccoli and alyssum DM in the treatments with both plant types by harvesting one adjacent alyssum-broccoli transplant pair from both rows of each bed (Fig. 1). Harvests occurred at least 1 m from the end of each bed and areas of beds with missing plants were avoided. Harvested plant tissue was oven-dried at 65 °C for at least 48 h until the weights had stabilized. Due to a shortage of drying oven space at the second harvest in 2009, the fresh weight of the 2 harvested broccoli plants for each plot was recorded, and one of these was randomly chosen for oven-drying to determine the percent dry matter that was used to estimate to dry matter of both harvested plants.

Prior to oven-drying the alyssum shoots from the final harvest in 2007 and 2008, the number of open inflorescences per alyssum transplant were counted to determine the relationship between alyssum transplant DM and the number of open inflorescences using regression analysis; open inflorescences were inflorescences (i.e., unbranched flowering stalks) with at least 1 open flower. The equation derived from the regression analysis was used to estimate the number of open inflorescences per ha and the number of open inflorescences per broccoli plant assuming the strip intercropping patterns for a 1 ha field described in 2.2 and Table 1.

At harvest 1 (2008) and harvest 2 (2007), prior to oven-drying, the number of open flowers on one randomly chosen flowering inflorescence was determined for each of the two harvested alyssum transplants for each treatment. This provided an average number of open flowers per flowering inflorescence across all replicates for each treatment, which was then multiplied by the number of flowering inflorescences for each transplant to estimate the number of open flowers per transplant at both harvests.

2.6. Statistical analysis

All analyses were conducted with SAS version 9.4 (SAS Inst. Cary, NC). Alyssum and broccoli DM data were checked to meet the assumptions of ANOVA and were transformed where necessary. Natural log transformation was used to homogenize the variance for alyssum DM at both harvests and for broccoli DM at harvest 1. The MIXED procedure was used for the ANOVA of alyssum and broccoli DM, whereby treatment, year, and their interaction were considered as fixed effects and block nested within year was a random effect. The MEANS procedure was used to calculate 95% confidence intervals (CI) of the response variables; for transformed variables, back-transformed means and CI are presented. As suggested by Drummond and Vowler (2011), I presented CI with the raw data graphically to illustrate the variability, skewness, and scatter of the data, and to provide a transparent and visual method to help readers make practical inferences.

Comparisons between treatment means with 95% CI within a harvest date can be made using the 'rule of eye' method whereby intervals that overlap with a mean are not different, and intervals that overlap by half of one interval arm are significantly different at $P \approx 0.05$ where sample sizes (n) are $\geqslant 10$ (Cumming, 2009); where n = 3, CI overlap can be 1 arm length for a significant difference of $P \approx 0.05$. Such comparisons are not adjusted to control the familywise error rate for multiple comparisons. Confidence intervals of the mean paired differences (i.e., effect sizes) between harvest 1 and 2 for alyssum and broccoli DM, and open alyssum flowers were calculated to illustrate the magnitude of change over time. Where CI are presented in text they are within square brackets, [ ]. The REG procedure was used to obtain the regression equation between open inflorescences and alyssum DM. Confidence intervals alone were used for comparisons between treatments of the number of flowering alyssum inflorescences per alyssum transplant at harvest 2 for 2007 and 2008, and the number of open flowers per alyssum transplant at both harvests.

3. Results

3.1. Alyssum shoot dry matter production

Alyssum shoot DM averaged across years at harvest 1 ranged from 4 g per transplant [3, 5; 95% CI] in the additive intercropping treatment to 14 g per transplant [9, 18] in A100 and there were clear differences between treatments and years (Fig. 2A); the lack of a significant treatment x year interaction ($F_{6,36} = 1.1$, $P = 0.4$) indicates that the treatments performed consistently across years. There was a general pattern of somewhat greater alyssum biomass during 2008, which is apparent in the raw data for that year (triangle symbols in Fig. 2A) and may have been because harvest 1 occurred later (42–43 DAT) in 2008 than in the other years (36–38 DAT). Despite the equal transplant density of alyssum and broccoli in B50A50D and B50A50S, averaged across years, alyssum was half as productive in B50A50S (mean, 6 g per transplants, [4, 7]) where it was planted in the same row with broccoli as in B50A50D where alyssum and broccoli occurred in different rows on the bed (mean, 12 g per transplant [10, 15]). The overlap in the CI for A100 and B50A50D suggests that alyssum grew equally well in these treatments up to harvest 1 (Fig. 2A).

The same general pattern for alyssum DM at harvest 1 also occurred at harvest 2, although the differences between treatments were even more apparent (Fig. 2B). From harvest 1–2, alyssum biomass more than doubled in A100 (27 g increase), compared with B50A50D (6 g increase), and the other treatments (B50A50S, 100B + 100A) where it did not change (Fig. 2C).

3.2. Broccoli shoot dry matter production

Broccoli shoot DM at harvest 1 ranged from an average across years of 25 g per transplant in B100 + A100 to 37 g in B50A50S, and the CI indicate that these two treatments differed from each other (Fig. 3A). There was also a clear pattern of lower broccoli DM during 2009 (see square symbols, Fig. 3A). Although the average DM of broccoli in B100 + A100 was lower than in B100, the overlapping CI suggest that broccoli was equally productive in both these treatments at harvest 1. At harvest 2, there were no apparent differences between years in broccoli DM that ranged from an average across years of 73–122 g per transplant and differed between several treatments (Fig. 3B). For example, broccoli DM was greatest in B50A50S, intermediate in B50A50D, and lowest in B100 and B100 + A100. Broccoli DM from harvest 1–2 more than doubled in all treatments with the largest change in B50A50S where there was an average increase of more than 80 g (Fig. 3C). At harvest 2, there was no evidence that additive intercropping affected broccoli growth because broccoli DM was essentially equivalent in B100 + A100 and B100.

3.3. Alyssum flowering

There was a positive, linear relationship between alyssum shoot DM and the number of open inflorescences per transplant (Fig. 4), and large differences between treatments that ranged from 2–204 open inflorescences per alyssum transplant in the additive treatment (B100 + A100) and A100, respectively. The linear relationship between alyssum DM and flowering agrees with previous work with alyssum-lettuce intercropping (Brennan, 2013).

The estimated average number of open alyssum flowers per alyssum transplant at harvest 1 ranged from 635 (A100 + B100) to 1980 in A100 (Fig. 5A), and despite the wide CI of A100, the majority of data suggest that A100 typically had at least twice as many open alyssum flowers as occurred in the other treatments at harvest 1. At harvest 2, there were clear differences between most treatments in the number of open alyssum flowers per transplant, with the greatest number of flowers in A100 (1863), followed by B50A50D (284), and B50A50S (20) and B100 + A100 (5) (Fig. 5B). There was strong evidence that the number of open alyssum flowers per transplant declined consistently by an average of more than 600 flowers for B50A50D, B50A50S and B100 + A100, whereas the wider CI of the difference for A100 provides little evidence of a change between harvests (Fig 5C). It is important to highlight that although alyssum DM in A100 increased by an average of 28 g per transplant from harvest 1–2 (Fig. 2C), the average number of open flowers per transplant differed relatively little between harvest 1 (1980 flowers) and harvest 2 (1863 flowers). This suggests that the regression equation between shoot DM and flowering at harvest 2 (Fig. 4) would not be an accurate way to predict the number of flowers at another time in the season. This is because the number of open inflorescences per transplant changed little between harvests as the inflorescences gradually elongated (accumulating DM) and shed mature seed from old flowers, while new flowers developed uninterruptedly on the distal end. From the point of first flower production a single unbranched alyssum flowering inflorescence of the 'Sweet' variety evaluated can elongate more than 70 cm while producing several flowers per cm of inflorescence (Brennan, unpublished data).

4. Discussion

4.1. Growth and competition in broccoli-alyssum versus lettuce-alyssum intercropping systems

Comparing the growth of alyssum in the present study with a similar study with romaine lettuce (Brennan, 2013) provides some useful insights on the competition and growth dynamics in vegetable-alyssum intercropping systems. For example, alyssum DM per transplant at harvest 2 in A100 (mean = 40, [33, 49]) with only alyssum on the bed in the present study with broccoli was similar to that for A100 intercropping in romaine lettuce (34, [27, 40] (Brennan, 2013), despite the much closer within-row spacing between alyssum transplants in the broccoli experiment ($\approx$16.5 cm) than in the lettuce experiment ($\approx$30 cm). This illustrates alyssum's growth plasticity and ability to be productive even when transplanted at lower densities in the A100 pattern. This is similar to how many cereal grasses are able to compensate for lower planting densities by producing more tillers or side shoots; however, the side shoots of cereals arise from the base of the plant below the soil surface, and the side shoots of alyssum arise at multiple points on the stem. This is an important and somewhat

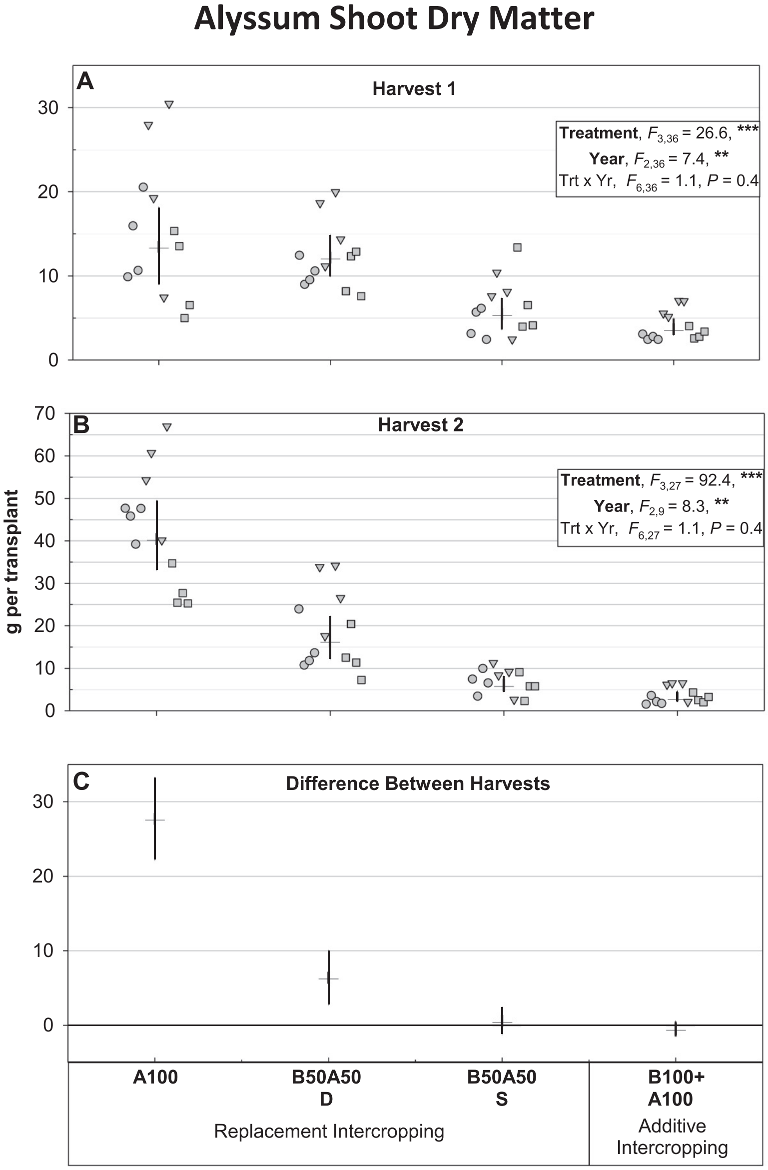

Fig. 2. Alyssum shoot DM production in four intercropping treatments in broccoli at two harvests (A, B) and their difference (C) during three years in Salinas, CA. Raw data points are symbol clusters around the mean and 95% CI across three years; means and 95% confidence intervals (CI) of the difference across years are also shown. Means and CI for alyssum DM averaged across years were back-transformed and were: A100 13.5 [9.0, 18.2], B50A50D 12.2 [10.0, 14.9], B50A50S 5.5 [3.7, 7.4], 100A + 100B 3.7 [3.0, 5.0] for harvest 1, and A100 40.4 [33.1, 49.4], B50A50D 16.4 [12.2, 22.2], B50A50S 6.0 [4.5, 8.2], 100A + 100B 3.0 [2.2, 4.5] for harvest 2. The means and CI for the difference between harvests were A100 27.7 [22.2, 33.2], B50A50D 6.4 [2.8, 10.0], B50A50S 0.6 [−1.2, 2.4], B100 + A100 −0.5 [−1.5, 0.5]. The raw data are in order from left to right for replicates 1–4 for 2007 (circles), 2008 (triangles), and 2009 (squares). Comparisons between treatments can be made using the 'rule of eye' method whereby intervals that overlap with a mean are not different, and CI that overlap by half of one interval arm are significantly different at $p \approx 0.05$ (Cumming, 2009). Harvest 1 occurred at 24–31 August (36–43 d after transplanting), and harvest 2 occurred at 16–28 September (59–66 d after transplanting). The box insert in A and B show the F statistics and significance of treatment, year and their interaction with significant effects in bold and where ** and *** are significant at the P < 0.01 and 0.001 levels, respectively. Fig. 1 provides more details on the intercropping patterns and treatment abbreviations.

unique characteristic of alyssum which makes it such as valuable insectary plant that can flower without interruption in vegetables like lettuce and longer season ones like broccoli.

The increased DM of broccoli in the B50A50S and D treatments compared with B100 at season-end, contrasts with the response of lettuce to alyssum in similar intercropping patterns (see Fig. 2B in Brennan, 2013). For example, lettuce in the same row as alyssum (i.e. L50A50S, where L = lettuce and A = alyssum) had equivalent final DM (≈50 g per transplant) as lettuce in L100 (lettuce on beds without alyssum). Whereas lettuce in a different row as alyssum (L50A50D) produced less DM (42 g per transplant). Furthermore, while there was no apparent difference in broccoli DM in the additive treatment (B100 + A100) versus B100, lettuce in a similarly additive intercropping treatment (L100 + A100) produced approximately 12 g less final DM than lettuce without alyssum (i.e. L100). Moreover, the alyssum shoot DM in A100 in broccoli was approximately 10 times greater than B100 + A100 near season-end, compared with A100 in lettuce that was approximately 2 times greater than in L100 + A100. These differences are likely due to the greater competitive ability of broccoli than lettuce over the season, and the closer within-row spacing in broccoli than lettuce. While the competitive ability of agronomic crops has received considerable research attention (Lemerle et al., 1996; Seavers and Wright, 1999; Worthington and Reberg-Horton, 2013), this is not the case with vegetables. I speculate that even at the same planting density, broccoli would have a greater competitive ability than lettuce

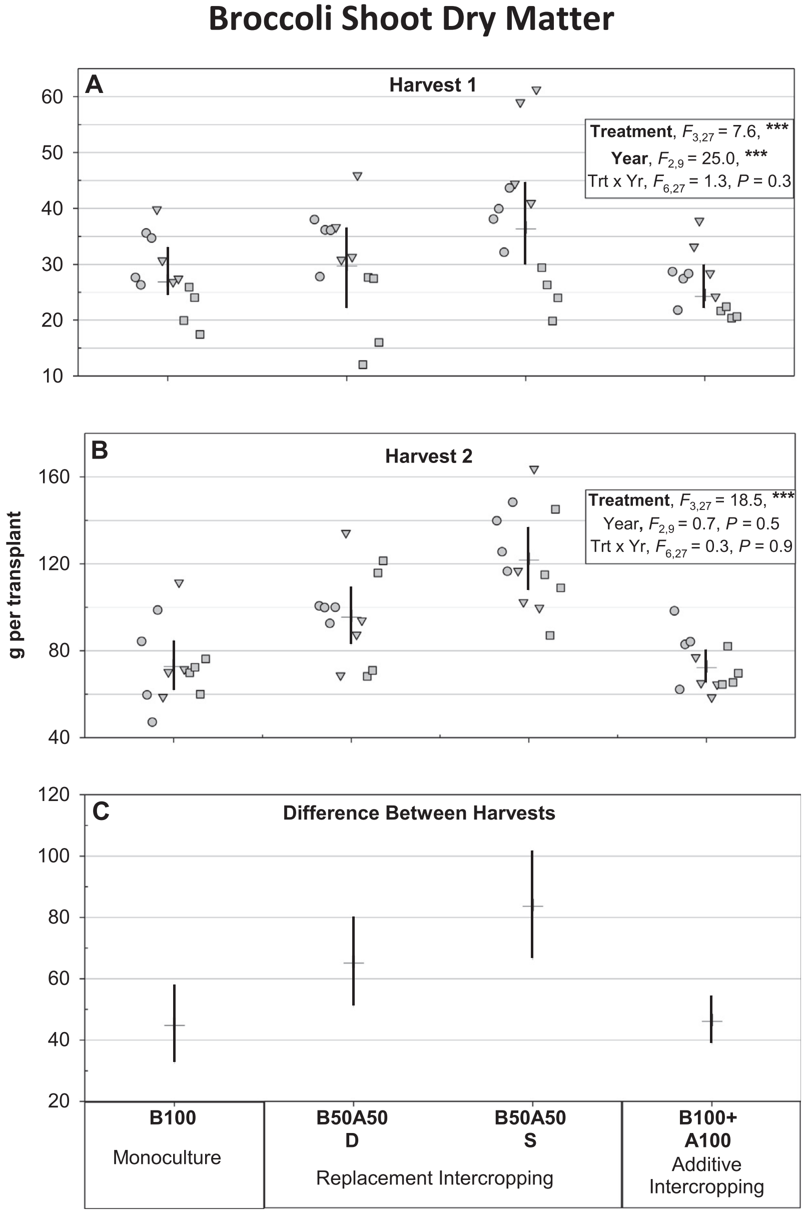

Fig. 3. Broccoli shoot DM production in monoculture broccoli and three intercropping treatments at two harvests (A, B) and their difference (C) during three years in Salinas, CA. Raw data points are symbol clusters around the mean and 95% confidence intervals (CI) across three years; means and 95% CI of the difference across years are also shown. Means and CI for broccoli DM at harvest 1 were back-transformed and were: B100 27.1 [24.5, 33.1], B50A50D 30.0 [22.2, 36.6], B50A50S 36.6 [30.0, 44.7], 100A + 100B 24.5 [22.2, 30.0]. Means and CI for harvest 2 were: B100 73.3 [61.9, 84.7], B50A50D 96.1 [82.9, 109.4], B50A50S 122.4 [108.0, 136.8], 100A + 100B 72.8 [65.2, 80.4]. The means and CI for the difference between harvests were B100 45 [33, 58], B50A50D 66 [51, 80], B50A50S 84 [67, 102], B100 + A100 47 [39, 54]. The raw data are in order from left to right for replicates 1–4 for 2007 (circles), 2008 (triangles), and 2009 (squares). Comparisons between treatments can be made using the ‘rule of eye’ method whereby CI that overlap with a mean are not different, and intervals that overlap by half of one interval arm are significantly different at $p \approx 0.05$ (Cumming, 2009). Harvest 1 occurred at 24–31 August (36–43 d after transplanting), and harvest 2 occurred at 16–28 September (59–66 d after transplanting). The box insert in A and B show the F statistics and significance of treatment, year and their interaction with significant effects in bold and where *** is significant at the $P < 0.001$ level. Fig. 1 provides more details on the intercropping patterns and treatment abbreviations.

because broccoli leaves are larger and more horizontal, and broccoli develops a taller canopy earlier.

4.2. Limitations, management implications, and practical application

A limitation of this study is that it does not provide information on optimal densities or arrangements of alyssum in broccoli to maximize biological control of aphids; the plant-insect interactions aspect of the intercropping systems were not possible to study in the relatively small area (48×15 m) of the field end where the study occurred. However, this study does provide practical and unique agronomic information on the growth and flowering dynamics of several potential broccoli-alyssum intercropping patterns that should be considered in efforts to design and evaluate land-efficient intercropping systems for broccoli. Designing reliable cropping systems for biological control of important pests such aphids is a complex process that will likely vary from site to site. Studies elsewhere (Berndt et al., 2002; Pfiffner et al., 2009) and the ongoing, commercial-scale, long-term systems experiment at this farm (Brennan and Boyd, 2012a) provide clear examples that providing an abundance of floral resources does not always ensure biological control of target pests. For example, an extremely heavy infestation of cabbage aphid occurred during the first broccoli crop in the long-term experiment

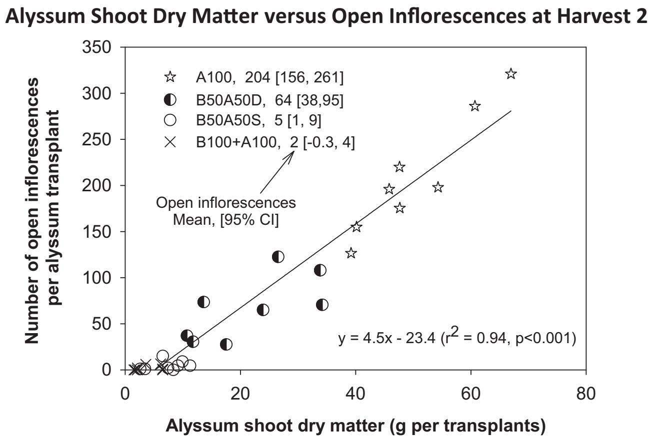

Fig. 4. Relationship between alyssum shoot dry matter and number of open inflorescences in four intercropping treatments of organic broccoli and alyssum in Salinas, CA in 2007 and 2008 at harvest 2 (59–61 d after transplanting). Data points represent the average of two transplants harvested from each of the four replicates each year. The numbers following the legend for each treatment are the mean and 95% confidence interval (CI) of the number of open inflorescences per alyssum transplant.

at the field of the present study in 2005. That year, alyssum was planted on 8 full beds interspersed among 40 beds of broccoli as also occurred with romaine lettuce during the first 2 years of this long-term experiment (Brennan, 2015). I speculate that the aphid problem, that rendered the broccoli unmarketable in 2005, was exacerbated by inadequate irrigation and fertility inputs. All subsequent commercial-scale broccoli crops at this site from 2006 to 2010 were marketable despite the reduction from 8 solid alyssum beds (i.e., A100) in 2005 and 2006, to 8 half beds of alyssum (i.e., B50A50D) in 2007–2010.

Of the 3 replacement intercropping patterns evaluated, the A100 pattern, where both lines on the bed were devoted to alyssum, appears to be the most efficient pattern overall for two main reasons. First, by 6 weeks after transplanting, A100 had produced approximately twice as many open alyssum flowers per alyssum transplant and thus per displaced broccoli transplant as both B50A50 patterns (Fig. 5A). Therefore, per alyssum transplant and per displaced broccoli transplant, A100 is the most cost-effective replacement pattern evaluated to provide floral resources for hoverflies and other important natural enemies of aphids on broccoli such as parasitoid wasps. Although the optimal number of alyssum flowers necessary to enable hoverflies to achieve adequate biological control of aphids in broccoli has not been studied, replacing (i.e., displacing) 1% of the broccoli transplants with alyssum (1% of 118,788; Table 1) using the replacement treatments would provide an estimated 2.4, 1.1, and 0.8 million open alyssum flowers per ha by 6 weeks after transplanting for A100, B50A50D, and B50A50S, respectively. Therefore to produce 5 million open alyssum flowers per ha by 6 weeks after transplanting would require displacement of 2520, 5520, and 7310 broccoli transplants using A100, B50A50D and B50A50S, respectively. Under these scenarios and assuming that each broccoli transplant produces one marketable head (i.e., flower bud), the A100 pattern could potentially produce 3000 more heads per ha than B50A50D (i.e., 5520–2520 = 3000) and 4790 more heads per ha than B50A50S. Organic broccoli produced from this research farm during the 3 years of this trial was sold for an average wholesale price of $13 per box for a 10 kg box with 14 bunches of heads per box. Assuming a typical bunch size of 3 heads per bunch, the A100 pattern could therefore potentially yield approximately $923 and $1481 greater gross sales than B50A50D and B50A50S, respectively; i.e., A100 produces 3000 more heads than B50A50D, 3000 heads ÷ 42 heads per box = 71 boxes at $13 per box = $923. A second major advantage of A100 over the other replacement treatment is that A100 is more likely to provide better broccoli plant size uniformity and thus more uniform heads through the field. The increased size of broccoli plants in both B50A50 patterns (Fig. 3B), approximately a week before the field was ready for commercial harvest, may hasten their maturity relative to the broccoli on beds without alyssum in the majority of the field. Scheduling the timing of broccoli harvests is far more complex than with vegetables like lettuce because broccoli tends to have much more variability of the maturity of the marketable component than occurs in lettuce (Brennan, personal observation). Therefore while lettuce is harvested in a single pass, most of the wholesale organic broccoli in this region is harvested in 2–3 separate passes over the field, several days apart, depending on the market price (Le Strange et al., 2010). Broccoli harvesting in California is costly (Dara et al., 2012; Le Strange et al., 2010; Tourte et al., 2004) and under typical market conditions for wholesale broccoli it would not likely be profitable to have to harvest mature heads from a relatively small portion of the field (i.e., 4%) with either B50A50 pattern in a separate pass before the rest of the field is ready to harvest.

In contrast to transplanted lettuce where additive intercropping with alyssum was recommended (Brennan, 2013, 2015), the additive intercropping approach alone is not as well-suited for broccoli because the vigorous broccoli plants, at the high density, suppressed alyssum growth and flower production especially during the later part of the season. Despite this intense competition, it is remarkable that alyssum in the additive pattern still produced relatively similar amounts of alyssum flowers as the B50A50 treatments up to the first 36–43 DAT (Fig. 5A). One potential strategy to efficiently utilize the additive approach in broccoli may be in conjunction with A100. For example, A100 could be applied on full beds or on several short sections of beds (i.e., 3–10 m long) to provide floral resources for beneficial insects through the whole season, and this could be augmented with an additive pattern, scattered through the field, specifically for early- to mid- season flower production. One way to place a large number of individual alyssum plants more evenly through a field using an additive pattern would be to insert individual alyssum transplants in a grid pattern (i.e., perpendicular to the bed direction, in one row, ≈every 10–20 m of row) as suggested for lettuce (Brennan, 2015). While hoverflies in vegetable fields are highly mobile (Gillespie et al., 2011; Wratten et al., 2003), distributing floral resources throughout the field early in the season may improve dispersion of adults through the field and thus facilitate aphid control evenly through the field. Even distribution of floral resources throughout a field may be more important for relatively small and potentially less mobile natural enemies of aphids such as parasitoids. Elegant research with the cabbage aphid parasitoid *D. rapae*, found that nectar provisioning close to aphids patches increased aphid parasitism, and retention and recruitment of parasitoids (Jamont et al., 2014). Furthermore, simulations suggest that scattering floral resources randomly through fields will increase parasitoid survival and aphid parasitism particularly early in the season (Vollhardt et al., 2010). From an anthropomorphic perspective, this combined strategy suggested here may be considered to provide evenly dispersed “temporary cafeterias” for natural enemies early in the season from the additive pattern, while A100 would provide food in the more “stable cafeterias” through the whole season after the temporary cafeterias have ‘gone out of business’ (i.e., been shaded out by the broccoli). An important advantage of this combined approach is that it could reduce the overall amount of alyssum residue to be incorporated into the soil at season-end because the additive alyssum would produce relatively little residue. A disadvantage to additive intercropping is that the alyssum would need to be inserted by hand between broccoli plants rather than by using a mechanical transplanter. However, additive

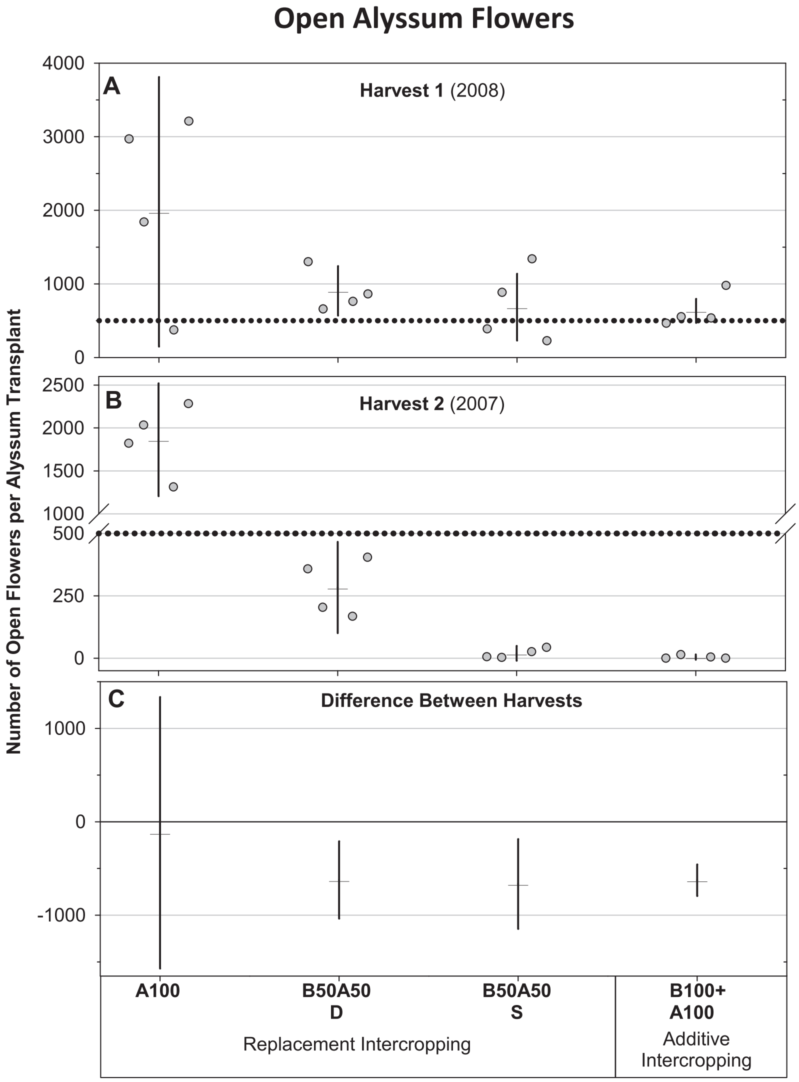

Fig. 5. Open flowers of alyssum per alyssum transplant in four intercropping treatments at two harvests (A, B) and their difference (C) during two years in Salinas, CA. Raw data points are symbol clusters around the mean and 95% confidence intervals (CI). The raw data are in order from left to right for replicates 1–4. Means and CI for open flowers at harvest 1 were: A100 1980 [148, 3812], B50A50D 906 [570, 1242], B50A50S 684 [230, 1138], 100A + 100B 635 [472, 798]. Means and CI for harvest 2 were: A100 1863 [1206, 2519], B50A50D 284 [100, 467], B50A50S 20 [−10, 49], 100A + 100B 5 [−6, 15]. The means and CI for the difference between harvests were A100 −118 [−1570, 1335], B50A50D −623 [−1036, −209], B50A50S −665 [−1145, −184], B100 + A100 −625 [−794, −457]. Comparisons between treatments can be made using the 'rule of eye' method whereby intervals that overlap with a mean are not different, and CI that overlap by half of one interval arm are significantly different at $p \approx 0.05$ (Cumming, 2009). Harvest 1 (A) occurred at 28–29 August (42–43 d after transplanting), and harvest 2 (B) occurred at 16 September (59 d after transplanting). Note that the scale of the y-axis differs between harvests and also in the upper and lower parts of the break in the y-axis for harvest 2. The horizontal dotted line at 500 flowers per transplant (A, B) is a reference to facilitate comparisons between harvests. Fig. 1 provides more details on the intercropping patterns and treatment abbreviations.

intercropping may be worthwhile if it allows growers to temporarily increase the number of alyssum flowers in a field without displacing broccoli. Furthermore, this additive pattern alone may be a low-cost, worthwhile way to augment floral resources for beneficial insects in conventional broccoli systems where the heavy use of broad spectrum insecticides is discouraged (Le Strange et al., 2010).

The radical difference between alyssum DM and flower production at season-end in the B50A50 patterns that differed only in the arrangement of the broccoli and alyssum (Figs. 2B, 4), contrasts with the results of my research with similar 50:50 patterns in romaine lettuce (Brennan, 2013). For example, in 50% romaine lettuce to 50% alyssum intercropping on the same bed, alyssum growth and flowering did not differ markedly when the alyssum was in the same or different line of the bed. Furthermore there was a relatively small difference in the number of open alyssum inflorescences per alyssum transplant in A100 beds in lettuce (168) versus an additive arrangement (L100 + A100) in lettuce (116), compared with the 100 fold difference (204 versus 2) between the number of open inflorescences per alyssum transplant in A100 in broccoli versus the additive broccoli alyssum pattern (Fig. 4). This difference in the performance of alyssum in broccoli in the present study versus alyssum in lettuce, illustrates the complexity of designing efficient intercropping systems in vegetables like lettuce and broccoli that differ in several regards (i.e., planting density, canopy structure, harvested product, and crop duration). Therefore, the results presented here are most directly applicable to broccoli production in common high density systems (>100,000 plants per ha) in California and Arizona, but not to lower density broccoli systems used elsewhere (Schellenberg et al., 2009; Ward et al., 2015; Warren et al., 2015).

For several years organic vegetable growers in California have relied heavily on alyssum as a dependable and relatively pest-free insectary plant in numerous crops. However, the recent discovery of an exotic stink bug (*Bagrada hilaris* Burmeister) in

California and neighboring States (Huang et al., 2014a; Lambert and Dudley, 2014) is concerning because alyssum, which is a brassica, is a host of this serious insect pest of several economically important brassica vegetables (Huang et al., 2014b; Joseph, 2014). This highlights the need for future research on non-brassica insectary plants for broccoli and other vegetables.

In conclusion, this study provides the first information on agronomic aspects of intercropping high-density, transplanted organic broccoli with alyssum for biological control of aphids. The A100 replacement intercropping pattern was the most efficient way to maximize alyssum flower production throughout the broccoli growth period and also provided the most alyssum flowers per alyssum transplant. The other replacement treatments with 50% broccoli and 50% alyssum transplants in different lines of the bed (B50A50D), or alternating in both lines on the bed (B50A50S) differed from each other with more alyssum DM in B50A50D, and more broccoli DM in B50A50S. However, both B50A50 patterns may be problematic because they increased broccoli plant size compared with broccoli on beds without alyssum (B100), which may complicate the timing of harvest. The additive intercropping pattern (B100 + A100) may be worthwhile to add floral resources to fields early in the season without displacing broccoli. More research is needed (1) to determine the optimal number of open alyssum flowers per ha that are necessary at various stages of the season to provide consistent biological control of aphids in broccoli, (2) to understand the movement of hoverflies and other natural enemies of aphids into and within fields from flowering hedgerows and whether biological control of aphids is influenced by various combinations of A100 and additive intercropping, and (3) to evaluate other non-brassica insectary species for intercropping with broccoli and other important vegetable crops.

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