# Effects of crop diversification levels and fertilization regimes on abundance of *Brevicoryne brassicae* (L.) and its parasitization by *Diaeretiella rapae* (M'Intosh) in broccoli

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**Abstract**

1 The effects of intercropping via competition on crop yields, pest [cabbage aphid *Brevicoryne brassicae* (L.)] abundance, and natural enemy efficacy were studied in the *Brassica oleracea* L. var. *italica* system.

2 From May to December 2004, insect populations and yield parameters were monitored in summer and autumn in broccoli monoculture and polyculture systems with or without competition from *Brassica* spp. (mustard), or *Fagopyrum esculentum* Moench (buckwheat), with addition of organic (compost) or synthetic fertilizer.

3 Competition from buckwheat and mustard intercrops did not influence pest density on broccoli; rather, aphid pressure decreased and natural enemies of cabbage aphid were enhanced in intercropping treatments, but this varied with the intercropped plant and season (summer vs. autumn).

4 In compost-fertilized broccoli systems, seasonal parasitization rates of *B. brassicae* by *Diaeretiella rapae* (M'Intosh) increased along with the expected lower aphid pressure compared with synthetically fertilized plants.

**Keywords** Cabbage aphid, crop yield, indirect effects, insect abundance, intercropping, natural enemies, nitrogen, organic fertilization, plant competition.

## Introduction

*Brassica oleracea* L. var. *italica* (broccoli) has been extensively used to investigate the role of crop diversification (e.g. intercropping) in reducing insect pest pressure, and the mechanisms accounting for such reduction (Hooks and Johnson, 2003): among the mechanisms are reduced pest colonization rate, reduced pest tenure time, oviposition interference, and increased mortality due to predators and/or parasitoids. These mechanisms underlie the natural enemy hypothesis (enhanced activity of natural enemies that reduces pest numbers in more diverse systems) and resource concentration hypothesis (a more diverse flora impairs the ability of insect pests to find and utilize its host plant) to explain lower pest populations in diversified cropping systems (Root, 1973).

The indirect role that inter-plant competition in intercropped systems plays in pest levels and dynamics has not been explored. Additive intercropping design experiments (i.e. the target crop has the same density in monoculture and in polyculture) introduce interspecific plant competition that impacts crop growth that may indirectly influence herbivore levels (Bukovinszky *et al.*, 2004). The role this plays has not been clearly elucidated because the widely-used additive designs incorporate the effects of plant competition that have not been separated from the effects of the intercropping on pest levels (Hooks and Johnson, 2003). In addition, cultural methods such as crop fertilization can affect pest pressure, and further confound the intercropping experiments and the interpretation of the results. It is known that soil fertility management may affect plant quality and may therefore affect insect pest abundance (Klostermeyer, 1950). Research shows that organically fertilized crops generally exhibit lower densities of several insect herbivores. Such reductions are commonly observed on organically grown crops, but the direct linkage to fertility is confounded by the increased abundance of natural enemies compared with conventional practices (Altieri *et al.*, 2005).

*Brassica* crops including broccoli are cultivated year round in the moderate climatic zones of the central California coast, and are attacked by cabbage aphid *Brevicoryne brassicae* (L.)

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(Homoptera: Aphididae), which is a specialist on the Brassicaceae. The aphid is an economic pest on broccoli because it infests the developing floral buds rendering the head they attack unmarketable. Cabbage aphids are attacked by the polyphagous parasitoid *Diaeretiella rapae* M'Intosh (Hymenoptera: Braconidae).

In the present study, an additive intercropping design experiment with broccoli as the target plant is used to test our hypothesis that crop diversity influences aphid abundance and parasitization in the absence of plant interspecific competition, and that such influence is mediated by the application of organic (compost) vs. synthetic fertilizer.

## Materials and methods

### Study site and design

This study was conducted at the University of California Agricultural Research Station (Albany, CA) (37°53′N, 122°19′W; 2 m a.s.l.). From May to December 2004, we replicated the same experiment twice: (1) May to August (summer) and (2) August to December (autumn).

The experiment was a two-way factorial (5 × 2; cropping system × fertilizer) in a completely randomized design, where the ten factor-level combinations (treatments) were replicated three times and applied to experimental plots of 3 m × 3 m size. Plots were separated by 1 m of bare soil.

The first factor consisted of five cropping systems: broccoli monoculture (Fig. 1A); broccoli intercropped with mustard, *Brassica* spp., with or without competition; and broccoli intercropped with buckwheat, *Fagopyrum esculentum* Moench, with or without competition. The additive design kept broccoli levels constant (ten sampled plants per plot; Fig. 1, dash circumscribed plants) using two spatial arrangements that introduced intercropping and/or interspecific competition (Fig. 1B,C). A total of 300 broccoli plants were sampled on each sampling date. Organically grown, 6-week-old broccoli seedlings (cv. 'Heritage', Santa Fe Nursery Inc., Salinas, California) were transplanted into plots on a 0.5 × 0.5 m row × plant grid (approximately 54444 plants/ha) on 22 May and 25 August. Intercropped plots were planted mid row with mustard or buckwheat (Peaceful Valley Farm Supply, Grass Valley, California) at the time the broccoli were transplanted in patterns that either increased (Fig. 1B) or did not affect (Fig. 1C) interspecific competition on the sample plants.

The second factor consisted of two types of fertilizer: synthetic fertilizer or compost applied at the same rate of 100 kg N/ha. The synthetic fertilizer (Best Sulphate of Ammonia, Pursell Industries Inc.) was incorporated into the soil in a small hole close to each broccoli plant immediately after transplanting. The compost (Grover Landscape Services) was added to the hole with a broccoli seedling. All of the plants were drip irrigated.

Because of the taller growth form of mustard and buckwheat, these intercropped plants were pruned 1 month after planting to avoid excessive shading and level of competition on slower growing broccoli. All plots and the 1-m interplot border were maintained weed-free by hand weeding.

### Sampling

All samplings and measurements were performed on five randomly selected broccoli plants per plot out of the 10 included in the plot sampling area (Fig. 1). Aphid and mummies were counted directly on three leaves per plant at 1-week intervals from 21 June, 30 days after transplanting (DAP), until 20 July in the first experiment, and between September 24 (30 DAP) and October 22 in the second experiment. Parasitization rates were assessed based on the number of mummified aphids: percentage parasitism = total parasitized aphids (immatures and apterous adults) × 100/ parasitized and nonparasitized aphids. Sampling started when the broccoli plants were well developed and intercrops plants had begun bloom. Broccoli plant height was also measured weekly as an indicator of interspecific competition. At the end of the season, the wet weight of broccoli head and plant (cut at ground level) was estimated on five plants per plot using an electronic balance (± 1 g).

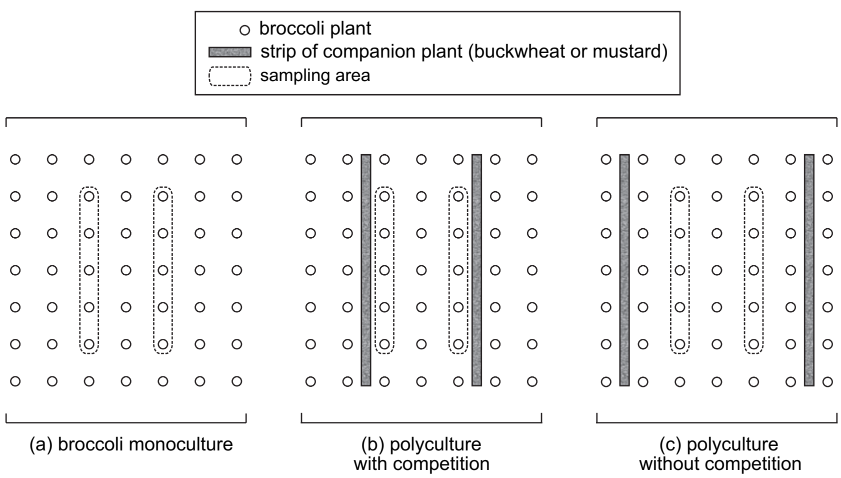


**Figure 1** Schematic representation of the additive intercropping design used to separate effects of crop diversity from the effects of competition.

### Statistical analysis

Because weekly aphids counts needed transformation to fit parametric assumptions, the cumulative number of counts per plot were used as measure of season long aphid pressure allowing the analysis of untransformed data. Seasonal parasitization data were also used for consistency.

The untransformed aphid data were analysed by two-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) as a post-hoc test when appropriate. Multiple regression analysis was performed on aphid data (Venables and Ripley, 2002; Faraway, 2004). The multiple regression analysis used dummy variables (values of 0 or 1 for absence or presence, respectively) for compost (*O*), mustard (*M*), buckwheat (*B*), and competition (*C*) to assess their influence on dependent variable, and the analysis used a stepdown method (Venables and Ripley, 2002) retaining only variables with slopes significantly greater than zero. Insect counts were analysed as counts per plot of five plants and three leaves per plant. Data were analysed using R statistical software (R Development Core Team, 2004; Faraway, 2004).

## Results

### Broccoli yield

In the summer experiment, cropping system did not significantly impact broccoli head biomass (ANOVA, $F_{4,20} = 1.24$, $P = 0.32$) and plant weight ($F_{4,20} = 1.28$, $P = 0.30$) (Fig. 2). In the autumn experiment, cropping system had a significant influence on both broccoli head ($F_{4,20} = 8.08$, $P < 0.001$) and plant weight ($F_{4,20} = 20.73$, $P < 0.0001$), reaching highest values in monoculture and buckwheat polyculture without competition (Fig. 2).

In summer, fertilization regime did not significantly influence broccoli head biomass ($F_{1,20} = 0.01$, $P = 0.91$, Fig. 3), but broccoli fertilized with compost had lower plant weight than synthetically fertilized broccoli ($F_{1,20} = 16.06$, $P = 0.0006$, Fig. 3). In autumn, broccoli yields were lower in compost-fertilized treatment than in synthetically fertilized plots (Fig. 3) both in terms of head biomass ($F_{1,20} = 19.05$, $P = 0.0003$) and plant weight ($F_{1,20} = 26.54$, $P < 0.0001$).

Multiple regression analysis failed to detect a significant treatment effect on head weight in the summer. However, in the autumn, head weight (*HW*) was significantly lower in broccoli plants fertilized with compost (*O*), intercropped with mustard (*M*), and subject to interspecific competition (*C*), whereas the interaction between organic fertilization and intercropping with mustard (*OM*) tended to increase head weight (Eq 1: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; $P$-levels valid for all equations).

$$HW_{\mathrm{Autumn}} = 322.4(^{***}) - 122.2\ O(^{***}) - 137.5\ M(^{***}) - 52.9\ C(^{*}) + 88.1\ OM(^{*}) \qquad (1)$$

$$R^2 = 0.70,\ F_{4,25} = 14.88,\ P < 0.0001$$

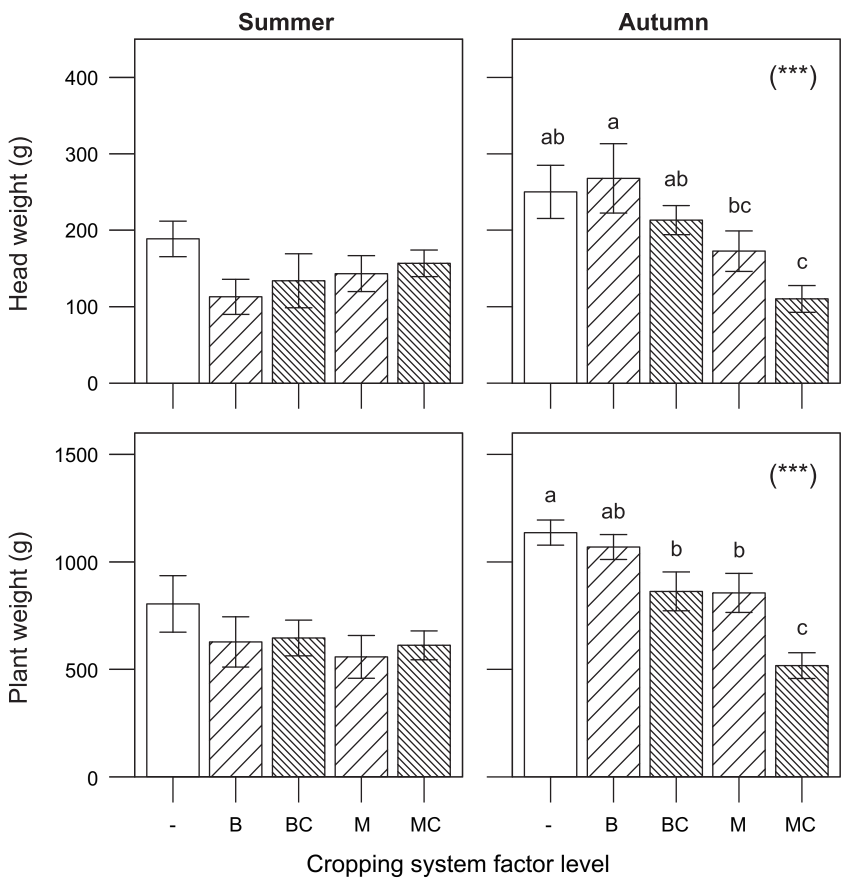


**Figure 2** Head (top) and plant (bottom) broccoli fresh weight (mean ± SE) at harvest as influenced by cropping system levels (−, monoculture; B, buckwheat polyculture without competition; BC, buckwheat polyculture with competition; M, mustard polyculture without competition; MC, mustard polyculture with competition) in two (summer and autumn) experiments at Albany, Calfornia, in 2004 (analysis of variance: ***$P < 0.001$; Tukey HSD: means with the same letter are not significantly different, $P < 0.05$).

 

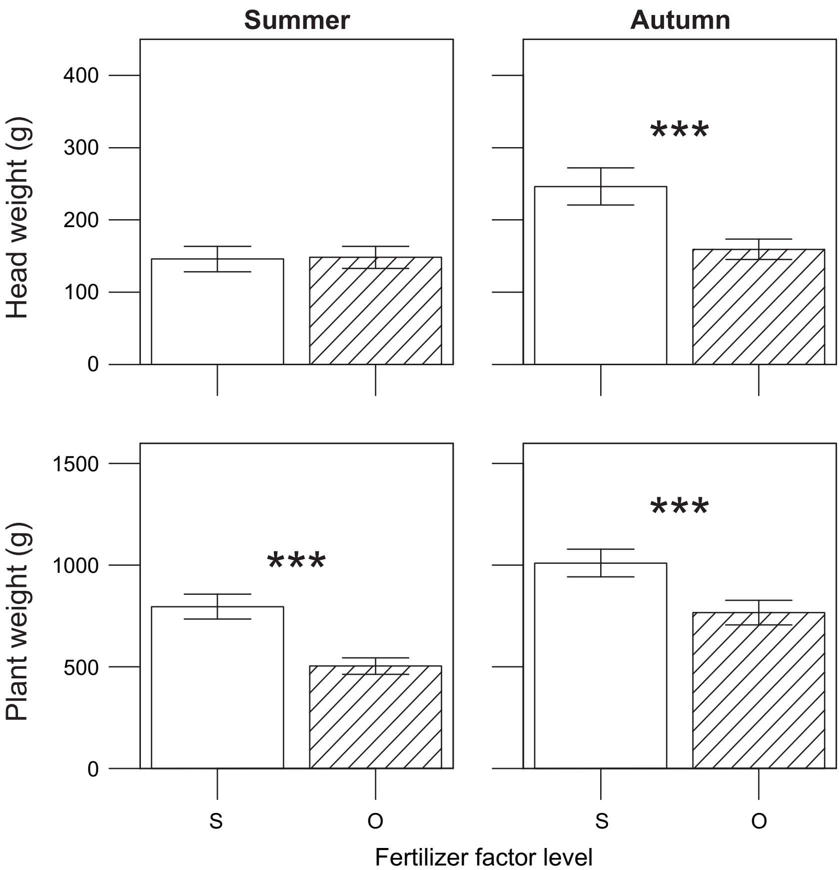


**Figure 3** Head (top) and plant (bottom) broccoli fresh weight (mean ± SE) at harvest as influenced by fertilizer type (S, synthetic fertilizer; O, organic fertilizer-compost) in two experiments (summer and autumn) at Albany, California, in 2004 (analysis of variance: $^{***}P < 0.001$).

Broccoli plant weight (*PW*) was lower in organically fertilized plots in the summer and in the autumn experiments (Eqs 2 and 3).

$$PW_{\text{Summer}} = 795.8(^{***}) - 292.4\,O(^{***}) \tag{2}$$
$$R^2 = 0.36,\ F_{1,28} = 15.87,\ P = 0.0004$$

$$PW_{\text{Autumn}} = 1238.6(^{***}) - 244.3\ O(^{***}) - 289.0\ M(^{***}) - 281.7\ C(^{***}) \tag{3}$$
$$R^2 = 0.82,\ F_{3,26} = 41.15,\ P < 0.0001$$

As with head weight, in the autumn, plant weight was significantly lower in broccoli plants intercropped with mustard and subject to interspecific competition (Eq 3).

As expected, plant height (*PH*) measured on the last sampling date increased significantly but equally (5.5 cm) in broccoli plants subject to interspecific competition in both the summer and autumn experiments.

$$PH_{\text{Summer}} = 38.7(^{***}) + 5.5\ C(^{***}) \tag{4}$$
$$R^2 = 0.15,\ F_{1,28} = 5.20,\ P = 0.030$$

$$PW_{\text{Autumn}} = 33.3(^{***}) + 5.5\ C(^{***}) \tag{5}$$
$$R^2 = 0.24,\ F_{1,28} = 9.24,\ P = 0.005$$

## Aphid abundance and parasitization rates

Cumulative aphid counts were significantly lower in composted broccoli plots compared with synthetically fertilized plots in the summer ($F_{1,20} = 17.48$, $P = 0.0004$) but not in the autumn ($F_{1,20} = 1.98$, $P = 0.17$) despite a similar trend (Fig. 4). In addition, polyculture plots also exhibited lower cumulative aphid counts than monoculture plots in the summer ($F_{4,20} = 4.47$, $P = 0.009$) but not in the autumn ($F_{4,20} = 0.32$, $P = 0.85$) despite a similar trend (Fig. 4). In the summer, broccoli interplanted and competing with mustard had the lowest aphid numbers, whereas broccoli competing with interplanted buckwheat had the lowest counts in the autumn (Fig. 4).

Cumulative aphid parasitization rates (arcsine transformed) were not significantly different among cropping systems either in summer ($F_{4,20} = 2.36$, $P = 0.08$) or autumn ($F_{4,20} = 0.42$, $P = 0.79$), but were significantly higher in composted broccoli than in synthetically fertilized plots in both seasons (Table 1).

Multiple regression analysis shows that cumulative abundance of aphids in the summer was lower when compost was used as opposed to synthetic fertilizer, and when intercropping with either buckwheat or mustard (Eq 6). However, the interaction of intercropping broccoli with either buckwheat (*B*) or mustard (*M*) with composting (*O*) significantly increased pest pressure (Eq 6).

 

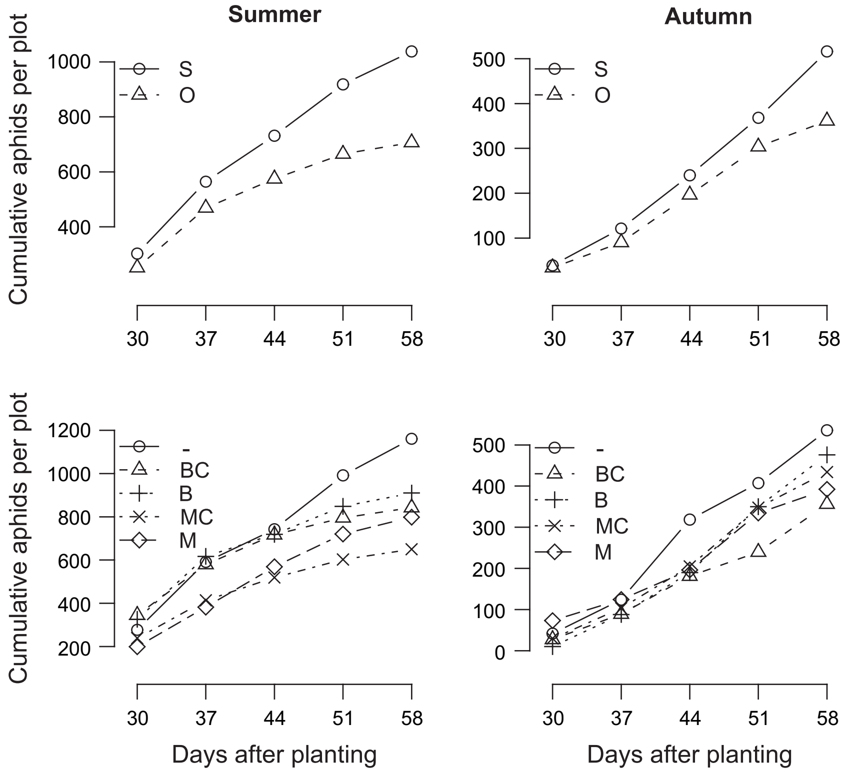


**Figure 4** Cumulative counts of aphids on five broccoli plants per plot at the different sampling dates as influenced by cropping system levels (–, monoculture; B, buckwheat polyculture without competition; BC, buckwheat polyculture with competition; M, mustard polyculture without competition; MC, mustard polyculture with competition) and by fertilizer levels (S, synthetic fertilizer; O, organic fertilizer-compost) in two (summer and autumn) experiments at Albany, California, in 2004.

$$Aphids_{\text{Summer}} = 1579.7(***) - 837.7\ O(***) - 633.2\ B(***) - 720.8\ M(***) + 697.5\ OB(**) + 568.3\ OM(*) \quad (6)$$

$$R^2 = 0.65, F_{5,24} = 9.07, P < 0.0001$$

Composting significantly increased seasonal aphid parasitization rates (*Par*) (arcsine transformed) in both the summer and autumn experiments (Eqs 7 and 8, respectively). Competition, however, significantly increased parasitization rates in the summer but not in the autumn.

$$Par_{\text{Summer}} = 0.027(*) + 0.040\ O(**) + 0.037\ C(**) \quad (7)$$

$$R^2 = 0.41, F_{2,27} = 9.69, P = 0.0006$$

$$Par_{\text{Autumn}} = 0.005(**) + 0.006\ O(*) \quad (8)$$

$$R^2 = 0.17, F_{1,28} = 5.81, P = 0.022$$

**Table 1** Seasonal cabbage aphid parasitism (mean ± SE) by *Diaretiella rapae* during summer and autumn experiments at Albany, California, in 2004

| | % Parasitism | |
|---|---|---|
| | Summer[a] | Autumn[b] |
| Synthetic | 4.2 ± 0.4 | 0.5 ± 0.1 |
| Organic | 8.3 ± 1.3 | 2.5 ± 1.4 |

[a]$P = 0.004$ synthetic vs. organic fertilizer ($F_{1,20} = 9.97$).
[b]$P = 0.03$ synthetic vs. organic fertilizer ($F_{1,20} = 5.14$).

## Discussion

The present study has addressed emerging questions in insect ecology and pest management, namely the effects of plant diversity (intercropping) and the resulting competition on pest and natural enemy abundance (Hooks and Johnson, 2003) as mediated by different fertilization regimes (compost vs. synthetic fertilizers) (Kumar *et al.*, 2004).

No evidence was found indicating that competition from intercropping influenced pest abundance. Broccoli yields were affected only in mustard intercrops and only in the autumn. Mustard is such a competitive plant (Daugovish *et al.*, 2003) that Kloen and Altieri (1990) found intersowing of this crop has to be delayed 1 week for broccoli yield not to be affected. Here, reduced plant biomass due to competition from mustard was observed. Although yield reductions in broccoli due to intercropping are not new (Hooks and Johnson, 2001, 2002), the effect of competition on aphid dynamics as mediated by reduced host-plant biomass had not been separated yet with a specific experimental design.

Intercropping significantly reduced pest pressure in the summer, but not in the autumn. Aphid reduction via intercropping with buckwheat and mustard has already been observed in other cruciferous crops (Kloen and Altieri, 1990; Hooks *et al.*, 1998), but we found that mustard exhibited a more marked effect than buckwheat. This is probably due to the trap cropping effect of mustard (Ludwig and Kok, 1998; Kloen and Altieri, 1990).

A positive effect of intercropping on aphid reduction was evident in the summer (Fig. 4, Eq 6), when the proximity of flowers (i.e. polyculture with competition) significantly enhanced aphid parasitization rates on nearby broccoli plants (Eq 7).

Monoculture and polyculture broccoli consistently had lower aphid densities and higher parasitization rates when fertilized with compost. Compost releases mineral nitrogen in the soil at a slower rate than synthetic fertilizer (Poudel *et al.*, 2002) and this has been related to lower foliar nitrogen content (Vagen *et al.*, 2004) leading to reduced pest incidence (Wermelinger, 1989). In the present study, synthetically fertilized broccoli produced more biomass, but also recruited higher pest numbers. Nevertheless, parasitism by *D. rapae* was greater in compost-fertilized plots.

In summary, intercropping and composting decreased pest abundance in broccoli in cropping systems with or without interspecific competition. In addition, depending on the intercropped plant and the growing season (summer vs. autumn), intercropping enhanced parasitism of cabbage aphid. The seasonal effectiveness of *D. rapae* was increased by composting despite lower aphid abundance in compost-fertilized broccoli.

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