Ponti L., Altieri M.A., Gutierrez A.P., 2007. Effects of crop diversification levels and fertilization regimes on abundance of *Brevicoryne brassicae* (L.) and its parasitization by *Diaeretiella rapae* (M'Intosh) in broccoli. Agricultural and Forest Entomology, 9: 209-214.
The definitive version is available at http://dx.doi.org/10.1111/j.1461-9563.2007.00330.x

Correspond to:
Miguel A. Altieri
University of California, Berkeley
Division of Organisms and the Environment
137 Mulford Hall-3114
Berkeley, CA 94720-3114
agroeco3@nature.berkeley.edu
Tel: 510-642-9802
Fax: 510-643-5438

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

Luigi Ponti[1], Miguel A. Altieri[1], Andrew Paul Gutierrez[2]

[1] Division of Organisms and the Environment, University of California, Berkeley

[2] Division of Ecosystem Science, University of California, Berkeley

**Running title:** Fertilization mediates aphid dynamics in broccoli

Word count: 3338

Authors' present address: Department of Environmental Science, Policy, and Management, 137 Mulford Hall, Berkeley, CA 94720-3114

# 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 fall 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. fall).
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, when compared to synthetically-fertilized plants.

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

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

In this 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-August (summer) and 2) August-December (fall). The experiment was a two-way factorial (5 × 2, i.e. 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 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, dash circumscribed plants in Fig. 1) using two spatial arrangements that introduced intercropping and/or inter-specific competition (Fig. 1b and c). A total of 300 broccoli plants were sampled on each sampling date. Organically grown, 6 weeks old broccoli seedlings (cv. "Heritage", Santa Fe Nursery Inc., Salinas, California) were transplanted into plots on a 0.5×0.5m 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) inter-specific 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 ® Sulfate of Ammonia, Pursell Industries Inc., Sylacauga, Alabama) was incorporated into the soil in a small hole close to each broccoli plant immediately after transplanting. The compost (Grover Landscape Services, Modesto, California) 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 one month after planting to avoid excessive shading and level of competition on slower growing broccoli. All plots and the 1 m inter-plot border were maintained weed-free by hand weeding.

### *Sampling*

All samplings and measurements were done on five randomly selected broccoli plants per plot out of the ten included in the plot sampling area (Fig. 1). Aphid and mummies were counted directly on three leaves per plant at one-week intervals from June 21, 30 days after transplanting (DAP), until July 20 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, as % parasitism = total parasitized aphids (immatures and apterous adults) × 100 / parasitized and non-parasitized 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).

### *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 analyzed with 2-way ANOVA and Tukey's honestly significant difference (HSD) as a post hoc test as appropriate. Multiple regression analysis was performed on aphid data (Venables & 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 & Ripley, 2002) retaining only variables with slopes significantly greater than zero. Insect counts were analyzed as counts per plot of five plants and three leaves per plant. Data were analyzed using the 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 (ANOVA, $F_{4,20} = 1.28$, $P = 0.30$) (Fig. 2). In the fall experiment, cropping system had a significant influence on both broccoli head (ANOVA, $F_{4,20} = 8.08$, $P < 0.001$) and plant weight (ANOVA, $F_{4,20} = 20.73$, $P < 0.0001$), reaching highest values in monoculture and buckwheat polyculture without competition (Fig. 2).

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

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

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

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

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

$$PW_{\text{Summer}} = 795.8^{(***)} - 292.4\,O^{(***)} \tag{2}$$

$R^2 = 0.36$, $F_{1,28} = 15.87$, $P = 0.0004$

$$PW_{\text{Fall}} = 1238.6^{(***)} - 244.3\,O^{(***)} - 289.0\,M^{(***)} - 281.7\,C^{(***)} \tag{3}$$

$R^2 = 0.82$, $F_{3,26} = 41.15$, $P < 0.0001$

As with head weight, in the fall plant weight was significantly lower in broccoli plants intercropped with mustard and subject to interspecific competition (eqn. 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 fall experiments.

$$PH_{\text{Summer}} = 38.7^{(***)} + 5.5\,C^{(*)} \tag{4}$$

$R^2 = 0.15$, $F_{1,28} = 5.20$, $P = 0.030$

$$PH_{\text{Fall}} = 33.3^{(***)} + 5.5\,C^{(**)} \tag{5}$$

$R^2 = 0.24$, $F_{1,28} = 9.24$, $P = 0.005$

### *Aphid abundance and parasitization rates*

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

Cumulative aphid parasitization rates (arcsine transformed) were not significantly different among cropping systems either in summer (ANOVA, $F_{4,20} = 2.36$, $P = 0.08$) or fall (ANOVA, $F_{4,20} = 0.42$, $P = 0.79$), but were significantly higher in composted broccoli than in synthetically fertilized plots in both seasons (Tab. 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 (eqn. 7). However, the interaction of intercropping broccoli with either buckwheat ($B$) or mustard ($M$) with composting ($O$) significantly increased pest pressure (eqn. 7).

$$Aphids_{Summer} = 1579.7^{(***)} - 837.7\,O^{(***)} - 633.2\,B^{(***)} - 720.8\,M^{(***)} + 697.5\,OB^{(**)} + 568.3\,OM^{(*)} \quad (7)$$

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

Composting significantly increased seasonal aphid parasitization rates (*Par*) (arcsine transformed) in both the summer and fall experiments (equations 8 and 9 respectively).

Competition, however, significantly increased parasitization rates in the summer but not in the fall.

$$Par_{Summer} = 0.027\,(*) + 0.040\,O^{(**)} + 0.037\,C^{(**)} \quad (8)$$

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

$$Par_{Fall} = 0.005^{(**)} + 0.006\,O^{(*)} \quad (9)$$

$R^2 = 0.17$, $F_{1,28} = 5.819$, $P = 0.022$

## Discussion and conclusions

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

No evidence was found that competition from intercropping influences pest abundance. Broccoli yields were affected only in mustard intercrops and only in the fall. Mustard is such a competitive plant (Daugovish *et al.*, 2003) that Kloen and Altieri (1990) found inter-sowing of this crop has to be delayed one 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 & 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 fall. Aphid reduction via intercropping with buckwheat and mustard has already been observed in other cruciferous crops (Hooks *et al.*, 1998; Kloen & Altieri, 1990), but we found that mustard exhibited a more marked effect than buckwheat. This is probably due to the trap cropping effect of mustard (Ludwig & Kok, 1998; Kloen & Altieri, 1990).

A positive effect of intercropping on aphid reduction was evident in the summer (Fig. 4, eqn. 7), when the proximity of flowers (i.e., polyculture with competition) significantly enhanced aphid parasitization rates on nearby broccoli plants (eqn. 8).

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 this study, synthetically-fertilized broccoli produced more biomass, but also recruited higher pest numbers. Nevertheless, parasitism by *D. rapae* was higher 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. fall), intercropping enhanced parasitism of cabbage aphid. The seasonal effectiveness of *D. rapae* was increased by composting despite lower aphid abundance in compost-fertilized broccoli.

## Acknowledgements

We thank Courtney Hall, Nicholas Knix, Jonathan Pilch, Paul Proge, Aida Gamal, Andre Monteiro, Mariana Portella, Maira Ribeiro, and Marcos Westphal, for their help in field preparation and data collection. We also thank the Statistics Department Consulting Service, University of California at Berkeley, for helpful suggestions on the analysis of data. Finally, we thank Maria A. Garcia for her helpful advice in experimental design. Luigi Ponti was financially supported by the Italian National Research Council (CNR n. 203.22 code 2) and the Division of Organisms and the Environment, University of California at Berkeley.

# References

Altieri, M.A., Ponti, L. & Nicholls, C. (2005) Enhanced pest management through soil health: towards a belowground habitat management strategy. *Biodynamics,* **253,** 33-40.

Bukovinszky, T., Tréfás, H., van Lenteren, J.C., Vet, L.E.M. & Fremont, J. (2004) Plant competition in pest-suppressive intercropping systems complicates evaluation of herbivore responses. *Agriculture, Ecosystems and Environment,* **102,** 185-196.

Daugovish, O., Thill, D.C. & Shafii, B. (2003) Modeling competition between wild oat (*Avena fatua* L.) and yellow mustard or canola. *Weed Science,* **51,** 102.

Faraway, J.J. (2004) *Linear Models with R*. Chapman & Hall/CRC, Boca Raton, Florida.

Hooks, C.R.R. & Johnson, M.W. (2001) Broccoli growth parameters and level of head infestations in simple and mixed plantings: impact of increased flora diversification. *Annals of Applied Biology,* **138,** 269-280.

Hooks, C.R.R. & Johnson, M.W. (2002) Lepidopteran pest populations and crop yields in row intercropped broccoli. *Agricultural and Forest Entomology,* **4,** 117.

Hooks, C.R.R. & Johnson, M.W. (2003) Impact of agricultural diversification on the insect community of cruciferous crops. *Crop Protection,* **22,** 223-238.

Hooks, C.R.R., Valenzuela, H.R. & Defrank, J. (1998) Incidence of pests and arthropod natural enemies in zucchini grown with living mulches. *Agriculture Ecosystems and Environment,* **69,** 217-231.

Kloen, H. & Altieri, M.A. (1990) Effect of mustard (Brassica hirta) as a non-crop plant on competition and insect pests in broccoli (Brassica oleracea). *Crop Protection,* **9,** 90-96.

Klostermeyer, E.C. (1950) Effect of soil fertility on corn earworm damage. *Journal of Economic Entomology,* **43,** 427-429.

Kumar, V., Mills, D.J., Anderson, J.D. & Mattoo, A.K. (2004) An alternative agriculture system is defined by a distinct expression profile of select gene transcripts and proteins. *Proceedings of the National Academy of Sciences of the United States of America,* **101,** 10535-10540.

Ludwig, S.W. & Kok, L.T. (1998) Evaluation of trap crops to manage harlequin bugs, *Murgantia histrionica* (Hahn) (Hemiptera: Pentatomidae) on broccoli. *Crop Protection,* **17,** 123-128.

Poudel, D.D., Horwath, W.R., Lanini, W.T., Temple, S.R. & van Bruggen, A.H.C. (2002) Comparison of soil N availability and leaching potential, crop yields and weeds in organic, low-input and conventional farming systems in northern California. *Agriculture, Ecosystems and Environment,* **90,** 125.

R Development Core Team (2004) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Root, R. (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs,* **43,** 95-124.

Vagen, I.M., Skjelvag, A.O. & Bonesmo, H. (2004) Growth analysis of broccoli in relation to fertilizer nitrogen application. *Journal of Horticultural Science and Biotechnology,* **79,** 484-492.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*. Springer-Verlag, New York.

Wermelinger, B. (1989) Respiration of the two spotted spider mite as affected by leaf nitrogen. *Journal of Applied Entomology,* **108,** 208-212.

# Tables

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

| | **% Parasitism** | |
|---|---|---|
| | *Summer* [a] | *Fall* [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 (ANOVA, $F_{1,20} = 9.97$).

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

# Figure legends

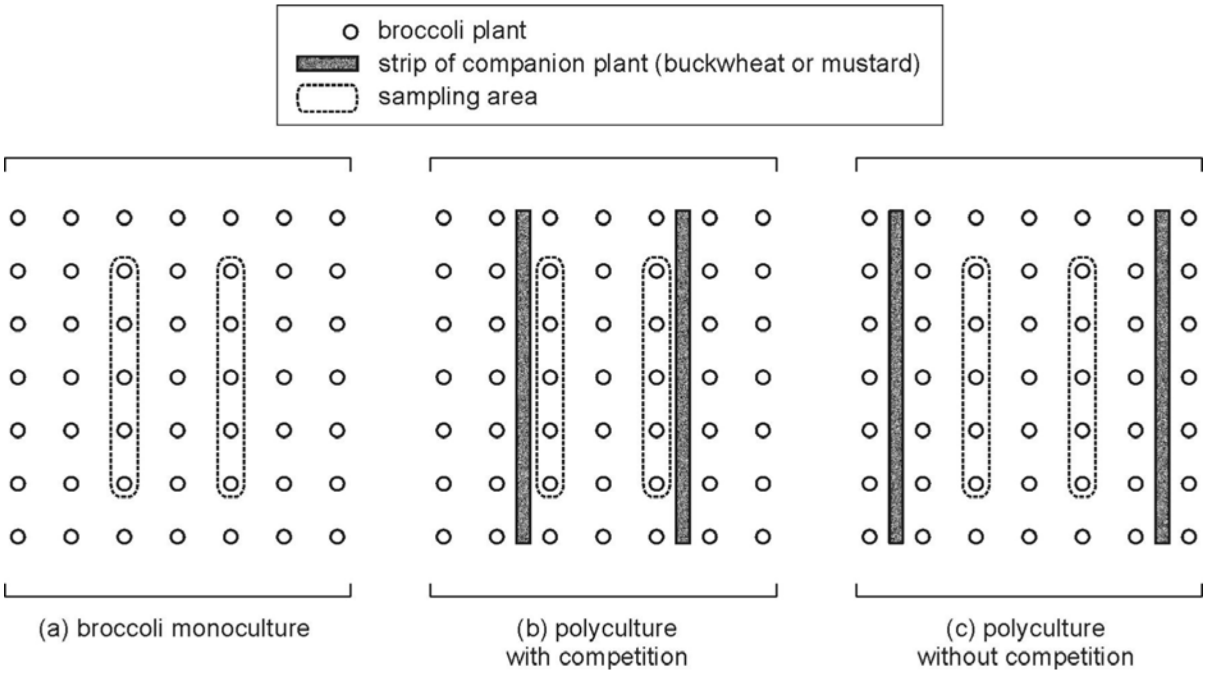
**Figure 1.** Schematic representation of the additive intercropping design used to separate effects of crop diversity from the effects of competition.

**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 fall) experiments at Albany, CA in 2004 (ANOVA: *** $P<0.001$; 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 fall) at Albany, CA in 2004 (ANOVA: *** $P<0.001$).

**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 fall) experiments at Albany, CA in 2004.

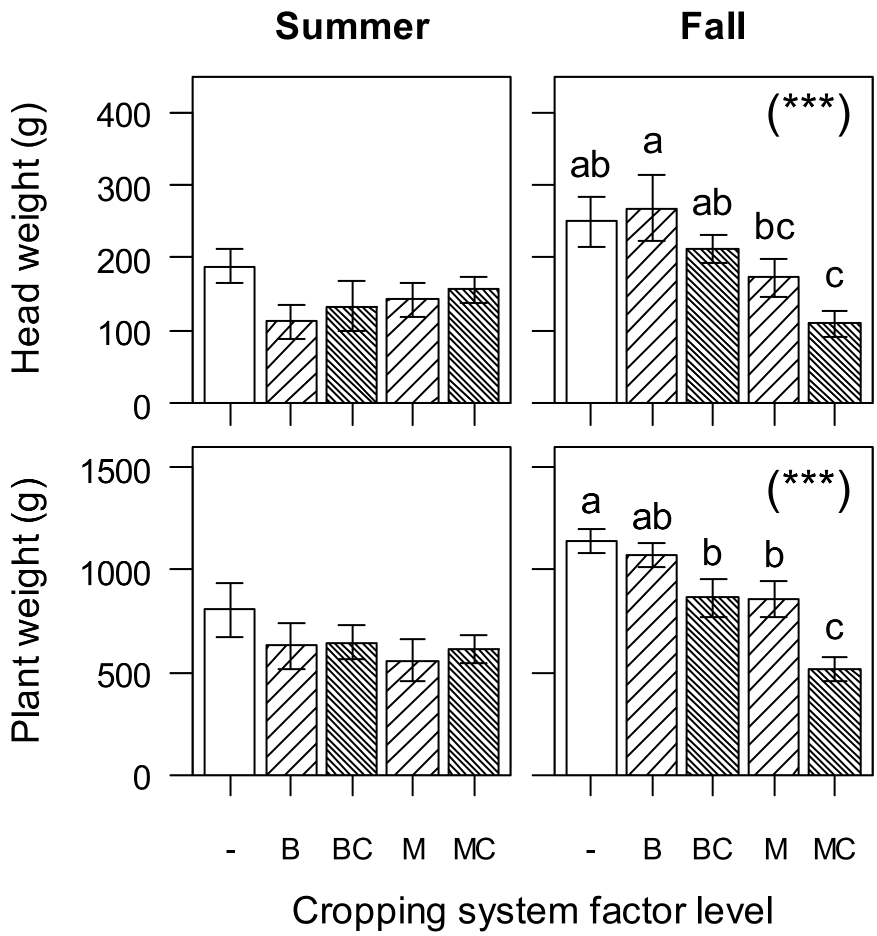
328




329

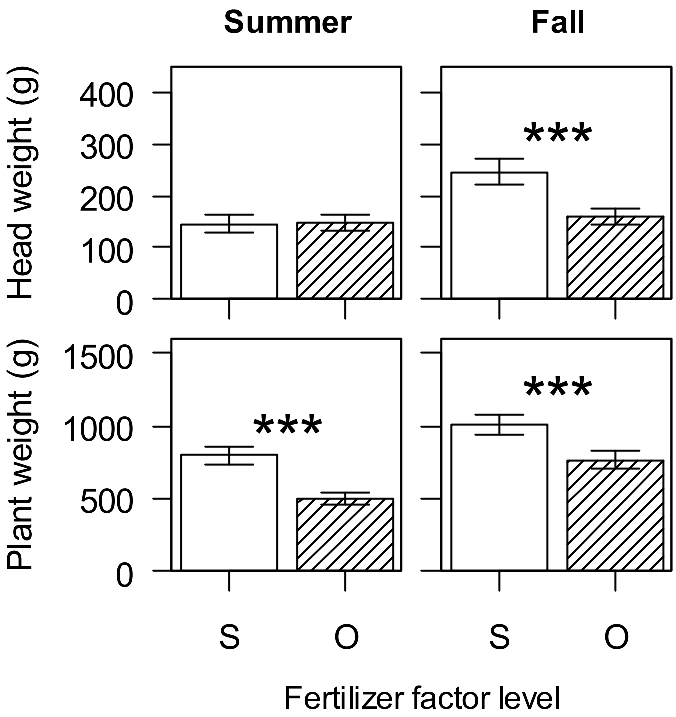
330

331 **(Figure 1)**


Summer
Fall
Head weight (g)
400
300
200
100
0
(***)
ab
a
ab
bc
c
Plant weight (g)
1500
1000
500
0
(***)
a
ab
b
b
c
- B BC M MC
- B BC M MC
Cropping system factor level

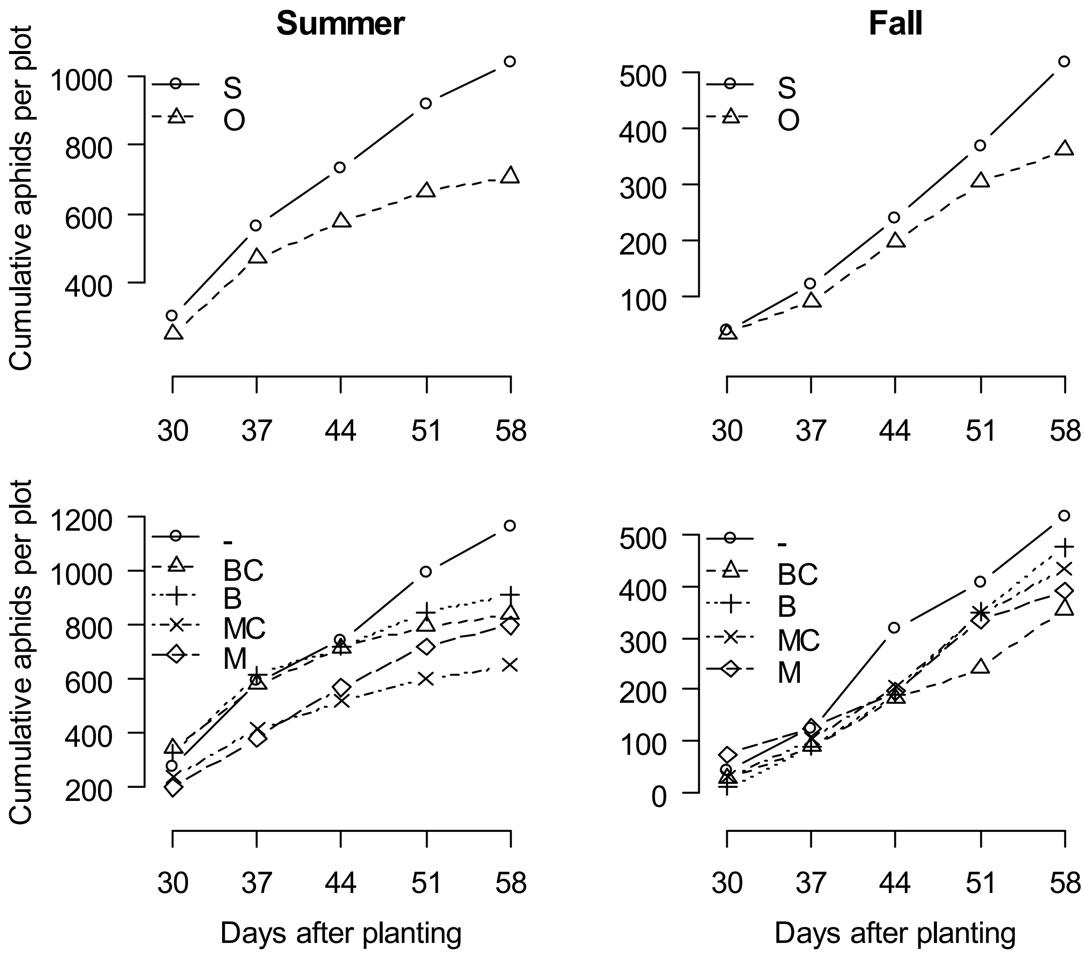
**(Figure 2)**

336




337

338 **(Figure 3)**


Summer
Fall
Cumulative aphids per plot
S
O
1000
800
600
400
500
400
300
200
100
30
37
44
51
58
Cumulative aphids per plot
1200
1000
800
600
400
200
-
BC
B
MC
M
500
400
300
200
100
0
Days after planting
Days after planting

**(Figure 4)**