

Selected article:

Diaz, M.F., Ramirez, A., Poveda, K. 2012. Efficiency of different egg parasitoids and increased floral diversity for the biological control of noctuid pests. *Biological Control*. 60:182-191

Introduction and Background

Currently, agricultural practices worldwide are becoming increasingly unsustainable. In response to increased population growth and higher food demands, agricultural intensification has continued to escalate, resulting in the transformation of complex landscapes characterized by resilience, low disturbance and high biodiversity to simplified agricultural landscapes characterized by high acreage, monoculture, and high levels of disturbance. Farming practices that emphasize higher production diminishes biodiversity and create conditions favorable for the establishment of insect pests (Letourneau 2005). Specialist herbivores are predicted to more easily locate and colonize large, dense, pure cropping systems compared to more diverse ecosystems (Root 1973). Additionally, highly disturbed cropping systems characterized by routine plowing and pesticide applications, contribute to the likelihood of pest outbreaks as natural enemies are more sensitive and recover more slowly after a disturbance event.

To mitigate crop losses due to pest insect outbreaks, management strategies are often pesticide based. Although they may be effective at killing pest insects, insecticides pose serious risks when use becomes routine and widespread. These risks include resistance development to pesticides, non-target effects on natural enemies, secondary outbreaks, contamination of water supply, and human health concerns (Aktar et al. 2009).

An alternative and potentially more environmentally sustainable means of managing insect pests relies on enhancing populations of natural enemies. Biological control, defined as the suppression of pest insects by antagonistic predators, pathogens and parasites, represents a fundamental method to suppress pest populations in agricultural fields (Driesche and Bellows 1996). Biological control began to receive widespread attention as an effective pest management strategy when suppression of the cottony cushion scale was demonstrated using its native natural enemies (Caltagirone 1987). Since then, much research has investigated the classical biological control approach in which non-native natural enemies are introduced to suppress an invasive species. Shortly thereafter, the augmentative approach, where natural enemies are inundatively released to quickly suppress insect pests, has been demonstrated to successfully reduce populations of pest insects. However, due to increased awareness of the risks of introducing species and their potential for native species displacement inherent in the classical biological control approach as well as repeated financial investments inherent in the augmentative biological control approach, the conservation approach to biological control has been receiving increasing attention (Parella et al. 1992, Elliott et al. 1996).

Conservation biological control, defined as habitat management to enhance natural enemies, represents a management strategy that poses the least environmental risk. This approach relies on locally present natural enemies to suppress pest insects. Thus the unintended impacts of introduced natural enemies and repeated financial investments for expensive inundative releases are minimized. Conservation biological control can be achieved through a variety of ways such as reducing pesticide use to

minimize non-target effects of natural enemies, reducing disturbance to areas inhabited by natural enemies (mowing field edges, for example) and crop diversification. Crop diversification, or increasing the diversity of plant species within a cropping system, can be implemented through the maintenance of undisturbed field borders, weedy field edges, trap crops, intercropping, or provision of flowering plants (Bach 1980, Thomas et al. 1991, Altieri 1999, Amaral et al. 2013).

In recent years the potential of conservation biological control to enhance natural enemy populations and effectiveness have been increasingly studied. Numerous studies have investigated the potential for crop diversification schemes to reduce the likelihood of colonization and establishment of pest species through disruption of pest host location strategies, creating unfavorable microhabitat for pests, and serving as a resource for natural enemies. Of these, resource provisioning, has received the most attention (Altieri 1999, Zhang et al. 2004, Heimpel and Jervis 2005, Zehnder et al. 2006, Letourneau et al. 2011, Géneau et al. 2013). By including flowering species in cropping systems, natural enemies are predicted to be more abundant due to the presence of floral resources that provide food (nectar, pollen, and alternative, prey) and refuge (Root 1973). By utilizing plant resources, natural enemies have been found to have increased longevity, fecundity, foraging activity, and rates of predation and parasitism (Lee and Heimpel 2005, Begum et al. 2006, Araj et al. 2011, Geneau et al. 2011, Hogg et al. 2011, Amaral et al. 2013).

Many studies show improved performance of natural enemies, primarily parasitoids, in the presence of flowering plant species and often reductions in pest populations. It is becoming increasingly apparent that floral resources can differentially impact natural enemies. Morphological characteristics of plant flowers such as chemical cues, flower color, aperture size, corolla depth, and nectar quality as well as insect morphology such as head size and mouthpart type can significantly influence attraction and utilization of a flowering resource (Vattala et al. 2006). When examining flowering plant species for their potential to maximize natural enemy performance, these factors in addition to their impacts on the targeted pest must be considered.

In the current article, Diaz et al. (2012) consider the potential of combining the augmentative and conservational approaches to biological control by investigating the impact of floral resource provisioning on performance of an inundatively released parasitoid in an effort to maximize their effectiveness. Additionally, the authors investigate the influence of provisioned floral plant species on the targeted pest insect.

Summary

In Diaz et al. (2012), the authors argue that pest management strategies consisting of routine applications of broad spectrum insecticides are largely unsustainable and contribute to the loss of functional biodiversity and ecosystem services provided by the natural environment. The authors maintain that reducing reliance on pesticides and focusing on alternative management strategies such as floral provisioning to enhance natural enemies may mitigate the deterioration of the ecosystem by promoting services such as pest suppression. Natural enemies, parasitoids for example, are capable of providing a significant pest suppression service. However, their success is largely dependent on the presence of undisturbed sites where food resources such as nectar and pollen are available. As these resources are predicted to improve parasitoid performance,

the authors suggest providing flowering species can function to increase their effectiveness.

In cape gooseberry (*Physalis peruviana*), a crop prone to damage by noctuid pests, the main avenue of management is pesticide applications. Diaz et al. (2012) attempts to investigate the potential of biological control using *Trichogramma* parasitoids. These egg parasitoids represent an effective pest management strategy. Therefore, the authors propose floral provisioning to prolong parasitoid persistence and increase their effectiveness in the field. Thus, the objectives of the study are to evaluate the effectiveness of *Trichogramma* egg parasitoids on two important gooseberry pests, investigate the impact of floral provisioning on parasitoid performance, examine the influence of flowering plants on parasitism rate in the field and finally, to examine the influence of floral resource provisioning on the targeted pest species.

The authors conducted a series of experiments at the lab, greenhouse, and field scales measuring parasitoid and herbivore performance parameters to test their hypotheses that floral resource provisioning can increase longevity and parasitism rates of *Trichogramma* egg parasitoids as well as pest fecundity. Initially, to determine the best candidate parasitoid, three species of *Trichogramma* (*T. atopovirilia*, *T. exiguum*, *T. pretiosum*) were screened in laboratory experiments for emergence, longevity, sex ratio, and rates of parasitism on *Spodoptera frugiperda* and *Copitarsia decolora*. From their results, *T. atopovirilia* was chosen as a model species. Next, to determine the potential for food resources to impact the parasitoid, the authors reared *T. atopovirilia* in the presence of an artificial diet and measured performance. Then, to examine how the presence of flowering plants affect parasitoid survival and parasitism rates, choice and non-choice experiments were conducted in laboratory and greenhouse settings using plants previously reported as resource provisioning for parasitoids. These species included borage, moricandia, sweet alyssum, coriander, white clover and red clover. Next, to demonstrate the impacts of floral provisioning at the field scale, experiments were conducted in established gooseberry fields where parasitoids were released and parasitism rates measured. While it is important to demonstrate that parasitoids can benefit from floral resources, it is equally important to assess impacts on the pest species. Therefore, to test the hypothesis that the pest, *S. frugiperda*, may also benefit from resource provisioning, the authors exposed it to floral resources and measured oviposition. Data from their experiments were analyzed using one-way analysis of variance (ANOVA), repeated measure ANOVA and mean comparison tests including Tukey's HSD and Kruskal-Wallis tests.

From their experiments, Diaz et al. (2012) demonstrated differential performance of parasitoids on two economically damaging species. *T. atopovirilia* was found to be the most effective agent and was chosen as the model species for subsequent experiments. The authors also demonstrated that resource provisioning with artificial diet as well as floral inflorescences of red clover can significantly increase the longevity and parasitism rates of *T. atopovirilia*. Positive effects of floral provisioning were confirmed when increased levels of parasitism were observed in field plots. Although red clover improved longevity and parasitism rates of the parasitoid, the fecundity of the pest, *S. frugiperda*, was also higher in the presence of red clover, borage and *Physalis*. From their results, the authors conclude that resources provided by flowering plants can increase parasitoid performance, however these same resources can also benefit the targeted pests.

Therefore, caution must be taken when selecting an insectary plant for the purpose of enhancing natural enemies.

Critique

Introduction

Despite a few spelling and grammatical errors, the article appears to be well written and presents a clear argument emphasizing the shift from unsustainable pest management practices towards lower risk strategies emphasizing enhanced natural pest suppression services. The authors also highlight the practicality of this approach to pest management. In developing countries, routine applications of pesticides may be expensive. Therefore, implementing insectary plants to support the parasitoid community represent a lower cost and more practical approach to pest management in the gooseberry cropping system. The objectives are clearly outlined and lead nicely into their experimental design.

Experimental Design and Methods

The experiments conducted are generally well designed, consisting of a logical flow of experiments conducted at multiple scales. This is especially important, as impacts seen at smaller scales in which the environment is largely artificial, may be overly emphasized. For example, parasitism may appear higher in laboratory petri dish trials compared to field experiments due to smaller foraging area in the laboratory setting (Höller and Haardt 1993). Additionally, the experimental design is also largely consistent with the nectar-provision hypothesis (Heimpel and Jervis 2005) which states that to demonstrate that a parasitoid is benefitting from floral nectar, the experimenter must demonstrate sugar limitation of the parasitoid, utilization of the floral resource, enhanced fecundity in presence of nectar, increased parasitism and reduced pest densities in the presence of nectar. Their experimental design considered each of these proposed issues.

While well designed, several improvements to their experimental design and methods could have strengthened the article.

In their initial laboratory experiments, the authors report using sentinel eggs of *S. frugiperda* and *C. decolora* that were approximately 48 hours old. This may have resulted in underestimation of the biological control ability of the selected species as parasitoids are well known to prefer and perform better on freshly laid egg masses. Liu et al. (1998) demonstrated significantly reduced rates of egg parasitism of *Ostrinia furnacalis* by the parasitoid, *Trichogramma dendrolimi*, in eggs aged 18 to 24 hours old compared to eggs aged the 0-6 hours old.

Following their initial laboratory experiments, Diaz et al. (2012) decided to use *T. atopovirilia* as their model species for subsequent experiments, citing higher proportion of emergence, longevity and parasitism compared to the other species. However, *T. pretiosum* was a similarly promising species. With the exception of emergence in the greenhouse, *T. pretiosum*, parameters did not significantly differ from *T. atopovirilia*. In fact, rates of parasitism by *T. pretiosum* on *C. decolora* were approximately two-fold higher than that exhibited by *T. atopovirilia*. The inclusion of *T. pretiosum* in subsequent experiments could have provided valuable insight into the influence of flowering resources on *Trichogramma* species with the potential to highlight differential performance at the species level and field scale performance.

During their artificial diet trials, a pollen control consisting of an artificial pollen diet, could have been included to demonstrate the relative influences of nitrogen-rich pollen versus carbohydrate-rich nectar in influencing the longevity and fecundity of the parasitoid. Zhang et al. (2004) observed increased longevity and fecundity of *Trichogramma brassicae* when supplemented with corn pollen compared to those reared on water alone. However, supplements including both corn pollen and honey supplements suggested higher parasitism and fecundity (Zhang et al. 2004).

In their floral provisioning trials, plant species selection was robust. Each species has been reported as beneficial to parasitoids and represent a diverse array of morphological characteristics such as color, flower aperture, and corolla depth that may vary in attractiveness and accessibility to parasitoids (Vattala et al. 2006). While coriander was included in the initial non-choice floral provisioning experiments, it was removed from subsequent experiments without justification. This represents a missed opportunity to generate data on the potential for coriander to improve *Trichogramma* longevity, fecundity, parasitism rates, as well as pest fitness.

I agree that it is important to demonstrate the positive effects of floral provisioning at the field scale. While I understand that there may be constraints associated with on-farm research, authors could have improved the experimental design of the field studies by deliberately establishing pure patches of floral resources and examining parasitism. As designed, the current treatments, red clover for example, underestimate the potential for the resource to support the parasitoid as these treatments were on average only fifty percent clover. Though significant differences were found using the current design, a study using pure patches would have highlighted the extent to which parasitoids could benefit from deliberately implemented flowering borders. Additionally, the non-resource control is comprised of approximately fifteen percent ground coverage of flowering species that could potentially influence parasitism. Alternatively the control treatment could have simply been mowed to provide a more accurate representation of field scale parasitism in the absence of a floral resource.

The experiments examining the influence of resource provisioning on herbivores are relatively novel and understudied. In the introduction the authors make it a point to highlight both *S. frugiperda* and *C. decolora* as significantly damaging to the gooseberry cash crop. However, their floral provisioning trials were only conducted on one species, *S. frugiperda*. It would have been interesting to see how *C. decolora* would have responded to the selected flowering plants. Data from these trials could have contributed potentially valuable information describing the effects of floral provisioning on an important pest species of a high value crop such as gooseberry that which could be applied to other crops attacked by these pests.

The statistical analyses used appear appropriate. One-way ANOVA is commonly used to compare means to detect significant differences. Additionally, these tests were followed by Tukey's HSD mean comparison tests to determine which means differed. Use of repeated measures ANOVA was also appropriate as it is commonly used to compare treatment means of a response variable measured over time. To meet the assumptions of ANOVA tests, data were transformed as appropriate. For pest provision trials, where the assumptions of ANOVA were not met, a non-parametric Kruskal-Wallis test was conducted to determine differences.

Results

Consistent with the parasitoid nectar provision hypothesis, results from their experiments suggested increased longevity in the presence of food sources (both artificial and red clover) compared with a control water diet, increased fecundity measured as parasitism rate in the presence of the floral resource as well as reduced pest numbers in the presence of floral nectar. The results provide fairly convincing evidence supporting the potential for floral resources to benefit parasitoids through increased longevity and fecundity. Additionally, they provide evidence suggesting non target effects of floral resources on the pest species. However, at certain instances in the manuscript, the results reported were not consistent with their figures. For example, the authors report *T. exiguum* and *T. atopovirilia* as both surviving longer than *T. pretiosum*. However, according to Table 2, only the longevity of *T. exiguum* was significantly higher than *T. pretiosum* whereas *T. atopovirilia* did not significantly differ from either. Similarly, for the choice experiments, the authors reported borage, alyssum, white clover, and red clover as significantly improving parasitism compared to control. However, according to Figure 4a, only the red clover significantly differed from the control treatment.

Discussion

With regards to validity of their conclusions, there are instances within the discussion where their claims are not necessarily supported by the data presented. For example, the authors conclude that *T. atopovirilia* is the most promising species to manage *S. frugiperda*. However, as previously mentioned, the data presented suggests no significant differences in parasitism rates between *T. atopovirilia* and *T. pretiosum* on *S. frugiperda*. Similar to other studies, this article demonstrates differential performance of natural enemies and selective use of floral resources. It is becoming increasingly apparent that successful implementation of floral resource provisioning programs for pest management will require extensive context specific knowledge of the biology and ecology of the organisms involved in a cropping system as the impacts of floral resource provisioning can vary depending on landscape, natural enemy morphology, floral morphology, and pest insect morphology. Previously, Vatalla et al. (2006) found increased longevity of the braconid wasp, *Microntonus hyperodae* in the presence of buckwheat, white mustard and coriander but not for red clover, white clover or phacelia, which are plants commonly reported to provide resources for parasitoids. They suggested that characteristics such as parasitoid head size and flower structure may impede access. Similarly, Geneau et al. (2011) found improved survival and fecundity *Microplitis mediator* and *Diadegma fenestrata* in the presence of buckwheat, cornflower, and the extrafloral nectaries of faba bean, but not in the presence of snapdragon or candytuft.

In addition to providing evidence suggesting that natural enemies can in fact, benefit from floral resource provisioning, the article demonstrates that resource provisioning can also benefit insects inhabiting other trophic levels, which is seldom examined. Kerhli and Bacher (2008) examined the influence of several plants species on the fitness of a parasitoid complex and its targeted pest, the chestnut leaf miner. Of the species screened, no flowering plant species benefitted parasitoids only and one species, cow parsley, benefitted both parasitoid and host. However, parasitoids benefitted up to eight times more than the chestnut leaf miner. Araj et al. (2009) found increased rates of

hyperparasitism of the parasitoid *Aphidius ervi*, an aphid parasitoid, in the presence of flowering buckwheat. However, the ability of *A. ervi* to suppress their aphid host was not significantly reduced in this study. Studies such as these demonstrate the importance of screening insectary plants for their influences at multiple trophic levels.

The authors provide an interesting solution to the issue of positive impacts of the flowering resource on the targeted pest species. They suggest floral provisioning may function as an “attract and kill” edge where herbivores are more likely to be attracted and oviposit. Consequently, as parasitoids are also attracted, the red clover could serve as a dead end host for the pests. This offers a solution to the limitations commonly encountered when considering crop diversification schemes. Generally when resource plants are provided, natural enemy abundance and parasitism rates are typically highest at field edges and decrease sharply towards the field interior (Landis and Haas 1992). Therefore, if a pest is attracted to edges where resource plant and enemy activity is higher, pest abundances are likely to be lower in the field interior where the cash crop is concentrated.

While the authors do demonstrate that parasitoids can benefit from floral resource provisioning, it is unclear if these benefits would translate into reduced damage and increased yields at the field scale. Therefore, future studies should be aimed at demonstrating the influence of red clover on *Trichogramma* parasitoids under field conditions, measuring the impacts on cash crop damage and yield. Additionally, in the many of their experiments, egg parasitism appeared to be very low (~20-30%) despite ample nutrients, hosts, and minimal foraging distance. In contrast, successfully released parasitoids, *T. ostrinae*, for example, exhibited parasitism rates ranging from 73-87% when inundatively released in China for corn borer management (Wang et al. 2005). In this case, the relatively low rates of parasitism may have been a function of host age. It remains unclear how economically feasible it would be to invest into establishing floral resources and purchasing biological control agents that parasitize such a low number of pest eggs. Additionally, future studies should evaluate the economics and practicality of provisioning argumentatively released natural enemies compared to conventional pesticide use.

Conclusions

Although numerous improvements could have strengthened the manuscript overall, the article represents a worthwhile contribution to field in that it demonstrates differential performance of natural enemies in the presence of floral resources at the laboratory, greenhouse, and field scales. Additionally, it demonstrates that the impacts of floral provisioning may influence other trophic levels, namely herbivores. Ultimately, the paper suggests that there is a “right” kind of diversity to provide when considering the use of companion plants to enhance natural enemies. Success of conservation biological control programs will continue to require intimate knowledge of the organisms involved in cropping systems as they may be differently affected by plant resources. As such, many plants will have to be screened for their influences at multiple trophic levels in different cropping systems.

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