

Companion Planting Effects of Insecticidal Marigolds and Nitrogen Fixing Legumes on Growth and Protection

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Abstract

In agricultural systems, intercropping is used to increase crop yield while simultaneously reducing herbivory. In order to test the effectiveness of three reputedly effective companion species, a garden plot at the University of Michigan's Biological Station was developed. This included *Tagetes patula*, a species known to exude a volatile insect repellent, along with *Phaseolus vulgaris* and *Pisum sativum*, two species of legumes that house nitrogen fixing bacteria. Measurements were taken over the course of several weeks on the percentage of leaf area lost due to insect herbivory, and growth in height for each plant. The results from the experiment show that there was a significant decrease in the amount of herbivory, as well as an increase in plant growth, between our intercropped and control plots. Overall the effectiveness of each plant species was supported by our data, showing that each species had a beneficial relationship with plants located in close proximity to them as thought by agriculturists for many years.

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Signed,



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Introduction

Companion planting has been used by farmers and household gardeners for many years as an effective strategy for increasing crop yields and decreasing pest incidence (Parker et al., 2013). When done correctly, the intercropping of various plant species has the potential of producing mutualistic relationships in which each species benefits. For instance, the intercropping of corn, maize, and squash was first developed by Native Americans and has been shown to produce higher yields and discourage insect herbivory (Risch, 1980). Although not commonly grown together, two plant species that are often used in similar systems are nitrogen-fixing legumes and insecticidal marigolds.

Leguminous plants have symbiotic bacteria that are highly valuable in agricultural systems given their widely demonstrated ability to fix nitrogen. Their root bacteria, *Rhizobia*, convert atmospheric nitrogen (N_2) into ammonia (NH_3) in exchange for protection and food supply from the plant (Peoples et al., 1995). With help from other bacteria in the soil, the ammonia is mineralized into ammonium (NH_4^+) and eventually converted to nitrate (NO_3^{2-}) in the soil. As a result of this process, *Rhizobia* not only increase the amount of nitrogen available to their host plant, but also to neighboring plants. In fact, a study by Peoples et al. (1995) found that crop yields in plants grown with legumes are often equivalent to those expected from applications of 30 to 80 kg of fertilizer-N ha^{-1} . Given that nitrogen is a key limiting nutrient in plant growth, this quality is highly practical for creating an efficient companion planting system.

Meanwhile, marigold species such as *Tagetes patula* have also shown promising results in companion planting (Hooks et al., 2010). Marigolds have been shown to behave as natural pest deterrents by secreting the toxic chemical α -terthienyl through their roots, and thus fending off surrounding threats (Gommers and Bakker, 1988). Additionally, certain species give off a

very pungent odor, which is thought to detract most above ground insects (Parker et al. 2013). Studies on the effectiveness of *Tagetes patula* have shown that the number of natural herbivores on companion plants were significantly lower than in equivalent monocultures of those same plants (Jankowska et al., 2009). In some cases, phytophagous organism density on non-marigold plants were noted as being reduced by more than 50% when grown in close proximity to *Tagetes patula* (Silveira et al., 2009). Such evidence for the effectiveness of these natural pesticides warrants further investigation about their uses and efficiency.

In order to better understand the effectiveness of these plants as companion plants, we performed an experiment in which *Tagetes patula* and two legume species, *Phaseolus vulgaris* and *Pisum sativum*, were planted in combination and assessed for their performance. By comparing how they grow together, as opposed to alone, we hoped to show that there was a significant difference in plant growth and herbivore protection. Given their unique characteristics, we hypothesized that plots containing both marigolds and legumes would experience greater overall plant growth than monocultures of these plants. The plot containing both plants should benefit from the fertilizing effect of the legumes as well as the pesticidal effect of the marigolds, while each of the other plots would only experience one of these benefits. Additionally, because of the pesticidal quality of marigolds, we hypothesize that any plot containing marigolds will experience a lower leaf surface area loss by herbivory than those without.

Materials and Methods

We conducted this experiment using a small garden plot (4m x 5.3m) located on the property of the University of Michigan Biological Station in Pellston, Michigan. This particular plot was well established and had been previously used for multiple growing seasons. In order to

ensure soil continuity throughout the area, we collected four initial soil samples and determined the baseline concentrations of nitrogen and phosphorous present in the soil. Each of the samples underwent both nitrogen and phosphorus salt extractions, and the extractions were given to the UMBS Chemical Lab for processing.

To establish our plot, we first planted a row of nitrogen fixing legumes which consisted of 12 bean plants (*Phaseolus vulgaris*) and 12 pea plants (*Pisum sativum*). The second plot, located 1.7 m away from the first, consisted solely of 12 marigold plants. These two plots would serve as the control plots for our study. Our experimental plot contained both marigolds and legumes (11 marigold, 19 legume), and were grown in parallel rows approximately 0.4 m apart. An effort was made to maximize the distance between marigold plots and the legume control plot in order to reduce the possibility of insecticidal effect spillover. To further decrease this possibility, a soil mound was built in between the marigold control and the legume control plot. Finally, a chicken wire fence was placed around the plot in order to prevent mammalian or non-insect herbivory (Figure 1).

Quantifying Effectiveness of Nitrogen Fixation on Growth

In order to test for the effectiveness of the legumes as nitrogen fixers, biweekly soil samples were taken to determine the amount of nitrogen in the soil. More specifically, we determined the levels of ammonium (NH_4^+) and nitrate (NO_3^{2-}) present, which are the forms of nitrogen most readily available for plants to use (Peoples et al., 1995). In addition, we observed the growth of each marigold plant by recording the height of the plant in centimeters and the number of buds and flowers present on each marigold.

Detecting Herbivory

To quantify herbivory on *Phaseolus vulgaris* and *Tagetes patula*, we recorded the number of leaves on each plant that exhibited any signs of herbivory. At the end of the experiment, we also determined the extent of herbivory by estimating the total missing-leaf area (cm²) on each individual plant. This process entailed measuring the radius of each missing-leaf area and approximating the area as either a circle (πr^2) or a rectangle, depending on its shape and location on the leaf.

Statistical Methods

To analyze our data, we performed Mann-Whitney U tests comparing both plant growth and missing-leaf area between the control and experimental plots. We chose to use Mann-Whitney U tests, rather than independent sample t-tests, because our sample sizes were small and normal distributions could not be assumed. Two of these tests were performed comparing the differences in marigold plant height and legume plant height between plots. Similarly, we performed two tests comparing the missing-leaf area in bean and marigold plants in both the experimental and control plots.

Results

Our first analysis comparing the growth rates of beans for each plot indicated that there was a significant difference between the two groups of beans ($\alpha = 0.05$). Our data showed that, on average, bean individuals co-planted with marigolds grew at a much faster rate than those by themselves. Afterwards, the data on marigold growth rates were examined using the same technique. The Mann-Whitney output for marigolds also showed that the two plots had a statistically significant variation in their growth rates (p-value < 0.05). The data suggest that

marigolds planted alongside beans displayed higher growth rates than those that were planted by themselves.

We performed another set of Mann-Whitney U tests in order to determine if there was any variability in leaf area lost between the species in the control and experimental plots. For the test performed on the beans, it was shown that there was a significant difference in the amount of area lost (p -value < 0.05). Our results suggest that the amount of herbivory on the beans in the plot with the marigolds was less than that found on the beans grown by themselves. However, our final test for the marigolds showed that there was not a significant difference in the amount of herbivory between the two plots at the $\alpha = 0.05$ level.

Our initial soil sample results show that before planting the initial concentrations of phosphate, nitrate, and ammonium were as follows: 1345.33 $\mu\text{g-P/g-soil}$, 257 $\mu\text{g-N/g-soil}$, and 32.13 $\mu\text{g-N/g-soil}$ respectively. The second test performed several days after planting showed a marked decrease in the amounts of both nitrogen compounds (Table 1). In the following analysis, nitrate concentrations in all of the soil samples continued to decrease, while the amount of ammonium in each of the plots increased an average of 16.82 $\mu\text{g-N/g-soil}$.

Discussion

Soil Nutrient Concentrations

From our initial soil samples, we found both nitrogen and phosphorus concentrations to be relatively equal throughout our plot. This was important in ensuring that each plot would start off with an equal amount of nutrients. Following the introduction of our plant species, we observed a steep decrease in the amount of ammonium and nitrate concentrations in the soil, which can be attributed to the uptake of nitrogen by the seedlings as they began growing. In the

subsequent trial, we found that nitrate concentrations continued to decrease while ammonium concentrations began to rebound in each of the legume plots (Figure 2). Increases in ammonium suggest nitrogen fixation is taking place in each of these plots. While both ammonium and nitrate are being produced in the soil, nitrate is more readily absorbed by the plants (Peoples et al., 1995). Therefore, we are witnessing nitrate being assimilated at a faster rate and continue to decrease in concentration (Figure 3), while ammonium has the ability to slowly increase. The same general trend was observed in the marigold control plot, although not to the same degree, which could be due to its close proximity to the bean plots.

Missing Leaf-Area and Herbivory Trends

Our findings suggest there was significantly more herbivory on *Phaseolus vulgaris* plants found inside the control plot than in our experimental plot, which supports our hypothesis that *Tagetes patula* should effectively decrease the missing-leaf area in surrounding legumes. Based on these results, it would appear that *Tagetes patula* was successful in deterring insect pests. Our results support the results of similar studies, in which the amount of pest incidence was significantly decreased in plants intercropped with *Tagetes patula* (Silveira et al., 2009). A controversy surrounding marigolds, and something we were unable to test for, is whether they are actually repelling insects or if they are interrupting the insects host finding and selecting behaviors (Parker et al. 2013). Future experiments with *Tagetes patula* should be directed more towards determining this mechanism, rather than further displaying its effectiveness.

Unlike the bean plants, we found there to be an insignificant difference in the amount of herbivory on marigold plants. Given that marigolds actively deterred pests and were equally present in both plots, there was no reason to suggest that herbivory would vary between them. However, this finding may contradict the widely demonstrated pattern of polycultures having

decreased levels of herbivory, when compared to similar monocultures (Parker et al. 2013). In other words, the presence of multiple species increases the opportunities that insect sensory cues are masked, or herbivore attention split, resulting in less overall herbivory for all plants involved (Parker et al. 2013). If this were the case, we would have expected there to be less overall herbivory in the intercropped plot. Although we had intended to compare herbivory on *Pisum sativum*, there appeared to be no signs of herbivory on these plants.

Plant Growth Rates

As mentioned in the results, we found highly significant differences in the growth rate of bean plants between the intercropped and control plots. This supports our hypothesis that predicted overall greater plant growth in the plot containing both *Tagetes patula* and our legume species. We believe this could be the result of decreased herbivore stress on the intercropped plants, thus allowing them to invest more energy into growth. In response to high levels of herbivory, plants are forced to make a tradeoff between growth and the production of defensive secondary compounds (Coley et al., 1985). Given the increased rates of herbivory across plots, individuals in our control plot may have been forced to increase defensive compound production and therefore temporarily limit their growth.

Our statistics also showed a significant difference in the growth rates of marigolds between our plots. Once again, this is strong evidence supporting our hypothesis that marigolds would experience greater growth rates when planted alongside the legume species. In the plots containing both legumes and marigold, the amount of plant-ready nitrogen continued to increase throughout the growing season. This leads us to believe that intercropped marigold plants were able to harvest more nitrogen and grow at a rate much faster than their monoculture counterparts.

Errors and Improvement for Future Experiments

Although we were able to produce significant results that supported our hypotheses, there are many potential errors that could have occurred throughout the experiment. As with most studies conducted in the field, human error must be considered. First, there may have been inconsistencies in measuring plant height or in detecting amounts of herbivory which may have affected our results. Also, there may have been mistakes in recording and inputting data. In addition to human error, it is possible that there were some flaws in our plot design. We were only permitted to utilize a limited amount of space in the garden, meaning that each of our experimental groups were relatively close in proximity. Even though we separated our two control groups from the experimental group with the soil mound, the effects of our experimental group may have extended to our controls. In the future, it would be ideal to have multiple plots within the garden so that there would be no chance of one group affecting another. Lastly, the time constraint of this study may have given us less significant results. Since we only had a few weeks to conduct our experiment, we were unable to allow our plants to reach full maturity. This study would be best replicated using a longer time period to allow for all of the plants to grow to their complete potential.

Improved Project Design

In performing this experiment, we encountered problems with non-insect herbivory that altered the course of our investigation. Accordingly, we thought it would be valuable to propose an alternate project design that would look at a more complex intercropping system. To do this, we could perform the same experiment but include a plant such as onion or garlic in each of the plots to deter mammalian herbivory. This would help us solely observe the effects of insect herbivory and nitrogen fixation without any mammalian intervention. Also, we could perform an

experiment that would test the effectiveness of a different method in deterring insect herbivory, such as trap cropping. Plants like nasturtiums have been shown to attract and trap pests on them, deterring these harmful insects and the potential damage they can cause from the plant you wish to protect (Riesselman, 2009). Therefore, intercropping these plants in the same fashion as our previous experiment could test if the trap cropping method is more effective at deterring herbivory than the volatile chemicals that marigolds give off.

References

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Figures and Tables

Table 1. Ammonium and nitrate concentration levels

Sample ID	Soil NH ₄ ⁺ -N (μg-N/g-soil)	Soil NO ₃ ²⁻ -N (μg-N/g-soil)
Nitrogen Baseline	32.13	257
Marigolds (5/28)	13.95	171.5
Beans (5/28)	10.2	127
Beans and Marigolds (5/28)	9.6	145
Marigolds (5/31)	26.3	110.95
Beans (5/31)	29.2	53.1
Beans and Marigolds (5/31)	28.7	66.05

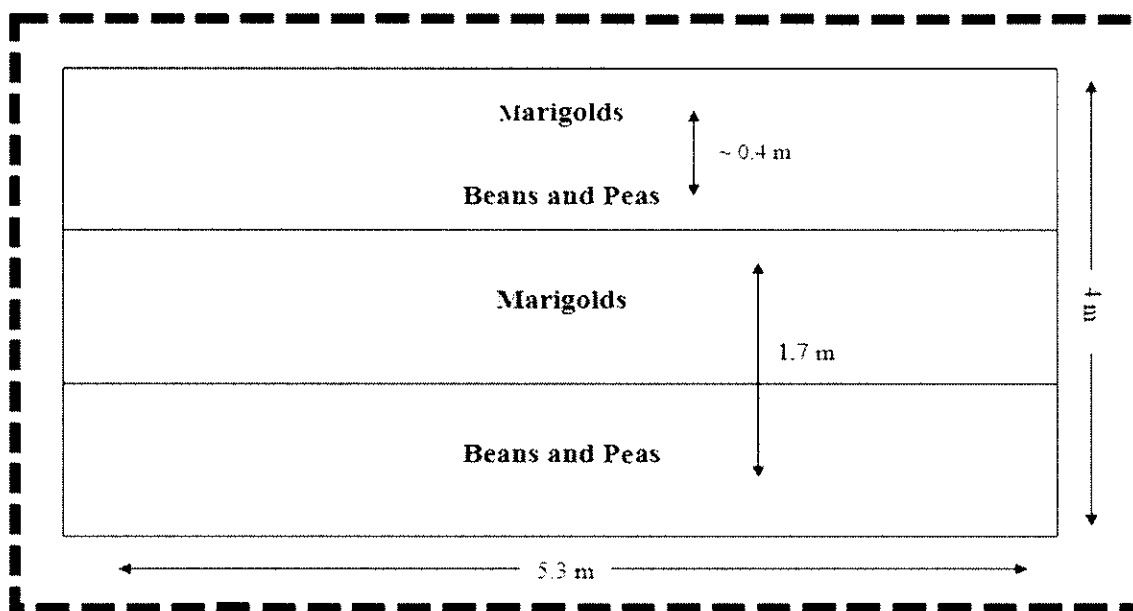


Figure 1. Arrangement of Plant Species

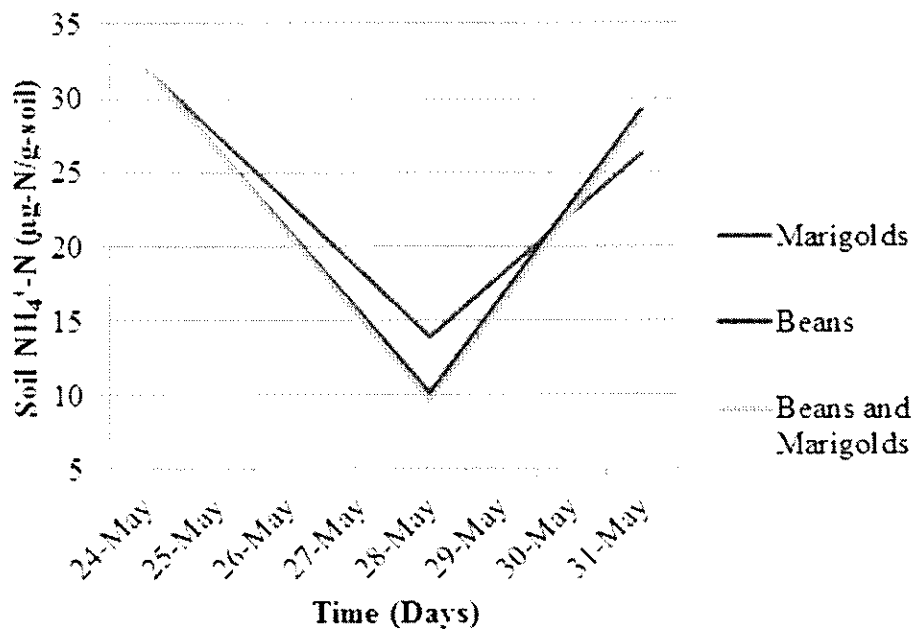


Figure 2. Variation in ammonium concentrations between each test plot

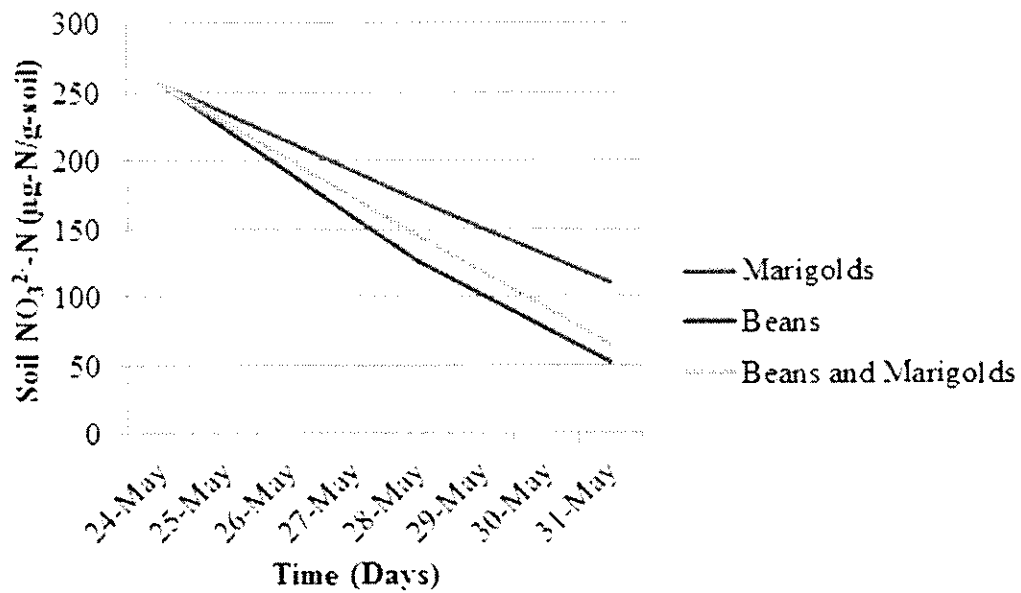


Figure 3. Variation in nitrate concentrations between each test plot

