APHIDS AND ANTS: MUTUALISTIC INTERACTIONS ON ASPEN TREES

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ABSTRACT

Mutualism is an interaction in which two species provide benefits for each other. The mutualistic relationship between *Chaitophorus populicola* aphids and several ant species on *Populus tremuloides*, quaking aspen, was investigated, in which aphids produce honeydew, a sugar-rich food for ants, and ants supposedly protect aphids from predators and help aphids maintain hygiene. Winged and unwinged aphid and ant populations were monitored for two weeks on trees in 25 sites at University of Notre Dame Ecological Research Center (UNDERC). Aphids completely disappeared on some trees during the initial two weeks, so of the remaining fourteen trees still hosting aphids, seven had attendant ants removed, and the tree trunks treated with Tanglefoot, a gluelike substance, to prevent ants from returning. Control and experimental aphid and ant populations were observed for two additional weeks. A significant positive correlation occurred between ant and aphid populations. Fewer aphids were found on stems with ants removed by Tanglefoot treatment, though this was insignificant. However, the relationship between overall ant presence and aphid population was significant; on average, aphid population size was three times higher on stems where ants were present. The hypothesis was supported; ant presence was associated with higher aphid population, as shown in this and other studies. Winged aphid abundance peaked an average of 5.3 days before a population crash event in an aphid-ant colony, indicating a response to environmental pressures such as overcrowding, plant quality, predation, and lack of ant attendance. Different forms of evidence from this and other studies contrast in determining whether mutualisms are always dependent on context. Results showed that aphids benefited overall from ant presence in this investigation. However, ants always benefited because they were free to leave the aphids when they no longer needed honeydew, while aphids did not, and aphid populations declined quickly without attendant ants.

The interaction between aphids and ants may more accurately be called a cross between mutualism and commensalism.

INTRODUCTION

Mutualism is a reciprocally beneficial relationship between organisms (Herre *et al*. 1999). Because the most studied relationships among ecosystems are those between predator and prey, mutualisms are often ignored or go unnoticed. This makes understanding mutualisms all the more important. The mutualism phenomenon is demonstrated between numerous species on earth, and is often the ecologically dominant foundation of entire ecosystems such as coral reefs (Boucher 1985, University of Arizona 2010).

Another well-documented example of protective mutualism is the relationship between certain species of ants and aphids, which is observed across a variety of ecosystems and locations. Generally, the larger ants offer protection from predators and disease, and clean the smaller aphids. In turn, aphids produce a sugar-rich substance known as honeydew, the waste product of an aphid's plant sap diet. Ants derive all or a large part of their nutrients from this honeydew as a source of food (Begon *et al*. 2011, Detran *et al*. 2010).

In recent years, plasticity in the expression of mutualisms and other species interactions has been recognized, and aphid and ant relationships are sometimes included in this type of case; ants and aphids may not always provide benefits for each other. Whether context dependency, the variability in species interactions due to a specific set of circumstances, is present in mutualism and all other species interactions is debated. While some support this claim (Agrawal et al. 2007, Bronstein 1994), others do not. Chamberlain and Holland (2009) deem that certain aphid-ant interactions are always beneficial to both species and not dependent on context.

Aphid-ant interactions have been observed on American or quaking aspens *(Populus tremuloides)* at UNDERC (University of Notre Dame Environmental Research Center), in the Upper Peninsula of Michigan. Attendant ants can be seen stroking the bodies of masses of aphids (*Chaitophorus populicola)*, with their antennae to encourage production of honeydew. In previous studies, ants have been shown to protect aphids by attacking potential predators. When larval predators of aphids were transferred to a bush with aphid colonies and attendant ants, the ants attacked the predator larvae immediately, and aphid population size remained stable (Rathcke *et al.* 1967).

In this study, the nature of the relationship between ants and aphids on aspen trees was investigated to determine whether they are involved in a mutualism in which each species provides clear benefits to the other. First, winged aphid morphs have been found to be become more abundant and disperse when under stressful conditions such as overcrowding, plant quality, and predation. Therefore the abundance of winged aphids was considered an indicator of environmental stress, and a sign that ants were not providing ample benefits to a colony (Kindlmann *et al.* 2002, Dixon 1977). Second, aphids can reproduce via viviparous parthenogenesis, without presence of eggs or larval stages. Population increase in aphids is straightforward, and aphids with high fitness will simply create copies of themselves without having to breed (Simon *et al.* 2002*)*. Population growth was quantified as a measure of fitness.

When both aphids and attendant ants are found together in a colony, their interactions should positively alter aphid population growth if the species interaction is mutualistic. When separated, negative or neutral population change should occur. I hypothesized that when present together, ants and aphids benefit each other through population growth. Specifically, I predicted that aphid abundance on an aspen tree increases with presence of ants and conversely decreases when ants are not present.

METHODS

Observational and experimental approaches were used to test this hypothesis. Thirty replicate aspen trees scattered throughout 25 locations at UNDERC were chosen and marked (Figure 1). The number of winged and unwinged aphids and ants on each of the trees were counted as the control group. All sites were monitored every other day for two weeks by counting all ants and both winged and unwinged aphids per colony, and noting any potential predators. Sites were compared rather than trees or colonies because several colonies were often found on a single tree, and therefore not independent of one another, and in some cases two trees were present per site, with similar environmental factors that could skew data toward certain sites.

After two weeks, aphids had completely disappeared from some trees, and only fourteen trees still hosted aphids. Seven of these remaining fourteen trees at thirteen sites were randomly chosen, stripped of ants, and covered with Tanglefoot. This sticky substance painted around the bases of trees was applied to prevent ants and other insects from crawling up stems (Contech Enterprises Inc.). These altered trees of just winged and unwinged aphids were counted as the experimental group, and the other seven trees left as the control group. Winged and unwinged aphids and ants on both groups of trees were counted over the course of the following two weeks. For each tree’s count of aphids, every colony per tree was monitored. On the last day, remaining ants were taken from their trees for identification. To evaluate possible lack of benefit from attendant ants, the highest number of winged aphids per colony was noted, and the mean number of days before a colonial population crash was calculated.

Using SYSTAT 13 software (Systat Software, Chicago, IL), data was first normalized using natural log transformation. To evaluate the relationship between mean ant and aphid abundance, I conducted regression analysis. Then, a one-way ANOVA test was used to determine possible differences between aphid abundance and Tanglefoot presence. Because some sites not treated with Tanglefoot hosted no attendant ants, a second ANOVA test was run, analyzing aphid abundance and ant presence rather than Tanglefoot presence, to include those colonies without ants.

RESULTS

Results showed that a positive linear relationship between ant and aphid abundance was observed, as calculated from data during the initial observational period (R^2=0.361, $p<0.001$). Ant abundance positively affected aphid abundance. Without ants, zero or only a few aphids were present usually. No cases were observed in which ants but no aphids were present. Ants were less numerous than aphids per colony, but a proportional linear relationship was noted (Figure 2).

A steady decrease in aphids was observed at a loss of all aphids on about 1.6 sites every other day with a standard deviation of 1.3 days, both before and after the Tanglefoot treatment on July 7 (Figure 3). New colonies established on trees during the study period are included. A population crash in this case is defined as the event in which aphid population dropped in number by more than one half in a two-day period. Abundance and proportion per colony of winged aphids were highest an average of 5.3 days before a population crash event in an aphid-ant colony, with a standard deviation of 3.3 days. Sites ranged from one to 15 winged aphids, and highest winged aphid abundance prior to population crash occurred in 41 of the 57 colonies.

Though aphid populations decreased on the seven sites treated with Tanglefoot and stripped of ants (Figure 4), aphid population size from sites treated with Tanglefoot was not significantly different from those seven left untreated ($F_{(1,12)}$=1.984, p=0.183). When untreated sites occurring without ants were grouped with Tanglefoot-treated sites, however, the difference between aphid population per site and ant presence was significant ($F_{(1,12)}$=11.724, p=0.005). Aphid population was approximately three times greater in sites where ants were present. Ant presence positively affected aphid abundance (Figure 5).

DISCUSSION

The results of this experiment support my hypothesis; ant presence does have a positive effect on aphid abundance on aspen trees. This was expected, as a number of other studies have shown similar results. It was interesting to find that removing ants from seven trees did not lead to a significant difference between those sites on which Tanglefoot was applied and those in which it was not. However, it was then noted that several of the trees, over time, hosted aphid populations without any ants present. So, rather than grouping the trees by Tanglefoot application, they were grouped by ant presence; then, aphid population size between trees with and without ants was significantly different.

This observation indicates that ant presence is dependent not only on Tanglefoot, but also on one or several other outside environmental factors. Predation may be one of these factors. Quaking aspens play host to at least 33 insect species, so it is likely that one or number of these preys upon ants that tend to aphid colonies (Jones *et al.* 2012). While counting aphid population, predators such as spiders and larval insects and dead ants hanging from webs, were often noted. Some of these predators, especially spiders, were ant-specific, leaving the aphids safe after trapping or eating their attendant ants. Also, ants do not constantly need honeydew, and may not always be present at a colony.

Before this investigation, I had presupposed that aphid colony populations would fluctuate but not disappear altogether. This assumption was quickly proven wrong, as multiple colonies would simply have disappeared every time aphids and ants were counted. How aphid species are able to survive at all became an important question. After further research, it was discovered that aphid survival could be explained by dispersal; aphids are able to spread from tree to tree because they have a winged morph. Aphids, although clonal, have been found to respond to environmental cues by switching from an unwinged reproductive mode, known as apterae, to a winged migratory mode, called alates (Dixon *et al.* 1993). They can, in times of environmental pressures, such as predation, low plant quality, overcrowding, and lack of ant attendance, adapt to grow wings and fly away (Dixon 1977). Winged aphids were often observed during this study, and on average, they were most abundant shortly before aphid population crashed. So, aphids did respond to environmental cues by developing wings. Some of these winged aphid increases and population crashes may indicate lack of ant attendance. This offers insight into population studies in aphid and general insect ecology; understanding dispersal mechanisms is necessary to correctly quantify certain populations, as estimating aphid populations has actually posed serious problems in investigations due to dispersal of winged aphids (Dixon 1977).

Mutualism has recently become known as a plastic interaction in many of its occurrences, and aphid and ant relationships are included among these. Context dependency, the variability in the outcome of species interactions due to biotic and abiotic circumstances, is considered by some to be ubiquitous in mutualisms and all other interactions between species (Agrawal *et al.*

2007, Bronstein 1994). This is debated; Chamberlain and Holland (2009) assert that certain aphid-ant mutualisms always benefit both species. In one study conducted by Offenberg, aphid-ant mutualism can quickly change to exploitation. When given sugar as an alternative food source to aphid honeydew, ants chose the alternative sugar and proceeded to eat the aphids instead, presumably as a source of protein (Offenberg 2001). So, mutualism is not a pact between the individuals of two species to always be there for one another, but an interaction arising from two species with the ability to provide necessary resources for the other. It can morph from a beneficial relationship for both species into a form of commensalism, in which one species benefits without affecting the other (Thompson 1988).

Ants do not always protect the aphids they associate with. In one qualitative study, various predators were presented to ant-aphid colonies, and the attendant ants often failed to protect their aphids (Rathcke *et al.* 1967). Predatory larval insects were observed with aphids in their mouths while the ants simply failed to react on more than one occasion during this study. In addition, with higher numbers of aphids per colony, benefits from ant tending are known to decrease or be nonexistent, and the effects of ants on aphids are most noticeable in populations of less than 30 aphids (Breton and Addicott 1992). Though results showed that aphids benefited overall from ant presence in this investigation in terms of population growth, it seems that ants always benefited, while aphid fitness did not always improve with attendant ants. Ants were free to leave the aphids when they no longer needed honeydew, while aphid populations suffered without attendant ants. The interaction between aphids and ants could more accurately be called a cross between mutualism and commensalism.

In summary, results supported the original hypothesis, and sites with ants present had aphid populations approximately three times higher than those without ants. This indicates that aphid-ant interaction is indeed a mutualism. However, increases in winged aphids and population crashes may be a response to lack of ant tending. Contrasting evidence between this and other studies debate whether this is always a mutualism; one could consider it a cross between mutualism and commensalism, with ants always benefiting and aphids only sometimes benefiting.

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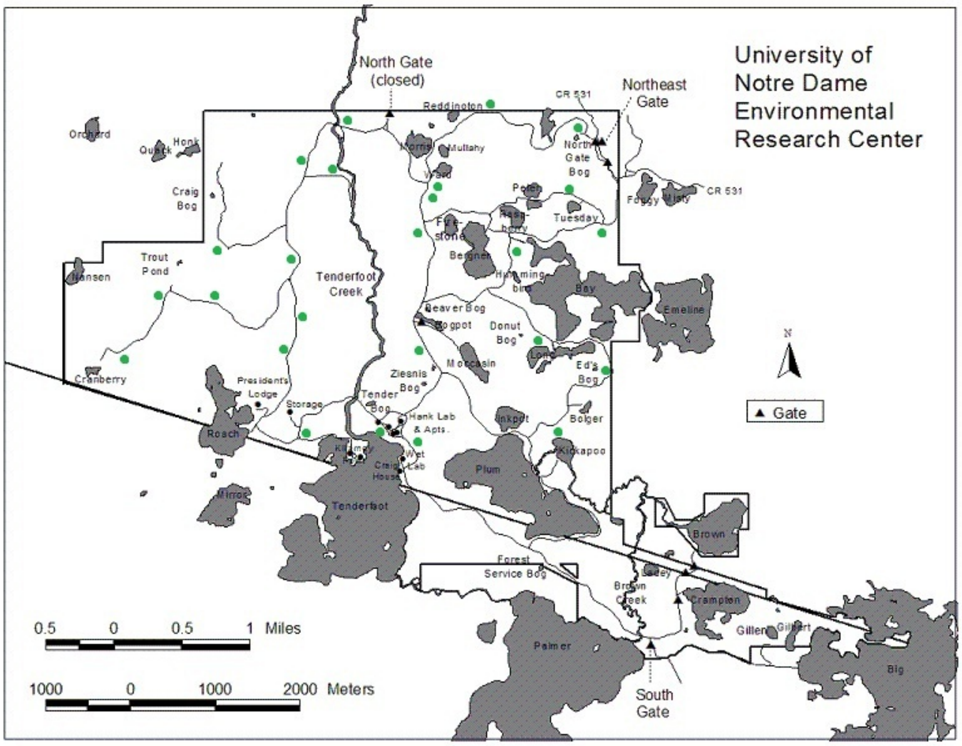

Figure 1. Map of sites at UNDERC, marked by green dots.

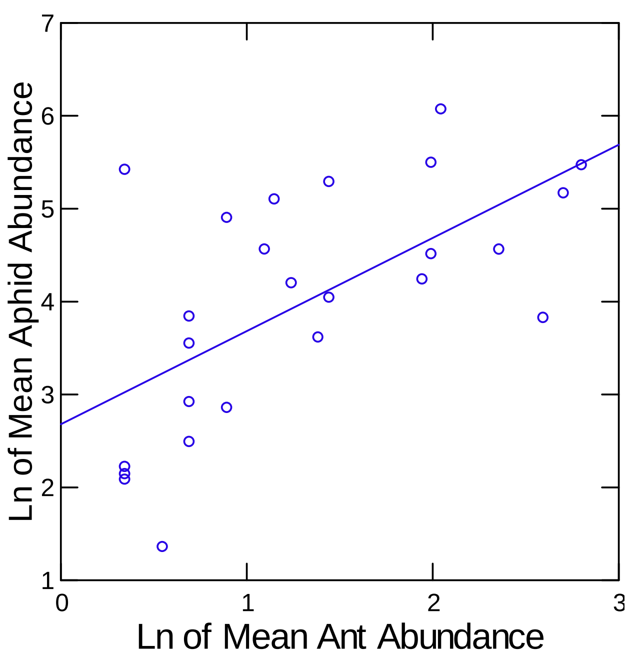

Figure 2. Natural log of mean ant abundance, for average of days 1 and 2, the days with the least

zeros. Days 1 and 2 were chosen because they included less zeros than all other days that tended to skew the data towards a larger difference.

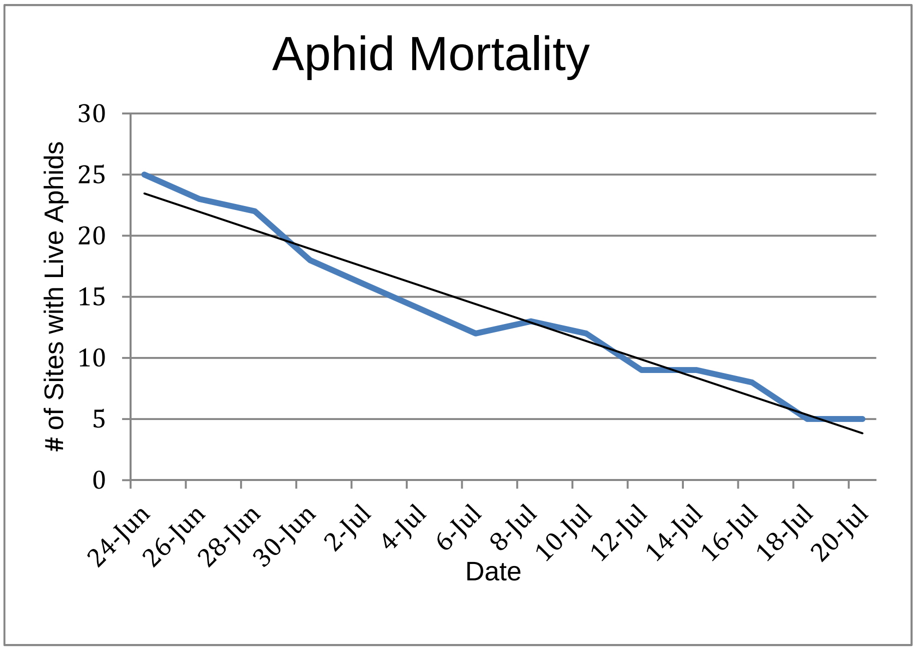

Figure 3. Number of sites with live aphids over four weeks, every other day, from June 25 through July 21. New colonies that appeared on trees throughout the study period are included.

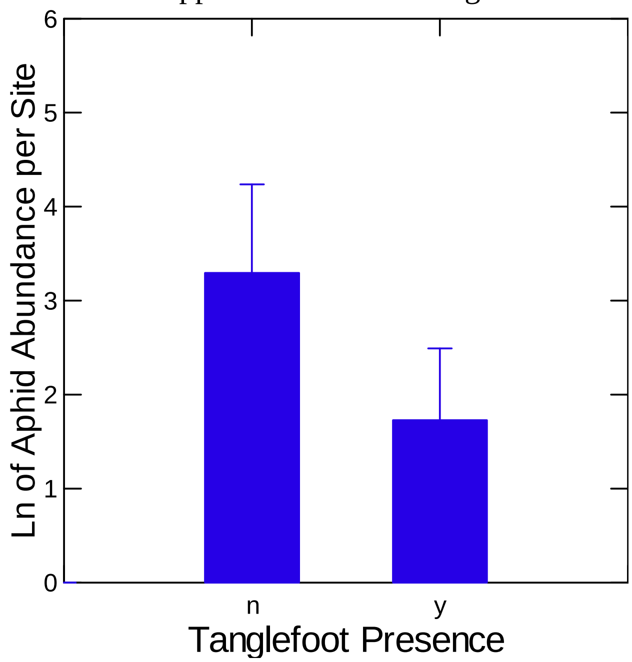

Figure 4. Natural log of aphid abundance per site for day 11 versus presence or absence of

Tanglefoot treatment. Day 11, July 15, was chosen a week after Tanglefoot application, when enough time had passed for the treatment to take full effect.

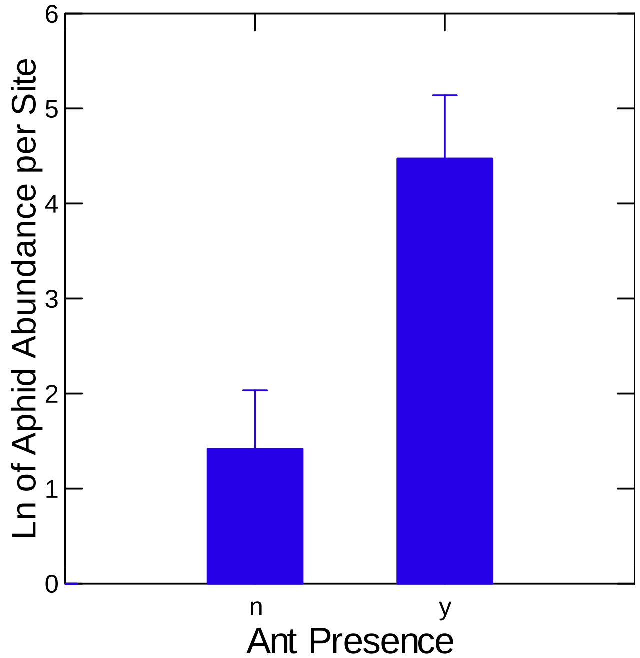

Figure 5. Natural log of aphid abundance per site for day 11 versus presence or absence of ants. Day 11 was chosen for comparison with Figure 3.