PROJECT REPORT

This is a final project report submitted
to the Organic Farming Research Foundation.

Project Title:

Harnessing aphid alarm pheromone to rid broccoli heads of aphids

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ORGANIC FARMING RESEARCH FOUNDATION

1. Project Summary

The purpose of this project was to find a way to make aphid-infested broccoli heads marketable by inducing the aphids to walk away in response to alarm pheromone. The project found no alarm behavior in cabbage aphids raised on crop plants, and the question of whether cabbage aphids raised on wild crucifers produce alarm pheromone remains unanswered.

2. Introduction to topic

One of the key pests of broccoli is the cabbage aphid, ***Brevicoryne brassicae*** L., which can cause economic loss by infesting the broccoli head (Nieto et al. 2006). Integrated Pest Management (IPM) guidelines insist that control measures be taken if even a few aphids are present when the head begins to form (Chaney & Natwick 2007). However, listed organic sprays are only partially effective and the natural enemies that organic farmers depend on are subject to natural variation in numbers (Nieto et al. 2006). The Good Humus farm was plagued by a bad infestation of broccoli heads one spring and the question came up regarding the possibility of removing the aphids post-harvest using alarm pheromone, the chemical signal released when an aphid is crushed, which signals nearby aphids to walk away or drop off (Nault et al. 1973).

A literature search revealed that cabbage aphids produce little to none of the usual aphid alarm pheromone, (E)-β-farnesene (EBF; Francis et al. 2004, 2005). However, studies of another crucifer specialist aphid – the turnip aphid, *Lipaphis erysimi* (Kaltenbach) – found that while it shows little alarm activity toward EBF (Dawson et al. 1987), it shows high alarm activity when exposed to EBF in conjunction with certain cruciferous compounds or whole extracts of cruciferous plant species. Indeed, when this aphid is crushed it releases several host-derived isothiocyanates. The profile of volatile compounds from crushed cabbage aphids is known (Francis et al. 2005) and these include the cruciferous compounds benzyl-isothiocyanate and benzylnitrile when the aphids are raised on white mustard (*Sinapis alba* L.), although not when raised on oilseed rape (*Brassica napus* L.; Francis et al 2004).

A preliminary field test suggested an alarm response in cabbage aphids. On a breezy day, a colony of cabbage aphids--characterized by their silvery-powdery exterior--infesting a volunteer *Brassica rapa* inflorescence was exposed to two crushed individuals from a nearby colony, leading to several members falling off or walking away. Another colony exposed to bits of moist soil gave no such response.

A project was fashioned wherein cabbage aphid alarm pheromone would be reproduced using a combination of the components of their volatiles. The most potent combination would be used to find an effective method to dislodge aphids from infested broccoli heads. In order to give growers more control over the process, the compounds would then be produced using plants that could be grown on the farm, such as German chamomile, Chinese cabbage, and giant goldenrod. Finally, the cost of removing aphids would be compared with the extra income possible from marketing the cleaned broccoli.

3. Objectives Statement

The original objectives were to 1) establish the existence of cabbage aphid alarm pheromone; 2) determine the compounds involved in the alarm response; 3) use the compounds to remove aphids from infested broccoli; 4) develop plant-based alarm pheromone from plants that can be grown on-farm; and 5) document labor and material costs of aphid removal. All of the latter objectives were dependent on success with the first objective, and as no alarm behavior was seen on field crops, an intermediate objective was established, that of testing for alarm behavior in aphids raised on a variety of wild cruciferous species.

4. Materials and Methods

Location. All research was conducted at the Good Humus farm, Capay, California, between February 2009 and January 2010.

Plants. Host plants for the aphids were diCicco broccoli (*Brassica oleracea*), Chinese cabbage (*B. rapa*), the second-generation offspring of an apparent cross of bok choy and rapini (*B. rapa*), field mustard (*B. rapa*), black mustard (*B. nigra*), the wild mustard *Hirschfeldia incana*, shepherd's purse (*Capsella bursa-pastoris*), and wild radish (*Raphanus sativus*). The broccoli was planted by the grower as a winter cover crop while the Chinese cabbage plants had been left in the field after the 2008 fall harvest. The bok choy-rapini hybrids were sown in a seedling tray on 21 February, and 50 of them were transplanted to the field on 28 March. The other plants, plus more bok choy-rapini, were sown in a seedling tray 27 September. Up to six of each were transplanted to the field on 14 November and 5 December, except for the field mustard, which did not germinate. The shepherd's purse and wild radish did not survive transplantation.

Aphids. Aphids naturally colonized the broccoli and Chinese cabbage. The other plants each received a single aphid raised indoors on radish and broccoli seedlings or found on market-purchased gailan (*B. oleracea*). The spring-planted bok choy-rapini hybrid plants received aphids on 26 April and were then covered with floating row covers. The fall-planted plants received aphids on 9 January and were covered with organza fabric, anchored with soil.

Pheromone release. In most instances aphids were crushed with forceps to induce release of alarm pheromone. On 21 March several inflorescences of diCicco broccoli with infestations of silvery-powdery aphids were clipped and brought indoors for testing. An inflorescence was placed on white paper and a crushed aphid held beside the aphid colony for 5 minutes while the aphid response was observed. The control was crushed fruit fly (*Drosophila melanogaster*) pupae. Five inflorescences were tested with aphids and four with fly pupae.

On 28 March aphids on broccoli inflorescences in the field were induced to release alarm pheromone by crushing or by the feeding of a soldier beetle or a ladybug larva, and the aphids' behavior was observed. No control was used on this date.

On 4 April varying numbers of aphids on Chinese cabbage inflorescences were crushed to induce alarm pheromone release and held next to nearby aphid colonies while the colonies' behavior was observed.

The spring transplants were inspected for aphids on 16 May, and the fall transplants inspected on 30 January.

5. Project Results

Of the aphid colonies on the clipped broccoli inflorescences, none came off in response to fly pupae, while a total of 2 started walking and 3 landed on the paper while crushed aphids were held nearby, out of colonies of dozens individuals, perhaps over one hundred.

Of the aphid colonies on the broccoli in the field, crushed aphids had no effect on one colony, and it was difficult to tell whether the few aphids seen walking in a second colony had started walking before exposure to the crushed aphids. The soldier beetle's feeding caused a few aphids on a colony to start walking, although the beetle also walked on the colony. The ladybug larva feeding caused no response from the other aphids.

The colonies of aphids on Chinese cabbage did not respond to a crushed aphid held nearby. When crushed aphids were placed directly on two other colonies, a total of six aphids began walking, but crushed fruit fly pupae placed directly on a colony caused three aphids to begin walking. A whole colony crushed and held next to a small colony of around 10 individuals caused only the lone winged adult to walk.

The spring transplants were exposed to predators and parasitoids due to tears in the row covers, and there were none of the silvery-powdery aphids surviving when the covers were removed. The fall transplants had a total of 6 aphid mummies and no living silvery-powdery aphids.

6. Conclusions and Discussion

At this point the existence of alarm pheromone in cabbage aphids is unproven. No statistical analyses were done because the effect would have to be large in order to be useful to growers. A good example of the effect of alarm pheromone was shown by another aphid species on sowthistle (*Sonchus* sp.) on the farm. When several crushed individuals were held near a colony, the living aphids leapt off as if they had been hit by a blast of air, an effect not seen when a clean glove was held near them.

It is conceivable that cabbage aphids, by sequestering glucosinolates from their hosts and converting them to thiocyanates, isothiocyanates, and nitriles when injured (Kazana et al. 2007), are defended well enough from predators that they have lost the alarm response. Indeed, the two-spot ladybug is unable to survive on cabbage aphids, and the growth of the seven-spot ladybug is inhibited when it is fed cabbage aphids exclusively (Pratt et al. 2008). However, the turnip aphid also is chemically defended but retains the alarm response (Dawson et al. 1987).

It is also possible that broccoli and Chinese cabbage lack the precursor compounds necessary for the cabbage aphid to produce alarm pheromone (Sharon Strauss, *pers. comm.*). The use of wild crucifers was intended to address this question, but the logical arena for investigating this basic question would be a more controlled setting such as a growth chamber. The requirement that all research be carried out on a certified organic farm meant that the aphids used for behavior studies were exposed to predators, parasitoids, and extreme temperatures.

One question that emerged from field observation of the system was why the natural enemies were able to remove all the aphids from the experimental plants but left the harvested cauliflower full of aphids. The aphid colonies reached very dense levels on the bolted broccoli cover crop and bolted Chinese cabbage by March, while the beneficials seemed to appear with warmer weather at the end of that month (Jeff Main, *pers. comm.*). The broccoli cover is planted for its known inhibition of soil pathogenic fungi (Koike & Subbarao 2000, Njoroge et al. 2009), but one wonders if its presence on the farm during the winter months allows the aphid population to build up. It would be interesting to compare aphid infestations in market broccoli and cauliflower from organic farms where broccoli cover crops are used versus those with no cole crops present during the winter, but the use of broccoli cover crops on farms that also market broccoli is rare (Mark Bolda, *pers. comm.*).

The question of whether aphids can be induced to leave a broccoli head by application of alarm pheromone remains unanswered. If cabbage aphids truly produce no alarm pheromone under any condition, that would be a fascinating evolutionary finding, but not helpful for growers. However, if there are conditions under which cabbage aphids produce alarm pheromone, such as a certain host, then the possibility remains for duplicating the alarm pheromone and testing whether it is effective in cleaning aphids from broccoli.

7. Outreach

There were no applicable findings from this study.

8. References

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9. Addenda

Fig. 1. Cabbage aphid colony on bolted broccoli.

Fig 2. Seedlings of the bok choy-rapini cross.

Fig. 3. Organza fabric covering the wild crucifers.

Fig. 4. Black mustard, *Brassica nigra*.