

## HOW DO PLANT DEFENSE COMPOUNDS INFLUENCE THE OVIPOSITION BEHAVIOUR OF SMALL CABBAGE WHITE BUTTERFLY *PIERIS RAPAE* (LINNAEUS)?

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### ABSTRACT

Jasmonic acid (JA) is an elicitor for induced plant defense. Herbivore attack causes an increase in endogenous JA in a plant, but exogenous JA treatment induces a similar set of compounds as an attack by an insect herbivore. In some plants, volatiles are induced after plant injury by herbivores and these volatiles repel the future herbivores.

But, do volatiles also have an effect on ovipositing herbivores? In this experiment the effect of JA (an inducing compound of volatiles) and attack of caterpillars on the oviposition behavior of the small cabbage white butterflies (*Pieris rapae*) on Brussels sprouts plants (*Brassica oleracea gemmifera*, cv. *Cyrus*) is investigated. A JA concentration of 0.1 mM and attack of *P. rapae* caterpillars negatively affect the oviposition behavior of the butterfly: *P. rapae* butterflies prefer to lay their eggs on untreated leaves. However, lower concentrations of JA did not have an effect on the oviposition preference of the butterflies.

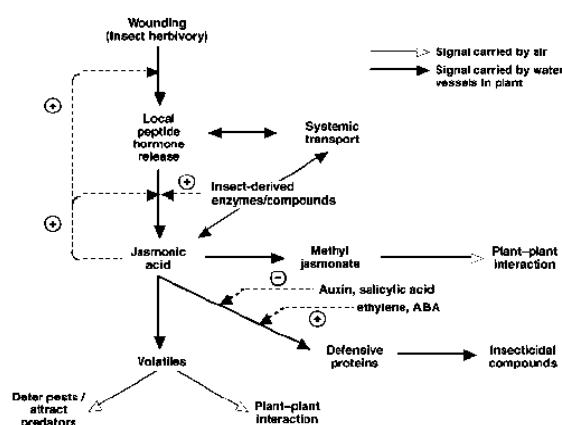
**Key words:** Jasmonic acid, oviposition, plant volatile, *Pieris rapae*

### 1. INTRODUCTION

Several studies demonstrated that herbivore-injured plants produce specific blends of volatiles which can be attractive to certain insect predators and parasitoids (Dicke, 1994; Turlings et al., 1995). Previous experiments from Steinberg et al. (1992) and Mattiacci et al. (1994) concluded that the parasitoid *Cotesia glomerata* is attracted by volatiles emitted by Brussels sprout after this plant is injured by *Pieris brassica* larvae.

Another study from Turlings (1990) concluded that seedlings, which were artificially damaged and treated with the regurgitant of *Spodoptera exigua* larvae on the damaged site, produced the same blend of volatiles as plants that are damaged by the caterpillars themselves. Later it was found out

that the chemical Jasmonic Acid (JA) plays a role in the induction. In Fig. 1 the major role JA plays in the production of signals is explained.



**Fig.1 Role of Jasmonic acid in production of signals (Gatehouse, 2002).**

Jasmonic Acid can be induced by several factors: mechanical wounding, water deficit, herbivore attack and attack by other pathogens. It can influence plant functions such as growth and development, protein storage, rate of assimilation, senescence and the most important one: defense against insects and microbes (Parthier, 1990; Herde et al., 1997; Staswick & Lehman, 1999). JA is also an important signal molecule, carrying information about injury and has been shown to be an essential regulatory component for the expression of direct and indirect defenses against herbivores (Thaler et al., 2002). Herbivore attack causes an increase in endogenous JA in the plant, but exogenous JA treatment induces a similar, but not identical set of compounds as an attack by an insect herbivore. JA has not been found to be directly toxic to herbivores (Thaler et al., 2001). In response to wounding, attack by a herbivore or treating the plant exogenously with JA, the JA concentration in the plant will usually increase, and lead to production of compounds involved in resistance to herbivores (Constabel et al. 1995). According to Bernasconi et al., 1998, emitted volatiles of herbivore-injured corn plants can repel corn leaf aphids. His team suggested that herbivores may be repelled by the odors, because herbivores could indicate that:

1. The plant has initiated production of toxic compounds against the presence of the herbivore.
2. These compounds act like a 'warning signal' to the surrounding that there are potential competitors present on the plant.
3. The warning signal attracts parasitoids and predators (Avdiushko et al., 1997).

In this experiment, this is going to be investigated for the oviposition behavior of cabbage white butterflies on Brussels sprout plants.

The goal of this experiment is to investigate the effect of Jasmonic acid on the oviposition of the white butterflies (*Pieris rapae*) on Brussels sprout plants (*Brassica oleracea gemmifera*, cv. *Cyrus*). This research project will deal with the effect of different concentrations of JA application and the effect on the oviposition preference of the butterflies when the plant is attacked by natural herbivores.

## 2. MATERIALS AND METHODS

### Plants

For this experiment leaves of Brussels sprout plants were used. These Brussels sprout plants (*Brassica oleracea gemmifera* L., cv. *Cyrus*) were sown and grown in a greenhouse ( $\pm 22^{\circ}\text{C}$ , 60% relative humidity, and a 16L: 8D photoperiod). During the experiment the plants were selected on the same phenotype, which means, that they had about the same size and amount of leaves. After this selection, for every replicate two leaves of the same position on two different plants were cut and placed in water or in Jasmonic Acid solutions. For example: one leaf on position 4 (fourth leaf on the stem) was cut from plant 1 and the other leaf was cut from plant 2 on position 4.

### Treatments

The Jasmonic Acid (JA) treatment was made by diluting pure JA (molar weight:  $210.3 \text{ g}\cdot\text{mol}^{-1}$ ) with tap water. Because JA has several effects (e.g. senescence) on the plants, relatively low (higher concentrations can be phytotoxic for the plant) concentrations were used in this experiment. These were made at three different concentrations:

- 0.1 mM Jasmonic Acid
- 0.01 mM Jasmonic Acid
- 0.001 mM Jasmonic Acid

But, since there was no difference in the results between 0.01 and 0.001 mM JA treatments, the 0.001 mM was later replaced by a dilution between 0.1 and 0.01 mM, 0.05 mM. Each of the leaves which were used for a treatment was put in a 15 or 20 ml solution (depending on leaf size, weather and availability of equipment). Before they were used in the experiment, they were for about 3 hours not in contact with the butterflies, so that the leaves had some time to take up the solution and the reaction within the leaf started. This was also to make sure that the butterflies didn't start to lay eggs on a leaf, while it hadn't taken up any JA yet. In every replicate there were two leaves used: one induced with JA and one control. The control leaf was cut from the plant at the same moment as the induced leaf, but was put in tap water instead of a JA dilution.

The fifth treatment of this experiment was infestation of Brussels sprout plants with caterpillars of the species *Pieris rapae*. Infestation with the caterpillars took place by placing 3 caterpillars on each leaf, but before they were cut and used in the experiment for 24 hours, the leaves were on the plant for 24 hours, as a precaution that the leaves won't wilt. When these leaves were put in the cages, the caterpillars were removed.

### Insects

Butterflies of the species *Pieris rapae* L. (*Lepidoptera: Pieridae*) about 6 days old, obtained from the laboratory, were used. This age of the butterflies was used, because only after 4 to 5 days after coming out of the pupae, they start to mate and to produce eggs. For every replicate, one male and one female of the same age were selected and put in the cages (greenhouse, 24°C, 60-70% humidity, and a 16L: 8D photoperiod). For every replicate, a new pair of butterflies was used.

The larvae which were used for the infestation of the cabbage plants for the natural herbivore attack were also from the species *P. rapae*. These larvae were about 1-3 days old.

### Set-up of the experiment

The experiment took place on the greenhouse (22°C, 60-70% humidity and sufficient light) of Wageningen University at the Binnenhaven. Every day 10 cages (at the end of the experiment 13, because more cages became available) were filled with one pair of *P. rapae* butterflies and 2 leaves: one treated leaf and one untreated (control) leaf. The treated leaves were placed at random in the cages. The butterflies fed on sugar water, and during late night/ early morning they were laying eggs on the leaves. Twenty-two hours after filling the cages, the amount of eggs on each leaf and position (upper side, lower side, glass) were counted and noted.

This was repeated 20 times for each treatment, to have sufficient amount of data for statistical analysis. Besides the number of eggs, which was counted and noted, the amount of solution taken up by the leaf was registered. Thus, it was possible to calculate the amount of JA taken up by the leaf in 24 hours.

At the end of the experiment, the oviposition behaviour of the butterflies was observed for about 1 hour after putting one infested and one control leaf in the cage, together with 3 female butterflies.

### Statistics

The statistical analysis took place by two different tests in the programme SPSS 11.0. For the analysis of the results of the oviposition behaviour, the Wilcoxon signed ranks test (non-parametric) test was used, and to analyse the difference between the water uptake between treated and control leaves, a Kruskal-Wallis Test was used.

### 3. RESULTS

During the experiment, it became clear that there were no clear differences between the oviposition behaviour on the replicates of 0.001 mM and 0.01 mM JA, and there was no difference in oviposition preference so far between treatment and control for 0.01 mM JA as well as 0.001 mM JA so it was decided to stop the treatment with 0.001 mM and replace it by a treatment of 0.05 mM JA.

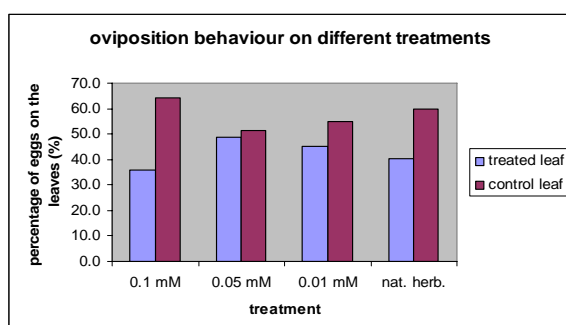


Fig. 2. Results oviposition behavior on different treatments

#### The influence of JA on water uptake

The uptake of water by the treated plants and by the control plants was measured after

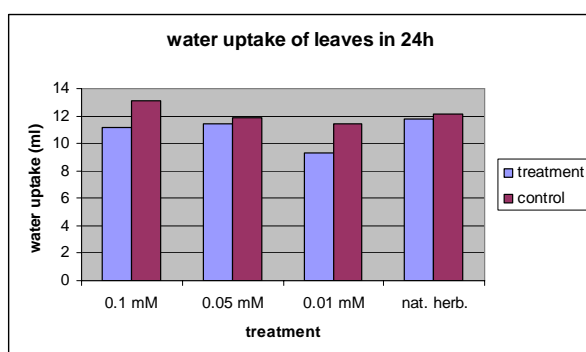


Fig. 3. Results water uptake of cabbage leaves in 24 hours

#### Oviposition behaviour of *P. rapae* butterflies on *B. oleracea gemmifera* leaves

The number of eggs laid on the treated leaf with 0.1 mM was significantly lower than on the control leaf ( $P = 0.021$ ). This was also noticed in the treatment with the natural herbivore ( $P = 0.018$ ). The treatments with 0.01 mM and 0.05 mM didn't show significant differences between the number of eggs laid on the treated leaf and the control leaf. (Fig.2)

No. of eggs on:	0.1 mM	0.05 mM	0.01 mM	nat. herb.
control	193	397	182	277
treatment	108	375	149	412

Treatment	Z-value	df	P-value
0.1 mM	-2.311	1	0.021
0.05 mM	-0.237	1	0.813
0.01 mM	-0.7	1	0.48
nat. herb.	-2.375	1	0.018

approximately 22 hours and it was seen that there was no significant difference in water uptake between a treated leaf and an untreated leaf ( $P$ -values were all above 0.05) (Fig.3).

	Z-value	df	P-value
Treatment	-2.17	1	0.03
Control	-2.996	1	0.003

Treatment	Chi-square	df	P-value
0.1 mM	2.12	1	0.15
0.05 mM	0.828	1	0.36
0.01 mM	3.204	1	0.073
Nat. herb.	0.066	1	0.8

### Oviposition behaviour: more on upper side or more on lower side?

All the eggs laid on both the treated and control leaves were counted and located: the upper side of the leaf, the lower side of the leaf, or on the glass.

As can be seen in Fig. 4 there is a significant difference between the number of eggs laid on the lower side of the leaf and the upper side of the leaf on both the treated and control leaves ( $P = 0.03$  on treated leaves and  $P = 0.003$  on control leaves).

*P. rapae* butterflies prefer laying their eggs at the lower side of the leaf. This is also seen in the visual observations, because when most of the butterflies were going to lay eggs, they sat at the edge of the leaf and curled their body down to oviposit on the lower side of the leaf.

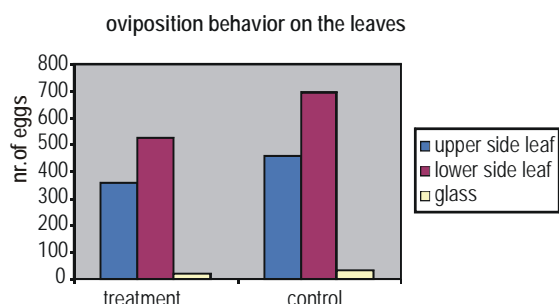


Fig. 4. Results of oviposition placement on leaves

## 4. DISCUSSION

Insects seek to distribute their eggs on the most suitable host plants that are available (Jaenike, 1978). Host encounter is followed by host assessment, which results in the decision of the female to accept or reject the oviposition resource based upon her assessment of the potential hosts' suitability. (Miller & Strickler, 1984; Singer, 1986). The performance of the larvae of herbivorous arthropods varies, depending on the quality of host plant. Larvae

of many insect species are unable to move from one host plant to another, and are thus forced to complete their development on the plant selected by their mother. Females preferentially oviposit on plants where offspring performance will be optimal (Nishida, 1995). Chinese cabbage (*Brassica campestris*) treated with JA (so artificially imitating that the plant was under attack by a herbivore) was less attractive for the diamondback moth (DBM) *Plutella xylostella* to oviposit than untreated cabbage plants, but this effect is plant-dependent. The same experiment concluded that JA-treatment of common cabbage (*B. oleracea*) made the plant more attractive to oviposit than untreated plants.

In this experiment, the oviposition behavior of *P. rapae* butterflies is affected by treatment with JA. When leaves were treated with 0.1 mM JA, the butterflies tend to not lay eggs on this infected leaf, but more on the untreated leaf, when they were able to choose between these two leaves. But, this phenomena is only seen at this JA concentration and not on lower JA concentrations, because there was no significant difference between the amounts of eggs laid on treated leaves with 0.01 mM and 0.05 mM and the untreated control leaves. The experiment was carried in the winter, and during winter volatile production is low. Maybe in the summer a lower concentration of JA could be sufficient. This phenomenon can be explained by the reasoning that a JA concentration of 0.1 mM is just high enough to start the production of signals, which repels herbivorous insects, but not high enough to have influence on other processes, like water uptake of the plant. Further investigation with higher concentrations of JA or longer period could give more information about senescence, growth, etc. By applying sufficient JA, the butterfly is warned by the plant that this plant

is stressed and the butterfly decides not to lay her eggs on this plant, because the conditions are not optimal for the development of the larvae.

This is also seen in the treatment with the natural herbivore. Because the plant is under attack by an herbivore, it starts to produce volatiles and the butterflies are warned not to lay the eggs on that plant, because there would be too much competition for the larvae. This could explain why the butterflies laid significantly more eggs on the untreated leaf than on the leaf which had been under attack by the caterpillars. But, this is probably not the only signal, maybe the feeding damage on the leaf also plays a role for the decision of the butterfly to lay her eggs on that leaf.

Not only has the status of the leaf, but also the position of the leaf had influence on the oviposition behavior of the butterflies. *P. rapae* butterflies namely laid significantly more eggs on the lower side of the leaves than on the upper side of the leaf. This is maybe because the conditions of the lower side of the leaf are more favorable for the eggs/larvae to develop. Although some cabbage leaves are more curled than others, which may make them more difficult for the butterflies to oviposit on the lower side of the leaves, they give better shelter against predators. Besides, the lower side of the leaf prevents eggs of being dried out/or burned by direct sunlight.

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