IMPACT OF PEST INSECT RESISTANT OILSEED RAPE ON HONEYBEES.

E. GRALLIEN, R. MARILLEAU, M-H PHAM-DELEGUE, A-L PICARD-NIZOU

Laboratoire de Neurobiologie Comparée des Invertébrés, INRA-CNRS (URA 1190), BP 23, La Guyonnerie, 91440 Bures-sur-Yvette, France

L. JOUANIN , F. MARION-POLL

Laboratoire de Biologie Cellulaire, Laboratoire de Phytopharmacie, INRA, Route de Saint-Cyr, 78026 Versailles cedex, France

ABSTRACT

A study was undergone to evaluate the effect of transgenic oilseed rape expressing protease inhibitors (PI) to confer pest-insect resistance on non-target insects, *i.e.* honeybees, which visit the crop. This investigation was focussed on the possible direct effects of the PI on the behaviour and physiology of bees. Experiments were conducted : (1) Under semi-natural conditions where foraging behaviour was observed in a choice situation between transformed and control plants. The number of visits as well as individual foraging strategies were recorded. In parallel, samples of nectars were collected for sugar composition analyses. (2) Under controlled conditions, where the effects of sugar solutions added with synthetic PI, were evaluated on associative conditioning performances and on sensory responses of the gustatory system.

INTRODUCTION

An alternative strategy for crop protection is the development of desease and insect-resistant crop plants, the possibilities of which have recently increased as a result of the advent of genetic engineering techniques. Plants with these new properties will need to be assessed for their specificity to target organisms, but also for their inocuousness to beneficial insects like the honeybee. Among the crop plants currently being genetically modified for increased insect and desease resistance, oilseed rape (*Brassica napus*) is of particular concern being very attractive to honeybees. The strategy used to confer insect resistance to oilseed rape is based on the expression of a gene coding for a protease inhibitor.

EXPERIMENTAL

Experiments on fresh plants

Plant material : One spring "00" line DRAKKAR and the progeny of its transformed derivative coded 70 OCI were used. A gene coding for a cysteine PI (OCI originating from rice) was placed under control of the constitutive 35S promotor of the Cauliflower Mosaic Virus, and then introduced into the oilseed rape by *Agrobacterium tumefasciens*-mediated transformation. All plants were grown in individual pots in a green house, in accordance with

the french rules on growing and handling genetically modified plants under confined conditions.

Behavioural observations : One-comb observation hives were used containing about 3,000 worker bees of *Apis mellifera mellifera*, a one-year-old queen and brood. Experiments were conducted under confinement in a climatised flight room. Five genetically modified plants and five control plants at a similar flowering stage were placed daily in the flight room between 14.00 h and 16.00 h. Each observation period lasted 45 minutes begining from the first landing on a plant by a forager and 2 or 3 replicates were performed each day. The number of bees visiting each line and the number of flowers visited were counted. With a mean of 18.54 (± 4.39) and 18.74 (± 6.70) visits per 50 flowers, respectively on the control and transformed plants, no significant differences between lines appeared.

At the same time, 45-minutes video recording were made of the flower scapes. Different variables of individual behavioural sequences (location on the plant, behavioural items such as searching, foraging, cleaning) were then analysed using an event recorder programmed with a behavioural analysis software. No difference was shown on the total duration of visits and on the mean time spent per flower between the two lines. Most bees visited 3 flowers successively, and the behavioural sequences built during these visits showed that, on transformed plants, one behavioural item (searching) was more frequent than on the control plants.

Nectar analysis : Nectar was sampled from genetically modified and control plants at a uniform flowering stage and the volumes of nectar secreted per flower were measured. Sugar composition was analysed using high performance liquid chromatography technique.

TABLE 1. Volumes and sugar concentrations of control and transformed oilseed rape nectars

	DRAKKAR	70 OCI	Signif. (Wilcoxon)
Volume (µl)	0.29 (± 0.07)	0.26 (± 0.08)	NS
Sucrose (g/100 ml)	0.28 (± 0.16)	0.48 (± 0.12)	$P<0.01$
Glucose (g/100 ml)	20.28 (± 5.74)	23.79 (± 3.29)	NS
Fructose (g/100 ml)	16.00 (± 4.47)	18.51 (± 2.43)	NS

Experiments on synthetic proteins

Proteins : Solutions of 30 % sucrose added with a cysteine inhibitor, egg white cystatin (Cys) at different concentrations (1, 5, 10 µg/ml) were prepared. Sucrose 30 % and water were used as controls.

Behavioural assay : We recorded conditioned proboscis extension in restrained individuals to 10 % linalool (an oilseed rape floral volatile) as the conditioning stimulus CS. The conditioning was performed using the different solutions (above) as the reward R. No effect of the Cys was found, compared to the control solution (Chi^2 test, 1 ddl), whatever concentration used along the acquisition (CS-R association) and the extinction (CS alone) phases (Figure 1).

Electrophysiological recordings : A preliminary study of detection responses of gustatory neuroreceptors, involved in the proboscis extension response located on the tarsi of prothoracic legs was conducted, using the solutions mentioned above as stimulations, and

sucrose 30 % and KCL 10^{-2} M as controls. Electrophysiological recordings were undergone using a reference electrode filled with KCL 10^{-2} M inserted ventrally in the inter-segment membrane, and a stimulation electrode filled with the test solutions added with KCL to insure electric conductance. The electrodes were connected to a preamplifier and an amplifier, and the responses were recorded using a microcomputer set with a data treatment software. Two types of sensilla were recorded (*sensilla basiconica* and *chaetica*). Action potential amplitudes were separated in 3 classes : high amplitude, less frequent (4.5 mV, 4 ms duration) ; middle amplitude, more frequent (1.5-1.8 mV, 3-4 ms) ; small monophasic potentials, not analysed. All types were observed with KCL stimulation, whilst high amplitude potentials were supressed by stimulating with sugar solutions (without difference between the different sugar solutions). The numbers of action potentials with middle amplitude obtained with the test solutions from *sensilla chaetica* are reported in Figure 2. No difference was shown according to the different solutions.

FIGURE 1. Acquisition (C1 to C3 trials) and extinction (T1 to T5 trials) for Cys added reward solutions.

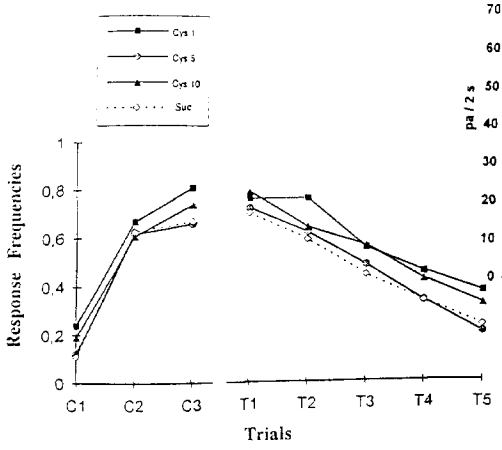
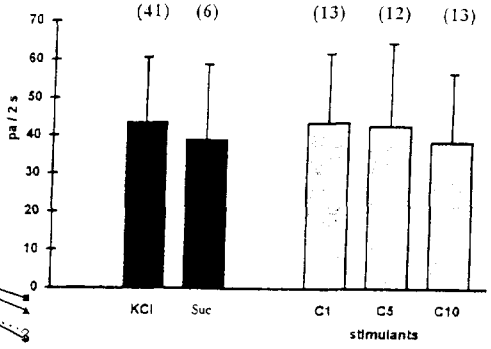


FIGURE 2. Number of action potentials (middle amplitude) recorded from *sensilla chaetica*



CONCLUSION

From the different biological assays and chemical analyses undergone, no drastic differences related to the plant transformation were found. However, the construction 70 OCI induces a rather low level of gene expression. Therefore this work is a contribution to the groundwork necessary for developping routine test procedures to assess biosafety of genetically modified plants on beneficial pollinating insects.

ACKNOWLEDGEMENTS

This work was partially funded by the company RUSTICA PROGRAIN-GENETIQUE. Plant multiplication was ensured by Station d'Amélioration des Plantes INRA, Rennes, France.