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RESEARCH ARTICLE

Survival and development of immature *Harmonia axyridis* (Pallas) feeding on *Chaitophorus populeti* (Panzer) propagated on transgenic *Populus alba* × *P. glandulosa*

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Abstract Laboratory feeding experiments with the poplar aphid, *Chaitophorus populeti* (Panzer), feeding on transgenic poplar (*P. alba* × *P. glandulosa*) varieties C13-5 and C013-5, were carried out to study the effect of transgenic poplar on the ladybird *Harmonia axyridis* (Pallas). The mortality and development time of the immature stages, the eclosion rate and body mass of *H. axyridis* were measured. The results indicated that *C. populeti* feeding on different varieties of transgenic plants had no statistically significant effect on the mortality of *H. axyridis* larvae. The development time of larval and pupal stages were not significantly different between the two transgenic poplars and a non-transgenic poplar. Furthermore, the body mass and eclosion rate did not show any difference between the *H. axyridis* feeding on aphids reared on transgenic plants and those from non-transgenic plants. It is suggested that transgenic plants have no deleterious effect on the predatory ladybird.

Key words transgenic *Populus alba* × *P. glandulosa*, *Harmonia axyridis* (Pallas), *Chaitophorus populeti* (Panzer), ecological risk

1 Introduction

The use of transgenic insect-resistant plants for controlling insect pests has attracted much attention in recent years. It has proven to be an effective approach for insect management (Poppy, 2000). Transgenic insect-resistant plants are expected not only to have the ability to resist the target pests, but also to be coordinated with the predatory enemies (Zhang et al., 2004). However, the wide use of this technology has raised concerns about its potential negative effect. Transgenic insect-resistant plants may have deleterious effects on beneficial predators by transmitting tonxin from the pest to the predator (Hilbeck et al., 1998). Although most studies have demonstrated that transgenic insect-resistant plants do not have any obviously harmful effects on the survival, development and reproduction of predatory enemies (Cui and Xia, 1999, 2000; Armer et al., 2000; Dong et al., 2003), some reports have shown that transgenic insect-resistant plants partially affect natural enemies (Hilbeck et al., 1998; Birch et al., 1999; Dutton et al., 2002). Therefore, ecological risk assessments are required. As an important part of the ecological risk assessment, the effect of transgenic insect-resistant plants on natural enemies should receive more attention.

Poplar (*Populus L.*) is a globally important forest component because of its rapid growth and suitability for cultivation on short rotations. Commercial poplar plantations have expanded rapidly in recent years due to increasing demand of fiber for paper and boards. The transgenic *Populus alba* × *P. glandulosa* is a new poplar variety which has, as an improved trait, resistance to the *Coleopterous* insects (Zhang et al., 2006). On the basis of several aspects, Hou (2008) evaluated the ecological risk of this transgenic poplar if the techniques were implemented, including an assay of the amount of expressed protein of the target gene, stability of foreign genes and others. However, the effects of foreign genes on the predatory enemies have not been investigated comprehensively. We carried out laboratory feeding experiments with the poplar aphid *Chaitophorus populeti* (Panzer), feeding on transgenic poplar (*P. alba* × *P. glandulosa*), in order to study the effect of transgenic poplar on the predator *Harmonia axyridis* (Pallas).

Previous studies about the effect of transgenic poplar on predatory enemies have mainly focused on the changes in population dynamics and predatory functions of the predatory enemy (Cui and Xia, 1999; Reed et al., 2001; Liu et al., 2003). Very few of these studies have concentrated on the biological characteristics of predators to assess the effect of transgenic plants on the development, growth and survival of predators, feeding on phytophagous insects on transgenic plants. In this study, we investigated the mortal-

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ity, time of development, pupal eclosion rate and body mass of newly emerged adults of *H. axyridis* feeding on *C. populeti* propagated on transgenic poplar and tried to determine the effect of transgenic poplar on the predatory ladybird.

2 Materials and methods

2.1 Plant materials

Transgenic poplars, *P. alba* × *P. glandulosa*, were planted in the Fangshan District, Beijing, in 2006. Two varieties, transgenic *P. alba* × *P. glandulosa* C13-5 and C013-5, were selected for the experiment. A non-transgenic *P. alba* × *P. glandulosa* variety served as control. The variety C13-5 was transformed with one foreign gene *BtCry3A* and the variety C013-5 with two foreign genes (*BtCry3A+OC-1*). All plants were grown under the same environmental conditions. According to the study by Hou (2008), the target genes have been verified to be in a stable presence in the genome of transgenic poplars using a PCR technique. The transgenic *P. alba* × *P. glandulosa* is suitable to be assessed further.

2.2 Insect species

Herbivore. The non-target herbivore *C. populeti* was used as prey. The aphids used in the experiments were obtained from the stand of the non-transgenic poplar (control) and the transgenic poplars C13-5 and C013-5 in Liulihe Town, Beijing, during July and August 2008.

Predators. The *H. axyridis* adults were collected from a poplar forest near the Chinese Academy of Forestry and reared on aphids for one generation in the laboratory in the middle of July, 2008. Eggs from the second generation were collected daily and maintained at 25–28°C until hatched. Neonate larvae (approximately 24 h old) were used for the experiments.

2.3 Laboratory feeding experiments

The experiments were conducted under a controlled environment at fluctuating temperatures of 25–28°C and 70% relative humidity. *C. populeti* collected from the two poplar varieties, C13-5, C013-5 and non-transgenic poplars, were fed with corresponding poplar leaves. Transgenic *Bt* poplar leaves or non-transgenic poplar leaves with aphids were placed in transparent plastic containers of 7 cm in diameter and 10 cm in height. The non-transgenic poplar was used as control. The newly hatched larvae of *H. axyridis* (approximately 24 h old) were introduced individually into the containers. The opening of each container was covered with medical gauze to allow air circulation. Ten to fifteen plastic containers were used in each treatment. Each treatment was replicated three times.

The aphids were replaced every 24 h and the number of aphids increased with the size and instar of *H. axyridis* (uneaten aphids from the previous day were removed). Stage-specific mortality and the developmental stage of immature *H. axyridis* were monitored and recorded daily, until the ladybird larvae died or developed into the adult stage. The developmental stage could be identified by finding the exuvium after each molt. Newly emerged adults were weighed immediately after their emergence.

2.4 Data analyses

Data on biological parameters of larval and immature stages (larval + pupal stage) were analyzed using one-way analysis of variance (ANOVA). The means were separated using multiple comparison tests (LSD, $p < 0.05$) for the mortality rate, developmental stages, eclosion rate and body mass.

3 Results

3.1 Effects of transgenic *Populus alba* × *P. glandulosa* on predator larval mortality change

The mortality of every development stage of *H. axyridis*, fed on the three prey types, is shown in Table 1. There was no statistically significant difference in mortality at each of the larval stages (i.e., first, second, third and fourth instar) of *H. axyridis* among the three prey types. For the entire larval stage, the mortality of *H. axyridis* larvae, fed on the two types of transgenic plant-fed aphids and non-transgenic plant-fed aphids was 10.81%, 17.65% and 15.38%, respectively. There was no significant difference in the total larval mortality among the three prey types ($p > 0.05$).

Table 1 Effect of transgenic poplar on mortality of each development stage of *H. axyridis*

Development stage	Larval mortality (%)		
	C13-5	C013-5	Control
1^{st} instar	2.7	8.82	5.13
2^{nd} instar	5.41	2.94	2.56
3^{rd} instar	0	2.94	5.13
4^{th} instar	2.7	2.94	2.56
Larval (total)	10.81±5.17	17.65±6.64	15.38±5.85

Note: Data in the table represent mean ± SE. Multiple comparison tests on total larval mortality data indicate no significant difference among the three treatments.

3.2 Effects of transgenic *Populus alba* × *P. glandulosa* on predator development period changes

The effect of *C. populeti* propagated on transgenic *P.*

alba × *P. glandulosa* on the time of development of *H. axyridis* is shown in Tables 2 and 3. There was no significant effect on development time of the 1st, 2nd, 3rd and 4th instars between the transgenic plant treatment and control ($p > 0.05$). Development time of the pupal stage was slightly but not significantly prolonged for larvae feeding on *C. populeti* from control (4.67±0.08 d) compared to *C. populeti* from the transgenic poplars (4.48±0.09 d and 4.50±0.11 d).

Table 2 Effect of transgenic poplar on time of development of *H. axyridis*

Development stage	Development time (d)		
	C13-5	C013-5	Control
1st instar	2.33±0.08	2.45±0.12	2.18±0.06
2nd instar	1.26±0.07	1.36±0.09	1.33±0.08
3rd instar	1.82±0.06	1.76±0.09	1.85±0.06
4th instar	4.33±0.10	4.35±0.11	4.30±0.09
Pupal	4.48±0.09	4.50±0.11	4.67±0.08

Note: Data in the table represent mean ± SE. Multiple comparison tests on development time data indicate no significant difference among the three treatments.

Table 3 Effect of transgenic poplar on time of development of larval and immature of *H. axyridis*

Variety	Larval duration (d)	Immature duration (d)
C13-5	9.82±0.16	14.32±0.17
C013-5	10.04±0.20	14.54±0.22
Control	9.69±0.13	14.35±0.11

Note: Data in the table represent mean ± SE. Multiple comparison tests on larval and immature duration data indicate no significant difference among the three treatments.

The total time of development of larval and immature *H. axyridis* can be seen in Table 3. Generally, the transgenic poplars had no significant effect on development time of the larval stage of *H. axyridis*. Similarly, total immature developmental times were not significantly different between the transgenic poplars and the non-transgenic poplar. *H. axyridis* feeding on the C13-5 and C013-5 transgenic poplars reached the adult stage 14.32±0.17 d and 14.54±0.22 d after the eggs were hatched and *H. axyridis* feeding on non-transgenic poplars reached the adult stage 14.35±0.11 d after hatching.

3.3 Effects of transgenic *Populus alba* × *P. glandulosa* on predator eclosion rate and body mass change

The eclosion rate and body mass of adults feeding on the three prey types are shown in Table 4. Most larvae pupated and developed into adults irrespective of the type of prey. There was no significant difference in eclosion rates of *H. axyridis* feeding on C13-5, C013-5 and control aphids (90.32%, 80.76% and 87.09%, respectively). The mean body mass of adult *H. axyridis* reared on C13-5 (24.39±0.26 mg) or C013-5 (23.95±0.43 mg) was slightly different but not significantly lower than that of adult *H. axyridis* reared on the control plants (25.07±0.52 mg).

Table 4 Effect of transgenic poplar on eclosion rate and body mass of *H. axyridis*

Variety	Eclosion rate (%)	Body mass (mg)
C13-5	90.32±5.39	24.39±0.26
C013-5	80.76±7.88	23.95±0.43
Control	87.09±6.12	25.07±0.52

Note: Data in the table represent mean ± SE. Multiple comparison tests on eclosion rate and body mass data indicate no significant difference among the three treatments.

4 Discussion

In the study of the effect of transgenic plants on predatory enemies, some previous investigations showed that transgenic plants do not have an adverse effect on predatory enemies by means of phytophagous insects (Lozzia et al., 1998; Riddick and Barbosa, 1998; Zwahlen et al., 2000; Zhang et al., 2004). On the other hand, some studies reached a different conclusion, showing that the *Bt* toxin in transgenic plants may have adverse effects on the predatory enemies through phytophagous insects (Hilbeck et al., 1998; Birch, 1999). These studies indicated that various transgenic plants have different effects on predatory enemies.

In our experiment, the *C. populeti* fed on varieties C13-5 and C013-5 had no significant effect on mortality of the *H. axyridis* and did not significantly affect the time of development of the *H. axyridis* during the larval and immature stage. In addition, the body mass of adults and eclosion rate of the *H. axyridis* were not affected significantly. This suggests that transgenic plants have no negative effect on the predatory ladybird.

Following are some reasons which have contributed to the conclusion of our laboratory experiment. It is possible that the potential transfer of toxic material from the transgenic plant via its aphid prey to the predator does not occur or, if it does, is not toxic to the predator. The research by Dutton showed that there was a small amount of *Bt* toxin inside the aphids after they ingested the transgenic *Bt* cotton, but the development, survival and reproductive ability of the predator *Propylaea japonica* (Thunberg) did not seem to have a significant effect when fed with these aphids in their laboratory feeding experiment. The study also mentioned that there was no clear, corresponding relationship between the effects of the transgenic *Bt* corns on *Chrysoperla carnea* and the amount of *Bt* toxin protein inside the prey (Dutton et al., 2002). On the other hand, they assumed that the *Bt* toxic material had been transferred into the insect predators through the prey, so that the quantity of expressed protein and the stability of the *Bt* toxins in predators may have resulted in the ineffectiveness of the *Bt* toxins to predators. According to the study of Yuan (2004), the amount of *Bt* insecticide protein (*BtIP*) in *P. japonica* showed significant differences on the growing stage of *P. japonica* by ELISA, where the *BtIP* could just be detected in the second and fourth larval stages, but not in the other stages of *P. japonica*. The corresponding laboratory feeding experiment in our study showed that the transgenic plants had usually no deleterious effect on the predatory ladybird. The quantity of expressed protein and the stability of the *Bt* toxins may cause different effects on the predator, such as a change in mortality, time of development and body mass.

Overall, no deleterious effects of the transgenic plants on the predators were found in this experiment; however, further studies must be conducted to verify these results. Safety assessment of transgenic plants is a process of long duration. The study on interactions between the predators and the herbivores not only provides useful information about the ecological safety assessment on a scientific basis, but is also a better way of future biological control for aphids. Thus the effect of transgenic plants on predators should be monitored over long periods of time.

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