PHENOTYPIC PLASTICITY IN THE RESPONSE OF APHIDS TO HOST PLANT QUALITY

THOMAS THIEME[1,*], BERND TRUBERG[2], and ANTONY F.G. DIXON[3,4]

[1] BTL Bio-Test Labor GmbH Sagerheide, Sanitz/Gr. Lüsewitz, Germany
[2] NORIKA GmbH, Sanitz/Gr. Lüsewitz, Germany
[3] Department of Biodiversity Research, Global Change Research Centre AS CR, Na Sádkách 7, 370 05 České Budějovice, Czech Republic
[4] School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, U.K.
* Corresponding author: tt@biotestlab.de

ABSTRACT

The objective of this study was first to determine whether the chemical defence of lupin is more effective against the generalist aphids that occur on lupins than the host specific, *M. albifrons*. Secondly, to determine whether the host specific aphid shows an increase in performance over time if it is reared on sweet lupin and whether the process is reversed when transferred back to a bitter variety of lupin. Of the lupin cultivars tested only those with strongly reduced alkaloid contents were acceptable as host plants by the generalist species of aphid tested, and only *M. albifrons* reproduced on the "bitter" cultivars. In order to define the performance of *M. albifrons*, developmental time, mean relative growth rate and number of embryos were recorded for two strains reared for several generations on a bitter cv. of lupin and then transferred for several generations to sweet lupin and then reared again on a bitter cv. of lupin. The performance of *M. albifrons* was not better on lupins rich in alkaloids. However, the results also indicate that this aphid can adapt and thrive on a previously resistant cultivar within five generations even when reproducing parthenogenetically and that this change is reversible.

Keywords: lupin, alkaloids, aphids, *Macrosiphum albifrons*, biological performance

Introduction

Host based genetic differentiation is well documented for many of the polyphagous aphid pests of agricultural and horticultural plants (Carletto et al. 2009 and references therein). In addition, there is an extensive and detailed literature on the phenotypic plasticity in host use by insects and aphids in particular (Dixon 1998; Whitman and Agrawal 2009). Most of that on aphids relates to changes that occur within, between or both within and between generations (Dixon 1998). Reports of changes occurring over time spans of more than two generations are rare. There are two reports of changes in terms of increase in performance (r_m and cumulative fecundity) on a poor quality host over periods of four generations (Micha 1989; Mackenzie 1990). As the aphids used in these studies reproduced parthenogenetically the changes are viewed as induced by a change in environmental conditions, which in both of the above cases was the transfer of the aphids to a poorer quality host. That is, a single genotype of two species of aphid exhibit a different phenotype (phenotypic plasticity) in terms of cumulative fecundity or r_m when in a different environment and more importantly their performance improved from generation to generation.

If the performance of aphids on poor quality hosts generally improves with time then this phenomenon could be important in the development of host races and speciation in aphids. In terms of applied biology it has important consequences for plant breeders that produce plants that are more resistant to pests mainly in terms of their food quality. Of the different legumes, lupin is the best protected by alkaloids from attack by herbivores. There are approximately 200 species in the genus *Lupinus*, of which *L. albus* (white lupin), *L. angustifolius* (narrow-leaved lupin), *L. luteus* (yellow lupin) and *L. mutabilis* (Andean lupin) are the best known and the seeds of which are often eaten by humans. The seeds of wild genotypes of *L. albus* mostly contain more than 1% alkaloids (Hanelt 2006), whereas sweet cultivars of this lupin contain a maximum of only 0.04% (Plarre 1999) to 0.06% (Ternes et al. 2005). Reports on the effect of the alkaloid content of lupins on aphid performance are contradictory. According to Gruppe and Roemer (1988) and Emrich (1990) *Macrosiphum albifrons* prefers bitter lupins, which are rich in alkaloids. Bournoville et al. (1988) and our observations indicate that *M. albifrons* can also produce large colonies on sweet lupins and cultivars extremely poor in alkaloids. Therefore, either (1) there are several different races of *M. albifrons* in Europe and/or (2) given time this aphid can adapt physiologically to a different level of alkaloid in its host plant; a phenomenon known as induction.

The objective of this study was first to determine whether the chemical defence of lupin is more effective against the generalist aphids that occur on lupins than the host specific, *M. albifrons*. Secondly, as it is reported that *M. albifrons* prefers bitter to sweet varieties of lupin, to determine whether it will show an increase in performance over time if it is reared on sweet lupin and whether the process is reversed when transferred back to a bitter variety of lupin.

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Material and Methods

Aphids

Multi-clonal strains of *Acyrthosiphon pisum, Aphis craccivora, A. fabae, Macrosiphum albifrons, M. euphorbiae, Myzus ornatus* and *M. persicae* were reared in an insectary. Two clonal strains of *M. albifrons* were reared, a holocyclic (strain P) and anholocyclic strain (strain G). Aphids of strain G were supplied by Dr. B. Emrich, University of Giessen. Strain P was established using aphids collected by Mr. T. Busch (Plant Protection Service Mecklenburg-Western Pomerania) in Potsdam.

Plants

The seeds of the plants used in the experiments (Table 1) and the information on their alkaloid contents were provided by Dr. P. Hanelt (Institute for Genetic and Crop Research, Gatersleben). All the plants were grown singly in 8-cm-pots and each covered with a glass-cylinder covered with gauze.

Experimental Design

Experiments to determine the development of the aphids on different cultivars of lupins at the 6 leaf stage were carried out in a green house at 20 ± 2 °C and a photoperiod of 16:8 h light. New-born larvae were caged individually on plants. Fourteen days later, all the aphids on each plant were counted. This procedure was repeated 25 times. All the aphids of *M. albifrons* used in this experiment were of strain P. Those transferred to lupins with high alkaloid content were previously cultivated for a total of three weeks on a bitter cultivar of *L. luteus* (Schwako) and those transferred to lupins with low alkaloid content were cultivated for a total of three weeks on a sweet cultivar *L. luteus* (Gülzower Süsse Gelbe).

To determine the biological performance of *M. albifrons*, these aphids were reared at 20 ± 2 °C and a photoperiod of 16:8 h light, for several generations on a bitter cv. of *Lupinus luteus* (Schwako). Newborn aphids were weighed 5–12 h after birth and transferred to the test plants. When they became adult 25 of these aphids were weighed, dissected to determine their embryo content and their developmental time noted. Mean relative growth rate (*mRGR*) was calculated using the equation in Chambers et al. (1985). Newborn larvae were then transferred to a sweet cv. of *L. luteus* (Gülzower Süsse Gelbe) and kept there for the next five generations, after which they were reared again individually on a bitter cv. of *L. luteus*. The *mRGR* of the aphids ($n = 25$) of each generation was calculated.

Statistical Analysis

To compare their biological performance the data on developmental time (*D*), mean relative growth rate (*mRGR*) and number of embryos in individuals of each generation (embryos) were transformed prior to the analysis (*D* as 1/square root, *mRGR* as square root and embryos as ln). As the transformed data were homogeneous in respect to their variances (analyzed using Levene's test for homogeneity) a GLM ANOVA (one-way analysis of variance; Hollander and Wolfe 1973), coupled with a pair wise comparison of means was performed (Bonferroni test).

All statistical evaluations were done using the computer program SAS 10. The values for means and standard errors are for untransformed data.

Table 1. Mean number of offspring produced within 14 d of birth on different lupins at 20 ± 2 °C. ($n = 25$, – initial number, 1 – 0–5, 2 – 6–10, 3 – 11–20, 4 > 20; *Ap-Acyrthosiphon pisum, Ac-Aphis craccivora, Af-A. fabae, Ma-Macrosiphum albifrons, Me-M. euphorbiae, Mo-Myzus ornatus* and *Mp-M. persicae*).

Lupin	*Ap*	*Ac*	*Af*	*Ma*	*Me*	*Mo*	*Mp*
Lupinus albus ssp. *albus* Cultivars							
Petkuser Bittere Weisslupine / bitter	–	–	–	4	–	–	–
Weisse Bitterlupine / bitter	–	–	–	4	–	–	–
Rimpaus Frühe Süsse Weisslupine / sweet	2	2	2	4	2	2	1
Neutra / sweet	3	2	3	4	2	2	2
Lupinus luteus Cultivars							
Schwako / bitter	–	–	–	3	–	–	–
Lüneberger Gelbe / bitter	1	–	–	4	–	–	–
Gülzower Süsse Gelbe / nearly sweet	3	2	2	4	2	2	1
Lupinus angustifolius Cultivars							
Gülzower Bittere / bitter	–	–	–	4	–	–	–
Müncheberger Süsslupine Blaue II / sweet	2	2	1	4	2	2	1

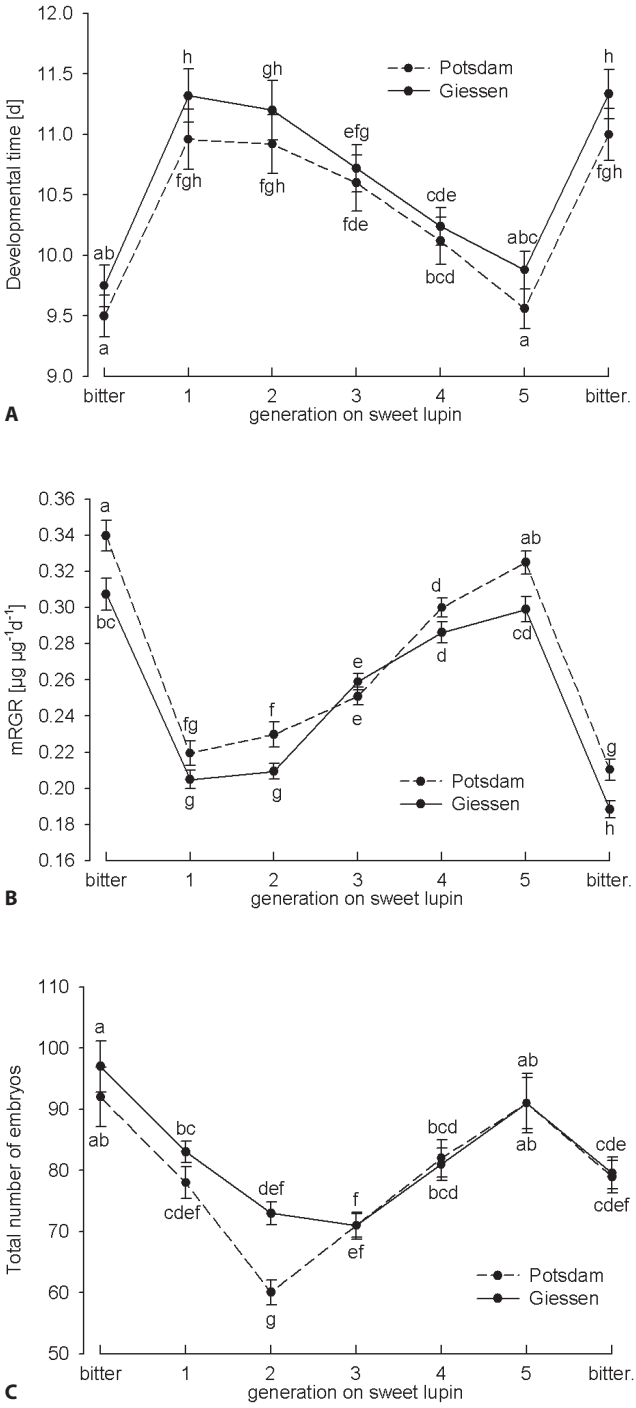

Fig. 1 Developmental time (A), mean relative growth rate (B) and total number of embryos (C) in viviparous apterous aphids of two strains of *Macrosiphum albifrons* reared on *Lupinus luteus* (bitter) and transferred and reared over a period of 5 generations on a sweet variety (sweet 1–5) and then transferred back to bitter lupin. (Values are means ± s.e.).

Results

Response of Generalist and Host Specific Aphids to Sweet and Bitter Lupins

Of the lupin cultivars tested only those with strongly reduced alkaloid contents were acceptable as host plants by all the species of aphid tested and *M. albifrons* (Table 1) was the only species that colonized and reproduced on the "bitter" cultivars, irrespective of the species of lupin.

M. albifrons was the only species that grew and reproduced consistently on both bitter and sweet lupins (Table 1). But these results differ from those of Berndt and Schliephake (2012) in that this aphid did not perform worse in terms of aphid population development on sweet lupin.

Response in Terms of Performance over Several Generations of the Host Specific Aphid when Reciprocally Transferred between Sweet and Bitter Lupins

As previously described for another two species of aphids, *Aphis fabae* and *Myzus persicae* (Mackenzie 1990), both strains of *M. albifrons* showed a change in performance with generation when transferred from sweet to bitter lupins and *vice versa*. After transfer from bitter to sweet lupin, the first generation took longer to mature than previously on bitter lupin (Fig. 1A). Differences in developmental time between generations were small and only partly significant. After transfer to a sweet cultivar of lupin strain G did significantly better but its *mRGR* was nevertheless significantly less than in the previous generation on bitter lupin. The total number of embryos was not significantly different in the two strains on bitter lupin. After transfer to sweet lupin strain P did significantly better in the second generation. The performance of the fifth generation did not differ significantly from the performance on bitter lupins (Fig. 1A–B).

The performance of the two strains of *M. albifrons* differed. There were significant differences in the *mRGR* of the aphids adapted to living on bitter and sweet lupins. There were more embryos, which indicate a higher potential fecundity, in the aphids of strain G reared on alkaloid-rich lupins (Fig. 1C). In addition the higher number of embryos per female in strain G was associated with small embryo size, which might indicate they will take longer to reach maturity. After transfer, however, aphids of both strains did badly and took several generations to adapt to the new host plant.

This study has shown that *M. albifrons* can colonize and adapt to lupin plants that are strongly protected by secondary plant substances. This is important, because the concentration of alkaloids in different species and cultivars of lupins differ.

Discussion

An analysis of 31 North American species of lupin yielded over 200 different alkaloids (Meissner and Wink 1992). In addition to the 2–6 main alkaloids in each species the remainder is made up of alkaloids that exist as by-alkaloids, which make up less than 1% of the total content of alkaloids.

The so-called sweet lupins differ from the wild or bitter lupins only in their alkaloid content, which is much

higher in the latter. Under natural conditions sweet lupins are eaten by sheep, hares, rabbits and insects, whereas bitter lupins are rarely eaten. The breeding of lupins for agriculture purposes has resulted in plants low in alkaloids but high yielding, which have almost no defense against insect herbivores. As a result lupin crops have to be protected with chemical insecticides and fungicides.

The method used to analyze the performance of *M. albifrons* did not indicate it preferred lupins rich in alkaloids (Table 1). Before the biological performance of all the strains was determined they were reared for several generations on the lupin cultivar on which they were to be tested. As described by Mackenzie (1990) for other aphid species, both of the strains of *M. albifrons* that were transferred between sweet and bitter lupins and *vice versa* (G and P) clearly showed an improvement in performance over a period of several generations (Fig. 1).

Differences in the biological performance of *M. albifrons* are related to their origins (Fig. 1A–C). Strain G showed no significant effect of the alkaloid content of the host plant on the increase in weight from birth to adult. The smallest increase in weight was recorded for strain P reared on the sweet lupin cv. Bornova. If *mRGR* is considered, which has the advantage of including not only the increase in weight but also the duration of development, then there are small differences. Compared with its performance on bitter lupin, strain P developed much faster on Bornova than strain G. The number of embryos indicates their potential fecundity. Strain G reared on alkaloid-rich lupin had the highest number of embryos but the lowest number with pigmented eyes (Fig. 1C). The high number of embryos per female in strain G was associated with their being smaller in size. According to Dixon (1998) small embryos become small larvae and take longer to develop from birth to adult than large embryos. This analysis of their biological performance indicates that *M. albifrons* has at least two different strategies as the aphids with reduced potential fecundity developed faster and grew faster (strain G) than those with a high potential fecundity (strain P).

In addition, there are several genetically different strains of *M. albifrons* (Blackman 1987; Guldemond et al. 1994), which plant breeders should be aware of when searching for varieties of lupin resistant to aphids. However, the results of this study also indicate that this aphid can adapt and thrive on a previously resistant cultivar within five generations even when continuously reproducing parthenogenetically and that this change is reversible, which makes it highly unlikely that this change was brought about by mutation. That is, continuously parthenogenetic polyphagous aphids can become ecologically specialized to living on different host plants by both induction, as reported here, and mutation. As induction occurs quite quickly it is likely that it occurs first and that mutations are more likely to affect their behaviour, in particular, their preference for feeding on a particular host plant, which results in a closer association between the aphid and a particular host plant and the evolution of a host race.

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