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Increased xylem ingestion and decreased phloem ingestion in the aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae) parasitised by *Aphidius ervi* (Hymenoptera: Braconidae)

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Abstract. The effects of parasitisation by *Aphidius ervi* on the feeding behaviour of the aphid *Acyrthosiphon pisum* were studied. There was progressive increase in the time devoted to xylem ingestion (G waveform) and concomitant decrease in time devoted to phloem ingestion (E2 waveform) in parasitized relative to unparasitized aphids, as the time from parasitisation increased. These changes are interpreted as a way aphids compensate for metabolic changes occurring during parasitisation.

INTRODUCTION

Parasites can alter the behaviour of their hosts (Combes, 1991; Poulin, 1994; Thompson & Kavaliers, 1994). These may result from pathological changes that counterbalance the detrimental effects of parasitisation (e.g. Karban, 1998), a host behavioural change induced by the parasitic organism to facilitate the successful completion of its life cycle (Brodeur & McNeil, 1989, 1992, 1994), or a combination of these processes.

Parasitised aphids show alterations in motor capacity, thermoregulation, host selection (Poulin, 1994; Honěk et al., 1998; Chow & Mackauer, 1999; Lagos et al., 2000) and in nutritional physiology (Rahbé et al., 2002). While it is known that after parasitisation aphids continue to feed and grow (Cloutier & Mackauer, 1979; Sequeira & Mackauer, 1992), increase their feeding rate significantly, and show important metabolic changes, nothing is known about the effect of parasitisation on their feeding pattern (Pennacchio et al., 1999; Falabella et al., 2000; Rahbé et al., 2002). In the present work, we explore the effects of parasitisation by *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiidae) on the feeding behaviour of the aphid *Acyrthosiphon pisum pisum* (Harris) (Hemiptera: Aphididae) using electronic recording. In particular, aphid feeding behaviour was recorded at different stages of parasitisation.

MATERIAL AND METHODS

Insect rearing

Aphids and parasitoids were collected from alfalfa (*Medicago sativa* L.) fields at the Instituto Nacional de Investigaciones Agropecuarias (INIA) at La Platina, Santiago, Chile. Parasitoids (*A. ervi*) and pea aphids (*A. pisum*) were maintained on potted alfalfa in a culture chamber at 20°C, 50% RH, and a 16L : 8D photoperiod.

Host parasitisation

Thirty apterous adult aphids from the stock colony were transferred to a single pot of alfalfa seedlings and after 12 h all adult aphids were removed, leaving only new-born first instars nymphs which were assigned to two treatments: parasitized aphids (n = 20), and unparasitized aphids (n = 20). In the case of parasitized aphids, 72 h after synchronisation a second instar nymph was individually placed in a Petri dish (4 cm diameter) containing a leaf of alfalfa. Thereafter, a CO_2-anaesthetised mated *A. ervi* female was introduced into the Petri dish and as soon as oviposition occurred the aphid was removed, thus avoiding superparasitisation. Parasitized and unparasitized aphids were individually confined in clip-cages on the leaves of potted alfalfa seedlings until their feeding behaviour was monitored. This was done with aphids whose feeding behaviour was recorded individually at five different periods: 72, 96, 120, 144, and 168 h after parasitisation. Once the monitoring of feeding behaviour finished, parasitized aphids were detached from the monitoring system and returned to clip-cages until the parasitoids emerged.

Monitoring of feeding behaviour

The feeding behaviour of parasitized and unparasitized aphids was monitored using the DC electropenetration graph technique (Tjallingii, 1978). A gold wire electrode (2 cm long, 25 µm diameter) was fixed to the dorsum of the aphid with conductive silver paint and a copper electrode was inserted in the soil of a potted plant. Both electrodes were connected to a DC electrical circuit designed to monitor stylet incursions inside plant tissues. When the aphid stylets penetrated into the plant tissues they closed the electrical circuit; the voltage changes produced were amplified and continuously recorded on a PC hard disk for later analysis. Different stylet activities and the location of stylets were inferred from specific patterns of voltage changes in the recorded signal (Tjallingii & Hogen Esch, 1993). Recordings were performed in such a way that parasitized and unparasitized aphids were simultaneously monitored. EPG recordings lasted for 8 h in each case.

Behavioural variables and statistical analysis

Typical waveform patterns associated with non-penetration (waveform usually designated by the letters NP), stylet pathway phase (waveforms A, B, and C combined, excluding waveform F), mechanical stylet difficulties during penetration (waveform F), xylem ingestion (waveform G), salivation into sieve elements (waveform E1) and phloem ingestion (waveform E2) were recognised (Tjallingii & Hogen Esch, 1993). The proportion of time devoted to different activities in parasitized and

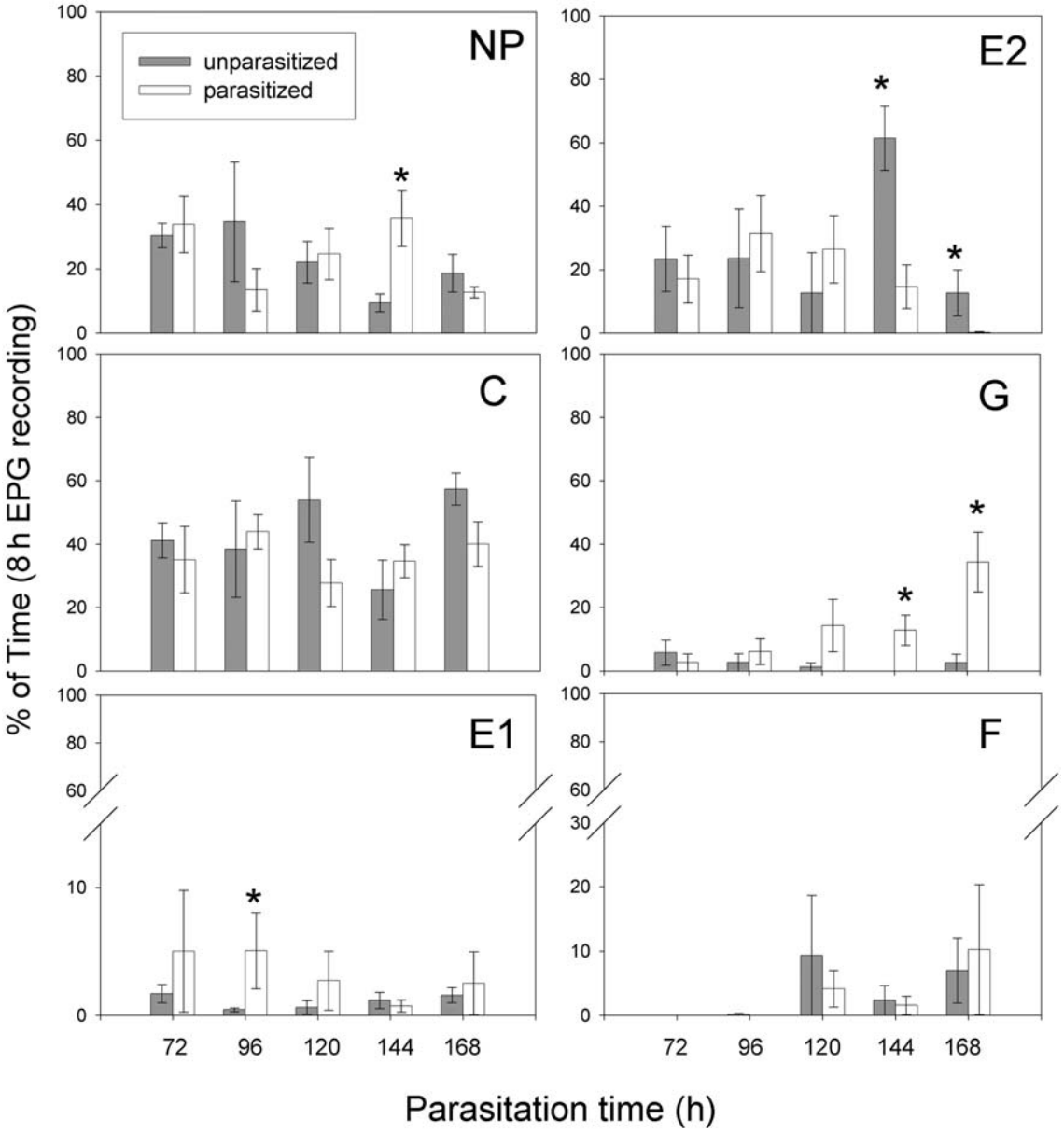

Fig. 1. Comparison of temporal changes that occurred in the times assigned to different kinds of feeding behaviour by *Acyrthosiphon pisum* parasitised by *Aphidius ervi*, relative to an unparasitised control. NP: non-penetration; C: stylet pathway phase; E1: salivation into sieve elements; E2: phloem ingestion; G: xylem ingestion; F: mechanical stylet difficulties during penetration. An asterisk indicates significant differences between treatment and control ($p < 0.05$).

unparasitized aphids was compared using the Mann-Whitney U non-parametric test.

RESULTS AND DISCUSSION

With increase in the time from parasitisation there was a progressive increase in the time devoted to xylem ingestion (G waveform) and a concomitant decrease in time devoted to phloem ingestion (E2 waveform) in parasitized relative to unparasitized aphids (Fig. 1). At 144 h after parasitisation, the time devoted to non-penetration behaviour (NP waveform) increased by a factor of three in parasitized aphids. However, 168 h after parasitisation, time devoted to non-penetration did not differ among parasitized and unparasitized aphids, suggesting that in the last phase of parasitisation aphids recover their willingness to penetrate plant tissues with their stylets. The only other significant change was an increase in the E1 waveform (salivation into sieve elements) in the first hours of parasitisation (Fig. 1). Lack of differences in the C waveform (stylet pathway phase) suggest that this activity, mostly devoted to exploration of the plant mesophyll in search of vascular tissue, is not significantly affected by parasitisation.

It has been shown that parasitisation induces metabolic and behavioural changes in the aphid host. For instance, the feeding rate of parasitized aphids increases significantly (Cloutier & Mackauer, 1979), and the composition of host haemolymph (e.g., amino acids, proteins, carbohydrates and osmolality), honeydew and fat body (e.g., glycogen) are usually altered after parasitisation (Cloutier & Mackauer, 1979; Slansky, 1986; Pennacchio et al., 1999; Falabella et al., 2000). These changes could be interpreted as a result of the differences in nutritional requirements of the parasitoid and its host. Indeed, during larval development parasitoids modify the internal nutritional physiology of

parasitized aphids by redirecting the biochemical pathways of amino acid production; thus, aphid bacterial endosymbionts (*Buchnera* spp.) are induced by parasitoids to substantially increase aromatic amino acid production (Rahbé et al., 2002). This change in amino acid composition could reduce the need of the aphid to ingest amino acids from plant phloem. The increased xylem ingestion and decreased phloem ingestion shown by parasitized aphids could be related to these homeostatic responses. For instance, an additional input of water could ensure the optimal absorption of sugars and amino acids through the epidermis of *A. ervi* larvae (Giordana et al., 2003). Alternatively, these changes could be simply a by-product of trauma and pathology suffered by parasitized aphids, rather than metabolic changes essential to support the nutrition and development of parasitoid larval stages.

Aphids in the late stages of parasitisation change their preference for feeding site and their dispersion behaviour, and either walk away from the plant or move to a more exposed part of a plant such as the upper surface of leaves (Chow & Mackauer, 1999), behaviour possibly related to the parasitized aphids having a decreased sensitivity to thermal differences (Lagos et al., 2000). These behavioural changes expose the aphid to strongly dehydrating conditions. Interestingly, xylem uptake is induced experimentally by fasting aphids under desiccating conditions (Spiller et al., 1990; Ramírez & Niemeyer, 2000), and the increased xylem ingestion by winged relative to unwinged aphids is thought to be due to dehydration during the teneral period (Powell & Hardie, 2002). Thus, the increase in xylem ingestion shown by parasitized aphids could also help them overcome the dehydration associated with the feeding site they choose.

In conclusion, this is the first study to address the changes in feeding patterns that occur in parasitized aphids and it shows that there is a decrease in phloem ingestion and increase in xylem ingestion, which may help parasitized aphid compensate for metabolic and behavioural changes occurring during parasitisation. Further studies are needed to better understand these changes in the feeding behaviour of parasitized aphids.

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