¿ ES LA INCORPORACIÓN DE LA EXPERIENCIA PREVIA UNA CARACTERISTICA ADAPTATIVA ?

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Resumen

En esta tesis se estudió el valor adaptativo de la capacidad de incorporar la experiencia previa (plasticidad fenotípica conductual) en la conducta alimentaria del áfido *Sitobion fragariae* (Walker). En experimentos de laboratorio se determinó que dentro de un genotipo la experiencia previa se presenta como un atributo que confiere distintos grados de variabilidad conductual en distintos niveles de su conducta alimentaria. La experiencia previa se asocia con facilitación de la alimentación en plantas hospederas más dotadas de defensas químicas. En plantas con mayores concentraciones de defensas la experiencia previa posibilita un acceso más rápido a la fase floemática. Comparaciones entre genotipos muestran variación inter-genotípica en la capacidad de incorporar la experiencia previa y en adecuación biológica. Sin embargo, aquellos genotipos que incorporan la experiencia previa no presentan mayor adecuación biológica. En forma diferente, el tiempo al primer acceso a la fase floemática se correlaciona negativamente con la adecuación biológica, lo que sugiere que la habilidad de acceder más rápido a los vasos floemáticos sería adaptativa. Esta tesis representa un esfuerzo por incluir a las conductas plásticas en el contexto más amplio de la plasticidad fenotípica como carácter adaptativo.

Sumary

The adaptive value of capacity to incorporate the previous experience (behavioral phenotypic plasticity) of the aphid *Sitobion fragariae* (Walker) was investigated. Laboratory experiments showed that previous experience seems to be a trait that introduce variability in different levels of feeding behavior. Within a genotype, the previous experience facilitate the feeding behavior on plants containing higher concentration of defenses. In plants containing higher concentrations of defenses, previous experience leads to a faster access to the phloem phase. Comparisons among genotypes showed inter-genotype variation in the capacity to incorporate the previous experience as well as in fitness. However, genotypes that incorporated previous experience did not show higher fitness. Differently, the time to a first access to phloem phase was negatively correlated with fitness, suggesting that a faster access to the sieve elements should be adaptive. This thesis represents an attempt to include plastic behaviors in the broader context of adaptive phenotypic plasticity.

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I. INTRODUCCIÓN GENERAL

ADAPTACIÓN Y MODELOS DE OPTIMIZACIÓN

Aun cuando la noción de que la conducta animal es el resultado de un mecanismo de adaptación y maximización de la descendencia goza de una amplia aceptación, su verificación empírica está mucho menos documentada. En este sentido, los modelos de optimización han logrado importantes avances en la contrastación empírica de las hipótesis adaptacionistas, particularmente aquellas relativas a los patrones de conductas reproductivas y alimentarias (Maynard-Smith, 1978, Stephens & Krebs, 1986; Parker & Maynard-Smith, 1990). Estos modelos establecen relaciones de costos y beneficios de una determinada conducta, asociadas a la maximización de la adecuación de corto y largo plazo producto de la selección natural (Mangel & Clark, 1986). Comúnmente, las predicciones de estos modelos se contrastan con observaciones empíricas (Maynard-Smith 1978; Orzack & Sober, 1994a). No obstante, esta perspectiva ha sido criticada, particularmente la teoría de forrajeo óptimo (Pierce & Ollason, 1987). Por ejemplo, se ha criticado la contrastabilidad de sus hipótesis y el carácter *ad hoc* de sus explicaciones cuando los modelos no son verificados. A pesar de sus limitaciones, la perspectiva optimizadora ha demostrado poseer valor heurístico (Stearn & Schmid-Hempel, 1987; Krebs & Kacelnik, 1991, Hughes, 1993; Ward, 1993).

Recientemente Orzack y Sober (1994a, 1994b) han señalado que, respecto de los modelos de optimización, debe verificarse la concordancia cualitativa y cuantitativa de los valores observados y los predichos por el modelo. El grado de importancia de la selección natural en la construcción del carácter dependerá también del grado de variación entre individuos respecto de tal concordancia (Orzack & Sober, 1994a; 1994b; 1996). La validez de las hipótesis adaptacionistas que sostienen la suficiencia explicativa de la selección natural en la evolución del carácter, así como su condición de óptimo local, dependerán del cumplimiento de estos requisitos. Sin embargo, Brandon y

Rausher (1996) han hecho importantes críticas a la propuesta de Orzack y Sober (194a). En particular, han señalado que la suficiencia explicativa de la selección natural y la condición de óptimo local son aspectos lógica y biológicamente independientes. Para Brandon y Rausher (1996) el criterio de suficiencia explicativa de la selección natural la entiende como causa histórica de la presencia del carácter (producto de la selección natural en el pasado), mientras que la condición de óptimo local apunta al proceso actual que generaría la predominancia del carácter relativo a fenotipos alternativos. La falta de disociación entre un concepto actualista y otro histórico de adaptación[1], produciría en la aproximación de Orzack y Sober (1994a) un sesgo en la confirmación de la hipótesis adaptacionista (Brandon & Rausher, 1996). Alternativamente, Brandon y Rausher (1996) proponen evaluar separadamente ambas componentes de adaptación. El concepto histórico, aunque abordable, sería empíricamente muy dificultoso. Respecto al del concepto actualista, la condición de óptimo local debe considerar la variación poblacional respecto del carácter, y evaluar experimentalmente el desempeño de los genotipos en distintos contextos ambientales. Brandon y Rausher (1996) valoran el aporte de Orzack y Sober (1994a) al estudio de adaptación dado que se enfatiza evaluar la variabilidad inter-individual, aspecto de creciente interés en el estudio de la

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[1] En biología evolutiva el concepto de adaptación ha estado permanentemente ligado a dificultades y ambigüedad tanto en su definición como en su evaluación (Cracraft, 1981; Mayr, 1982; Gould & Vrba, 1982; Sober, 1984; Stephens & Krebs, 1986; Gans, 1988; Sherman, 1988; Reeve & Sherman, 1993; Brandon, 1996). Su denotación y connotación depende del ámbito fenomenológico desde donde emerge (Reeve & Sherman, 1993). Subdisciplinas abocadas al estudio de la historia evolutiva de un carácter (e.g. paleobiología) adoptan una definición *histórica* o *retrospectivista* de adaptación ("*adaptación es un carácter construido por la selección natural para su rol actual*" [Gould & Vrba, 1982]), definición que se concentra en el contraste de hipótesis relativas a la participación de la selección natural en la construcción del carácter, enfatizando la trayectoria filogenética de los atributos individuales y sus relaciones de ancestro/descendiente. Por otro lado, disciplinas interesadas en explicar la existencia de fenotipos, particularmente de atributos sin registro histórico (e.g ecología conductual), adoptan una *definición actualista* de adaptación ("*adaptación es una variante fenotípica que resulta en una adecuación biológica mayor entre un conjunto especificado de variantes en un ambiente dado*" [Reeve & Sherman, 1993]), enfatizando su interés por explicar la predominancia de algunos fenotipos por sobre formas alternativas. Ambos conceptos de adaptación se diferencian en entender la adaptación como *producto* de un proceso evolutivo, como es el caso de la perspectiva histórica, o bien como el *proceso* mismo que explica los sesgos en la representación de fenotipos y las diferencias en la adecuación biológica, en el caso de adaptación actualista (Sober, 1993; Brandon, 1996).

adaptabilidad de las conductas (Ward, 1993; Nonacs & Dill, 1993; Carriere & Roitberg, 1996; Schmitz *et al.*, 1998).

ADAPTACIÓN Y VARIACIÓN FENOTÍPICA

Estudiar el valor adaptativo de un caracter conductual bajo un concepto exclusivamente actualista de adaptación, implica establecer su variación inter-individual y su relación con adecuación biológica. En el caso del estudio de la adaptabilidad de conductas plásticas, tales como el aprendizaje o la motivación, existe una correspondencia con el estudio de la adaptabilidad de la plasticidad fenotípica (Jaenike & Papaj, 1992; Stephens, 1991; 1993; Carriere & Roitberg, 1996). Sin embargo, a pesar del evidente paralelo, los atributos conductuales plásticos no han sido incorporados en los estudios formales de plasticidad fenotípica, los que se han concentrado principalmente en atributos morfológicos (Dukas, 1998).

La plasticidad fenotípica, definida como la variación en la expresión fenotípica de un genotipo como consecuencia de su interacción con el ambiente (Bradshaw, 1967; Schlichting, 1986, Cheplick 1991; Stearns, 1992; Gomulkiewicz & Kirkpatrick, 1992; Via, 1994), no supone una relación "uno a uno" entre genotipo y fenotipo, en tanto que considera el rol del ambiente en el desarrollo de los fenotipos. La plasticidad fenotípica suele ser evaluada mediante la construcción de la *norma de reacción* de un genotipo, la cual se refiere a los fenotipos posibles producidos por un genotipo en un rango ambiental (Via, 1994). La situación inversa, producción de fenotipos similares a partir de distintos genotipos, se conoce como *canalización* o norma de reacción plana (Stearns & Kawecki, 1994). Esta última situación es relevante en términos evolutivos dado que por *canalización* se puede producir el mismo fenotipo con independencia del ambiente, (Roff, 1997), retardando el efecto de la selección natural (Cheplink, 1991). Aun cuando la plasticidad fenotípica y la norma de reacción puede estar bajo control genético y por tanto evolucionar como un carácter *per se* (Schlichting & Pigliucci, 1993; Via, 1993), la plasticidad fenotípica puede afectar la evolución del fenotipo integrado (Bradshaw, 1967). La plasticidad fenotípica podría jugar un importante papel en los patrones de uso de un recurso, particularmente en ambientes heterogéneos (Levins, 1968; West-Eberhard, 1989; Thompson 1991). Similarmente, desde el contexto de la teoría de forrajeo óptimo el aprendizaje sería ventajoso dado que permitiría obtener información respecto de la naturaleza del ambiente (Stephens, 1987; Bernstein, *et al.,* 1988; Cuthill *et al.*, 1990; Dukas & Real, 1993).

Dado que para investigar la plasticidad fenotípica de un carácter conductual se requiere exponer a un genotipo a distintos ambientes, su valor adaptativo en términos de un concepto actualista de adaptación implicará entonces evaluar además su relación con adecuación biológica.

INCORPORACIÓN DE LA EXPERIENCIA PREVIA COMO UN CARÁCTER ADAPTATIVO DE LA CONDUCTA

En esta tesis se estudia el valor adaptativo de una conducta plástica: la conducta de aprendizaje. Sin embargo, a pesar de su menor valor intuitivo se prefiere el término *incorporación de la experiencia previa* en lugar de aprendizaje, debido a que el primero representa un nivel fenomenológico desligado de connotaciones respecto del nivel causal subyacente (Stephens, 1993)[2]. En este sentido, *la capacidad de incorporar la experiencia previa alude a la expresión de distintos fenotipos conductuales dependiendo de la interacción entre genotipo y ambiente.* Tal interacción puede ocurrir a lo largo de la ontogenia o en un período de ella.

Estudiar el valor adaptativo de la capacidad de incorporar la experiencia previa implica: 1) determinar la expresión de fenotipos conductuales de un genotipo en

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[2] "*I focus in the behavioral change caused by experience, and I am not concerned with the* mechanisms *that an animal uses to change its behavior in light of experience; experience can be used to chenge behavior via some*

distintos ambientes (norma de reacción), y 2) determinar la relación entre esta variación y la adecuación biológica de los genotipos.

ADAPTACIÓN EN ORGANISMOS CLONALES

Los organismos clonales con módulos de vida separada son ideales para estudiar la respuesta de un genotipo a distintos contextos ambientales (Schlichting, 1986; Via, 1994). Las plantas con fases de crecimiento vegetativo han sido muy utilizadas para estudiar la relación entre fenotipo y genotipo en un contexto evolutivo (Bradshaw, 1967; Cheplick, 1991). Entre los animales que se han utilizado para estudiar la interacción entre genotipo y medio ambiente en la expresión de fenotipos de historia de vida ligados con etapas específicas del ciclo de vida de desarrollo, destaca los rotíferos, briozoos, anémonas, cladoceros y áfidos (Hughes, 1989). En el caso particular de los áfidos la conducta alimentaria es uno de los aspectos más relevantes de la interacción entre éstos insectos y su planta hospedera (Dixon, 1998). Dada la brevedad del periodo de vida de los áfidos, la experiencia previa tiene una rápida expresión en los fenotipos conductuales (De Barro *et al.*, 1993; Mackenzie & Guldemold, 1994; Dixon, 1998). Este fenómeno es aún más relevante debido a que los áfidos presentan desarrollo partenogenético con generaciones "telescópicas", esto es, cada ejemplar hembra porta en su interior las dos siguientes generaciones, lo que se traduce en un fuerte efecto maternal (Dixon, 1998).

INCORPORACIÓN DE LA EXPERIENCIA PREVIA COMO UN CARÁCTER ADAPTATIVO DE LA CONDUCTA ALIMENTARIA EN ÁFIDOS

Los áfidos son insectos herbívoros que se alimentan en la mayoría de los casos del floema de plantas. Se han descrito hasta la fecha aproximadamente 5300 taxa válidos a

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complex cognitive process or via some extremely simple stimulus-response scheme. Strictly speaking, my interest is in behavioral phenotypic plasticity " Stephens (1993) p. 209.

nivel específico o subespecífico (Remaudière & Remaudière 1997) distribuidos fundamentalmente en regiones templadas (Heie 1994). La mayor parte de las familias de plantas vasculares son utilizadas como hospederos por los áfidos y es así como en áreas templadas una de cada cuatro especies de plantas presenta áfidos asociados (Dixon 1994, Dixon 1998). La especialización es la característica más relevante de la interacción entre áfidos y plantas, ya que la mayoría de las especies de áfidos utilizan como hospederos tan sólo a un género o a lo más a una familia de plantas (Eastop 1973). Esta asociación estrecha entre áfidos y plantas ha conducido a considerarlos como "botánicos" expertos en el reconocimiento de su planta hospedera (Hille Ris Lambers 1979).

La reproducción a través de partenogénesis cíclica alterna eventos de reproducción sexual con la producción de varias generaciones partenogenéticas. En esta modalidad reproductiva cada áfido lleva en su interior a las próximas dos generaciones de su progenie, lo que se traduce en que los procesos de crecimiento somático y reproducción sean simultáneos. La principal consecuencia de estas características de los áfidos es la capacidad para explotar rápidamente su planta hospedera a través de múltiples generaciones de corta duración que no pueden sobrevivir alejadas del hospedero por mucho tiempo. Tanto la modalidad reproductiva como la especialización en la utilización de sus plantas hospederas refleja una interacción estrecha entre áfidos y plantas, en la cual las características químicas de la planta han sido destacadas como uno de los factores causales más relevantes (van Emden 1978).

Una vez que los áfidos han seleccionado un hospedero, insertan su estilete en el tejido vegetal (conducta de prueba) para acceder a vasos floemáticos (Dixon, 1998). Por el canal salival de su estilete, los áfidos secretan dos tipos de saliva con distintas funciones: i) un tipo de saliva forma una vaina rígida en la ruta recorrida por el estilete, ii) otro tipo de saliva contiene pectidasas que facilitan el paso del estilete por entre las células. A través del canal alimentario la savia floemática y el contenido xilemático es extraída hacia el tubo digestivo desde los vasos floemáticos y xilemáticos, respectivamente. Debido al pequeño tamaño de los áfidos su interacción con la planta es difícil de estudiar por simple inspección visual. Afortunadamente una técnica electrofisiológica, conocida como EPG, basada en la facilidad del paso de la corriente eléctrica entre el áfidos y la planta ha sido diseñada por McLean y Kinsey (1964). Esta técnica ha sido importante para conocer la conducta de alimentación de áfidos, así como también los efectos de algunos componente de la planta (ceras epicuticulares, metabolitos secundarios, etc.) y otros factores de resistencia de vegetales en la conducta de áfidos (Tjallingii, 1995). La relación entre la experiencia previa de los áfidos y el patrón de incursión del estilete ha sido escasamente estudiado. Montllor *et al*., (1983) informasron que ejemplares del biotipo C de *Schizaphis graminum* (Ronfani) previamente expuestos a plantas de sorgo, al estar nuevamente en contacto con sorgo disminuyeron el tiempo de la primera ingestión sostenida de savia floemática de vasos floemáticos, y también aumentaban el tiempo de ingestión. Estos experimentos fueron realizados con individuos de un mismo genotipo, por lo que la disminución en el tiempo de acceso y aumento en la duración de la alimentación en los vasos floemáticos fue substancialmente debida al efecto de la experiencia previa en la conducta alimentaria. Por otro lado, Prado y Tjallingii (1997) estudiaron el efecto de la interrupción de la alimentación de *Aphis fabae* en habas luego de 48h de alimentación continua sobre este hospedero. Estos autores determinaron que el tiempo de inico de la ingestión floemática era menor si las interrupciones eran más breves (1 min versus 100 min). Estos resultados sugieren que el efecto de la experiencia tiene una corta duración. Es importante resaltar que esta escala de tiempo es muy relevante para la vida de los áfidos. Por ejemplo, los áfidos demoran en promedio 2 horas en acceder a una ingestión floemática, y dado que la vida media de los áfidos es de aproximadamente 30 días (720 h), esta actividad equivale al 0,27 %. En el caso de tiempo de vida promedio el ser humano (ca. 65 años), este porcentaje equivale a 0.18 años (ca. 2 meses) buscando alimento.

La conducta de búsqueda de vasos floemáticos parece ser entonces muy sensible a la experiencia previa y debiera ser un atributo ventajoso. Sin embargo, los estudios de la relación entre la experiencia previa y la conducta alimentaria provienen de un contexto agronómico, y no abordan el problema de la adaptabilidad de las conducta plásticas en áfidos. Estudiar el valor adaptativo de la capacidad de incorporar la experiencia previa en áfidos implica entonces: 1) investigar la capacidad de incorporar la experiencia previa de distintos genotipos en distintos ambientes, y 2) determinar la relación entre esta variación y la adecuación biológica. Para el caso de la conducta alimentaria de los áfidos, el ambiente esta constituido principalmente por las características de la plantas hospederas, cuyas características químicas resultan relevantes la para la biología de los áfidos (Dixon, 1998). Es importante señalar que en áfidos el "individuo evolutivo" corresponde al "clon" o "genet", constituido por ejemplares genéticamente idénticos de vida separada. Por este motivo, la medida de adecuación biológica se centra en el crecimiento poblacional del clon (Janzen, 1977; Hughes & Cancino, 1985; Tuomi & Vuorisalo, 1989; Eriksson, 1993; Dixon, 1998).

II. OBJETIVOS GENERALES

En tesis se investiga el valor adaptativo de los atributos conductuales plásticos. En particular, se estudia la experiencia previa en relación con la conducta alimentaria del áfido *Sitobion fragariae* (Walker). Se evalúa la adaptación en un contexto actualista comparando los fenotipos conductuales dentro y entre genotipos en distintos ambientes y se examina su correlación con la adecuación biológica.

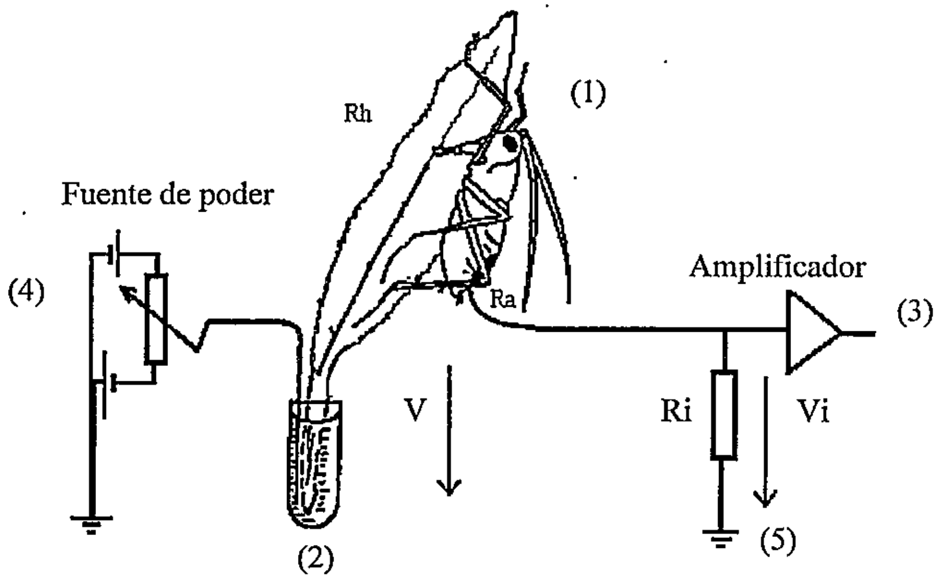
III. HIPÓTESIS DE TRABAJO

1. Si la experiencia previa en una determinada planta hospedera afecta la conducta alimentaria, entonces debiese observarse diferencias en la conducta alimentaria entre áfidos con y sin experiencia previa en ella.
2. Si la experiencia previa es una característica adaptativa, entonces dentro de un genotipo la experiencia previa debería mostrar una correlación con adecuación biológica.
3. Si la experiencia previa es una característica adaptativa, entonces debería existir variación genética respecto de la capacidad de incorporar la experiencia previa.
4. Si la experiencia previa es una característica adaptativa, entonces aquellos genotipos capaces de incorporar la experiencia previa (plasticidad fenotípica conductual) debería mostrar mayores valores de adecuación biológica.

IV. METODOLOGÍA GENERAL

GRÁFICOS DE ELECTROPENETRACIÓN (EPG)

La ruta que sigue el estilete de los áfidos en el interior de los tejidos vegetales no puede ser observada exteriormente. En 1964, McLean y Kinsey publicaron la técnica de monitoreo eléctrico de la conducta de alimentación y salivación de áfidos, técnica conocida como EPG (*Electropenetration Graph*). Esta técnica consiste en conectar al áfido a un circuito eléctrico (DC o AC dependiendo de las características del amplificador de señales utilizado) que incluye la planta sobre la cual el animal se encuentra. Esto es posible si un electrodo constituido por un fino alambre de oro (10-25 µm de diámetro) es adherido al dorso del áfido mediante un adhesivo conductor (pintura de plata líquida), y otro electrodo es conectado al substrato donde crece la planta y a una fuente de poder de 12 volts (Figura 1).



Gráficos de electropenetración (EPG)

Figura 1. Diagrama del circuito para el registro EPG. Un electrodo es conectado al dorso de un áfido (1), en tanto que otro electrodo es conectado al sustrato donde crece la planta (2). El sistema incluye una fuente de poder ajustable (4) y un amplificador (3), ambos instrumentos conectados a tierra (5).

Una vez que el estilete del áfido penetra en el tejido vegetal, el circuito eléctrico es cerrado, y entonces pueden ser registrados los patrones de ondas producidos por las variaciones de voltaje debidas a la resistencia variable de la interfaz estilete-planta. Existen estudios (Tjallingii & Esch, 1993) que han correlacionado los patrones de ondas con la posición y actividades del estilete en la planta. Estos se basan en cortes seriados que se obtienen anestesiando rápidamente a un áfido cuando éste generaba un determinado patrón de ondas, y luego observándolos al microscopio electrónico. El monitoreo puede ser registrado por medio de un computador, con el cual las señales pueden ser analizadas detalladamente. En una penetración es posible distinguir conductas tales como incursiones en el espacio intercelular-intramural (ondas A,B y C, Figura 2) en la epidermis o el mesófilo. También es posible reconocer incursiones intracelulares caracterizadas por una rápida caída de potencial (pd's), así como ingestión en los vasos xilemáticos (onda G), problemas de penetración del estilete (onda F), e incursiones en los vasos floemáticos (onda E). Se distinguen dos ondas floemáticas, llamadas E1 y E2, que corresponden a salivación al interior de los vasos floemáticos e ingestión pasiva de floema, respectivamente (Figura 2).

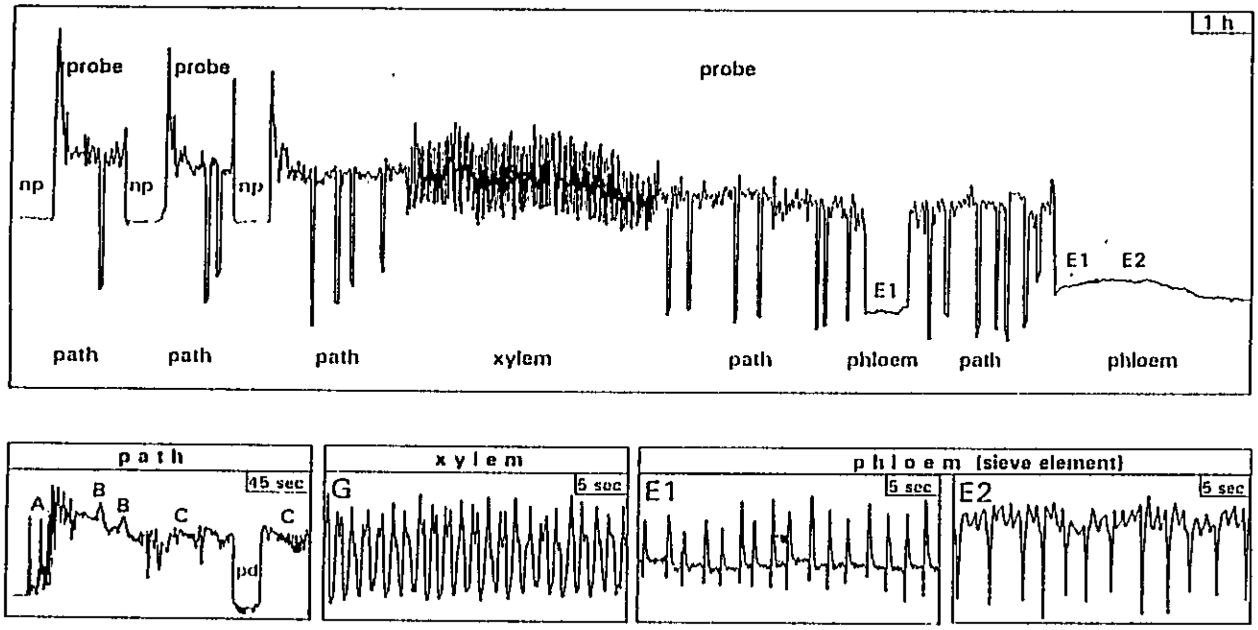


Figura 2. Esquema de los patrones de ondas más característicos del registro de EPG (panel superior) y su denominación estandar (se ha preferido mantener la nomenclatura original en inglés). Se muestran tres periodos de prueba (paneles inferiores), el último de lso cueales culmina con acceso a los vasos floemáticos. **np**: No-penetración del estilete en el tejido vejetal; **path**: Incursiones intercelulares con salivación en células de la epidermis y mesófilo (subdivididos es A, B y C); **xylem**: Ingestión en vasos xilemáticos; **pd**: Incursiones intracelulares; **E1**:Patrón de salivación en elementos cribosos; E2: Ingestión de floema en los elementos cribosos. (Tomado de Prado 1997).

CAPÍTULO 1

LA INFLUENCIA DE LA EXPERIENCIA PREVIA Y DEL HAMBRE EN LA CONDUCTA DE PRUEBA DE *SITOBION FRAGARIAE* (WALKER) (HEMIPTERA: APHIDIDAE)

THE INFLUENCE OF PREVIOUS EXPERIENCE AND STARVATION ON THE PROBING BEHAVIOR OF *SITOBION FRAGARIAE* (WALKER) (HEMIPTERA: APHIDIDAE)

Claudio C. Ramírez & Hermann M. Niemeyer

Abstract

It was hypothesized that (1) previous experience of aphids in a host plant leads to differences in their probing behavior relative to aphids without previous experienced on it, and (2) starvation modifies these experience-induced differences. Using electronic recording, the probing behaviour of the aphid *Sitobion fragariae* (Walker) on wheat *Triticum aestivum* L. and oat *Avena sativa* L. was examined, comparing aphids with or without previous experience on a given host, and with or without a period of starvation before the evaluation of probing behavior. All comparisons were performed within a single aphid clone in order to minimize the effect of genetic variation. Unstarved aphids with previous experience in wheat showed shorter time in F waveform (mechanical difficulties of the stylet during penetration) as well as longer time in pathway phase when probing in wheat relative to aphids without previous experienced on it. These differences were not observed when probing was assessed in oat. Starved aphids showed increased time devoted to xylem ingestion regardless of previous experience, as compared with unstarved aphids It seems that starvation reoriented the probing behavior in order to maintain the water balance.

Keywords: probing behavior, previous experience, starvation, mechanical difficulties, xylem ingestion

Introduction

Aphids are a group of sap feeding insects which, during their exploration of plant tissues in order to reach their feeding sites, penetrate into cells of the epidermis, mesophyll, and vascular bundle (Pollard, 1973; Dixon, 1985). Xylem penetration elicits ingestion of nutrient and water, while sieve element access is followed by sap ingestion (Spiller *et al.*, 1990; Tjallingii, 1987). These probing behaviors depend on many internal and external factors (Dixon, 1985; Klingauf, 1987). Within internal factors, previous experience or short-term conditioning and starvation have been shown to affect aphid probing behavior. Thus, aphids with experience in a host were able to find the phloem more rapidly and sustain longer feeding periods than aphids without such previous experience (Montllor *et al.*, 1983), while starved aphids showed earlier initiation of plant penetration with shorter probing periods (Klingauf, 1987; Powell, 1993; Prado, 1997, but see Hardie *et al.*, 1992). However, the responses of aphids to the simultaneous effect of both internal factors, have not been reported.

In other groups of arthropods such as beetles (Hileman *et al.*, 1995), hymenopterean parasitoids (Grasswitz & Paine, 1993), and mites (Zhang & Sanderson, 1992), foraging responses have been shown to be more influenced by starvation than by previous experience. We hypothesized that previous experience of aphids in a host leads to differences in the probing behavior relative to aphids without previous experience on it, and that starvation would modify these experience-induced differences. Therefore, we compared the probing behavior in a given host plant of the aphid *Sitobion fragariae* (Walker) with or without previous experience on it, and with or without a period of starvation before the evaluation of probing behavior. All comparisons were performed within a single aphid clone in order to minimize the effect of genetic variation.

Materials and Methods

Aphids

Individuals used were from a clone of *S. fragariae* derived from a single virginoparous apterous individual collected from a grass field in central Chile. The aphids were cultured for about 10 to 12 generations on oat seedlings (*Avena sativa* cv. Nehuen) growing in plastic boxes in a room at 20 ± 2 °C and 16:8 (L:D). The identity of the aphid was determined with RAPDs and PCR procedures according to Figueroa *et al.*, (1998).

Behavioural monitoring of feeding behavior

The Electrical Penetration Graph (EPG) technique (Tjallingii and Hogen Esch, 1993) to record aphid probing behavior was used. A gold wire electrode (2 cm long x 25 µm diameter) was fixed to the dorsum of the aphid with conductive silver paint. A copper electrode was inserted in the soil of a potted plant. Both electrodes are connected to a DC electric circuit designed to monitor aphid stylet incursions inside plant tissues (Tjallingii and Hogen Esch, 1993). When the aphid stylets penetrate into the plant tissues they close the electrical circuit and the voltage changes are amplified and continuously monitored. All signals are recorded on a PC hard disk for later analysis. The stylet activity and stylet tip position can be inferred from specific patterns of voltage changes in the recorded signal (Tjallingii and Hogen Esch, 1993). The effect of experimental stress during EPG manipulation and recording has been shown to be non-significant (Tjallingii, 1986; Prado, 1997).

Experiment 1 (host transfer of unstarved aphids)

This experiment was performed in order to compare the probing behaviour in a host plant between aphids with or without previous experience on it. The host plant used to test previous experience was wheat (*Triticum aestivum* cv. Millaleu), and oat (*A. sativa* cv. Nahuen) was used as control host. Thus, transferences from wheat to wheat (W-W), oat to wheat (O-W), as well as oat to oat (O-O) and wheat to oat (W-O) were performed.

Apterous adult aphids from the monoclonal colony maintained on oat were transferred separately to potted seedlings of wheat or oat in the 2-leaf growth stage. After 24h all adult aphids were removed and all new-born first instars were maintained in each host plant until they reached the reproductive stage. This period of time was labelled "previous experience" on the respective host plant. Two groups of aphids were obtained: 1) with previous experience on wheat, and 2) with previous experience on oat. Subsequently, electrical monitoring of the feeding behaviour was performed on individuals from each group of aphids. Aphids were connected to the EPG electrode and then kept for one hour in a Petri-dish before being transferred to wheat or oat seedlings (2-leaf growth stage). This last transference divided each group of aphids into two new groups, configuring a total of four different experimental treatments: 1) experience in wheat and assessment in wheat (W-W), 2) wheat and oat (W-O), 3) oat and wheat (O-W), and 4) oat and oat (O-O). Continuous monitoring of feeding was performed during 8h.

Experiment 2 (host transfer of starved aphids)

This experiment was performed in order to evaluate whether any behavioural differences found in Experiment 1 could be effected by starvation. The experiment involved the same steps of Experiment 1, with the exception that aphids were kept under starvation in the Petri dish with moist filter paper for 14h before being connected to the EPG electrode, individually transferred to wheat or oat seedlings (2-leaf growth stage), and monitored for 8h as in Experiment 1.

Behavioural variables

Each EPG profile obtained from Experiments 1 and 2 was analysed with a software designed *ad hoc* (WinEPG; Flores *et al.*, in preparation). Typical waveform patterns associated with non-penetration (NP), pathway phase (C), mechanical difficulties during stylet penetration (F), xylem ingestion (G), phloem access (E1) and phloem ingestion (E2) were recognised (Tjallingii, 1987). The accumulated time allocated to each of these activities was estimated in both experiments.

Results

Experiment 1 (host transfer of unstarved aphids)

Unstarved aphids with previous experience on wheat showed less time in mechanical difficulties during stylet penetration (F waveform) and longer time in pathway phase (C waveform) in wheat relative to aphids feeding in wheat but with previous experience on oat (W-W and O-W comparison in Table 1). On the other hand, no significant differences were observed in the activities performed in oat between aphids with different previous experience (O-O and W-O comparison in Table 1).

Times to first phloem phase (first appearance of E1waveform) and to sustained phloem ingestion (first appearance of E2 waveform longer that 10 min) were not affected by previous experience in wheat or oat (Fig. 1), although a trend to show faster first phloem phase was observed in wheat after previous experience on it (W-W and W-O in Fig.1).

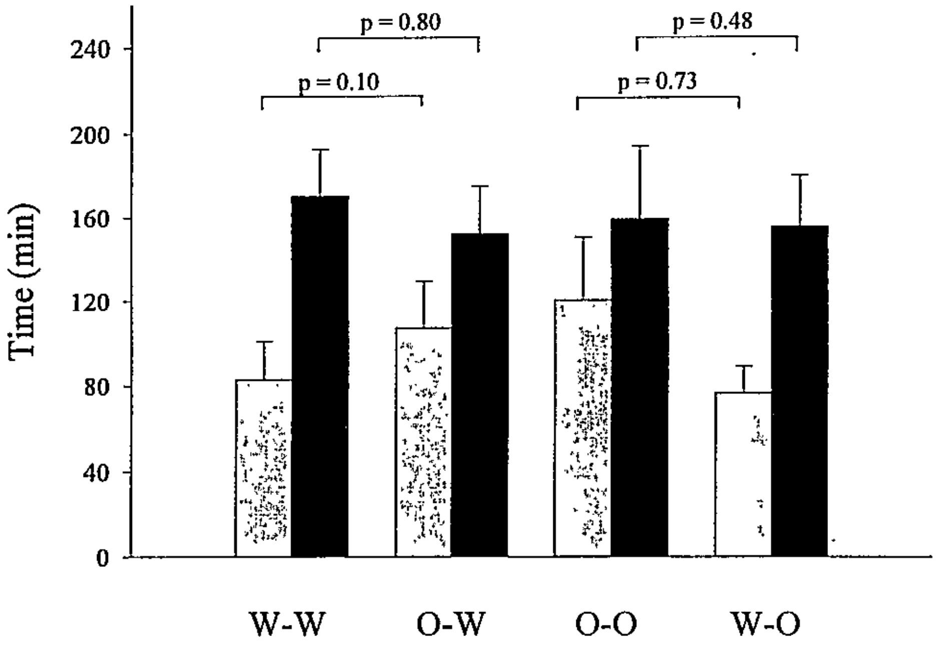


Figure 1. Time from the start of the experiment to the first phloem phase (☐) and first sustained phloem ingestion (■) by the aphid *S. fragariae* on wheat (W) or oat (O) after previous experience on each host. Bars represent standard error (n = 15). P-values were calculated with Mann-Whitney U test.

Table 1. Proportion of time allocated to different activities during 8h of electrical monitoring of the feeding behaviour of *S. fragariae* in the Experiment 1 (a) and Experiment 2 (b). C, intercellular pathway; E1 salivation into sieve elements; E2 phloem ingestion; G, ingestion from the xylem, NP, non-probing ; F, difficulties during stylets penetration. Probability values (P-values) were obtained after MANOVA (Experiment 1: Lambda Wilks=0.46, P=0.006; Experiment 2: Lambda Wilks=0.54, P=0.28). Different letters in column G and F of the mean that the differences among the values are significant at P < 0.05 (*a posteriori* Tukey HSD test). The asterisks mean that the values of the variable between the equivalent treatment of Experiment 1 and Experiment 2 are significantly different at P < 0.05 (t-test).

Treatment of experience	Time allocated (%) to different activities during 8h of recording of feeding behaviour						
	n	C	E1	E2	G	NP	F
a) *Experiment 1 (without starvation)*							
W-W	15	31.6 ± 4.1	9.5 ± 2.0	40.8 ± 5.8	4.8 ± 0.9 b	12.7 ± 4.0	0.6 ± 0.4 (6) a
O-W	15	17.1 ± 2.1	5.8 ± 1.4	55.1 ± 4.0	2.8 ± 1.4 ab	5.4 ± 1.2	13.8 ± 4.4 (9) b
O-O	15	27.9 ± 6.5	4.7 ± 1.2	47.3 ± 9.2	5.5 ± 3.5 ab	12.2 ± 2.2	2.4 ± 1.4 (4) a
W-O	15	22.7 ± 3.0	8.8 ± 1.6	55.5 ± 5.9	0.8 ± 0.4 a	10.3 ± 2.5	1.9 ± 0.7 (7) a
P-level		0.09	0.11	0.27	0.02	0.15	0.0008
b) *Experiment 2 (with starvation)*							
W-W	15	17.8 ± 1.7 *	11.8 ± 1.9	49 ± 4.5	15 ± 4.0 *	6.2 ± 1.2	0.2 ± 0.2
O-W	15	20.3 ± 2.3	9.5 ± 1.4	54.3 ± 1.8	10.4 ± 1.9 *	4.8 ± 1.4	0.7 ± 0.3 *
O-O	15	25.1 ± 4.9	14.4 ± 5.9	43.7 ± 5.8	9.9 ± 2.2	5.5 ± 1.5 *	1.4 ± 1.0
W-O	15	18.8 ± 2.9	11.4 ± 2.5	56.3 ± 4.2	7.9 ± 1.8 *	4.5 ± 1.5 *	1.1 ± 0.5
P-level		0.45	0.72	0.24	0.07	0.47	0.80

Experiment 2 (host transfer of starved aphids)

No significant differences were found in any of the probing activities by starved aphids independently of experience and/or feeding host (W-W and O-W, and O-O and W-O comparisons of Experiment 2 in Table 1).

Comparison of activities in Experiments 1 and 2

The time in pathway phase in wheat after the period of previous experience on wheat was significantly reduced in starved aphids (comparison of activity C in treatment W-W between Experiments 1 and 2 in Table 1). The period of starvation led to a significant increase in the time in xylem ingestion in wheat (comparison of activity G in treatments W-W and O-W between Experiments 1 and 2 in Table 1), and also in oat oat albeit only when they had previous experience in wheat (comparison of activity G in treatments W-O between Experiments 1 and 2 in Table 1). Starved aphids with previous experience in oat showed significantly lower mechanical difficulties during stylet penetration on wheat (comparison of activity F in treatment O-W between Experiments 1 and 2 in Table 1). On the other hand, starvation significantly reduced the non-probing time in oat in both treatments of previous experience (comparison of activity NP in treatments O-O and W-O between Experiments 1 and 2 in Table 1).

Discussion

The results show that, within a clone, specific activities of probing behaviour were affected by previous experience. Thus, aphids with previous experience in wheat and tested in wheat showed shorter time in mechanical difficulties of the stylets during penetration and a longer time in pathway phase than aphids without previous experience in wheat. On the other hand, differences were not observed in aphids with experience in oat.

It is likely that the differences in the time showing mechanical difficulties may be attributed to different host handling experienced by aphid. In other words, previous experience may induce abilities which improve the handling of the host plant by the aphids. However, since times to first phloem phase were not affected by previous experience in wheat or oat, this enhanced host handling did not have a positive effect in the phloem stage. Montllor *et al.,* (1983) reported a shorter time to achieve a first sustained phloem ingestion in sorghum by individuals of the aphid *Schizaphis graminum* (Rondani) with previous experience on sorghum, although in this case the presence of mechanical difficulties was not reported. This variability in the effect of previous experience of feeding by aphids may be related with: 1) the aphid species studied which may vary in their capacity to be affected by previous experience, 2) the clones or biotypes used which could also be more or less sensitive to previous experience, and 3) the host plants used which may differ in their capacity to affect future aphid behaviour. Further research is needed to elucidate the relative importance of this source of variance on aphid behaviour.

The pathway phase activity corresponds to extracellular position of the stylets during exploration of the plant tissues with continuous secretion of saliva (Pollard, 1973), this activity being occasionally interrupted by F waveforms occurring at extracellular level (Tjallingi, 1987). Therefore, the decrease in the time allocated to pathway phase in wheat shown by aphids without previous experience on it (C waveform in the W-W and O-W treatments of the Experiment 1), seems to be a consequence of the advent of mechanical difficulties of the stylet (more time in F waveform).

The decrease in the non-probing time of starved aphids suggests that under this stress condition aphids spend more time inside the plant than on its surface. However, this decrease was independent of previous experience and significant only in oat but not in wheat (comparison of activity NP between Experiments 1 and 2 in Table 1), suggesting a differential suitability between both hosts. Nevertheless, due to the larger variations associated with this parameter, conclusions from non-probing behaviour must be taken with caution (Tjallingii, 1994).

The time devoted to xylem ingestion increased after starvation both in wheat and oat (although non-significantly in the O-O treatment) and regardless of previous experience. Given that greater duration of xylem uptake of dehydrated aphids has been reported earlier (Spiller *et al.*, 1990), this suggests that starvation more critically affected *S. fragariae* by desiccation stress than by nutrient stress. Our experimental procedures did not allow the comparison of variables such as rate of phloem ingestion, which could have been modified by starvation. However, ingestion rate in aphids is fairly constant, and differences in sap intake appears to be achieved mainly by changes in the duration of the ingestion period (Tjallingii, 1994).

In summary, in this aphid-plant combination our results showed that intraclonal probing behaviour is affected by experience and starvation. Lack of previous experience in a host may elicit difficulties in plant penetration, and aphids subjected to starvation reorient their behaviour to the maintenance of their water-balance.

Acknowledgements

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CAPÍTULO 2

EFECTO DE LA EXPOSICIÓN PREVIA A ÁCIDOS HIDROXÁMICOS EN LA CONDUCTA DE PRUEBA DEL ÁFIDO *SITOBION FRAGARIAE* (WALKER) EN PLÁNTULAS DE TRIGO.

THE EFFECT OF THE PREVIOUS EXPOSURE TO HYDROXAMIC ACID IN THE PROBING BEHAVIOR OF THE APHID *SITOBION FRGARIAE* (WALKER) ON WHEAT SEEDLINGS

Claudio C. Ramírez, Paula P. Caballero & Hermann M. Niemeyer

Abstract

We hypothesized that aphids after previous exposure to hydroxamic acids (Hx), a family of secondary plant compounds deleterious to aphids, are able to reduce their subsequent exposure to them. This hypothesis was tested by evaluating the time to produce salivation into a sieve element (SSE) by the aphid *Sitobion fragariae* (Walker) on seedlings of two wheat cultivars of *Triticum aestivum* differing in their concentration of Hx. The total time to produce a first SSE was significantly longer in the high-Hx cultivar; however, the subsequent second SSE (first SSE after interruption of probing) in this cultivar was significantly reduced, reaching the level observed in the low-Hx plants. Therefore, a strategy to reduce the exposure to secondary compounds was observed only in the second SSE in high-Hx plants. When the experimental plant was replaced by a new unattacked plant after the first SSE, aphids did not change the behavior described, thus excluding an aphid-induced plant susceptibility. Number of cell punctures and accumulated duration was not affected by previous exposure to Hx, neither in low nor high Hx cultivars. Total time and pathway time but not cell punctures, seem to be the variables affected by previous exposure to Hx.

Keywords: Aphid, experience, probing behavior, sieve elements, salivation, cell punctures, hydroxamic acids, EPG, *Sitobion fragariae, Triticum aestivum.*

Introduction

Aphids are specialized feeders that probe through the plant epidermis and mesophyll in order to access their final feeding site, the sieve elements. Aphid probing behavior in both susceptible and resistant host plants has been frequently reported (Sharks and Chase, 1979; Montllor *et al.*, 1983; Montllor and Tjallingii, 1989; Dorschner and Baird, 1989; Morgham *et al.*, 1994, Webster *et al.*, 1993; Caillaud *et al.*, 1995). Probing behavior in resistant plants is characterized by shorter probes, longer non-probing times and shorter phloem ingestion times than in susceptible plants, suggesting that aphids are able to detect plant resistance factors and display aversive behavior.

In the cases of some cereals, the presence of hydroxamic acids (Hx) in the epidermis, mesophyll and sieve elements (Argandoña *et al.*, 1987; Niemeyer, 1991) has been suggested as the main resistance factor against aphids (Niemeyer & Pérez, 1995). Thus, aversive behaviors (Argandoña *et al.*, 1983; Givovich and Niemeyer, 1995; Mayoral *et al.,* 1996), as well as demographic parameters of aphid biology (Thackray *et al.*, 1990; Niemeyer, 1991; Givovich and Niemeyer, 1994) have been correlated with Hx. The mechanism by which Hx are recognized by aphids is rather speculative. In an intact plant, Hx are present as glucosides, which are enzymically hydrolyzed to aglucones when the tissue is injured and intracellular compartmentation is destroyed (Hofman and Hofmanova, 1971). Aglucones exert stronger effects on the behavior and performance of aphids than the respective glucosides (Corcuera *et al.*, 1985). Damage to mesophyll cells by the stylets on their way to the sieve elements has been described and finely studied using light and electron microscopy (Pollard, 1973; Spiller *et al.*, 1985; Tjallingii and Hogen Esch, 1993). It was shown that all types of cells show punctures and the highest number of damaged cells are found inside the vascular bundle, although the magnitude of cellular damage seems to be moderate (Brzezina *et al.*, 1986; Tjallingii and Hogen Esch, 1993). Concerning aphid testing capacity, it has been shown that there are no chemoreceptors present on the aphid labium or the stylets (Wensler, 1977; Tjallingii, 1978), and that gustatory organs are located in the epipharyngeal wall and are stimulated by ingesta (Wensler and Filshie, 1969). Hence, detection of secondary metabolites by aphids must occur through the sampling of plant contents.

The aim of this work was to study the effect of the previous exposure to Hx on aphid probing behavior. We have chosen, using the electrical penetration graph technique (EPG), the time taken by an aphid to produce the first E1 waveform (first phloem phase) as standard parameter. This parameter has been pointed out as an indicator of the location of non-sieve element factors affecting plant acceptance by aphids (Tjallingii, 1995). E1 has been correlated with salivation into the sieve elements (SSE hereafter) (Prado & Tjallingii, 1994), suggested to be involved in suppressing the wound reaction of the sieve elements (Tjallingii & Hogen Esch, 1993). We hypothesize that after previous exposure of aphids to Hx during their path through the epidermis and mesophyll in their route to the sieve elements, they are able to reduce their subsequent exposure to them, particularly in high-Hx plants. We tested this hypothesis by evaluating the time needed to produce a first SSE by the aphid *Sitobion fragariae* (Walker) on two wheat cultivars differing in their concentration of Hx, and comparing it with the time needed to produce a subsequent SSE after interruption of probing. We also investigated whether differences in such behavioral responses are accounted for by the aphids intrinsic probing ability or by changes in the plant´s susceptibility induced by the aphid. With this aim, the experimental plant was substituted by a new unattacked plant after the first SSE by the aphid.

Methods And Materials

Insects

Individuals of the aphid *S. fragariae* were collected from wild annual Poaceae in central Chile. From a single collected individual, a colony of clones was created and kept on oats (*Avena sativa* L. cv. Nehuén) under laboratory conditions, at 20 ± 2 °C and 16:8 (L:D) photoperiod.

Plants

Cultivars of the wheat *Triticum aestivum* L. differing in Hx concentration were used in order to expose aphids to plants with different levels of these plant secondary compounds. The first leaf of 7-day old seedlings was used. Chemical analysis showed that Hx concentration in the first leaf was significantly higher in cv. Naofén than in cv. Millaleu (1.94 ± 0.31 mmol/kg fresh weight, $n = 8$, and 1.09 ± 0.30 mmol/kg fresh weight [mean ± S.E.], $n = 8$, respectively; $F (1,14) = 29.21$, $n = 16$, $p < 0.00009$).

Experiments

In both wheat cultivars, probing behavior of the aphid was electronically monitored and when a first SSE occurred (determined by the observation of an E1 waveform), the aphid was carefully separated from the plant and then immediately returned to it. Thus, the stylets were not allowed to stay in the lumen of the penetrated sieve element cell, and a new probing sequence was forced to start. The interruption lasted less than 5 seconds. The experiment continued until a new SSE was observed (observation of a new E1 pattern) in the manipulated aphid.

A second similar set of experiments was performed, but in this case the experimental plant was substituted by another non-attacked plant once the aphid showed its first SSE. Such design allowed the control of a possible aphid-induced change in the plant due to the stylets' activities inside the plant tissues, a change that could be influencing the subsequent SSE. The plant used for replacement was of the same cultivar and developmental stage as the original plant. A total of 10 replicates in each experiment was performed.

Monitoring of probing behavior

The probing behavior of each experimental aphid was electronically monitored using electrical penetration graphs (EPG). A gold wire electrode (25 μm diameter) was fixed to the dorsum of an apterous adult aphid with conductive silver paint. Another electrode was inserted in the soil of the potted plant. Both electrodes were connected to a DC electric circuit designed to monitor aphid stylet's incursions inside plant tissues (Tjallingii, 1978). When the aphid stylets penetrate into the plant tissues, they close the electrical circuit and the voltage changes are amplified and continuously monitored. All signals were directly displayed on screen and recorded on a PC hard disk for detailed analysis with the EPGview software with a sampling rate of 100 Hz (Flores *et al.*, 1998, submitted). Different stylet activities and the location of the stylets' tip produce specific patterns of voltage changes in the recorded signal (electrical penetration graph) so the stylets' activity and stylets' tip position can be judged from the displayed signal (Tjallingii and Hogen Esch, 1993). With this technique, waveforms associated with non-probing (NP), cell punctures (pd's), pathway activities composed by intercellular-intramural advancement with salivary sheath formation (C), xylem ingestion (G), difficulties in the stylet during probing (F) and salivation (E1) in the sieve elements can be determined (Tjallingii and Hogen Esch, 1993).

Statistical analysis

The times taken by aphids from their placing in the plant to the production of SSE (dependent variable) were compared with a three-factor ANOVA with repeated measures analysis. Factors were labelled as "Hx, "SSE's" and "Plant". Each factor had two levels. Factor "Hx" included cultivar with low and high Hx concentration; "SSE's" included first and second SSE; "Plant" factor included conditions where the experimental plant was or was not substituted by another non-attacked plant once the aphid showed its first SSE. Non-probing time, pathway time, number of cell punctures and their accumulated duration were similarly analyzed as dependent variables with ANOVA for repeated-measures. Tukey HSD test for multiple comparisons was also performed. Data were transformed (log (x+1)) if ANOVA assumptions were violated.

Results

Table 1 shows that the variation in the total time to produce SSE was significantly affected only by the factor "SSE's" and by its interaction with the factor "Hx" (SSE's * Hx). In other words, total time to produce a SSE significantly differed between first and second SSE, and was also modulated by the Hx concentration. The "Plant" factor was not significant, indicating that plant substitution did not affect aphid behavior as measured by this variable. In the high-Hx cultivar, the total time needed to achieve a first SSE was significantly longer than the same period for the low-Hx cultivar (Fig.1A). Aphids showed a significantly shorter total time to achieve a second SSE with respect to the first SSE only in the high-Hx cultivar (Fig. 1A). On the other hand, the time to achieve the second SSE was not significantly different in both cultivars (Fig.1A).

Considering only the pathway time (C pattern) before a SSE as the dependent variable, the same trends were observed (Fig. 1B). In this case however, only the interaction "SSE's * Hx" was significant (F(1,36) = 4.62, p=0.038, three-factor ANOVA with repeated measures analysis). Similarly, for non-probing time a significant source of variation was the interaction "SSE's * Hx" ; however, this effect was accounted for by a significant difference in duration between non-probing time before the first SSE in low and high-Hx cultivars, (14.3 ± 3.1 and 32.6 ± 6.6 min for low and high-Hx respectively, p = 0.04 Tukey HSD test). In contrast, patterns G and F did not show significant differences associated with plant substitution, Hx or SSE (data non shown). The number of cell punctures (pd's) showed a significant effect only in the

"Hx" factor (F(1,36)=4.74, p=0.036, three-factor ANOVA with repeated measures analysis).

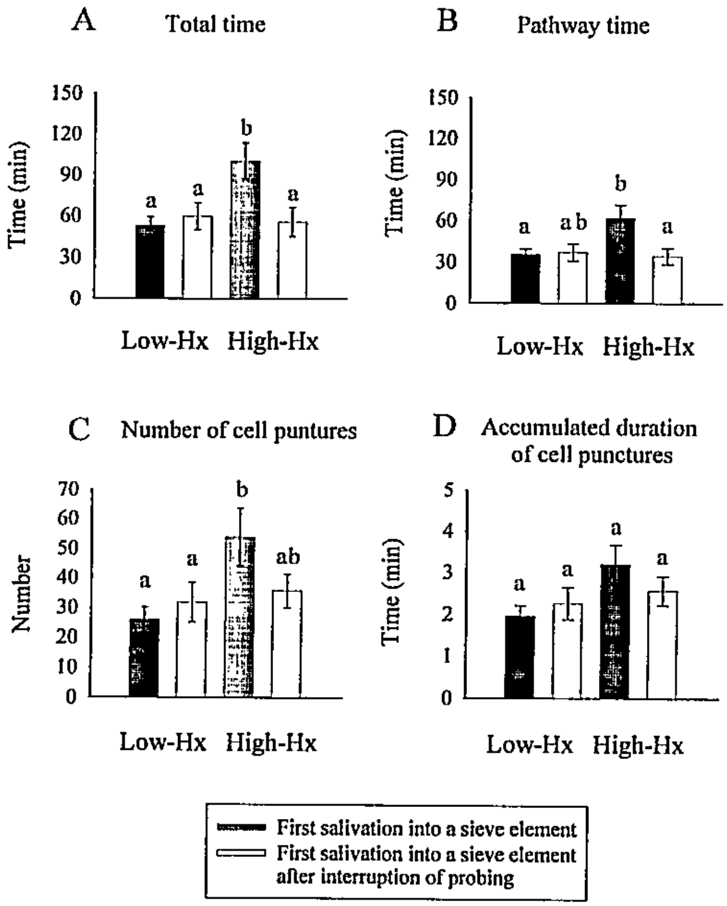

Figure 1. Time to first SSE (dashed area) and to first SSE after interruption of probing (white area) by the aphid *S. fragariae* in wheat cultivars with low- and high-Hx concentrations. Total time before SSE (A), time in pathway activities (B), number of cell punctures (C) and duration of cell punctures (D) are shown. Bars represent standard errors for n = 20 in each case. Different letters among bars mean that the differences among the values are significant at $p < 0.05$ (Tukey HSD multiple comparison test).

TABLE 1. Three-way ANOVA with repeated-measures of the total time to produce SSE (dependent variable). See *Statistical analysis* in the text for details.

Sources	Effect		Error		F	p-level
	df	MS	df	MS		
Plant	1	0.046	36	0.099	0.472	0.496 ns
Hx	1	0.255	36	0.099	2.573	0.117 ns
SSE's	1	0.406	36	0.069	5.882	0.020 s
Plant * Hx	1	0.138	36	0.099	1.386	0.246 ns
Plant * SSE's	1	0.014	36	0.069	0.208	0.650 ns
Hx * SSE's	1	0.579	36	0.069	8.392	0.006 s
Plant * Hx * SSE's	1	0.104	36	0.069	1.513	0.226 ns

Since the Hx * SSE interaction was not significant, this effect was due mostly to a larger difference between number of cell punctures before the first SSE in low and high-Hx cultivar (25.9 ± 4.17 and 53.8 ± 9.7 min for low and high-Hx respectively [mean ± standard error], p = 0.04 Tukey HSD test) than before the second SSE (31.8 ± 6.7 and 35.7 ± 5.7 low and high-Hx respectively [mean ± standard error], p = 0.04 Tukey HSD test). The number of cell punctures was not different between the two periods before SSE within each cultivar (Fig. 1C). The accumulated duration of cell punctures did not show significant differences associated with Hx or SSE (Fig. 1D) or with plant substitution.

Discussion

In the high-Hx plants, the first SSE was produced after a longer probing period than in the low-Hx plants (Fig.1A). A similar significant effect of plant resistance on the times required to the first phloem access has recently been observed in a different aphid-plant combination (Chen *et al.*, 1997). Interestingly, in the high-Hx plants, shorter times to second SSE were recorded (Fig. 1A). Since time to achieve a second SSE in the high-Hx cultivar did not differ from the time devoted to the same activity in the low-Hx cultivar (Fig. 1A), it seems that aphids are able to overcome the high concentration of Hx during this period and consequently behave, at least in terms of their time assignation, as if they were on the low-Hx plants. Moreover, the significant decrease in the time to the second SSE in the high-Hx cultivar was attributable to a decrease in pathway activities but not to changes in other EPG patterns, suggesting that the main factor affecting plant acceptance is encountered during passage through the intercellular and intramural plant compartments in the high-Hx cultivar. Furthermore, the fact that our experimental design did not allow phloem ingestion (E2 waveform), supports our proposal that pre-phloem ingestion factors are mainly involved in the behavioral changes described.

The fact that the decrease in the time to produce a second SSE was not affected by a substitution of the experimental plant for an unattacked one, confirms that such time changes are accounted for by a change in the aphid's intrinsic ability to probe in the plant's tissues rather than by an increase in plant susceptibility induced by the aphid during probing prior to its first SSE. This result is similar to the lack of aphid-induced plant effect on the behavior of the aphid *Rhopalosiphum padi* feeding on wheat (Prado & Tjallingii, 1997).

The number of cell punctures did not show significant differences between first and second SSE in both cultivars. Thus, it appears that interruption of feeding "resets" the pattern of cell punctures. This result is in agreement with those of Prado (1997) who found that the exploratory behavior of aphids seems to be "reset" by short interruption intervals (1 min.). Total time and pathway time but not cell punctures seem to be the variables affected by previous exposure to Hx.

These facts support the hypothesis that after previous exposure to Hx during their route to the sieve elements, aphids employ a strategy of reducing their exposure to Hx, particularly when they face higher concentrations of them. This mechanism implies that during the first experience previous to salivation in sieve cells aphids recognize the presence of Hx, particularly if present at sufficiently high concentration. Such perception may initially elicit an avoidance behavior, mainly expressed as longer periods of pathway activities. This may be considered a "training" period within a simple learning process. Afterwards, during the path to a subsequent salivation into sieve cells, exposure to Hx is reduced through shorter duration of pathway activities as a result of the learning. An alternative explanation to the pattern observed is a mechanism of physiological adaptation to Hx by aphids, i.e. the deterrent effect of Hx decreases after exposure to them. However, because cell punctures may elicit hydrolysis of Hx from glucosides to the more toxic aglucones when the tissue is injured (Hofman and Hofmanova, 1971), the trend to show a lower number of cell punctures after the first SSE in the high-Hx cultivar seems to fit better a mechanism involving a reduction of the aphids' exposure to Hx.

Finally, the mechanisms proposed here should be understood as part of a variety of mechanisms that aphids may use in the field in order to overcome plant resistance factor such as secondary compounds.

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CAPÍTULO 3

VARIACIÓN INTRACLONAL INDUCIDA POR LA EXPERIENCIA EN EL USO DE HOSPEDERO EN EL ÁFIDO *SITOBION FRAGARIAE* (WALKER)

EXPERIENCE-INDUCED INTRACLONAL VARIATION IN HOST USE WITHIN A CLONE OF THE APHID *SITOBION FRAGARIAE* (WALKER).

Claudio C. Ramírez & Hermann M. Niemeyer

Introduction

Many ecological factors have been mentioned as underlying the intraspecific specialization in host use within a polyphagous insect species (Futuyma & Moreno 1988, Jaenike 1990, Bernays & Chapman 1994). Genetic variation between individuals and the effects of the experience are two factors that have received increased attention (Via 1994). Several studies over a broad range of insect taxa have provide support for each these two mechanisms (see for example, Papaj & Lewis 1993, Szentesi & Jermy 1990), while a smaller number of studies have assessed the relative contribution of each mechanism to the host plant use (Rausher 1984, Via 1991). In the case of aphids (Hemiptera:Aphididae), some studies have evaluated genetic variation in host use (Mackenzie 1996, De Barro *et al.*, 1995), experience-mediated differences in host use (Robinson 1993, Montllor 1993, McCauley 1990a, 1990b), while others studies have designed experiments that directly evaluate the genetic and experience components of fitness (Via 1991, De Barro *et al.*, 1995). The main protocol of this approach is based on comparing the performance of individuals from different habitats in reciprocal transplant experiments (Via 1994). Following this protocol, Via (1991) did not find a significant effect of experience on the performance of the pea aphid *Acyrtosiphum pisum* after three generations on alfalfa or clover, although a clear trend was observed. Differently, De Barro *et al.*, (1995) provided evidence that maternal experience of the grain english aphid *Sitobion avenae* can influence offspring performance on wheat and cockfoot. Similarly Douglas (1997) showed that a clone of *Aphis fabae* affiliated to

Vicia faba improved its performance on the secondary host-plant, *Tropaeolum majus*, after 5-9 generations of previous experience on it. Mackenzie (1996) reported progressive improvement in the performance of *A. fabae* clones across multiple parthenogenetic generations on an initially unfavourable host. However, although behavioral constrains are the first steps that aphid must confront when arriving to a host plant (Kingauf, 1987), much less is known about the behavioural mechanism which may be underlying this experience-mediated improvement of in the performance.

The aim of this study was to describe the effect of experience on the feeding behaviour and fecundity of the aphid *Sitobion fragariae* (Walker). We compared the feeding behaviour and fecundity in a host plant between aphids with or without previous experience on it, and associate this behavior with fecundity. All comparisons were performed within a single aphid clone in order to minimize the effect of genetic variation. Our qualitative prediction was that previous experience in a host would imply a facilitation in feeding behaviour on that host relative to the behaviour on non-experienced host. Such differences in behaviour should match with fecundity.

Materials and Methods

Aphids

Individuals used were from a clone of the blackberry-grass aphid *Sitobion fragariae* (Walker) derived from a single virginoparous aptera collected from annual grasses in central Chile during the early summer of 1995. The aphids were cultured for about 10 to 12 generations on oat seedlings (*Avena sativa* c.v. Nehuen) under laboratory conditions, at 20 ± 2 °C and 16:8 (L:D) in plastic boxes.

Behavioral monitoring procedure

The Electrical Penetration Graph (EPG) technique (Tjallingii 1978) to record the aphid probing behaviour was used. A gold wire electrode (2 cm long x 25 μm diameter) is fixed to the dorsum of the aphid with conductive silver paint. Another electrode is inserted in the soil of a potted plant. Both electrodes are connected to a DC electric circuit designed to monitor aphid stylet incursions inside plant tissues (Tjallingii 1978). When the aphid stylets penetrate into the plant tissues they close the electrical circuit and the voltage changes are amplified and continuously monitored. All signals are recorded on a PC hard disk for later analysis. Different stylet activities and the location of the stylet tip produce specific pattern of voltage changes in the recorded signal, so that the stylet activity and stylet tip position can be inferred from the recorded signal (Tjallingii & Hogen Esch 1993).

Host transfer experiment

This experiment was performed to compare the feeding behaviour in a host plant between aphids with or without previous experience on it. The test host plant was wheat (*Triticum aestivum* cv. Millaleu), and oat was used as control host. Apterous adult aphids from the monoclonal colony maintained on oat were transferred separately to potted seedlings of wheat and oat in the 2-leaf growth stage. After 24h all adult aphids were removed and all new-born first instars produced were maintained in each host plant until they reached the reproductive stage (generation 1). This period of time was labelled "previous experience" on the respective host plant. Two groups of aphids were obtained: 1) with previous experience in wheat, and 2) with previous experience in oat. Subsequently, electrical monitoring of the feeding behaviour was performed on individuals from each group of aphids. Aphids were kept for one hour in a Petri-dish after being connected to the EPG electrode, and subsequently transferred to wheat or oat seedling (2-leaf grown stage). This last transference divided the groups labelled "previous experience" into two new groups, configuring a total of four different experimental treatments: 1) from wheat to wheat (W-W), 2) from wheat to oat (W-O), 3) from oat to wheat (O-W), and 4) from oat to oat (O-O). Continuous monitoring was performed during 8h.

Behavioral variables

Each EPG profile were analyzed with the WinEPG software (Flores *et al.*, 1998, in preparation). Typical waveform pattern of non-penetration (NP), salivation (A and B), pathway phase (C), cell punctures (pd, potential drops), mechanical difficulties during stylet penetration (F), xylem ingestion (G), phloem access (E1) and phloem ingestion (E2) were recognized (Tjallingii 1988). The accumulated time assigned to each these activities was estimated in both experiments.

Fecundity

To measure fecundity, as in the experiment of behavioral monitoring apterous adult aphids with previous experience in wheat or oat were transferred to wheat or oat, performing also four cross combinations (W-W, W-O, O-W and O-O). Each transferred aphid was individually paced inside clip-cages on separated potted plants and reared under laboratory conditions at 22 ± 2 °C and 16:8 (L:D) in plastic boxes, and the number of new nymph produces every two days during 14 days was determined.

Statistical analysis

Squared Mahalanobis distance (SMD) from a stepwise discriminant analysis was used to compare to the multivariate behaviour performed in each experimental group. This analysis enabled us also to determine which behavioural variables discriminate between comparing groups (Caillaud *et al.*, 1995). Tukey HSD test was used for *a posteriori* comparisons.

Results

Host transfer experiment

The pattern of probing behaviour of the four treatments (groups) was compared with a forward stepwise discriminant function analysis. A significant discriminant function accounting for 74,6 % of total variance was found (Wilks' Lambda = 0.537, F (9,126) = 3.65 p = 0.0004). Table 1 shows the Mahalanobis distance between groups. No significant differences were found in the behaviour on wheat and oat after previous experience in the respectively plant, (W-W) and (O-O) treatment (Table 1). There were also non significant differences in the behaviour on oat between aphids that previously

Table 1. Squared Mahalanobis distance (SMD) between treatment groups obtained from a forward stepwise discriminant analysis. Asterisks shows significant differences ($p < 0.05$). The higher the Mahalanobis distance, the higher the global differences the between two comparing groups. Variables included in the analyses are described in the text.

		O-W	O-O	W-O
W-W	SMD	3.476	0.686	0.571
	F ratio, df = (3,52)	7.520	1.411	1.327
	P	0.00028 *	0.249	0.275
O-W	SMD		2.601	2.308
	F ratio, df = (3,52)		5.210	5.159
	P		0.003*	0.003 *
O-O	SMD			1.158
	F ratio, df = (3,52)			2.478
	P			0.071

experienced oat (O-O) or wheat (W-O), although in this case the level of non significance was marginal (Table 1). Conversely, the behaviour on wheat between aphids that previously experienced wheat (W-W) or oat (O-W) was significantly different. The behaviour of aphids transferred from oat to oat (O-O) or wheat (O-W) resulted in significant differences (Table 1), while the inverse combination of previous experience in wheat and a posterior transference to wheat (W-W) to oat (W-O) did not produce significant differences.

The stepwise discriminant analysis included in the model only three out of the seven variables used, which in order of the magnitude of the standardized canonical coefficient are: time occupied in mechanical difficulties during stylet penetration (F), salivation in the sieve elements (E1) and time in phloem ingestion (E2)(F=-0.99, E2=-0.47, E1=0.03). This three variables are the ones that allow a discrimination between the four groups.

Fecundity

A significant higher number of nymph was observed in those aphid reared upon oat than in wheat (Fig.1). In those aphid reared on wheat, the fecundity in oat and wheat was not significant different. The same was shown for the one reared upon oat, although a trend to higher fecundity on oat was observed.

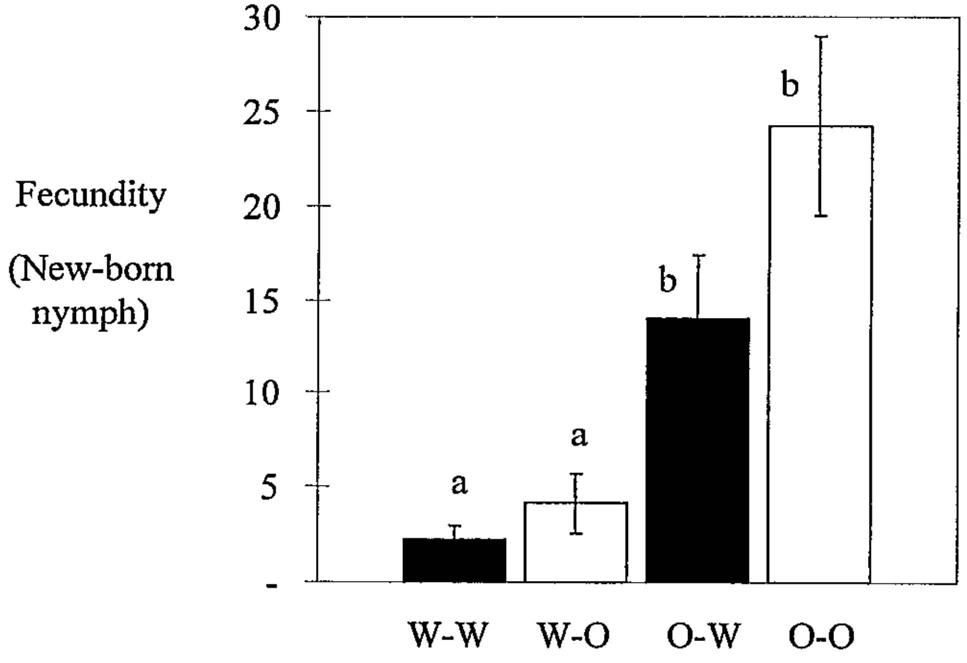

Figure 1. Fecundity of *S.fragariae* in relation to previous experience treatment. W-W, experience in wheat and fecundity in wheat; W-O, experience in wheat and fecundity in oat; O-W, experience in oat and fecundity in wheat; O-O, experience oat and fecundity in oat. N = 10 in each group. Different letters in the bars mean that the differences among the values are significant at $p < 0.05$ (Tukey *a posteriori* test).

Discussion

The multivariate behaviour in relation the effect of the previous experience depend of the host plant were such developmental experience was performed. This is the case of wheat where the multivariate pattern of feeding behaviour differs depending of the previous experience on it. This is not the case of oat where the pattern did not differ regardless previous experience. Such difference in the consequences of previous experience suggest that both host differs in the ability to induce a imprinting.

The main behavioral variable accounting for this differences was time occupied in mechanical difficulties during stylet penetration (F). In relation to this behavior electron microscope sections, after stylet cutting during F waveform, have shown that during this behaviour the stylets is in cell walls over long distance (Tjallingii 1987). It has been also suggest that this behaviour is associated with mandibles and maxilla dislocation (Prado 1997), and with "grooming behaviour" corresponding to an aphid attempting to clear its stylet from clumsy compounds (Caillaud *et al.*, 1995). It is likely that time showing this behaviour may be due to differences in the handling abilities induced by the experience with this host. Similar mechanism may be occurring with the variation in the salivation into the sieve elements (E1) and phloem ingestion (E2), but in this case al the level of interaction with the sieve elements.

These differences in the multivariate pattern of feeding behaviour did not matched with the reproductive outcome after experience. Similar result were found by Mccauley *et al.* (1990a) for the aphid greenbugs *Schizaphis graminium,* but in this case with time to achieve phloem ingestion. Thus, it is suggested that behavioral variation caused by experience is not explaining variation in fitness, at least in a intra-clonal level. It is likely that the significantly higher fecundity following experience on oat may be genetically determined, being the clone studied specialized to oat and hence performing poorly on other hosts due to trade-off of plant utilization (Via, 1991; Mackenzie, 1996). However, this may not be the case since *S. fragariae* use blackberry *Rubus fruticosus* as a primary host, although it also alternate with various Poeacea species (especially cocksfoot grass *Dactylus glomerata* L.) (Blackman & Eastop, 1984) including oat and wheat during plantations season and wild annual grasses (Starý, 1993; Starý *et al.*, 1993). Alternatively, since relationship between nutritional quality and fecundity has been well described in aphids (Dixon, 1985), oat could simply represent a host with higher nutritional quality relatively to wheat, in which case our results shows that experience could have reinforce the higher oat profitability. In this sense our shows that *S. fragariae* is more specialized to oat that to wheat.

The present work have shown that within a clone the history of rearing produce variation in the reproductive pattern. Behavioral variables studied seems not to be related with that reproductive outcome. A host profitability-variation hypothesis need to be tested in order to understand such fecundity variation.

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CAPÍTULO 4

VALOR ADAPTATIVO DE LA EXPERIENCIA PREVIA EN LA CONDUCTA DE BÚSQUEDA DEL FLOEMA EN UNA ESPECIE DE ÁFIDO

ADAPTIVE VALUE OF PREVIOUS EXPERIENCE IN THE PHLOEM SEARCHING BEHAVIOUR OF AN APHID SPECIES

Ramírez, C.C & H.M. Niemeyer

Abstracts

The hypothesis that aphids are able to use previous experience in order to reduce time to reach a phloem phase in a host plant may constitute an adaptive traits was tested. We (1) evaluate the effects of the previous experience on time to achieve a first phloem phase and to compare the time elapsed between two consecutive phloem phases, (2) compare this behaviour within and between individuals (genetical individuals or clones), and (3) evaluate its correlation with a parameter of fitness (intrinsic rate of population increase, r_m). Electronic monitoring was used to record the feeding behaviour. Two out of three clones showed a decrease in the time between two consecutive phloem phases; however these clones showed the lowest r_m. Time to achieve a first phloem phase varied between clones and was negatively correlated with r_m., while time to the achieve a second phloem phase did not showed variation between clones and was not correlated with r_m. This result suggest that a shorter time to the first phloem seems to be an adaptive trait and it should be selectively maintained over their alternatives phenotypes.

Introduction

Aphids are specialised plant-sucking insects. Once settle, the aphid penetrate with their stylets deeply into the plant tissues searching for cells to feed on, especially the sieve elements (Dixon 1985; Klingauf 1987; Tjallingii 1995). During this period of searching the aphid's stylet moves actively following intercellular path but also performing cells punctures in different cells. A phloem phase is initiated by a prolonged puncture into sieve elements which always involves a short period of salivation into their lumen usually followed by the phloem ingestion (Tjallingii 1995). The duration of the entire process of searching until a phloem ingestion is achieve may takes for long, depending of the combination of aphid-plant species.

Prolonged periods of searching may be adverse for the energetic or water balance of the aphid, especially for the two generations of embryos carried by a parthenogenetic female (telescoped generations), thus, there could be an adaptive advantage in being able to initiate a phloem phase quickly (Dixon 1985). If this trait is adaptive, holding the history-free concept of adaptation of Reeve & Sherman (1993) which emphasising the phenotype existence understanding adaptation as a phenotypic variant that result in the highest fitness among a specified set of variant in a given environment, it is expected to find a positive association between reaching the phloem phase early fitness. Early phloem phase may be obtained by achieving a faster first phloem phase, or alternatively by a simple form of learning during the searching experience so that after a first experience in a phloem phase the second phase could be reached comparatively in a lesser time. In order to study the plausibility of these mechanisms and their association with fitness we (1) evaluate the time to reach a first phloem phase between different individuals, and (2) also study the effects of the previous experience by comparing the time elapsed between two consecutive phloem phases among individuals, associating in both cases the intrinsic rate of population increase among this individuals.

It is important to mention that aphids are clonal organism and therefore the concept of individuality is not applicable straightforwardly (Janzen 1977; Hughes & Cancino 1985; Tuomi & Vuorisalo 1989; Eriksson 1993; Dixon 1994). The organism developed from a zygote (the genet) is able to reproduce by cyclic parthenogenesis leading to a genetically identical progeny of discrete and functionally independent units (ramets). The ramets originated from a single genet will be hereafter indistinctly refereed as "clone" or "genetical individual". In this sense, because genetic variation occurs among clones (apart from the variation due to somatic mutations), this unit is considered of evolutionary relevancy. More than an obstacle, this particular features of aphids facilitate the experimental manipulation of within-and between individual variation of a behavioural trait.

Materials and Methods

Clones

The aphid studied was *Sitobion fragariae* (Walker) commonly found in cultivated and in wild annual Poaceae in temperate climates. We collected aphids from different localities of central Chile, and each aphid was allowed to propagate parthenogenetically on wheat (*Triticum aestivum* c.v. Millaleu) growing in plastic boxes in a room at 20 ± 2 °C and 16:8 (L:D). Five clones lines were subsequently studied.

Electronic monitoring

The sieve element searching behaviour of each experimental aphid was electronically monitored using electrical penetration graphs (EPG). This technique consist in to fix a gold wire electrode (25 μm diameter) to the dorsum of an apterous adult of the aphid with conductive silver paint. Another electrode is inserted in the soil of the potted plant. Both electrodes are connected to a DC electric circuit designed to monitor aphid stylet's incursions inside plant tissues (Tjallingii 1978). When the aphid stylets penetrate into the plant tissues they close the electrical circuit and the voltage changes are amplified and continuously monitored. All signals are directly displayed on screen and recorded on a PC hard disk for detailed analysis with and *ad hoc* software, WinEPG (Flores *et al.*, 1998, in preparation). Different stylet activities and the location of the stylets' tip produce specific patterns of voltage changes in the recorded signal (electrical penetration graph) so the stylets' activity and stylets' tip position can be judged from the displayed signal (Tjallingii & Hogen Esch 1993).

Experiment of searching behaviour of sieve elements

To evaluate the time between two sequential phloem phases each aphid was continuously monitored and when a first phloem phase occurred, event that was determined by the observation of a E1 waveform, the aphid was carefully separated from the plant and then immediately returned to it. The interruption lasted less than 5 seconds. The experiment continued until a new phloem phase was observed (observation of a new E1 pattern) in the manipulated aphid. This procedure was performed in 10 aphids of each clone.

Intrinsic rate of population increase

To estimate the intrinsic rate of population increase of a clone, a group of adult aphids were kept for 12h in a host plant and subsequently 10 newly-born nymphs produced during that period were individually confined to the plant by a clip cage and maintained under controlled conditions of temperature and photoperiod (20°C and 16L:8D). The intrinsic rate of population increase (r_m) was determined by the equation of Wyatt & White (1977) : $r_m = 0.738 (\ln M_d)/T$, were M_d is the number of nymph produced in the time equivalent to the pre-reproductive period (T), i.e. the time from the birth to the initiation of nymph production. During that period newly-born nymphs were removed after every observation in order to avoid a crowding effect in the reproductive output.

Results

Significant variation in the time to a first phloem phase was observed among the three clones studied (Kruskal-Wallis test: $H_2 = 7.14$, $P < 0.05$). Two out of the three clones studied (Cl-1 and Cl-0 in Fig.1b) showed significant differences in the time to achieve a first and second phloem phase, being in both cases significantly lower the time to the second phloem phase (Fig. 1b). The clone Cl-2 did not showed significant differences between time to first and second phloem phase ($U=17$, $P=0.22$). However, the reduction in the time to a second phloem phase showed by clones Cl-1 and Cl-0 was caused by its higher time to achieve a first phloem phase. Differently, time to the second phloem phase showed no significant variation between clones (Kruskal-Wallis test: $H_2 = 0.63$, $P > 0.05$).

The intrinsic rate of population increase (r_m) between clones (Fig. 1a) varied significantly (Kruskal-Wallis test: $H_2 = 9.12$, $P < 0.05$). Interestingly r_m was significantly inversely correlated with time to the first phloem phase (Spearman rank correlation: $r_s = -1.0$, $N = 3$, $P < 0.01$), while it was not correlated with the time to the second phloem phase (Spearman rank correlation: $r_s = -0.1$, $N = 3$, $P > 0.10$).

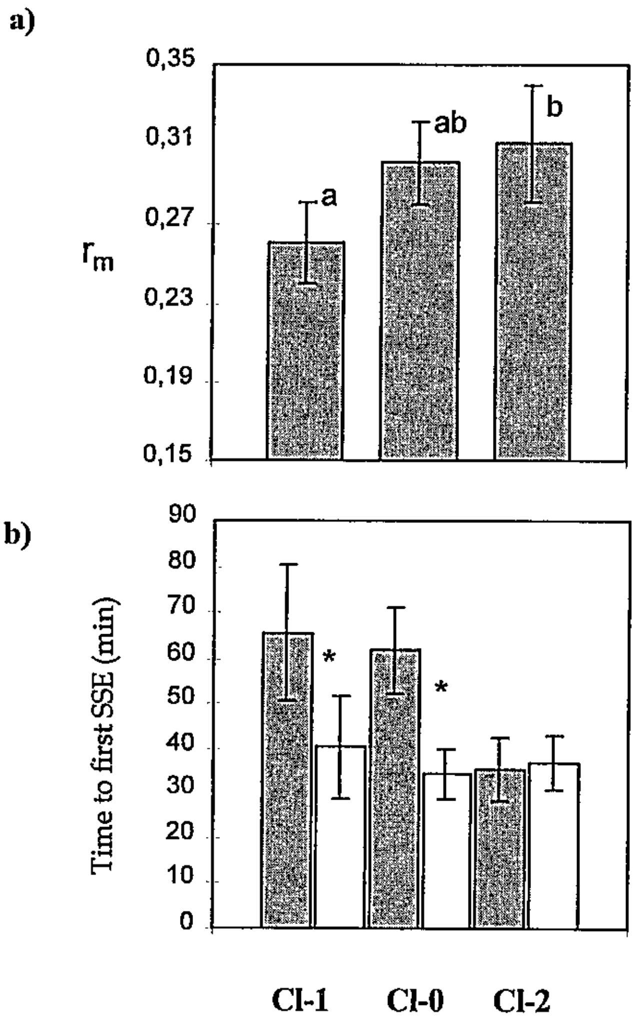

Figure 1. Mean (± S.E) of (a) intrinsic rate of population increase, r_m, and (b) time to achieve a first (▨) and second (☐) phloem phase of five clones of the aphid *S. fragariae* on the wheat *T. aestivum*.

Discussion

Two out of the three aphids clones studied (Cl-1 and Cl-0) were able to show a lower time between two consecutive phloem phases, and therefore they seems affected by previous experience. However, this clones showed the lowest r_m, while the clone showing no difference between time to each phloem phase (Cl-2) showed higher r_m. Therefore, due to this inverse relation with fitness its suggested that the ability to be affected by previous experience is not adaptive. However, since time to the second phloem phase did not varied between clones (ca. 35 min in average), the decrease in the time between two consecutive phloem phases showed by clones Cl-1 and Cl-0 was due to the longer time needed to achieve the first phloem phase rather than to a decrease in the time to achieve a second phloem phase (Fig.1b). In other words, this clones were slower in their ability to reach a first phloem phase, but in their second searching period they were able to start a new phloem phase in a time comparable to the one showed by all other clones. Differently, the clone Cl-2 did not showed differences in the time to achieve both phloem phases and may be considered insensitive to previous experience.

Interestingly, the time to the first phloem phase showed a negative correlation with the r_m between clones, while time to the second phloem ingestion showed no correlated with fitness. Therefore, a shorter time to the first phloem instead of the ability to be affected by experience seems to be the adaptive trait, and it should be selectively maintained over their alternatives phenotypes.

Differently to the present work, McCauley *et al.,* (1990) found no significant relationship between any aspect of feeding behaviour and fecundity. However, most of the previous studies in the effect of experience in aphids have used different “biotypes”, category which may be genetically heterogeneous (Via 1991), and also the identity of clones compared has been stated phenotypically (e.g. performance) instead of genetically. These restrictions may have obscured the inter-clonal variability and dismiss the magnitude of the genetic component on behaviour and fitness. Considering the genetic identity between clones De Barro (1995) reported that conditioning in *Sitobion avenae* (Fabricius); however in this case the variable affected was performance over generations.

A limitation of the present work it could be related with the fact that the experimental aphids were wired and hence confined to a relatively small area of the leaf, thus decreasing the possibility of a free choice of the plant tissue. However, because the wire effect was present in all cases, it should have affected equally times to first and to second phloem phases both within and between genotypes. On the other hand, the effect of the wiring has been shown to produce no significant changes in the sequence and time course of probing events relative to free aphids (Prado, 1997, Tjallingii, 1986). On the other hand, another weakness may come from inter-individual variation in the host plant; however, to evaluate behaviour were same seedling of the same stage and of the same cultivar, thus minimising it effect.

Finally, it is likely that the phloem searching behaviour is one of many traits affecting direct or indirectly aphid fitness. In particular, it may be indirectly correlated with a morphological traits such as body size (Dixon & Kindlman 1994; Via & Shaw 1996). Although in the current study the age of aphids was controlled and hence variation in size was minimised, in natural conditions such correlation could be taking place.

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ANEXO 1

PATRÓN TEMPORAL DE INCURSIONES CELULARES POR EL ÁFIDO *SITOBION FRAGARIAE* (WALKER) Y ARREGLO CELULAR EN EL TEJIDO FOLIAR DEL TRIGO DE INVIERNO *TRITICUM AESTIVUM*

PATTERN OF CELL PUNCTURES BY THE APHID *SITOBION FRAGARIAE* (WALKER) AND CELL ARRANGEMENT IN THE LEAF TISSUES OF THE WINTER WHEAT, *TRITICUM AESTIVUM* L.

Claudio C. Ramírez, Nancy Olea and Hermann M. Niemeyer

Abstract

The pattern of cell punctures in the electrical penetration graph from the start of a probe leading to the first phloem phase by the aphid *Sitobion fragariae* (Walker)(Hemiptera: Aphididae) on two cultivars of the wheat, *Triticum aestivum* L., differing in hydroxamic acid concentration was studied. In addition, the spatial arrangement of cells in the leaf of the host plant was histologically observed and the number of cells between the epidermis and phloem counted. The results suggest a feeding deterrent effect of hydroxamic acid and a constant stylet puncturing activity into plant cells.

Keywords: *Sitobion fragariae*, cell punctures, EPG, leaf tissue, wheat.

Introduction

Aphids are a group of specialised phloem-feeding insects (Dixon, 1985). During penetration into plant tissues before achieving a phloem phase, the aphid's stylets produce a variable number of cell punctures (Pollard, 1977). Light and electron microscopy studies coupled with electrical penetration graphs (EPG) show that nearly every cell in the epidermis, parenchyma and vascular bundles encountered by the stylet before a phloem phase occurs, is punctured (Spiller *et al.,* 1985; Kimmins, 1986;

Tjallingii & Hogen Esch, 1993). The phloem phase starts with a short period of salivation into a sieve element (E1 waveform in EPG records), which is usually followed by phloem ingestion (Prado & Tjallingii, 1994).

The aim of this work is to explore a possible relationship between the pattern of EPG-recorded cell punctures in the first probe ending in a phloem phase and the histologically-estimated numbers of cells from the epidermis to a sieve element, and the eventual effect of the plant chemistry on the parameters studied. The system studied included the aphid *Sitobion fragariae* (Walker) on two cultivars of the wheat *Triticum aestivum* L. differing in hydroxamic acid (Hx).

Materials and Methods

Insects

Apterous virginoparous individuals of a single clone of the aphid *S. fragariae* (Walker) were kept on oat (*Avena sativa* L. cv. Nahuén) under laboratory conditions, at 20 ± 2 °C and 16:8 (L:D) photoperiod. Probing behaviour was monitored through electrical penetration graphs or, EPG's (Tjallingii, 1978). Waveforms have been correlated with the position of the stylet in the plant tissue (Tjallingii, 1978); consequently, stylet activities can be assessed in detail. In our study, it was assumed that each potential drop (pd) in the signal recorded revealed a cell puncture (penetration through the plasmalemma), whether it occurred in cells of the epidermis, mesophyll or sieve elements (Tjallingii, 1985). Aphids were withdrawn from the stock culture and hooked to the EPG system. Activities were followed until a phloem phase was observed. This period included a variable number of probes, comprising the last one which ended in a phloem phase, as judged from the observation of the E1 waveform. Probes in which xylem ingestion occurred were excluded from the analysis.

Plants

The first leaf of 7-day old seedlings of wheat *T. aestivum* cvs. Millaleu and Naofen, were used for aphid stylet monitoring and histological preparations. Chemical analysis showed that Hx concentration was significantly higher in cv. Naofén than in cv. Millaleu (1.94 ± 0.31 mmol/kg fresh weight, n = 8, and 1.09 ± 0.30 mmol/kg fresh weight, n = 8, respectively; F (1,14) = 29.21, n = 16, p = 0.00009).

In order to estimate the number of cells from the epidermis to the sieve elements, histological preparations of unharmed seedlings of both cultivars were produced. Segments corresponding to the upper third of the leaf (n = 22 leaves of each cultivar), were fixed in 3-4% formaldehyde in 0.1 M sodium phosphate pH 7.2. To improve infiltration, the segment was kept in the fixative under vacuum for 24 h. After rinsing and dehydration with ethanol, samples were infiltrated under vacuum with EPON 812 resin, and 2 μm thick transversal sections of the embedded segments were stained with 1% toluidine blue in 1% sodium borate. The sections were mounted on glass slides for light microscopy observations. One section was then chosen for further studies which showed sufficient incorporation of staining agent. The number of cells intercepted by an imaginary transect (Fig. 1B) drawn from a point between two epidermal cells to the closest sieve element in each leaf section of the upper surface was counted. The central and the two adjacent vascular bundles were used for countings in each section.

Pattern of cell punctures and number of cells in the plant tissue

In order to describe the pattern of cell punctures, each probe ending with a phloem phase was split into two parts of equal duration after substracting the duration of pd's, and the number of cell punctures in each period was determined. Similarly, the transect used to count the number of cells in the leaf tissue was divided into two segments of equal length, allowing the characterisation of the "external part of the mesophyll", which included cells from epidermis and mesophyll, and the "internal part of the mesophyll", which included mesophyll cells of the outer and inner bundle sheath and vascular cells until the sieve element (Fig. 1B).

Results and Discussion

Mean duration of cell punctures did not differ significantly between the two parts of the recording in the low-Hx cultivar (Table 1), while in the high-Hx cultivar cell punctures were significantly shorter during the first part of the recording (Table 1). Moreover, during the first part of the probes, a significantly lower number of cell punctures was observed in the low-Hx cultivar than in the same period in the high-Hx cultivar ($p=0.03$, t test for independent samples; not shown in Table 1). These results suggest a deterrent effect of Hx at the level of epidermis and/or mesophyll as has been suggested earlier (Givovich & Niemeyer, 1991; Mayoral *et al.*, 1996). In support of this suggestion of feeding deterrency by Hx, the number of probes from the beginning of recording to the probe ending in a phloem phase was significantly higher in the high-Hx than in the low-Hx cultivar (4.70 ± 0.58 and 7.22 ± 0.91 in low- and high-Hx cultivars respectively; $p=0.02$, t test for independent samples), and the total accumulated probing time before the probe ending in a phloem phase (16.0 ± 3.2 and 41.8 ± 7.8 min in low-and high-Hx cultivars respectively; $p=0.004$, t test for independent samples). It is important to note that the comparisons discussed are not distorted by the duration of the probe ending in a phloem phase, since these did not differ between cultivars (Table 1).

The number of cell punctures increased significantly as penetration progressed within the probe ending in a phloem phase (Table 1), and the number of cells estimated histologically was higher in the internal part of the transect than in its external part in both cultivars (Table 1). Moreover, the estimated number of punctures per cell did not differ significantly along the transect/probe studied in both cultivars (ratios in Table 1).

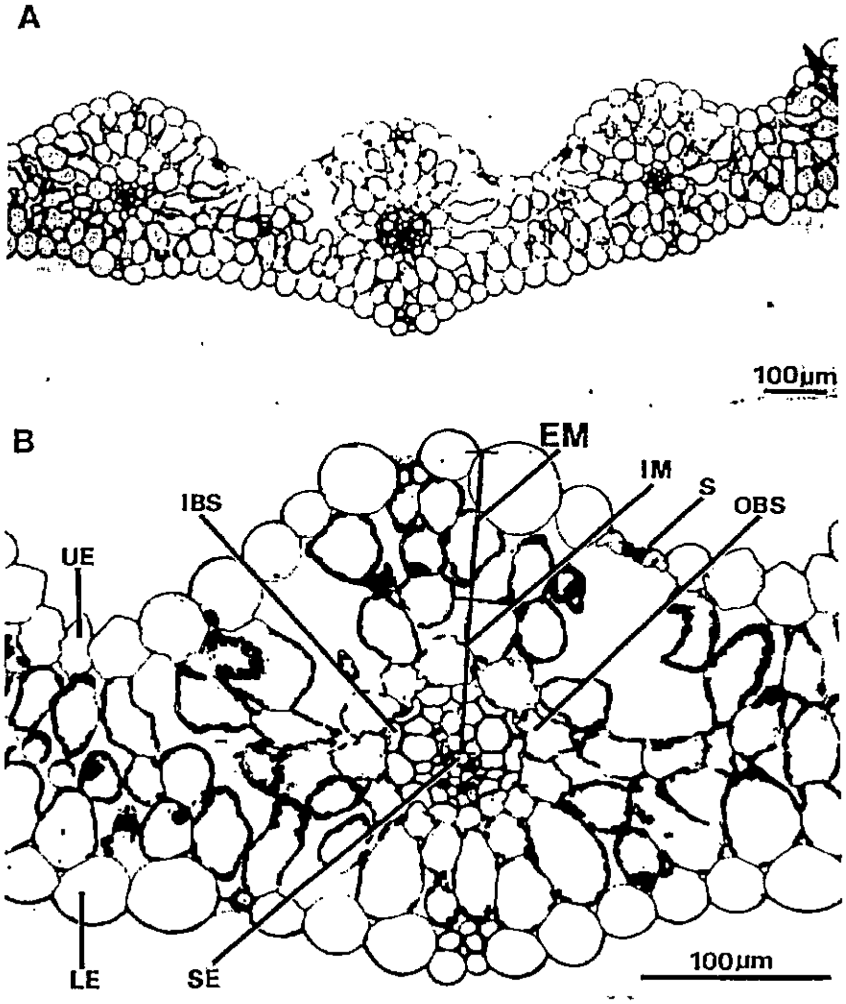

Figure 1. Light microscopy preparations of a transversal section of the leaf of a wheat *T. aestivum* seedling. Since no differences between cultivars were apparent, only cv. Millaleu is shown. **A**: central and two lateral veins; arrows indicate the veins sampled. **B**: detail of central vein. Transects studied included the external mesophyll (EM) and internal mesophyll (IM) of the upper surface of the leaf (adaxial surface). UE = upper epidermis, LE = lower epidermis, OBS = outer bundle sheath, IBS = inner bundle sheath, S = stoma, SE = sieve element.

TABLE 1. Pattern of cell punctures from EPG recording of the first probe ending in a phloem phase (a) and number of cells estimated histologically along a defined transect (b). Mean and standard errors are shown. p-level calculated with t-test for dependent samples or independent samples (duration of the probe).

Parameter	Low Hx (n = 19)			High Hx (n = 16)		
a) EPG recordings						
	First part of recording	Second part of recording	p-level	First part of recording	Second part of recording	p-level
Number of cell punctures	5.9 ± 1.1	9.3 ± 1.4	0.04	8.3 ± 1.6	12.0 ± 2.7	0.03
Duration of cell punctures (sec)	5.1 ± 0.14	5.0 ± 0.08	0.63	4.6 ± 0.08	5.0 ± 0.11	0.04
Duration of the probe (min)	22.6 ± 3.6			23.8 ± 3.1		0.34
b) Histological analysis						
	External part of transect	Internal part of transect		External part of transect	Internal part of transect	
Number of cells	1.6 ± 0.03	2.3 ± 0.04	0.0001	1.7 ± 0.03	2.4 ± 0.04	0.0001
c) Ratios						
Estimated number of punctures per cell	3.6 ± 0.8	4.0 ± 0.9	0.94	4.9 ± 1.2	5.0 ± 1.8	0.98

Given aphids seem to follow a trial-and-error procedure in which nearly every cell encountered in punctured (Tjallingii & Hogen Esch, 1993), this suggests a constant rate of stylet puncturing activity within any given cell. We do not at present have an explanation to this; however, not quite unexpectedly, different aphid-plant combinations yield different quantitative results. For example, in *Aphis fabae*/*Vicia faba*, Tjallingii & Hogen Esch (1993) report ca. 1.4 punctures per cell in the vascular bundle.

The results obtained rest on one important constraint, which is that the stylet penetration was associated with a unidimensionally-evaluated pattern of inner structure of the leaf (number of cells encountered along a straight transect), whereas stylets penetrate plant tissue tridimensionally (Pollard, 1977). This parsimonious approach serves as an initial point of reference for further increases of complexity and realism. Comparison between the pattern of cell punctures and plant tissues with different cell arrangements (i.e. stem, leaf) in other aphid-plant systems would shed further light into the mechanism of aphid stylet penetration into plants.

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ANEXO 2

SALIVACIÓN EN LOS ELEMENTOS CRIBOSOS EN RELACIÓN A LA QUÍMICA DE LA PLANTA: EL CASO DEL ÁFIDO *SITOBION FRAGARIAE* (WALKER) Y DEL TRIGO DE INVIERNO *TRITICUM AESTIVUM* L.

SALIVATION INTO SIEVE ELEMENTS IN RELATION TO PLANT CHEMISTRY: THE CASE OF THE APHID *SITOBION FRAGARIAE* (WALKER)(HEMIPTERA:APHIDOIDEA) AND THE WINTER WHEAT *TRITICUM AESTIVUM* L.

Claudio C. Ramírez & Hermann M. Niemeyer

Abstract

Extended sieve element salivation (E1 waveform in the electrical penetration graph) is a characteristic activity during early sieve element punctures, particularly in resistant plants. In order to explore a chemically-mediated mechanism of resistance associated with sieve element salivation, we compared the pattern of feeding behaviour of the aphid *Sitobion fragariae* (Walker) on two cultivars of the wheat *Triticum aestivum* L. with different concentrations of hydroxamic acids (Hx). During 24 h of electronic monitoring, aphids dedicated over 50 % of the total time to phloem ingestion from the sieve elements. Total time allocated to E1 in the experiment, time to first E1 within the experiment, time allocated to E1 before a sustained phloem ingestion (E2) and the contribution of sieve element salivation to the phloem phase (E1/[E1+E2]) were significantly higher in the high-Hx cultivar. The increased salivation in plants with higher contents of Hx suggests the existence, at least in this system, of a chemically-mediated sieve element constraint.

Keywords: sieve element salivation, aphid, resistance, wheat, *Sitobion fragariae*, *Triticum aestivum*

Introduction

Feeding behaviour of cereal aphids has been intensively studied in relation to plant resistance. Comparison of feeding behaviour in susceptible/resistant plants as well as in host/non-host plants has been used to explore the plant factors accounting for the resistance (Spiller, 1988; Girma *et al.*, 1992; Caillaud *et al.*, 1995, Mayoral *et al.*,1996). Recently, studies using electrical monitoring (EPG) have reported increased salivation into sieve elements (E1 pattern) (see review in Prado, 1997), as well as delay in sieve element ingestion (Givovich & Niemeyer, 1995; Caillaud *et al.*, 1995) of aphids feeding on resistant plants. In order to explore a chemically-mediated mechanism associated with sieve element salivation, we studied the feeding behaviour of the aphid *Sitobion fragariae* (Walker) on two cultivars of wheat, *Triticum aestivum* L. varying in their content of hydroxamic acids (Hx), with special emphasis on EPG parameters related to salivation in sieve elements. Several investigations on Hx have addressed their role in the resistance of wheat against aphids (Niemeyer, 1990; Nicol *et al.*, 1992; Givovich *et al.*, 1994; Mayoral *et al.*, 1996, Nicol & Wratten, 1997).

Materials and Methods

Insects

A clonal colony of *S. fragariae,* derived from an individual collected in central Chile, was established in the laboratory on oat (*Avena sativa* L. cv. Nahuén) at 20 ± 2 °C and L16:D8 photoperiod.

Plants

The first leaves of 7-day old seedlings of cultivars Naofén and Millaleu of the wheat *Triticum aestivum* L. were used. Chemical analysis showed that Hx concentration (determined as the glucoside of DIMBOA following the procedure described by

Weibull and Niemeyer, 1995) in these leaves was significantly higher in cv. Naofén than in cv. Millaleu (1.94 ± 0.31 mmol/kg fresh weight, n = 8, and 1.09 ± 0.30 mmol/kg fresh weight, n = 8, respectively; F (1,14) = 29.21, n = 16, p < 0.001).

Experimental set-up

Each experimental aphid was monitored during 24 h using the EPG technique (Tjallingii, 1978). All signals were recorded on a PC hard disk and analysed with the EPGview software (Flores *et al.*, 1998, unpublished). Detailed assessment of aphid activities can be achieved since during aphid probing the location of the stylet tips and their activities produce recognisable patterns of voltage changes in the recorded signal (Tjallingii & Hogen Esch, 1993; Prado & Tjallingii, 1994).

Parameters used to study probing behaviour

Aphid feeding behaviour was continuously monitored for 24 hours on Millaleu (low-Hx) and Naofén (high-Hx) seedlings. A total of 23 recordings were performed for each cultivar. The following wave forms were recorded and recognised: non-probing (NP), pathways activities (C), salivation into a sieve element (E1), ingestion from the sieve elements (E2), xylem ingestion (G), and difficulties during stylet penetration (F). Timing of the occurrence of each pattern also allowed the evaluation of the following parameters related to activities in sieve elements: 1) proportion of the time allocated to each activity, 2) time to first E1 within the experiment, 3) total duration of E1 periods within the experiment, 4) number of E1 periods, 5) mean duration of E1 periods, 6) duration of E1 periods before the first sustained E2 (i.e. E2 for more than 8 minutes) in the experiment, 7) duration of E1 periods before the first sustained E2 within a probe (a probe is a continuos period without withdrawal of the stylets from the plant), 8) mean duration of the E1 period preceding the first sustained E2, 9) contribution of total E1 to total phloem phase (E1/[E1+E2]), and 10) time to the first sustained E2 in the experiment.

Results and discussion

The major aphid activity during 24 h of electronic monitoring was phloem ingestion, to which aphid dedicated over 50 % of the total time (Figure 1). The number of aphids showing phloem phase was not significantly different between cultivars (20 out of 23 and 21 out of 23 in low and high-Hx cultivar, respectively; Z= 0.94, P = 0.34, test for two proportions [Zar, 1996]). Similarly, the proportion of time devoted to phloem ingestion did not show differences associated with Hx variation, a result comparable with those reported for other cereal aphid species (Girma *et al.*, 1992; Givovich & Niemeyer, 1996; Mayoral *et al.*, 1996). Surprisingly, xylem ingestion (G pattern) was significantly higher in the low-Hx cultivar, although the number of aphids showing G pattern was not significantly different (12 out of 20 and 8 out of 21 in low and high-Hx cultivar, respectively; Z= 1.09, P = 0.27, test for two proportions [Zar, 1996]). This result are in contradiction with other studies in other cereal aphid species (Givovich & Niemeyer, 1995). It is likely that in the present system, xylem ingestion was reduced simply as a consequence of the higher proportion time devoted to E1 in high-Hx cultivar (Figure 1).

Focusing on sieve element salivation, the high-Hx cultivar showed significantly higher values than the low-Hx cultivar in the following E1-related parameters (see Table 1): time to first E1 within the experiment, total duration of E1 periods, duration of the E1 periods before the first sustained E2 and contribution of sieve element salivation to the phloem phase. Clearly, Hx affect process of salivation into the sieve elements.

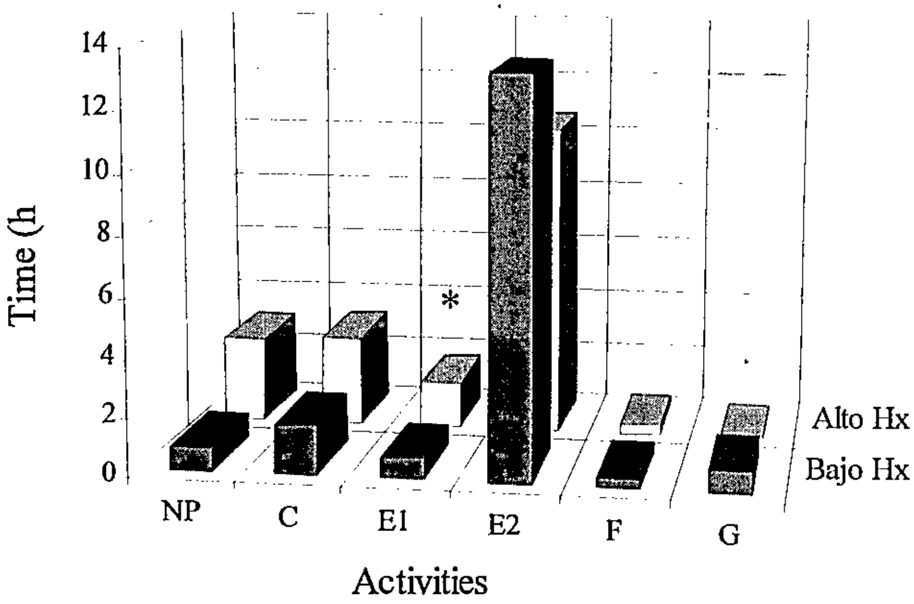

Figure 1. Proportion of the time (%) allocated to each activity during 24 h of EPG recording of probing behaviour. Asterisks indicate significant differences P < 0.05, Mann-Whitney U test. NP: non-probing; C: pathways activities; E1: salivation into a sieve element; E2: ingestion from the sieve elements; G: xylem ingestion; F: difficulties during stylet penetration.

Table 1. EPG parameters of feeding behaviour related to salivation and ingestion in the sieve elements over 24 h reording period. Probabilities were calculated using a Mann-Whitney test.

EPG Parameter	Low Hx n = 20 (X ± SE)	High Hx n = 21 (X ± SE)	P	
2. Time to first E1 (min)	69.8 ± 10.4	140.5 ± 36.2	0.033	
3. Total duration of E1 periods (min per 24 h) *	44.5 ± 10.2	102.2 ± 16.0	0.002	
4. Number of E1 periods (#)	5.1 ± 0.9	8.4 ± 1.6	0.260	ns
5. Mean duration of E1 periods (min)	9.9 ± 1.1	28.4 ± 9.1	0.170	ns
6. Duration of E1 before first sustained E2 (min)	30.3 ± 7.1	54.7 ± 9.7	0.041	
7. Duration of E1 before first sustained E2 within a probe (min)	25.7 ± 5.8	46.6 ± 9.3	0.077	ns
8. Mean duration of the E1 preceding first sustained E2 (min)	11.1 ± 1.6	30.2 ± 9.0	0.123	ns
9. Contribution of E1 to phloem phase (%)	11.2 ± 4.4	19.3 ± 4.9	0.018	
10. Time to first sustained E2 (min)	228.6 ± 46.5	291.2 ± 54.5	0.251	ns

* : Figure 1 shows this values in terms of the proportion of the time allocated to each activity.

Since Hx are mainly present in meristematic, epidermal, mesophyll and vascular tissue (Epstein *et al.*, 1986, Argandoña *et al.*, 1987), it is reasonable to speculate that Hx is perceived by the aphid during cell punctures in their route to sieve elements, and causes a delay in the time needed to attain a first sieve element salivation due to its deterrent effect (Niemeyer & Perez, 1995; Ramírez *et al.*, 1998, unpublished). Brief cell punctures in the sieve elements may also occur (Tjallingii & Hogen Esch, 1993) and can contribute in a such delay. On the other hand, the increased duration of salivation periods seems more likely to be the result of Hx in the phloem sap (Givovich *et al.*, 1994; but see Caillaud & Niemeyer, 1996). However, a direct causal link between the Hx content and E1 duration must be made with caution, because the Hx content of extracts of macerated whole leaves does not correlate strongly with content in the phloem sap (Givovich *et al.*, 1994).

In conclusion we have shown that Hx are associated with a delay in the time to start the process of salivation in the sieve elements and with a increase in the process of salivation itself, suggesting that these compounds may be acting both at the level of the epidermis/mesophyll and the phloem.

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ANEXO 3

EFECTO DE LA EXPERIENCIA EN LA PREFERENCIA DE OLORES DEL ÁFIDO DE LOS CEREALES *SITOBION FRAGARIAE* (WALKER) (HEMIPTERA: APHIDIDAE)

THE EFFECT OF EXPERIENCE ON ODOUR PREFERENCE OF THE CEREAL APHID *SITOBION FRAGARIAE* (WALKER) (HEMIPTERA : APHIDIDAE) AND CEREALS

Claudio C. Ramírez *, Paula P. Caballero & Hermann M. Niemeyer

Introduction

The experience-induced changes in behavior have been described in many different insect species (Szentesi & Jermy, 1990; Papaj & Lewis, 1993; Bernays & Chapman, 1994), including aphids (Apablaza & Robinson, 1967; Hubert-Dahl, 1975; Schweissing & Wilde, 1979; Robinson, 1993; Lushai *et al.,* 1997). Studies on aphids involved offering the complete host plant as a choice stimulus to groups of conditioned aphids. This design has two limitations: (1) it does not allow for the identification of the host cue or cues whose perception is affected by previous experience; and (2) it is restricted to group behaviour, thus masking the decision-making process at the individual level.

Host-finding by the winged morph of aphids is influenced by environmental factors such as gravity, light, temperature, relative humidity, wind; and by host plant properties such as shape, colour and odour (Dixon, 1985; Klingauf, 1989). It is possible that the response to any of these host properties may be increased through relevant experience. In particular, host odour selection has been shown to be important in the short distance attraction to potential hosts by winged aphids (Visser, 1986; Pickett *et al.*, 1992; Nottingham & Hardie, 1993; Pettersson, 1994). However, the relationship between experience of an individual aphid on a host, and the subsequent odour-based selection has not been reported, although odour-conditioned responses of the aphid *Cryptomyzus korschelti* Bormer have been studied under no-choice conditions (Visser & Taanman, 1987).

The aim of this work is to study the odour-based host preference of winged individuals of the aphid *Sitobion fragariae* (Walker) after rearing (i.e experience) on different host plants.

Materials and Methods

A clone of *S. fragariae* from a stock colony kept on oat (*Avena sativa.* L.cv. Nahuén) was maintained for at least three generations on seedlings of three different host plants: wheat (*Triticum aestivum* L. cv. Millaleu), rye (*Secale cereale* L.cv. Tetra) and barley (*Hordeum vulgare* L. cv. F.Union). This rearing time was used as experience treatments on each host plant, exposing the aphids to the whole plant from nymph to adult. Pots with about 30 to 40 seedlings covered with transparent plastic cages were used to rear the aphids. They were kept in a room at 23 ± 2°C and a photoperiod of 16L:8D photoperiod. Parthenognetic alate adults from these cultures were used in the behavioural choice tests. Genetic identity of the aphid studied was determined with RAPDs and PCR analysis according to Figueroa *et al.*, (1998).

A Pettersson's olfactometer (Pettersson, 1970) was used as the odour test chamber. The arena consisted of a central zone and four arm zones. White paper was placed on the floor of the arena to facilitate walking by the test aphid and also to ascertain its position throughout the experiment. The olfactometer was surrounded by a white paper cylinder (h = 10 cm) in order to homogenise lighting and avoid the visual stimuli of the aphid with the plants. Each arm had an inlet where odours were introduced. Two of the olfactometer arms were connected with plastic tubes to a glass belljar which contained a pot with about 30 seedlings of the host plant whose odours were to be tested (wheat, rye or barley). The other two arms were connected to another belljar which contained another pot with 30 seedlings belonging to the alternative host plant whose odours were simultaneously tested. A vacuum pump exhausted air from the centre of the arena at a flow of 300 ml/min. Air-tight seals and an air filter at the inlet of each belljar were used to avoid external odours during the experiments. The central zone contained both host plant odours and represented the decision making area. The seedlings used were at decimal growth stage 11, first leaf unfolded (Zadoks *et al.*, 1974). Oat was used as the alternative host plant in the choice experiments for the three hosts tested.

In each experiment one winged aphid was introduced into the central zone of the olfactometer and, after 5 minutes of adaptation time, the aphid was visually monitored for 15 minutes and time spent in each zone was recorded with the help of computer software (The Observer, Noldus Corp.). The experiments were performed in a room at 23 ± 2°C. The relative position of the belljars was interchanged after every repetition. The whole setup of belljars containing the seedlings and the test aphid were replaced at every repetition to avoid pseudoreplication (Hulbert, 1984).

Results were analysed using nonparametric statistic (Wilcoxon one-tailed rank-sum test for two groups), the total time spent in the two treatment arms being compared with the total time spent in the two control arms.

Results

When aphids reared on wheat were offered the choice between odours coming from wheat or from oat, they spent significantly longer times in the olfactometer arms receiving odours from wheat seedlings (Fig. 1A). In contrast, experience on rye led to a significantly higher preference for oat odours in comparison to rye odours (Fig 1B). When experienced on barley, aphids did not show a significant preference between odours coming from barley or oat (Fig. 1C). To test whether the responses shown by aphids with experience on wheat and on rye were due to the effect of experience rather than to experience-independent

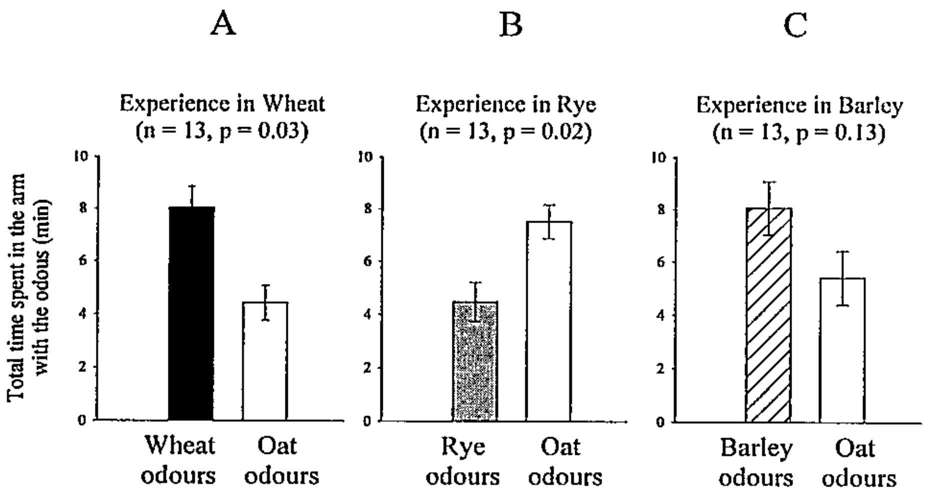
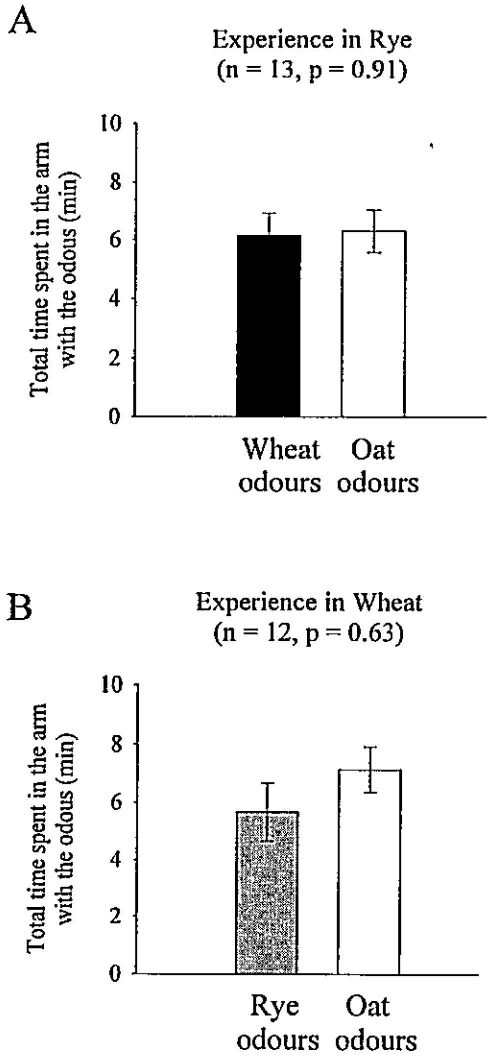


Figure 1. Total time spent (min) in the arm of the olfactometer receiving odours from the plant tested by winged aphids after experience in three different host plants : wheat (A), rye (B) and barley (C). Time in the central zone is not plotted (see test). Bars shows one standard error, and the p value for Wilcoxon matched pairs test. n = 13.



Experience in Rye (n = 13, p = 0.91)

Experience in Wheat (n = 12, p = 0.63)

Figure 2. Total time spent (min) in the arm of the olfactometer receiving odours from the wheat or oat plant by winged aphids after experience in wheat (A) and oat (B). Bars shows one standard error, and the *p* value for Wilcoxon matched pairs test. n = 20.

attraction/repellence by host odours, aphids with experience on each of these hosts were tested against odours of the other host with oat as a control. Aphids with experience on rye (Fig. 2A) or wheat (Fig. 2B) did not show a preference for odours of the other host. Time spent in the central zone was always significantly lower that time spent in the control or stimuli arms (data no shown).

Discussion

The results show that odour selection is affected by experience. Given the genetic homogeneity of the aphids studied -they belong to a single clone- and that the three host plant species tested led to different odour choices (preference, avoidance or no-choice), our data suggests that the type of response to these odours is related to the species of host plant on which aphids developed. Thus, while wheat generates a preference for its odours, rye elicits an aversion behaviour towards its odours, and barley does not lead to a biased behaviour. The fact that aphids with experience on rye did not show any preference between wheat or oat odours (Fig. 2A), suggests that the only causal factor in the positive preference to wheat observed may be identified as experience, instead of a specific arresting mechanism in wheat (attraction/repellence effect). The same argument can be used in the case of aphids with experience on wheat which did not show preference for rye or oat odours (Fig 2B). Experience seems to be the main factor explaining the positive and negative odour-based preferences observed.

Previous studies reporting increased preference for a host after experience (Apablaza & Robinson, 1967; Hubert-Dahl, 1975, Schweissing & Wilde, 1979, Robinson, 1993) were not designed to distinguish the particular host cue involved, and hence the relevance of olfactory cues could not be ascertained. For example, Schweissing & Wilde (1979) and Robinson (1993) evaluated preference after 24 h and 48 h of exposure to the whole plant, respectively. This time period was presumably long enough to involve the pooled effects of olfactory, visual, mechanical and gustatory activities for selection, because the olfactory stimulus appear to be important only in the first minutes of the host plant selection process (Visser, 1986).

Our results are in agreement with those of Lushai *et al.,* (1997), who found an important environmental effect in the host selection by the aphid *Sitobion avenae* (Fabricius). In particular, we found that experience may affect the ability to select a host based exclusively in the perception of its odours. Such phenotypic variation caused by experience may be important in the generation of intraclonal differentiation and enhance intraspecific variation, but such consequences need further studies.

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DISCUSIÓN GENERAL

V. DISCUSIÓN GENERAL

La hipótesis relativa al efecto de la experiencia previa en una determinada planta hospedera sobre la conducta alimentaria (Hipótesis 1), fue puesta aprueba al criar áfidos de un mismo genotipo sobre plantas trigo (*Triticum aestivum* L.) o en avena (*Avena sativa* L.). No obstante que en término generales la conducta global fue similar, se observó una reducción de las dificultades de penetración del estilete en trigo sólo en áfidos que se habían desarrollado previamente en trigo, mientras que no se observó lo mismo en áfidos con experiencia previa en avena (ver transferencias trigo-trigo *versus* avena-trigo en Experimento 1, Capítulo 1). Por lo tanto se confirmó la Hipótesis 1. No obstante, esta conducta fue sensible al estado fisiológico de los áfidos, particularmente a la falta de ingestión. Se observó que, independiente de la experiencia previa, los áfidos reorientan su patrón de alimentación en función de mantener su balance hídrico (ver Experimento 2, Capítulo 1).

Dado que la experiencia previa se asoció con diferencias en las propiedades de la planta, particularmente en el caso del trigo, se estudió con más detalle la relación entre conducta alimentaria y la química interna de esta planta, así como su estructura morfológica interna (Anexo 1). Se estudió el caso particular de los ácidos hidroxámicos [Hx] presentes en distintas concentraciones en dos cultivares del trigo *Triticum aestivum* L., compuestos conocidos como importantes agentes de resistenacia del trigo. Se determinó que el patrón temporal de incursiones intracelulares al interior del tejido vegetal depende principalmente de la morfología interna de la planta (ver Figura 1, Anexo 1). Por otro lado, en el Anexo 2 se muestra que los áfidos son capaces de responder conductualmente a la química de la planta, variando la duración de la primera fase floemática (ver Figura 1, Anexo 2). Una mayor concentración de Hx se correlaciona con una mayor duración del periodo de salivación en los vasos floemáticos (ver resultados y discusión, Anexo 2).

Una vez establecida con detalle la relación de los áfidos y la química de la planta, se retomó la Hipótesis 1. Con este conocimiento se estudió el efecto de la experiencia previa dentro de un clon en plantas de trigo que varían en sus propiedades químicas. En el Capítulo 2, se muestra que al variar la química de la planta hospedera la conducta alimentaria de los áfidos, en particular el tiempo de inicio de la fase de alimentación en el floema, se reduce cuando los áfidos han experimentado previamente tal fase, habilidad que se presenta sólo en las plantas más dotadas de Hx (ver Figura 1, Capítulo 2). Esto se verifica en el hecho de que la reducción en el tiempo de acceso a la fase floemática es mayor mientras mayor es la concentración de Hx presentes en el trigo, independiente de algún posible cambio en la fisiología de la planta (ver Tabla 1, Capítulo 2).

La hipótesis que relaciona la conducta y adecuación biológica (Hipótesis 2) fue puesta a prueba en el Capítulo 3. Desde una perspectiva multivariada, se observó que en ejemplares pertenecientes a un mismo genotipo la variación fenotípica conductual producto de la experiencia previa no se correlaciona con la adecuación biológica. Las diferencias en el patrón reproductivo parecen relacionarse con la calidad del recurso (susceptibilidad).

EXPERIENCIA PREVIA Y SU SIGNIFICADO FISIOLÓGICO

La posibilidad de (1) mostrar menores dificultades en la penetración del estilete como producto de la experiencia (Capítulo 1), (2) flexibilizar la conducta de búsqueda de los vasos floemáticos bajo condiciones de estrés hídrico y reorientar la actividad hacia la búsqueda de los vasos xilemáticos (Capítulo 1), así como (3) mostrar mayor salivación en los vasos floemáticos en plantas más defendidas (Anexo 2), y (4) acceder a los vasos floemáticos en un menor tiempo como producto de la experiencia previa en plantas más defendidas (Capítulo 2), serían respuestas cuyo significado se enmarca en el contexto de la mantención del equilibrio fisiológico. A este nivel los áfidos muestran una flexibilidad que les permite responder en el corto plazo a condiciones internas y externas adversas, y mitigar los efectos negativos de las restricciones fisiológicas en la tasa de crecimiento poblacional. Este es el caso de la mayor salivación en los vasos floemáticos, actividad que podría contrarrestar las respuestas defensiva de carácter inducido de la planta hospedera (Anexo 2). A pesar de que los áfidos pueden responder conductual y fisiológicamente a la presencia de constituyentes químicos de la planta, la ruta que sigue el estilete al interior del tejido vegetal esta principalmente determinada por la organización de éste (Anexo 1). Este hecho suguiere que la componente morfológica de la interacción podría establecer limitaciones a la capacidad de respuesta de los áfidos.

Dado el particular modo de vida de los áfidos, la posibilidad de disminuir los efectos negativos de una planta hospedera sobre la fisiología de éstos, puede traer más beneficios que lo observado para otros organismos. Kindlmann & Dixon (1994) ha estimado que pequeñas diferencias en la tasa de crecimiento son enormemente amplificadas por medio de la partenogénesis cíclica, lo que permitiría compensar las perdidas en relación con la tasa de crecimiento obtenida en un hospedero más benigno. Sin embargo, en esta tesis estos aspectos no fueron abordados y su relación con la plasticidad fenotípica conductual no se conocen.

VARIACIÓN FENOTÍPICA CONDUCTUAL EN UNA PERSPECTIVA EVOLUTIVA

La hipótesis de variación entre genotipos debida al efecto de la experiencia previa fue confirmada (Capítulo 4). En algunos genotipos la experiencia previa de inicio de la fase de alimentación en los vasos floemáticos no favorece un acceso más rápido en su segunda instancia. Por otro lado, aquellos genotipos en que la experiencia previa si favorece un acceso más rápido a los vasos floemáticos no presentan adecuación biológica mayor. Sin embargo, en los genotipos estudiados la adecuación biológica se correlacionó inversamente con el tiempo a la primera fase floemática, de tal forma que un primer acceso más rápido se correlaciona con mayor adecuación biológica. Independiente de la experiencia previa, un acceso más rápido a los vasos floemáticos se asocia con mayor adecuación.

Bajo una perspectiva basada en teoría de decisiones, Stephens (1993) establece las condiciones bajo las cuales la plasticidad fenotípica conductual sería favorecida. Su modelo predice que, en organismos sin sobreposición de generaciones, el "aprendizaje" se favorece sólo cuando la predictibilidad del recurso es alta dentro de una generación (durante la ontogenia) y baja entre generaciones (madres/hijas). Este modelo se ajusta a las condiciones de los áfidos de los cereales, dado que en ellos efectivamente el grado de sobreposición de generaciones es bajo (Dixon, 1998) y la predictibilidad del recurso dentro de una generación es alta. No obstante, para los áfidos la predictibilidad entre generaciones podría ser baja o alta dependiendo de la intensidad de la dispersión antes del inicio del periodo de crecimiento partenogenético. Por otro lado, se ha descrito que la capacidad de dispersión de los áfidos es baja (Loxdale *et al.*, 1993) por lo que el modelo de Stephens (1993) podría aplicar para áfidos. Para el caso de *S. fragariae*, las ventajas de la plasticidad fenotípica se verificarán en el ámbito fisiológico; sin embargo, dada la falta de correlación entre plasticidad y adecuación queda aun abierta la interrogante sobre la mantención evolutiva (entre generaciones) de este atributo.

Una explicación alternativa más radical a la falta de correlación entre plasticidad y adecuación es la adoptada por Via (1993), quien señala que no cabría esperar adaptabilidad de la plasticidad ya que ella no estaría sujeta a selección natural como un carácter *per se*, sino que más bien sería una propiedad emergente resultante de la selección sobre un conjunto de caracteres en ambientes variables (Via, 1993). Sin embargo, cada día hay más adherentes a la idea de que la plasticidad parece poseer una base genética y evolucionar como carácter *per se* (Via *et al.*, 1995). Suponiendo que la plasticidad en el caso de *S. fragariae* tenga control genético, entonces la falta de correlación con adecuación implicaría que la plasticidad conductual no sería un carácter sobre el cual la selección natural opera en el presente. De modo diferente, y dado que mostró variación entre genotipos y se asoció con mayor adecuación biológica, el tiempo de acceso a la primera ingestión floemática sería un atributo adaptativo y favorecido en el presente por la selección natural.

Recientemente se ha sugerido que la plasticidad fenotípica conductual en áfidos podría tener una incidencia en la formación de razas en poblaciones simpátricas (Via, 1991; Mackenzie & Guldemold, 1994; Guldemold & Mackenzie, 1994; Mackenzie, 1996). La plasticidad fenotípica conductual permitiría que un clon mejorase gradualmente la utilización de un recurso inicialmente adverso, situación que produciría aislamiento reproductivo y eventualmente interrupción del flujo génico entre poblaciones que ocupan hospederos distintos en una misma área (Mackenzie & Guldemold, 1994). Evidencias de este hecho son la presencia de correlación genética negativa en la utilización de hospederos. en algunas especies de áfidos (Via, 1991; De Barro *et al.*, 1993, Mackenzie, 1996; Sandstrom, 1996), y el mejoramiento en la utilización de un hospedero adverso (Mackenzie, 1996; Douglas, 1997). Por otro lado, la plasticidad conductual de los áfidos podría genera un aumento en las probabilidades de permanencia por reforzamiento de permanencia o preferencia de un hospedero determinado hospedero. Esta posibilidad encuentra sustento parcial en el fenómeno de inducción de preferencia desarrollada por *S. fragariae* (ver Anexo 3), el cual presenta inducción de preferencia por volátiles de trigo luego de experiencia previa en esta planta. No obstante, este fenómeno puede variar dependiendo del grado de resistencia del hospedero (ver Anexo 3). La plasticidad conductual podría tener consecuencias evolutivas al reforzar la aceptación de un hospedero adverso o un nuevo hospedero.

¿Cómo se relacionan estos resultados con la historia natural de *S. fragariae* ?. *S. fragariae* es un áfido recientemente introducido en Chile (Remaudiere *et al.*, 1993). Se estima que habría llegado a Chile en el año 1967, año en que fue reportado por primera vez *S. avenae* (Walker), especie bajo lo cual habría permanecido taxonómicamente asignado y sólo recientemente diferenciado (Remaudiere *et al.*, 1993). A diferencia de muchos áfidos de los cereales, *S. fragariae* desarrolla un ciclo de vida holocíclico con alternancia de hospedero. Al inicio del otoño se producen los morfos sexuales y el apareamiento sexual se desarrolla sobre su hospedero primario *Rubus fruticosus*. Durante primavera-verano los huevos que han sobrevivido al invierno generan linajes clonales que se reproducen partenogenéticamente sobre varias especies de gramíneas, entre las que se encuentran las plantaciones de trigo (Blackman & Eastop, 1984; Remaudiere *et al.*, 1993; Starý *et al.*, 1994). Considerando estos atributos es posible especular respecto de las consecuencias ecológicas de las conductas determinadas en el laboratorio. Supóngase una plantación homogénea de un trigo resistente (e.g. cv. Naofen) que es colonizada continuamente y en forma aleatoria desde el inicio de la estación (primavera) y hasta el final de ella (verano) por distintos genotipos de *S. fragariae*. De acuerdo a los resultados obtenidos en esta tesis, y presumiendo que (1) los factores bióticos y abióticos permanecen constantes durante la estación y que (2) cada genotipo se reproduce exponencialmente sin limitaciones de recurso, entonces aquellos genotipos que acceden más rápido a los vasos floemáticos desarrollarán una mayor tasa intrínseca de crecimiento poblacional relativo a genotipos más lentos en acceder a los vasos floemáticos. Si la probabilidad de arribo a la plantación no se relaciona con la capacidad de acceder a los vasos floemáticos y ésta es igual para todos los genotipos, entonces al final de la estación debería observarse una mayor proporción de aquellos genotipos que acceden más rápido a los vasos floemáticos. Si el carácter no esta ligado al sexo y la mortalidad de los huevos no se relaciona con éste carácter, entonces al inicio de la siguiente estación permanecerá la mayor representatividad de los genotipos capaces acceder a los vasos floemáticos en menor tiempo y mostrar tasas de crecimiento mayores. Si durante la recombinación génica producida durante la fase sexual se generan genotipos que expresen menor tiempo de acceso a los vasos floemáticos y mayores tasa de crecimiento poblacional, entonces estos genotipos rápidamente serán expandidos y su probabilidad de permanecer serán mayores. Este modelo representa exactamente las ventajas evolutivas de la partenogénesis cíclica, en la cual la fase sexual genera variabilidad que se "prueban" en distintos ambientes, y las formulas exitosas se expanden rápidamente cuando las condiciones son favorables (Maynard-Smith, 1998). Para algunas especies de áfidos, las permanencia temporal de un genotipo pueden verse prolongada dado que bajo ciertas circunstancias un genotipo puede eludir la recombinación sexual, y pasar el invierno asexualmente y multiplicarse nuevamente en la estación favorable (Dixon, 1998). Los supuestos del modelo señalado no son del todo irreales, particularmente en condiciones de cultivos. Las plantaciones representan ambientes altamente homogéneos; la probabilidad de arribo de los áfidos es efectivamente aleatoria (Ward, 1993); de no mediar control de plagas, la gran concentración de recursos permite crecimientos exponenciales. Por otro lado, la posibilidad de pasar el invierno en hierbas perennes, favorecería la permanencia de un genotipo exitoso. En el caso particular, *S. fragariae* se ha descrito que presenta un amplio rango de hospederos secundarios entre los cuales se encuentran plantas perennes (Remaudiere *et al.*, 1993). Por lo tanto, atributos asociados con altas tasas de crecimiento poblacional tienen una alta probabilidad de fijarse en la población.

PROYECCIONES EN EL ESTUDIO DE LA PLASTICIDAD FENOTIPICA CONDUCTUAL Y VALOR ADAPTATIVO.

Para entender los patrones de conducta alimentaria de los áfidos se requiere enfatizar aun más las comparaciones inter-individuales. En esta tesis los genotipos comparados pudieron no ser representativos de la variación que puede presentarse en la naturaleza, la cual puede ser aun mayor en poblaciones de especies holocíclicas. Del mimo modo es necesario explorar los patrones de conducta y su relación con adecuación biológica en otras especies de áfidos. Es probable que la relación entre conducta alimentaria y adecuación biológica sea muy distinta entre las especies de áfidos endémicas y las introducidas.

La ausencia de correlación entre la conducta plástica y adecuación requiere ser estudiada más afondo, particularmente en relación con la reproducción con generaciones telescópica. En este sentido, es necesario establecer si las consecuencias en la adecuación biológica de una determinada conducta plástica pueden ser expresadas en la segunda o tercera generación. Del mismo modo es necesario extender los parámetros de la adecuación biológica a evaluar (e.g. tamaño corporal de la crias). También es necesario explorar las consecuencias que puede tener un rápido acceso en los vasos floemáticos. Es importante establecer cuanto de éste atributo depende de la constitución de la planta hospedera, y cuanto depende de la habilidad de un genotipo o de una especie de áfido.

Similarmente, es necesario entender la relación entre la conducta alimentaria y su carácter adaptativo en el contexto de la llamada "fidelidad" de los insectos a la planta hospedera. Este fenómeno suele dar lugar a un compromiso ("trade-off") en la capacidad del insecto para utilizar plantas de otra especie distinta de aquella a la que se es fiel. La experiencia previa podría facilitar y reforzar la fidelidad a una especie de planta en particular, y dependiendo del grado de variabilidad intrapoblacional, la diferenciación intraespecífica de los insectos podía verse incrementada originando razas nuevas y eventualmente originando especies nuevas vía especiación simpátrica (Jaenike y Papaj 1992, Mackenzie 1996). En el caso particular de los áfidos, como se ha visto antes, los cambios en la selección del hospedero, la conducta alimentaria, y especialmente la fecundidad mediados por la experiencia previa, pueden influir en la fidelidad a una planta y, en consecuencia, ser causa importante de su evolución.

En resumen, desde en punto de vista conceptual, esta tesis estudia la adaptabilidad de una conducta desde un concepto actualista de adaptación, aspecto de creciente necesidad en biología evolutiva (Reeve & Sherman, 1993). Tal perspectiva es más afín con los estudios de adaptación centrados en la variación fenotípica, esto es, adaptación local y plasticidad fenotípica, que con los estudios de optimización (Brandon & Rausher,1996). Por otro lado, los estudios de plasticidad fenotípica se han concentrado principalmente en atributos morfológicos, y no han enfatizado los atributos conductuales de los organismos (Stephens, 1991; 1993; Dukas, 1998). De este modo, esta tesis representa un esfuerzo por incluir a las conductas plásticas en el contexto más amplio de la plasticidad fenotípica como carácter adaptativo.

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