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EVOLUCIÓN DE ESTRATEGIAS DE DEFENSA MIXTAS EN PLANTAS ANTE SUS HERBÍVOROS

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PRESENTA

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Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 20 de abril de 2009, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** del (la) alumno (a) **GARRIDO ESPINOSA ETZEL** con número de cuenta **96312865** con la tesis titulada: **"EVOLUCIÓN DE ESTRATEGIAS DE DEFENSA MIXTAS EN PLANTAS ANTE SUS HERBÍVOROS."**, realizada bajo la dirección del (la) **DR. JUAN ENRIQUE FORNONI AGNELLI:**

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Resumen

En los últimos años, varios trabajos teóricos han propuesto que la presencia de niveles intermedios de resistencia y tolerancia representa una estrategia evolutivamente estable. Sin embargo, la evidencia empírica con la que se cuenta hasta el momento parece contradecir algunos de los supuestos de estos modelos. En este trabajo se presenta evidencia experimental sobre el efecto de ambas defensas en el desempeño de los herbívoros; el papel que juegan los procesos de adaptación local y la selección dependiente de la frecuencia en el mantenimiento de niveles intermedios y, el efecto de un tercer interactuante en la expresión de la tolerancia. Se encontró que la resistencia afecta negativamente el desempeño de los herbívoros mientras que la tolerancia no tiene efecto alguno. Además, las plantas expresaron mayor tolerancia cuando sus herbívoros se encontraban localmente adaptados. Se encontró también que la resistencia y la tolerancia están bajo selección dependiente de la frecuencia positiva y negativa respectivamente. Finalmente, la colonización por micorrizas disminuyó la expresión de la tolerancia. Dado que el beneficio de la resistencia disminuye con la adaptación local de los enemigos naturales y, que la tolerancia se encuentra bajo selección dependiente de la frecuencia negativa, la asignación simultánea a resistencia y tolerancia podría representar la estrategia de defensa más estable en condiciones naturales.

Summary

Over the last decade, several theoretical studies have proposed that the presence of intermediate levels of resistance and tolerance represents an evolutionary stable strategy. However, most empirical evidence is at odds with theoretical assumptions. In this dissertation, I present empirical evidence about the differential effect of both defenses on herbivore performance; the role played by herbivore local adaptation and frequency-dependent selection on the maintenance of intermediate levels of defense and, the effect of a third interacting species on the expression of tolerance. I found that resistance had a negative effect on herbivore performance while tolerance did not affect herbivore performance at all. Additionally, host plants expressed higher tolerance when their local herbivores were locally adapted. I also found that resistance and tolerance were under positive and negative frequency-dependent selection respectively. Finally, mycorrhizal colonization decreased the expression of host tolerance to aboveground defoliation. Given that resistance could eventually become ineffective when herbivores become locally adapted and, that tolerance is under negative frequency-dependent selection, the presence of mixed defense strategies could represent the most feasible strategy to cope with natural enemies under natural conditions.

Introducción General

Uno de los grandes retos en ecología evolutiva es entender cómo las interacciones bióticas determinan la abundancia, distribución y expresión fenotípica de los organismos. En condiciones naturales, los individuos interactuan con una gran diversidad de organismos: recursos, competidores, mutualistas, enemigos naturales, entre otros (MacArthur, 1972). En este sentido, el estudio de las interacciones bióticas consiste, básicamente, en el análisis de la respuesta fenotípica de los individuos de una especie en relación a los fenotipos de otros interactuantes (Agrawal, 2001). Particularmente, las plantas y sus enemigos naturales han sido uno de los sistemas biológicos más utilizados para estudiar los procesos coevolutivos como resultado de una “carrera armamentista” (van Valen, 1973; Janzen, 1980; Futuyma & Slatkin, 1983). En esta dinámica, tanto las plantas como sus consumidores responden de manera recíproca a la variación fenotípica de la especie con la que interactuan. Dado que este tipo de interacción produce efectos negativos recíprocos en las especies interactuantes, la metáfora de la "carrera armamentista" hace referencia a que ambas especies interactúan en un callejón donde la única salida es la evolución de defensas y contra-defensas que reducen los efectos negativos recíprocos de ambas especies (Janzen, 1980).

Históricamente, el desarrollo teórico y experimental de la ecología evolutiva de las interacciones planta-enemigos naturales se ha basado en el supuesto de que los caracteres de resistencia –aquellos que evitan o reducen el daño– son la única respuesta evolutiva de las plantas ante las presiones de selección impuestas por sus consumidores (Fritz & Simms, 1992). Sin embargo, a principios de la década de los noventa, cuando comenzamos a entender cómo evolucionan los caracteres de resistencia ante las presiones de selección de los enemigos naturales, nos enfrentamos al desafio de incorporar ideas y evidencia nuevas que sugerían que las plantas también son capaces de tolerar el daño además de resistir.

Estrategias de defensa en plantas

Se consideran mecanismos de defensa a aquellos atributos de las plantas, involucrados en la interacción con sus consumidores, que les confieren beneficios en términos de éxito reproductivo en presencia de daño (Karban & Baldwin, 1997). En general, las plantas pueden responder al daño mediante mecanismos de resistencia y/o de tolerancia (Strauss & Agrawal, 1999). Los caracteres de resistencia son aquellos que evitan la pérdida de tejido foliar (Fritz & Simms, 1992) y/o reducen el desempeño y preferencia de los enemigos naturales (Karban & Baldwin, 1997). Por el contrario, los caracteres de tolerancia no evitan que las plantas sean consumidas sino que reducen el efecto negativo del daño sobre su éxito reproductivo (Strauss & Agrawal, 1999). Los mecanismos de defensa pueden ser constitutivos o inducidos, dependiendo de si su expresión está condicionada o no a la presencia de daño (Karban & Baldwin, 1997; Strauss & Agrawal, 1999; Strauss *et al*., 2003). Cuando la expresión de las defensas no depende de la ocurrencia de daño, se considera que la respuesta es constitutiva; mientras que, cuando la expresión de las defensas incrementa después del daño, se considera que la respuesta es de tipo inducida.

Resistencia al herbivorismo.

La mayoría de los estudios sobre la adaptación de las plantas a sus consumidores se han enfocado en la evolución de rasgos de resistencia. La resistencia puede ser definida como una forma de defensa cuyos componentes incluyen: la producción de una gran diversidad de metabolitos secundarios; la presencia de tricomas foliares o espinas y, cambios en la dureza de las hojas (Fritz & Simms, 1992). Todos estos componentes reducen la intensidad del daño, afectando negativamente el desempeño y la adecuación de los insectos herbívoros (Awmack & Leather, 2002).

Tolerancia al herbivorismo.

Inicialmente, la habilidad de ciertas plantas para crecer o reproducirse después de que han sido expuestas a sus consumidores fue investigada por agricultores que trataban de estimar los costos económicos del herbivorismo en cultivos de importancia económica (Painter, 1958). Comúnmente el término compensación se ha considerado un sinónimo de tolerancia (Rosenthal & Kotanen, 1994; Strauss & Agrawal, 1999). Sin embargo, la compensación se refiere a la respuesta promedio, generalmente positiva, de las plantas después de que han sufrido algún tipo de daño en carácteres relacionados con el crecimiento, la biomasa o producción de semillas (McNaughton, 1983); por lo que este término no hace referencia al parentesco genético del material vegetal a partir del cual se estima la tolerancia (Paige & Whitham, 1987; Maschinski & Whitham, 1989). Sólo cuando el grado de compensación es evaluado en términos de éxito reproductivo de un grupo de individuos relacionados genéticamente, los términos tolerancia y compensación pueden considerarse sinónimos (Fornoni *et al*., 2003a).

Mecanismos de tolerancia. A pesar de que los mecanismos involucrados en la expresión de la tolerancia no han sido completamente dilucidados, revisiones recientes mencionan una serie de atributos de las plantas que se asocian de manera correlativa con la tolerancia (Rosenthal & Kotanen, 1994; Strauss & Agrawal, 1999; Stowe *et al*., 2000; Fornoni *et al*., 2003a). Estos caracteres pueden clasificarse en dos grandes grupos: aquellos involucrados en la fisiología vegetal y, los relacionados con la arquitectura vascular (Tabla 1). De esta forma, la pérdida de tejido vegetal puede tolerarse mediante una redistribución de recursos o con el aumento en la tasa fotosintética o de crecimiento. Sin embargo, aún cuando los recursos sean suficientes y adecuados, la tolerancia puede verse limitada por el número de meristemos apicales que sobrevivieron al daño. Por lo tanto, ambos grupos de caracteres son probablemente complementarios en la determinación de la respuesta de tolerancia de las plantas.

El nivel y la composición de las defensas presentes en las plantas es, en gran medida, resultado de su interacción con los enemigos naturales (Marquis, 1992; Seger, 1992). Es decir, el contexto biótico en el cual crecen y se reproducen las plantas juega un papel fundamental en la evolución de sus defensas. Diversos estudios muestran que los herbívoros ejercen fuertes presiones de selección sobre los caracteres defensivos de sus plantas (Berenbaum *et al*., 1986; Simms & Rausher, 1989; Fritz & Simms, 1992; Rausher, 1992; Rausher, 1996; Mauricio & Rausher, 1997; Shonle & Bergelson, 2000; Valverde *et al*., 2001; Fornoni *et al*., 2003b). Por lo anterior, se considera que la expresión de las defensas es adaptativa y funciona de forma estratégica para responder a la presencia de los enemigos naturales. Dado que la estrategia defensiva de una planta es, en la mayoría de los casos, un conjunto de caracteres defensivos, se ha desarrollado el concepto de síndrome de defensa (Agrawal & Fishbein, 2006). Es decir, el síndrome de defensa presente en una planta puede incluir tanto caracteres de resistencia (*e.g.* tricomas, alcaloides, etc.) como caracteres de tolerancia (*e.g.* mayor crecimiento, reasignación de recursos, etc.). En particular, el concepto de síndrome hace referencia a que interacciones ecológicas específicas pueden producir conjuntos de caracteres defensivos similares. De esta forma, las características defensivas de las plantas pueden converger debido a que las características bióticas en las cuales evolucionan son similares.

Evolución de estrategias de defensa mixtas

Dado que la resistencia y la tolerancia parecen cumplir la misma función –reducir el efecto negativo del daño sobre la adecuación– es lógico hacerse las siguientes preguntas: ¿la resistencia y la tolerancia representan estrategias redundantes? ¿son estrategias mutuamente excluyentes? aquellas plantas que presentan ambas estrategias ¿están mejor defendidas? Estudios teóricos sugieren que la resistencia y la tolerancia representan mecanismos redundantes (Simms & Triplett, 1994; Fineblum & Rausher, 1995; Mauricio *et al*., 1997). El razonamiento detrás de esta

Tabla 1. Familias o especies de plantas en las que se han cuantificado respuestas al herbivorismo natural o artifical. Información obtenida de Rosenthal y Kotanen (1994), Strauss y Agrawal (1999) y Stowe *et al*. (2000).

Mecanismos de Tolerancia		**Familia / Especie**	**Referencia**
Fisiológicos	Almacenamiento de recursos en la raíz	Gramíneas	Briske et al., 1996
		Themeda tiandra	Danckwerts, 1993
		Arctium tomentosum	Heilmeier et al., 1986
		Asclepias syriaca	Hochwender et al., 2000
	Incremento en la tasa fotosintética	*Phaseolus vulgaris*	Wareing et al., 1968
		Poaceas	Nowak & Caldwell, 1984
		Gramíneas	Welter, 1989
		Salix planifolia	Houle & Simard, 1996
		Abutilon theophrasti	Mabry & Wayne, 1997
		Asteráceas	Meyer, 1998
	Incremento en la tasa de crecimiento	*Themeda tiandra*	Danckwerts, 1993
		Lycopersicon esculentum	Welter & Steggall, 1993
		Salix planifolia	Houle & Simard, 1997
		Gossypium hirsutum	Rosenheim et al., 1999
	Redistribucion de recursos	*Isomeris arborea*	Krupnick et al., 1999
		Artemisia tridentata	Bilbrough & Richards, 1993
	Incremento en la absorción de recursos	Gramíneas	Briske et al., 1996
		Abutilon theophrasti	Mabry & wayne, 1997
		Sporobolus oiclados	Chapin III & McNaughton, 1989
	Reactivación de meristemos	*Pastinaca sativa*	Hendrix & Trapp, 1989
		Palmas	Grubb, 1992
		Piper arieianum	Mauricio et al., 1993
		Raphanus raphanistrum	Lehtila & Strauss, 1999
		Ipomopsis aggregata	Paige, 1999
	Floración tardía	*Gentianella campestris*	Lennartsson et al., 1997
Arquitectura	Protección de meristemos	Gramíneas	Coughenour, 1985
	Modificación en la arquitectura	*Abies concolor*	Shea, 1989
		Urtica dioica	Mutikainen et al., 1994
		Zea mays	Rosenthal & Welter, 1995
		Gossypium hirsutum	Rosenheim et al., 1997

hipótesis es el siguiente. Los genotipos completamente resistentes no obtendrían beneficios de expresar mecanismos de tolerancia ya que la probabilidad de ser dañados es muy baja. Por otro lado, aquellos genotipos tolerantes no expresarían más beneficios por ser resistentes debido a que el daño no reduce significativamente su adecuación. Si además, consideramos que ambas estrategias representan un costo para los individuos que las expresan (revisado en Núñez-Farfán *et al*., 2007) entonces, presentar niveles máximos de ambas estrategias representaría un costo total mayor que los posibles beneficios obtenidos. Por lo tanto, bajo esta hipótesis, la selección natural debería favorecer genotipos completamente resistentes o tolerantes y no a aquellos que presenten ambas estrategias.

Evidencia empirica indica, sin embargo, que en condiciones naturales las plantas usualmente expresan niveles intermedios de resistencia y tolerancia; es decir, las plantas expresan estrategias de defensa mixtas (revisado en Núñez-Farfán *et al*., 2007). Dicho patrón de asignación sugiere que las estrategias de defensa mixtas podrían representar equilibrios evolutivamente estables mantenidos por selección natural o, ser el resultado de restricciones genéticas y/o ecológicas a la evolución de estrategias puras de resistencia o tolerancia. En los últimos años, varios trabajos teóricos han modelado la evolución conjunta de la resistencia y la tolerancia para entender el mantenimiento de estrategias mixtas (Fineblum & Rausher, 1995; Mauricio *et al*., 1997; Jokela *et al*., 2000; Roy & Kirchner, 2000; Tiffin, 2000; Restif & Koella, 2003; Fornoni *et al*., 2004a). Paralelamente al desarrollo de estos estudios, se fue acumulando evidencia empírica sobre las posibles restricciones a la evolución de ambas estrategias y sobre el valor adaptativo de cada una de ellas (Fineblum & Rausher, 1995; Mauricio *et al*., 1997; Mestries *et al*., 1998; Stowe, 1998; Pilson, 2000; Roy & Kirchner, 2000; Stinchcombe, 2002; Fornoni *et al*., 2003b; Weinig *et al*. 2003; Fornoni *et al*., 2004b; Leimu & Koricheva, 2006; Boege *et al*., 2007; Stevens *et al*., 2007). Sin embargo, dicha evidencia empírica reveló una fuerte inconsistencia con los supuestos y predicciones teóricas (ver Tabla 1 del Capítulo I). Esta inconsistencia y, por momentos, contradicción entre la teoría y la evidencia empírica fue la motivación de este trabajo.

En esta tesis no sólo se propone una nueva dinámica ecológica-evolutiva para entender el mantenimiento de estrategias de defensa mixtas, sino que se genera evidencia empírica que nos lleva un paso adelante para entender la evolución conjunta de la resistencia y la tolerancia como resultado de las presiones de selección que ejercen los enemigos naturales. Específicamente, se considera cómo los procesos de adaptación local y de selección dependiente de la frecuencia actuando sobre la resistencia y la tolerancia, modifican el valor adaptativo de ambas estrategias defensivas. Incorporar dichos procesos puede ayudarnos a entender más claramente la evolución y el mantenimiento de niveles intermedios de resistencia y tolerancia en poblaciones naturales.

En el primer capítulo de esta tesis se presenta un análisis teórico para explicar el mantenimiento de estrategias de defensa mixtas en condiciones naturales. La dinámica evolutiva que surge de este análisis está basada en el efecto diferencial que tienen ambas estrategias de defensa en la respuesta evolutiva de los enemigos naturales (Espinosa & Fornoni, 2006). El análisis incorpora el proceso de adaptación local de los enemigos naturales, el efecto de la selección dependiente de la frecuencia sobre ambas defensas y el efecto que tendría la presencia de un tercer interactuante sobre la evolución conjunta de la resistencia y la tolerancia. Finalmente, se plantean diversos escenarios ecológicos bajo los cuales podrían evolucionar estrategias puras de resistencia o de tolerancia o estrategias mixtas. Este capítulo concluye con predicciones específicas sobre los niveles de adaptación local de los enemigos naturales y el valor adaptativo de las estrategias de defensa.

Para la parte experimental de esta tesis (últimos cuatro capítulos) se utilizó el sistema de estudio compuesto por la planta *Datura stramonium* y su herbívoro especialista *Lema trilineata*. Mejor conocida en nuestro país con el nombre común de toloache, *D. stramonium* es una hierba anual erecta que alcanza hasta los dos metros de altura y que se propaga exclusivamente por semillas. Esta especie sostiene una comunidad grande de herbívoros, parásitos y patógenos: herbívoros foliares especialistas y generalistas, un depredador de semillas, nemátodos de raíz, varias especies de hongos, bacterias y virus (Weaver & Warwick, 1984; Núñez-Farfán & Dirzo, 1994). *Lema trilineata* (Coleoptera: Chrysomelidae) es considerado el herbívoro foliar más importante de *D. stramonium*. Las larvas y adultos de este coleóptero consumen una gran cantidad de tejido foliar llegando a producir defoliaciones del 100% (*obs. pers.*). Estudios previos indican que la pérdida de área foliar reduce la adecuación de *D. stramonium* (Valverde *et al*., 2001), mientras que la resistencia de esta especie afecta negativamente el desempeño de *L. trilineata*.

El Capítulo II (Espinosa & Fornoni, 2006) presenta un estudio sobre los efectos diferenciales que tienen ambas estrategias de defensa en el desempeño de los enemigos naturales –supuesto en el que se basa el desarrollo teórico de esta tesis. Este capítulo representa la primera evidencia experimental de que la tolerancia, a diferencia de la resistencia, no afecta negativamente la sobrevivencia ni el desempeño de insectos herbívoros (Weis & Franks, 2006). Este resultado sugiere que mientras la resistencia favorecería una carrera armamentista, la tolerancia podría atenuar este proceso. Se plantea también que, el efecto diferencial que ambas defensas tienen sobre el desempeño individual de los enemigos naturales podría modificar su dinámica poblacional. De esta forma se espera que, cuando la frecuencia de genotipos resistentes sea alta, el tamaño poblacional de los enemigos naturales disminuya (Underwood & Rausher, 2000; Thaler *et al*., 2001). Por el contrario, el tamaño poblacional de los consumidores podría aumentar con la frecuencia de genotipos tolerantes (Roy & Kischner, 2000; Espinosa & Fornoni, 2006). Finalmente, se propone que cuando la carga de herbívoros –y por lo tanto, el daño que presentan las plantas huésped– aumente, el valor adaptativo de la tolerancia podría disminuir.

El tercer capítulo explora experimentalmente los patrones de adaptación local de los enemigos naturales y de sus plantas huésped. En particular, se evalúa si el componente biótico del ambiente puede generar procesos de adaptación local

(Crémieux *et al*., 2008; Biere & Verhoeven, 2008). Hasta el momento, ningún estudio había evaluado el efecto del ambiente biótico sobre los patrones de adaptación local de ambas especies interactuantes. Los resultados de este trabajo muestran variación geográfica en los niveles de adaptación local de los enemigos naturales (Thompson, 2005). Es decir, las poblaciones de herbívoros presentan todo el rango de posibilidades –desde adaptación local hasta situaciones donde los herbívoros nativos tienen un mal desempeño en su lugar de origen. Por el contrario, los resultados de este capítulo no presentan evidencia de adaptación local de las plantas huésped hacia sus enemigos naturales. Sin embargo, se muestra que en aquellas poblaciones donde los enemigos naturales están más adaptados a sus plantas huésped, éstas presentan mayores niveles de tolerancia. Lo anterior sugiere que cuando los enemigos naturales están localmente adaptados –y por lo tanto, la resistencia ya no evita o reduce el daño–, la tolerancia representaría la estrategia de defensa más factible ante la presencia de los enemigos naturales (Jokela *et al*., 2000).

En el Capítulo IV se evalúa experimentalmente si la resistencia y la tolerancia se encuentran bajo selección dependiente de la frecuencia. Se evalúa además, si diferencias en la densidad de herbívoros –y, por lo tanto, en la cantidad de daño– podrían ser uno de los mecanismos mediante los cuales los enemigos naturales ejercen una presión de selección dependiente de la frecuencia. Este capítulo representa el primer estudio en detectar selección dependiente de la frecuencia sobre la resistencia y la tolerancia simultáneamente. Los resultados obtenidos muestran que, contrario a las predicciones teóricas, la resistencia se encuentra bajo selección dependiente de la frecuencia positiva. Es decir, el éxito reproductivo de los genotipos resistentes es mayor cuando la frecuencia de éstos es alta. Por el contrario, se muestra que la tolerancia está bajo selección dependiente de la frecuencia negativa. Esto es, cuando los genotipos tolerantes son raros –se encuentran en baja frecuencia–, su éxito reproductivo es mayor. Se muestra también que la densidad de herbívoros y el nivel promedio de daño que presentan las plantas aumenta con la frecuencia de genotipos tolerantes. En general, los resultados de este capítulo indican que la selección dependiente de la frecuencia puede mantener la variación en las defensas de las plantas y, también podría explicar la presencia de niveles intermedios de resistencia y tolerancia.

En los últimos años, se ha reconocido el papel fundamental que juega la interacción entre los componentes edáficos y aéreos de los ecosistemas (*feedbacks between below- and aboveground components*) en determinar la abundacia de las plantas y sus interacciones con otros miembros de la comunidad (Van der Putten *et al*., 2001; Wardle *et al*., 2004). En el último capítulo de esta tesis, se evalúa si la interacción planta–micorriza puede condicionar o modificar la expresión de la tolerancia a la defoliación. Los resultados de este capítulo muestran que existe un nivel óptimo de colonización por micorrizas. Este resultado apoya predicciones teóricas que no habían sido corroboradas experimentalmente (Gange & Ayres, 1999). Además se muestra, por primera vez, que la expresión de la tolerancia a la defoliación disminuye cuando la densidad de colonización aumenta. Estos resultados indican que la colonización por micorrizas no siempre representa un beneficio para la planta huésped y que, por el contrario, podría restringir la evolución de la tolerancia a la defoliación. En conjunto, la evidencia experimental que se presenta en los últimos cuatro capítulos de esta tesis permite validar la propuesta teórica que se plantea en el primer capítulo.

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Capítulo 1

Ser resistente o tolerante:

la evolución de estrategias de defensa mixtas

Manuscrito Preparado para *Oikos*

To be resistant or tolerant: the evolution of mixed defence strategies

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Summary

Theory predicts that resistance and tolerance represent mutually exclusive strategies of host defence. However, empirical evidence reveals that individual hosts simultaneously allocate resources to both mechanisms. Understanding the maintenance of this defence pattern remains controversial because empirical evidence is at odds with theoretical assumptions. Here, we present a novel host-enemy dynamic that considers: the differential selective effect of each defence strategy on natural enemies; the process of local adaptation of the natural enemies to their hosts and the effect of negative and positive frequency-dependent selection acting on resistance and tolerance respectively. Our analysis suggests that a mixed pattern of defence allocation could be evolutionary stable because of the differential dynamic that each strategy has in the interaction with natural enemies.

In general, plants and animals defend themselves from the consequences of their natural enemies (herbivores, pathogens and parasites) in at least two ways. While resistant hosts reduce damage or infection, tolerant ones buffer fitness loss due to damage (Núñez-Farfán et al. 2007; Råberg et al. 2007). Theoretical work suggests that because resistance and tolerance seem to have the same function —to reduce the negative effects of natural enemies on fitness—, they might represent redundant mechanisms against the same selective pressure (Simms & Triplett 1994; Fineblum & Rausher 1995; Mauricio et al. 1997). In other words, a host that is completely resistant would not benefit from also being tolerant because it would have a very low probability of being attacked, and a host that could completely tolerate herbivory or infection will not gain any benefit from being resistant given that damage does not significantly reduce its fitness. If we also consider that both defence mechanisms have significant fitness costs (reviewed in Nuñez-Farfán et al. 2007), then having maximum levels of both defensive mechanisms would represent to the host a greater total cost than expressing just one but it would not report any additional benefit. Thus, under this hypothesis, natural selection should favour the allocation of resources to either resistance or tolerance but not to both (Simms & Triplett 1994).

Recent evidence reveals, however, that individual hosts usually allocate resources simultaneously to both resistance and tolerance mechanisms (Mauricio et al. 1997; Pilson 2000; Medel 2001; Fornoni et al. 2003a; Leimu & Koricheva 2006). That is, individual hosts usually express a mixed pattern of defence allocation (Núñez-Farfán et al. 2007). Because natural selection act on both resistance and tolerance, this allocation pattern could be described as a Mixed Defence Strategy (MDS). Understanding the maintenance of MDS in natural populations has become the aim of substantial theoretical work in the last decade. Some of these theoretical studies have proposed that MDS constitute an Evolutionary Stable Strategy (ESS) maintained by natural selection (Mauricio 2000; Tiffin 2000; Restif & Koella 2003; Fornoni et al. 2004a). Alternatively, other models have explained the presence of MDS due to genetic and/or selective constraints to the evolution of complete resistance or complete tolerance (Fineblum & Rausher 1995; Mauricio et al. 1997; Roy & Kirchner 2000; Fornoni et al. 2004a). In a recent study, Núñez-Farfán and collaborators (2007) reviewed the conditions promoting the evolutionary stability of MDS given the available empirical and theoretical evidence. Specifically, they reviewed the assumptions and predictions of studies modelling the joint evolution of resistance and tolerance. While some of the assumptions of these models have been validated, most empirical evidence is at odds with theoretical assumptions (Table 1). Thus, considering the evidence available so far it is still not possible to conclude whether MDS are evolutionary stable or not (Núñez-Farfán et al. 2007).

Are mixed defence strategies evolutionary stable?

Despite the efforts made to understand the maintenance of MDS in natural populations, we are still far from answering this question either because more empirical evidence is necessary to improve and validate theoretical assumptions or because theoretical explanations have not explicitly considered other biological aspects of victim-exploiter interactions. Here, we argue that future theoretical and empirical studies aimed at understanding the evolutionary stability of MDS would benefit from considering the following biological processes: (1) the differential effect of each defence strategy upon the evolutionary response of natural enemies (Stinchcombe 2002a; Espinosa & Fornoni

2006), (2) the process of local adaptation of the natural enemies to their hosts (Mopper & Strauss 1998; Lively & Dybdahl 2000), (3) the effect of negative frequency-dependent selection acting on resistance traits (Dybdahl & Lively 1998; Brunet & Mundt 2000) or on traits that confer both resistance and tolerance (*e.g.* slow rusting; Roy & Kirchner 2000) and (4) the effect of positive frequency-dependent selection acting on tolerance (Roy & Kirchner 2000; Restif & Koella 2004).

Host defences and the evolutionary responses of natural enemies

It has recently been demonstrated that tolerance, unlike resistance, does not negatively affect individual herbivore survival and performance (Espinosa & Fornoni 2006). This differential effect of both defence strategies could lead to different evolutionary responses of the natural enemies (Rausher 2001; Stinchcombe 2002a). While host resistance could favour an arms-race coevolutionary process, host tolerance could lessen this process and instead it could lead to an increment upwards in the natural enemies' population size. Given that resistance reduces natural enemy survival and performance (Bernays & Chapman 1994; Karban & Baldwin 1997; Awmack & Leather 2002), it could also negatively affect natural enemy demography and thus their population size. Empirical evidence shows that host resistance reduces the abundance of natural enemies within a population (Underwood & Rausher 2000; Thaler et al. 2001). Thus, it seems reasonable to suppose that in those populations where most hosts allocate all the available resources to resistance the selective pressures upon their natural enemies will increase, promoting a reduction in the enemy population size.

On the other hand, the absence of a negative effect of tolerance upon natural enemies individual performance suggests that tolerance may lead to an increase in the growth rate of natural enemies and thus on its population size (Roy & Kirchner, 2000; Espinosa & Fornoni 2006). The latter could happen if tolerance mechanisms involve an increase in the amount of tissue available for future infection or damage. However, no empirical study has evaluated the demographical effects, if any, of tolerance upon natural enemies. Although the idea of differences in quality-quantity of resources available for the natural enemies between resistant and tolerant hosts is not new (Stinchcombe 2002a; Espinosa & Fornoni 2006, Weis & Franks 2006), it has not been incorporated into theory yet. Moreover, if more tolerant hosts represent higher quality-quantity food source than less tolerant ones, then variation in tolerance expression could be positively correlated with the potential increment in the natural enemies´ population size.

Negative frequency-dependent selection, natural enemies' adaptation and host resistance

Several evidence indicates that herbivores, pathogens and parasites adapt rapidly to their hosts, reducing the fitness of the most abundant resistant host genotype within the population (Chaboudez & Burdon 1995; Ebert & Hamilton 1996; Mopper & Strauss 1998; Roy 1998; Lively & Dybdahl 2000). This context-dependent process could explain why damage or disease does not dissappear completely from the host population or only for a short period of time until their natural enemies evolve counter-defences. Despite the empirical evidence, most models developed until now have considered that resistance effectiveness does not change through time (Simms & Rausher 1987; Fineblum & Rausher 1995; Abrahamson & Weis 1997; Tiffin 2000; Fornoni et al. 2004a; but see Jokela et al. 2000; Roy & Kirchner 2000). However, whenever natural enemies experience stronger selection pressures (Abrams 1986; Vermeij 1994; Brodie & Brodie 1999) or have shorter generations times than their hosts, they are more likely to become locally adapted to their hosts than vice versa (Hafner et al. 1994; Kaltz & Shykoff 1998; Zhan et al. 2002). Thus, maximum benefits of resistance will be expected when natural enemies are not locally adapted to their host. Under a local adaptation scenario, the mean damage upon resistant hosts will increase resulting in a decrement of resistance benefit.

Positive frequency-dependent selection and host tolerance

Competitive optimization models proposed until now have considered that the benefit of being tolerant increases with the frequency of tolerant hosts within the population (Roy & Kirchner 2000; Restif & Koella 2004). In other words, it has been supposed that tolerance benefit is under positive frequency-dependent selection. In Roy and Kirchner´s model (2000) tolerance prolonged the survival of infected hosts, thus keeping the disease in the population longer and increasing the risk of exposure to disease for both resistant and tolerant hosts. This dynamic increases the advantage of tolerant hosts relative to non-tolerant ones. Thus, as the frequency of tolerant hosts increases, the overall incidence of infection also increases, thereby the fitness advantage of tolerant hosts over non-tolerant genotypes increases as well. This model suggests that the evolution of tolerance could be described as a positive feedback loop that leads to the fixation of tolerant alleles within a population. However, once tolerance alleles become fixed, futher increments in the amount of damage would eventually reduce tolerance benefit (Fornoni & Núñez-Farfán 2000; Fornoni et al. 2003a; Hutha et al. 2003). That is, tolerance net benefit could increase at low damage levels but after reaching a threshold point in damage it could start decreasing. A decrement in tolerance capacity could have higher fitness costs if damage occurs before reproduction. Hence, the maximal benefit of tolerance would be attained at low to moderate levels of damage within the population.

The maintenance of Mixed Defence Strategies

Taking into account the above processes, we present the following dynamic describing the maintenance of MDS in natural populations. Consider a host population exposed to natural enemies. Those host genotypes that allocate all their available resources to resistance would have higher fitness than those allocating all to tolerance, if the benefit of resistance at reducing natural enemies damage or infection is higher than the benefit of tolerating damage (Mauricio et al. 1997; Fornoni et al. 2004a). However, because resistance exerts selective pressures upon natural enemies to overcome this type of defence, a process of local adaptation within the enemy population could be favoured. This dynamic could result in an arms-race coevolutionary process mediated by host resistance and natural enemies local adaptation (Fig. 1). That is, when the benefit of the most abundant resistant genotype decreases, due to the natural enemies' adaptation, hosts should escalate their previous level of resistance or evolve new defensive traits that would help them overcome their natural enemies. This response could be either by increasing the allocation to existing resistant characters (*e.g.* increment of metabolite concentration) or with a novel resistance mechanism. This new resistant phenotype would again promote the evolution of counter-defence mechanisms in their natural enemies and so on. Thus, the adaptive value of host resistance would depend primarly on the reduction of its benefit imposed by natural enemies' adaptation (Simms & Rausher 1987; Jokela et al. 2000). So far, we have laid out an argument that many others have observed or commented on (Roy & Bierzychudek, 1993; Dybdahl & Lively, 1995; Lively & Dybdahl 2000). Our argument is that this dynamic could be eventually altered if the effectiveness of host resistance is reduced and host tolerance became the only profitable strategy to cope with an increasing amount of damage (Jokela et al. 2000) (Fig. 1).

Under a local adaptation scenario, natural selection could favour any host genotype that allocates more defence resources to tolerance rather than to resistance traits affecting the arms-race coevolutionary dynamic. This mutant tolerant genotype could be favoured because although resistant and tolerant hosts could express equivalent costs of defence, the tolerant genotypes would have higher fitness benefit than the resistant ones due to lower fitness losses imposed by damage or infection. This fitness benefit would promote an increase in the frequency of tolerant genotypes within the population. As the frequency of tolerant genotypes increases, the natural enemy population size and the level of damage would also increase. However, an increasing amount of damage could reduce the host capacity for tolerating damage. Thus, tolerance benefit would eventually decrease with damage (Fig. 1).

Considering the above scenario it is worth highlighting that the reduction in tolerance benefit could be decelerated by either any external factor that regulates the natural enemies population size or by increasing allocation to tolerance. For example, even when natural enemies become locally adapted to their host resistance, the presence of a third trophic level (herbivore parasites, parasitoids or predators) could diminish the abundance of natural enemies (reviewed in Halaj & Wise 2001), thereby ameliorating the amount of damage hosts receive. Additionally, the presence of mutualistic associations, such as mycorrhizal fungi, could increase the tolerance level a plant can express if the fungi indirectly alter plant storage patterns or if they increase plant access to scarce or immobile soil minerals, thereby allowing the plant to better overcome tissue loss after damage (Borowicz 1997; Kula et al. 2005; Bennett et al. 2006) (Fig. 1).

Given that resistance could eventually become ineffective when herbivores become locally adapted and that tolerance benefit decreases with damage, both exclusive allocation of resources to tolerance and exclusive allocation to resistance are expected to be evolutionary unstable. In other words, host populations should be composed by genotypes expressing MDS. Hence, in a population where natural enemies are locally adapted (that is, when the benefit of being resistant is low), a mutant genotype capable of allocating more of its resources to tolerance rather than to resistance mechanisms will have a fitness advantage over resistant hosts. The latter will be true if hosts do not evolve novel resistance mechanisms. However, due to a great asymmetry in evolutionary potential between hosts and their natural enemies it is reasonable to expect that allocating resources to tolerance mechanisms will be the most profitable strategy to follow until a more efficient resistant mutant appears within the population. On the other hand, when the frequency of tolerant hosts within the population is high, any other genotype allocating resources to resistance mechanisms would have higher fitness benefits because it could prevent damage. Given that tolerance benefit depends indirectly on the extent of the local adaptation level of the natural enemies and that this depends on the status of the coevolutionary arms-race driven by resistance, then the specific proportion of resources allocated to resistance and tolerance is likely to change through evolutionary time. Thus, the optimum MDS could corresponds to a dynamic ESS.

Perspectives

The analysis presented here provides new insights for understanding the presence of intermediate levels of resistance and tolerance in natural populations. It has been proposed that the coevolutionary process acting on resistance alone could explain the maintenance of variation in this strategy. We argue that considering how tolerance modifies the coevolutionary process can account for the maintenance of the variation in the expression of MDS. Moreover, this analysis leads to the formulation of two specific predictions that could be examined within populations. First, because resistance is affected by the enemy adaptation and tolerance benefit decreases with the amount of damage, it is expected that natural selection would favour those genotypes that follow the least frequent combined strategy of resistance and tolerance allocation within a population. We are aware of no study that has manipulated the frequency of resistant and tolerant patterns of allocation to evaluate its selective value. In this sense, artificial selection experiments represent a promising tool to generate lines with different patterns of resource allocation to resistance and tolerance (see Stowe 1998). Second, if across population variation in the pattern of defence allocation is mainly determined by natural selection rather than other evolutionary processes, a negative correlation is expected between resistance effectiveness and natural enemies extent of local adaptation. On the other hand, the correlation is expected to be positive between tolerance benefit and natural enemies adaptation (Núñez-Farfán et al. 2007). The analysis presented here was formulated based on the premise that those natural enemies with sexual reproduction usually have higher potential for adapting rapidly to their hosts than vice versa. Although this pattern is usually true for plant-herbivore and host-parasite interactions, in those systems where the latter premise is not satisfied (*e.g.* some plant-pathogen interactions) the benefit of being resistant could be maintained for a longer evolutionary time.

However, most of the best exemplified cases of host-exploiter coevolution corresponds to systems where natural enemies have higher potential for a coevolutionary response.

A corollary of our analysis is that if resistance and tolerance are redundant defence mechanisms, the presence of intermediate levels of both resistance and tolerance would imply that natural enemies could adapt to their host resistance more slowly than when hosts defend themselves through complete resistance. In this sense, and as a consequence of the dynamic proposed above, expressing MDS could represent the most feasible strategy to additionally compensate the lower evolutionary rate of hosts to cope with the selective pressures imposed by their natural enemies. When the mean population pattern of defence is biased toward resistance, the intensity of the antagonistic interaction will increase; conversely, a pattern of defence biased toward tolerance will slow down the coevolutionary process (Roy & Kirchner 2000). Hence, the presence of a selection mosaic upon resistance could also be explained by the state of the interaction in the coevolutionary process.

Understanding how genetic variation is maintained in natural populations still remains as a central goal of evolutionary biology (Futuyma 2005). For the case of host-enemy interactions, negative frequency-dependent selection has been the most commonly invoked mechanism for explaining the maintenance of genetic variation in host defence (Frank 1996). Empirical evidence have provided support for this expectation in those cases of simple polymorphic expression of resistance but not for polygenic resistance traits. In most cases however, host defences are complex suites of traits with polygenic inheritance (Seger 1992; Roy & Kirchner 2000). Roy and Kirchner (2000) indicated that polygenic traits appear to behave in similar ways in theoretical models. The question remains, however, whether reality behaves as models do. More empirical work is needed to examine quantitative variation in plant defence integrating the dynamics of resistance and tolerance. Moreover, because natural enemies drive the evolution of host defences it is important to evaluate changes in their populations and their co-evolutionary response as a result of changes in host traits.

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Table 1. Review of the assumptions of theoretical studies modelling the evolution of resistance (R) and tolerance (T). Empirical studies (intraspecific) supporting or not the theoretical assumptions are also listed. NA: absence of empirical evidence.

Mixed Defence Strategies as	**Assumptions**	**Empirical evidence**	
		unsupported	**supported**
Evolutionary Stable	costs or benefits of R and T are non-linear functions of allocation to defence (Fornoni et al. 2004a; Restif & Koella 2004)	Stinchcome 2002b Weinig et al. 2003 Fornoni et al. 2004b Baucom & Mauricio 2008†	Mauricio et al. 1997 Pilson 2000 Bergelson et al. 2001¶
	costs of R and T differ within populations (Tiffin 2000; Fornoni et al. 2004a)	NA	Pilson 2000
	benefits of R and T are more-than-additive (Fornoni et al. 2004a)	Mauricio et al. 1997 Weinig et al. 2003 Agrawal et al. 2004* Fornoni et al. 2004b	NA
	if resistance and tolerance are genetically linked such that the same trait affects both R and T	NA	slow rusting; see Roy & Kirchner 2000 Mestries et al. 1998
Evolutionary Unstable	R and T are alternative redundant strategies if (**a**) there is a negative genetic correlation between R and T (Simms & Triplett 1994; Fineblum & Rausher 1995; Abrahamson & Weiss 1997; Roy & Kirchner 2000) or if (**b**) benefits of R and T are less than additive (Mauricio et al. 1997)	(**a**) Leimu & Koricheva 2006¶ Boege et al. 2007 Stevens et al. 2007 (**b**) Mauricio et al. 1997	(**a**) Fineblum & Rausher 1995 Stowe 1998 Pilson 2000 Fornoni et al. 2003b Baucom & Mauricio 2008† (**b**) Agrawal et al. 2004* Baucom & Mauricio 2008†

* Study about R and T to frost damage
† Study about R and T to herbicide
¶ Reviews

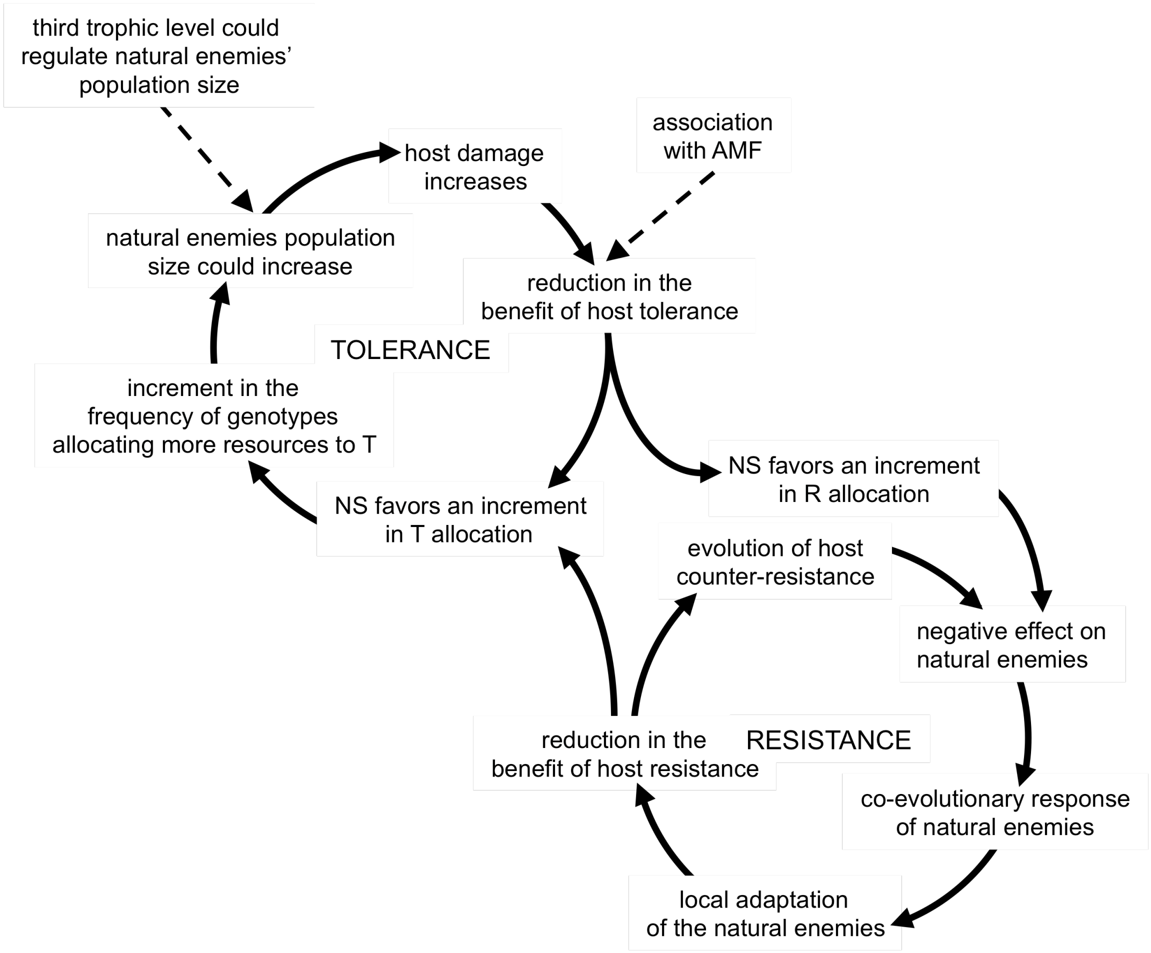

Figure 1. Schematic representation of the evolutionary dynamic driven by host defences and natural enemies. While host allocation to resistance (R) could be determined by the extent of the natural enemies' local adaptation, allocation to tolerance (T) may depend on the frequency of hosts allocating resources to tolerance and on the mean damage level experienced by hosts. Discontinuous arrows indicate the potential effects of a third party species (*e.g.* third trophic level and arbuscular mycorrhizal fungi AMF) on the evolutionary stability of host tolerance. Arrows connecting both dynamics represent shifts in the allocation patterns of host defence. NS: natural selection.

Capítulo 2

La tolerancia no ejerce una presión de selección sobre los enemigos naturales

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Host tolerance does not impose selection on natural enemies

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Summary

- Coevolution between hosts and their natural enemies is believed to operate through the evolution of resistance traits. Although the importance of tolerance to natural enemies as an alternative defensive strategy has been recognized, there is still no consensus about the possible role of host tolerance in the evolutionary outcome of the interaction.
- Here, using bioassay experiments, we tested the hypothesis that variation in host tolerance among selected plant genotypes could impose a selection pressure upon a specialist herbivore.
- Tolerance did not affect herbivore larvae survival, weight gain, efficiency of food consumption, total food consumption, developmental time and adult mass. These results therefore do not support the hypothesis that host tolerance could affect natural enemy performance. However, resistance did negatively affect herbivore larva survival. Genetic variation in herbivore larva survival was detected, thus suggesting the potential for a coevolutionary response.
- Our results indicate that host tolerance would reduce opportunities for a coevolutionary response by the natural enemies of the host. Contrary to predictions from previous models, our results suggest that host tolerance may constitute an evolutionarily unstable defensive strategy.

Key words: coevolution, defense, host–enemy interactions, insect–plant interactions, resistance, tolerance.

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Introduction

Coevolutionary theory applied to antagonistic interactions (plant–herbivore, plant–pathogen, predator–prey or host–parasite) has been developed under the assumption that interacting species exert reciprocal negative genetic effects (Janzen, 1980; Futuyma, 1998). Occurrence of coevolutionary responses among species requires two conditions to be fulfilled: (1) each species involved in an interaction must affect the performance of the other species with which it interacts, and (2) genetic variation in those traits involved in the interaction must exist for a response to natural selection to occur. The validity of these conditions has been well supported by empirical work indicating that hosts have evolved resistance traits that negatively affect the performance of their natural enemies (Rausher, 1996; Agrawal, 2001). These resistance traits were shown to constitute the selective pressure upon enemies to evolve counter-resistance traits (Berenbaum & Zangerl, 1998; Geffeney *et al.*, 2002; Ratza *et al.*, 2002; Thrall & Burdon, 2003; Allen *et al.*, 2004).

More recently, there has been recognition that hosts also defend themselves through mechanisms of tolerance that reduce or buffer fitness losses after damage (Strauss & Agrawal, 1999; Jokela *et al.*, 2000; Stinchcombe & Rausher, 2002; Fornoni *et al.*, 2003a, 2004b) or infection (Roy & Kirchner, 2000; Hansen & Koella, 2003; Restif & Koella, 2003, 2004). Thus, while host resistance reduces the probability of being damaged or infected, host tolerance reduces the negative impact of damage on fitness (i.e. reduces the cost of damage or virulence). Although several studies have advocated the presence of fitness benefits and costs of tolerance (Stowe *et al.*, 2000; Koskela *et al.*, 2002; Stinchcombe & Rausher, 2002; Stinchcombe, 2002a; Fornoni *et al.*, 2004b), no study has determined whether the defensive strategy of tolerance results in a different coevolutionary response from the response expected to be produced by resistance (discussed in Stinchcombe, 2002b).

Theoretical models have recently incorporated the evolution of both tolerance and resistance as alternative defensive strategies. These studies assume that, unlike resistance, tolerance does not exert negative effects upon enemy development and performance (Roy & Kirchner, 2000; Tiffin, 2000; Stinchcombe, 2002b; Fornoni *et al.*, 2004a). In other words, tolerance may not constitute a selective pressure upon natural enemies (Stinchcombe, 2002b). In contrast, the first attempt to model a coevolutionary dynamics with tolerance and resistance as host defense mechanisms predicted that tolerance could produce a different pattern of evolution of enemy traits from that expected for resistance (Restif & Koella, 2003). Therefore, that tolerance can constitute a selective pressure on natural enemies is still an untested assumption of previous theoretical models (Tiffin, 2000; Stinchcombe, 2002b; Restif & Koella, 2003; Fornoni *et al.*, 2004a). If higher levels of tolerance correspond to higher levels of host quality (i.e. more nitrogen content associated with compensatory photosynthesis in plants) (Stinchcombe, 2002b; but see Gassmann, 2004), tolerant hosts may select for higher levels of infection or consumption among natural enemies (Restif & Koella, 2003). Under this condition, an association between tolerance and the enemy traits involved in the interaction must exist (see Stinchcombe, 2002b). Although there is evidence of a negative correlation between resistance level and damage (reviewed in Marquis, 1992), no study has ever determined whether an association between tolerance and consumption by natural enemies exists (Stinchcombe, 2002b).

In this study, we tested the hypothesis that host tolerance can impose a selective pressure on the natural enemies of the host. Specifically, we used bioassay experiments with a plant–herbivore system involving the annual plant *Datura stramonium* and its specialist leaf beetle *Lema trilineata* to determine whether plant tolerance directly influences herbivore larva survival, weight gain, efficiency of food consumption, total food consumption, developmental time and adult mass. Finally, the presence of genetic variation and correlations among herbivore traits was also examined in order to explore the potential for an evolutionary response by the herbivore.

Materials and Methods

Datura stramonium L. (Solanaceae) is the host of the folivorous leaf beetle *Lema trilineata* (Olivier) (Chrysomelidae) from Mexico to Canada. All the larval stages of this herbivore occur on the leaf tissue of the host plant, and can consume almost 100% of individual plants (J. Núñez-Farfán, UNAM, Mexico City, Mexico, pers. comm.). Herbivore damage can reduce plant fitness (Núñez-Farfán & Dirzo, 1994). *D. stramonium* contains tropane alkaloids and foliar trichomes that function as components of resistance against herbivory (Shonle & Bergelson, 2000; Valverde *et al.*, 2001). Plant material used in the present study was gathered from a population of *D. stramonium* in Central Mexico (18°N, 99°W), for which the existence of additive genetic variation and genotypic selection acting on tolerance and resistance to folivorous herbivores had previously been demonstrated under natural field conditions (Fornoni *et al.*, 2003b, 2004b). From this data set, two groups of host lines were selected, each represented by four genotypes (full-sibs). These two groups showed significant differences in tolerance ($F_{7,51} = 5.55$; $P < 0.0001$) but similar levels of resistance ($F_{7,59} = 0.98$; $P = 0.4480$), as estimated from damage under natural field conditions (Fornoni *et al.*, 2003b). For the purpose of the present study, our choice of host lines reduced by approximately 50 times the variation in resistance relative to that in tolerance ($\mathrm{CV}_{\mathrm{resistance}} = 2.48\%$ and $\mathrm{CV}_{\mathrm{tolerance}} = 104.68\%$). This manipulation ensured the absence of a correlation between tolerance and resistance ($r = -0.1228$; $P = 0.7932$) and increased the power to detect an effect of tolerance. Using this plant genetic material, two bioassay experiments were performed during 2002–2003 under laboratory conditions. Thirty seeds from each host line were sown in the glasshouse of the Ecology Institute [Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico] to obtain six to eight adult plants per line planted in 4-l pots filled with potting soil. This procedure was repeated for each of the bioassay experiments described below.

Experiment 1

This experiment was designed to determine whether tolerance background (low- vs high-tolerance host lines) affects herbivore larva survival, weight gain, efficiency of food consumption, total food consumption, developmental time, and adult mass. Also, we wished to determine whether this effect relies on the expression of a plastic (after damage) or a constitutive (before damage) component. Because of the late germination rate, only seven host lines were used in this experiment. Before flowering, all the leaves of half of the plant replicates from each host line were artificially defoliated to 50%, following the methodology used by Fornoni & Núñez-Farfán (2000). After the phenostage at which the defoliation treatment was applied, subsequent leaves produced by defoliated and nondefoliated plants were used to feed an experimental population of the herbivore *L. trilineata.* This phenostage (flowering stage) corresponds to the time at which *L. trilineata* starts consuming its host plant in the field. Although lines had *a priori* reduced variation in resistance, this defensive trait was also measured using the reciprocal of the proportional amount of damage inflicted by individual larvae upon each host line. The experimental population of *L. trilineata* was composed of a sample of 621 individuals collected from a population located in the El Pedregal de San Angel Preserve in the Valley of Mexico (19°N, 99°W). Larvae were taken to the laboratory and reared at 25°C with a 12 : 12 h photoperiod. Each larva was reared in a 250-cm^3 plastic pot filled with 80 cm^3 of sterilized soil and covered with a mesh. Pots were watered (10 ml) every 2 d until the start of the pupal stage. Each larva was fed using one of the plant replicates of each host line. Fresh leaf squares of 5 cm^2 in each pot were replaced every 2 d and stored for estimation of the amount of leaf tissue consumed by the larvae. This area of leaf tissue was similar in terms of mass among host lines, as no significant differences were previously detected in specific leaf weight ($F_{7,395} = 1.63$; $P = 0.1228$). The experiment was continued for 24 d, until all adults had emerged.

During the experiment, the following variables were measured: herbivore larva survival (days to death), weight gain, efficiency of food consumption, total food consumption, developmental time and adult mass. Weight gain (g d^{-1}) was estimated as the proportional increment in mass between the second and fourth larval stages relative to the number of days between the two larval stages. This time interval corresponds to the larval stages with the highest rate of consumption (EGE & JF, unpublished data). Because of the absence of differences among host lines in specific leaf weight (see above), the efficiency of food consumption (g cm^{-2}) was estimated as the ratio of weight gain relative to the amount of consumed leaf area. After 3 wk at 25°C, dry leaf area consumed was calculated by adding together the leaf areas consumed from all the 5-cm^2 squares of leaf tissue given to each larva. For each leaf tissue square, leaf area consumed was measured using Digital Image Analysis Systems (WinDias Basic; Delta-T Devices Ltd, Cambridge, UK). Total food consumption (cm^2) was estimated by adding together the total leaf areas consumed by each larva. Developmental time corresponds to the number of days the larva takes to reach the adult stage. Adults were weighted (g) as an estimate of size at maturity. Leaf area consumed during the first larval stage was used to estimate host line resistance to the experimental population of the herbivore. Host line resistance was estimated as the average of the proportion of leaf area consumed by each larva.

As we used fewer plants than the number of larva replicates assigned to each combination of tolerance level and defoliation treatment, the results were analyzed as a split plot design following the model: herbivore performance = tolerance + defoliation treatment (tolerance) + resistance + error (Crawley, 1993, pp. 51–52). Survival analysis was performed following the Cox regression model (Cox, 1972). For the other response variables, analyses were performed with the analysis of variance (ANOVA) (type III SS) option in PROC GLM (SAS, 1999). Weight gain was square root transformed, developmental time was transformed as the inverse of the square root, and efficiency of food consumption was log-transformed to improve normality. The ANOVA for efficiency of food consumption was performed without including resistance as a covariable as damage was used to estimate the dependent variable. For all the variables except survival, the total number of larvae included in the analysis was reduced because of mortality.

Experiment 2

This experiment was designed to estimate the presence of genetic variation in the herbivore population for the same traits as measured in experiment 1. For this experiment, a similar set of host plant replicates to that used in experiment 1 were grown in the glasshouse and used to feed herbivores from an experimental population of 709 larvae (31 maternal half-sib families × 22.87 ± 4.20 eggs per family) obtained from the same site as used previously. Larvae were randomly assigned to one of 20 blocks within the laboratory and maintained under the same conditions as described above. The experiment was continued for 33 d, until all adults emerged. Except for the analysis of genetic variation in survival, larval mortality reduced to 15 the number of families that had sufficient numbers of replicates (8–18 larvae per family) for the analysis of genetic variation on continuous and meristic variables. For these analyses, herbivore genotype was considered a random factor, and resistance was included as a covariable. The analyses of the continuous and meristic variables were performed with the ANOVA (type III SS) option in PROC GLM (SAS, 1999). Phenotypic and genotypic correlations were estimated among all pairs of variables using the Pearson correlation coefficient in JMP (SAS, 1995). Only the variables that showed genetic variation were included in the correlation analysis. Mean herbivore larva survival was only included in the estimation of genotypic correlations.

Results

The findings of the present study indicate that tolerance and resistance exert different effects upon herbivore larva survival (Table 1). No evidence of an effect of tolerance (before and after defoliation) on herbivore larva survival was detected

Table 1 Results of the χ^2 survival analysis using the proportional hazard model

	d.f.	χ^2 likelihood-ratio	*P*
Tolerance	1	0.002	0.9599
Defoliation (tolerance)	2	2.658	0.2647
Resistance	1	104.107	< 0.0001

N = 621.
d.f., degrees of freedom.

(Table 1). Mean survival time (± standard error) was 8.15 ± 0.58 and 9.85 ± 0.55 d for insects grown on low- and high-tolerance hosts, respectively. The survival analysis had a power of 0.90 (holding $\alpha = 0.05$) to detect a difference in mean survival time greater than 1.7 d between levels of tolerance (Collet, 2003, p. 300). Despite the previous absence of differences in resistance among selected host lines at their site of origin, significant differences in resistance were detected with our experimental herbivore population ($F_{6,614} = 10.82$; $P < 0.0001$). After the bioassay experiment, the estimation of plant resistance increased the coefficient of variation in resistance from 2.4 to 8.4%. Surprisingly, this small amount of variation was negatively associated with herbivore larva survival ($r = -0.96$; $P = 0.0003$, $N = 7$) (Fig. 1). Tolerance level, defoliation treatment and resistance did not affect the other herbivore characters measured [degrees of freedom are 1, 2, 1 and 122 for tolerance, defoliation (tolerance), resistance and error source of variation, respectively; all $F < 3.1022$; all $P > 0.0870$]. Power analyses indicated that, if the true sizes of the treatment effects are as small as those estimated in this experiment, we would have needed sample sizes 30–2968 times larger (depending on the trait) to obtain statistical significance (holding $\alpha = 0.05$ and $1\text{-}\beta = 0.80$). Given that our experiment was contrived to enhance any possible effect of tolerance, our nonsignificant results for insect survival, growth and performance give reasonable confidence that the corresponding differences are likely to be very small.

Genetic variation was detected for herbivore larva survival ($\chi^2_{30} = 64.72$; $P = 0.0002$), developmental time ($F_{14,122} = 2.80$; $P = 0.0234$) and adult mass ($F_{14,122} = 2.36$; $P = 0.0453$). Correlation analyses among herbivore characters revealed a positive phenotypic correlation between adult mass and developmental time ($r = 0.25$; $P = 0.0006$). No genetic correlations between variables were detected. Although this result suggests that selection imposed by host resistance would not affect the evolution of other characters besides survival, it should be treated with caution given the sample size ($N = 15$) available for these analyses.

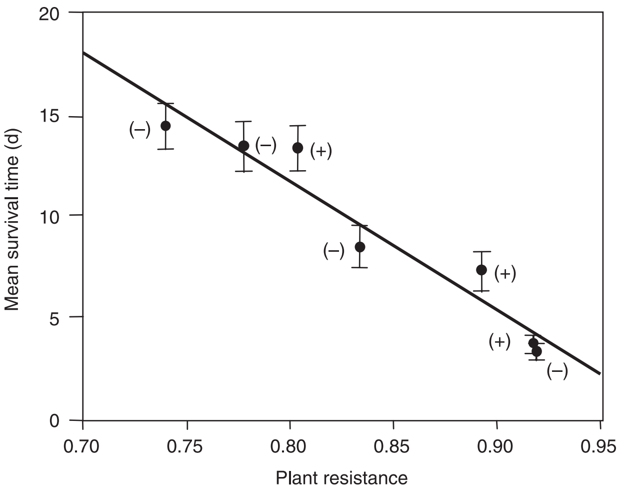

Fig. 1 Negative correlation between plant resistance and herbivore larva survival. +, Plant families with high tolerance levels; –, plant families with low tolerance levels. No evidence of a negative correlation between resistance and tolerance was detected ($r = 0.49$; $P = 0.2562$).

Discussion

The results obtained in the present study support the hypothesis proposed by Stinchcombe (2002b) that host tolerance could relax the selective pressure on natural enemies. Specifically, plant tolerance did not affect herbivore larva survival, weight gain, efficiency of food consumption, total food consumption, developmental time and adult mass. As these herbivore traits are usually affected by plant quality (Scriber & Slansky, 1981; Weilbull, 1987; Taylor, 1989; Moran, 1992; Wheeler & Halpern, 1999; Awmack & Leather, 2002), our results suggest that tolerance mechanisms may not necessarily be related to plant quality (Stinchcombe, 2002b). Instead, a negative correlation between plant resistance and herbivore larva survival was detected. In addition, the presence of genetic variation in survival among herbivore families suggests the potential for a coevolutionary response.

Two points should be considered in the interpretation of the absence of an effect of tolerance on herbivore performance. First, a genotype × environment interaction in the phenotypic expression of tolerance could have reduced the differences in tolerance among selected host lines, increasing the probability of detecting a nonsignificant effect. Hence, further effort should be devoted to examining the possible existence of an effect of tolerance on herbivore performance in the field. Secondly, as we were not able to determine whether genetic variation in the plasticity of herbivore traits existed across tolerance levels, we cannot rule out the possibility that host tolerance could affect herbivore performance. If genetic variation in plasticity of insect traits is present, host tolerance would select an evolutionary change in the herbivore.

Our results suggest that, for the *D. stramonium–L. trilineata* system, the host defensive strategy is expected to have contrasting effects on herbivores and hence on the coevolutionary process; while resistance would promote a coevolutionary response, tolerance would not. Although this idea has been previously proposed (Rosenthal & Kotanen, 1994; Rausher, 2001; Stinchcombe, 2002b; Fornoni *et al.*, 2004b), its evolutionary implications have attracted little attention.

Jokela *et al.*'s (2000) model predicted that the adaptive value of tolerance would increase when natural enemies become locally adapted to the level of resistance of their host population (i.e. when the host receives an increasing amount of damage). Their study described the conditions under which a tolerant mutant could invade a population but did not explore whether tolerance could be evolutionarily stable.

Other models have suggested that the conditions for invasion and fixation of tolerant mutants depend on the relative values of the costs and benefits of tolerance (Tiffin, 2000; Fornoni *et al.*, 2004b). Therefore, previous models considered tolerance and resistance as alternative strategies in terms of fitness and also assumed that tolerance levels were proportional to the amount of damage. Based on these assumptions, these models predict that host tolerance would be an evolutionarily stable defensive strategy. While some evidence supports the expectation that tolerance and resistance could function as alternative mechanisms of defense (van der Meijden *et al.*, 1988; Valverde *et al.*, 2003), there is no empirical evidence supporting the assumption that host tolerance could be a linear function of the amount of damage.

Our results suggest that, if tolerance does not affect enemy consumption negatively, an increase in the enemy load would possibly increase the amount of damage, thus reducing the host capacity for tolerance. Although the exact shape of the relation between tolerance and damage has not been deeply examined, it is reasonable to expect that under low levels of damage tolerance would increase (Hutha *et al.*, 2003; see del-Val & Crawley, 2005). As damage increases, tolerance will finally reach its maximum and any further increase in the amount of damage will reduce the benefits of tolerance because of internal/external constrains. Recent studies have indicated that hosts probably face limits on their maximum tolerance because of resource limitation (Fornoni *et al.*, 2004a) and/or physiological and morphological constraints (Hochwender *et al.*, 2000). Thus, the adaptive value of tolerance may be related to variation in the enemy population size. This possible association could explain temporal and spatial fluctuations in selection of host tolerance and the presence of intermediate levels of tolerance observed in natural populations.

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Capítulo 3

Adaptación local: considerando simultáneamente a los herbívoros y a sus plantas huésped

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Local adaptation: simultaneously considering herbivores and their host plants

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Summary

1. Although the phenomenon of local adaptation has received much attention, studies evaluating the contribution of the biotic environment to the patterns of local adaptation for both interacting species are still scarce. Here, we evaluated how the biotic environment affects the patterns of reciprocal local adaptation in an herbivorous insect and its host plant.

2. To test for herbivore and plant local adaptation, two four-by-four cross-infestation experiments were performed. The first one was done in the laboratory to estimate herbivore performance while the second one was done under semi-natural conditions to estimate herbivore population growth rate and sex ratio as well as plant damage, seed production and tolerance to herbivory.

3. Although we found geographic variation in the extent of herbivore local adaptation – from adapted to maladapted–, there was no evidence of plant adaptation to its specialist herbivore. Interestingly, tolerance was higher in those populations where the native herbivores were locally adapted.

4. These results suggest that tolerance is an interaction trait whose expression may depend on the extent of herbivore adaptation. If plants can modulate their tolerance response to the presence of their native herbivores, tolerance could compensate for the higher evolutionary potential of the natural enemies.

Introduction

How natural selection acts on traits to produce adaptations to different environments remains as a central question in evolutionary biology. In particular, the biotic component of the environment experienced by every organism is usually variable in space and time (Thompson, 2005). This is particularly true for coevolutionary victim-exploiter systems, where each species constitutes an ever-changing environment to which its opponent has to adapt (Gandon & Michalakis, 2002). The geography of coevolutionary responses may lead to variation among populations in the patterns of local adaptation for both the victim and the exploiter (Futuyma & Slatkin, 1983). At a given point in time, patterns of local adaptation would either show local adaptation of the victim or the exploiter, but not of both, because of differential migration rates, generation times, population sizes and the time-lagged cycles typical of coevolutionary processes (Gandon *et al*., 1996; Gandon & Michalakis, 2002). Although local adaptation seems to be a common phenomenon among plants (Linhart & Grant, 1996) and animals (Greischar & Koskella, 2007), studies evaluating the biotic component of local adaptation among interacting species are still scarce.

Reciprocal transplant experiments have been the standard approach to test for local adaptation. This experimental design allows the examination of the relative importance of the interaction between genetic and environmental variation in determining individual fitness (Antonovics & Primack, 1982). However, when individuals are transplanted to a different environment, and their fitness is lower than that achieved by the native genotypes, such pattern of local adaptation can be the result of (1) differences in some abiotic component of the environment (*e.g.* temperature, altitude, humidity) between the native and the foreign site or (2) differences in the genetic composition of the interacting species (Kawecki & Ebert, 2004). It is also possible that phenotypic plasticity in those traits involved in the interaction may reduce the chances of detecting a significant pattern of local adaptation. Thus, this experimental approach is not sufficient to determine the causes of adaptation because neither the physical nor the biotic environment is usually manipulated (Biere & Verhoeven, 2008; Crémieux *et al*., 2008). To determine whether biotic interactions account for patterns of local adaptation, the fitness consequences of the interaction for both species must be examined controlling for the effects of the physical environmental context (Biere & Verhoeven, 2008).

In a classic arms-race coevolutionary process, when natural enemies are locally adapted to their hosts, its performance is expected to be higher than that achieved by foreign enemies. In turn, native hosts would suffer higher levels of damage than foreign hosts because of reduced efficiency in host resistance (Lively & Dybdahl, 2000). Under this scenario, it has been theoretically suggested that hosts would defend themselves through a tolerance response to ameliorate the negative effect of natural enemies (Jokela *et al*., 2000). On the other hand, when hosts are locally adapted to their natural enemies, they are expected to experience lower amounts of damage (*i.e.* higher levels of resistance) than foreign nonadapted hosts. Thus, native natural enemies would present lower performance than foreign enemies. Testing for local adaptation among interacting species and its relationship with the expression of specific traits involved in the interaction can help us evaluate if these traits play a major role in the evolutionary outcome of the interaction. To our knowledge, there have been no attempts to evaluate this association.

Using an herbivorous insect−plant system, we first determined if both herbivore and plant populations are genetically differentiated in those traits involved in the interaction. Second, we evaluated the extent of local adaptation of the herbivore to its host plant; the reciprocal question, whether the host plant is adapted to its herbivore was also addressed. Finally, we explored possible associations between the extent of herbivore local adaptation and variation in host traits involved in the interaction. To answer these questions, two four-by-four cross-infestation experiments were performed in a common garden (*i.e.* under similar abiotic conditions).

Materials and Methods

Study System

Datura stramonium L. (Solanaceae) is a hermaphroditic annual plant that grows on disturbed areas, from tropical forests to xerophytic shrublands. This species experiences from 10 to 70% of individual foliar damage (Valverde *et al*., 2001). The main folivorous insect of this plant is the specialist beetle *Lema trilineata* (Olivier) (Coleoptera: Chrysomelidae). All but the pupa stage of this herbivore occur on the leaf tissue of its host, where it can survive and reproduce for up to four generations per season (*pers. obs.*). Previous studies indicate that herbivore damage reduces *D. stramonium* fitness (Valverde *et al*., 2001) while plant resistance has a negative effect on *L. trilineata* performance (Espinosa & Fornoni, 2006).

Experimental design

Four populations in Central Mexico were chosen: Pedregal (19.32°N, 99.19°W), Teotihuacan (19.68°N, 98.86°W), Tula (20.05°N, 99.35°W) and Esperanza (18.85°N, 97.37°W). Geographic distances between populations varied from 52 to 245 km. To test for herbivore and plant local adaptation, two four-by-four cross-infestation experiments were performed during 2005 and 2006. The first one was done under laboratory conditions to estimate herbivore individual performance while the second one was performed under semi-natural conditions to simultaneously estimate herbivore population growth rate and sex ratio as well as plant damage, seed production and tolerance to herbivory.

Experiment 1. During the summer of 2005, seeds collected in 1999 from 30 maternal families per population were germinated. Two weeks after germination, 20 plants per population (N = 80) were individually transplanted into 4-liter pots, filled with potting soil, and placed in a greenhouse at the Instituto de Ecología (UNAM). Simultaneously, around 100 adults of *L. trilineata* from each of the same populations were collected and taken to the laboratory. Herbivores were allowed to reproduce for one week to obtain approximately 30 clutches per population (N = 827). After eclosion (August, 2005), each clutch was divided in four groups so that each group could be fed with leaves from each plant population. There were at least six larvae per clutch per plant population. All larvae were individually reared and its survival was checked daily until adult emergence (see Espinosa & Fornoni, 2006 for a description of the rearing technique). Individual performance was estimated as the product of the following relativized variables: survival probability, efficiency of food consumption, inverse of developmental time and adult mass. For all these variables, a significant interaction between herbivore and plant population was detected (results not shown; all $F > 1.96$; all $P < 0.0408$). Efficiency was estimated as weight gained between leaf area consumed. Weight gain was estimated as the increment in mass between the second and fourth larval stages between the number of days between these two stages. Leaf area consumed by each larva was calculated using a Digital Image Analysis System (WinDias Basic; Delta-T Devices Ltd, Cambridge, UK). Developmental time was considered as the number of days between hatching and adult emergence. Adults were weighted to the nearest mg using a digital balance (OHAUS).

Experiment 2. The following year (August 2006), 60 plants per population (N = 240) were obtained as described above, taken to a common garden and placed inside 60 mesh-cages (65 × 70 × 75 cm). Simultaneously, herbivores were collected as described in the first experiment and allowed to reproduce in the laboratory. After hatching, larvae originating from each experimental population were randomly placed at a constant density (three larvae per leaf) –which control for plant size and minimize intra-specific competition– on plants from all four populations. A control treatment (plants without herbivores) was also included. Each plant-herbivore combination included four plants within a cage (four plants × five treatments × three replicates × four populations). Two months later (October 2006), when all the adults from the initial larvae emerged and reproduced, total number of eggs, larvae from all instars and adults per combination treatment were counted. Afterwards, adults were placed in vials filled with alcohol at 70% and sexed in the laboratory. At this time, when plants had ended flower production, all leaves and fruits were cut and stored in paper bags during three weeks at 25°C. Plant damage was estimated using a Digital Image Analysis System (WinDias Basic; Delta-T Devices Ltd, Cambridge, UK), and total seed number was counted. Herbivore population growth rate was estimated as the ratio between final and initial number of first-instar larvae. Because there were eggs and larvae from different instars at the end of the experiment, we expressed all these life stages in terms of first-instar larvae using the transition probabilities obtained in the laboratory. Tolerance to herbivory was estimated for each plant-herbivore combination as the difference in seed production between damaged and undamaged (control) plants relative to the amount of damage experienced.

Statistical Analyses

All herbivore variables were analysed with a two-way ANOVA including herbivore, plant population and their interaction as sources of variation. Herbivore individual performance was ln-transformed to improve normality. Because there was only one replicate per combination treatment for the variables of herbivore population growth rate and tolerance to herbivory, the significance of the interaction term was calculated following the procedure proposed by Tukey for a two-way ANOVA without replication (Kutner *et al*., 2005; pp. 880-891). Herbivore sex ratio was analysed using a nominal logistic regression. Finally, plant damage and seed production were analysed with an ANCOVA including the same factors mentioned above and initial plant size as a covariate. All analyses were performed in JMP 7.0 (SAS, 2007).

Patterns of Local Adaptation. To test for local adaptation, we followed the local-foreign criterion –which emphasizes the comparison among herbivore or plant populations within habitats–, testing the hypothesis that herbivores or plants perform better at their native site relative to foreign genotypes (Kawecki & Ebert, 2004). Because differentiation in performance among populations can biased the results obtained when using this criterion (Thrall *et al*., 2002), to test for herbivore local adaptation performance was standardized for each herbivore population. Similarly, to test for plant local adaptation seed production was standardized for each plant population. If the interaction between herbivore and plant population was significant after this standardization, separate one-way ANOVAs for each population were performed followed by contrasts between sympatric (control) and each allopatric combination (Dunnett´s test).

Coefficient of Local Adaptation. In order to describe the variation in the extent of local adaptation among herbivore and plant populations, we estimated the degree of local adaptation for each population with the following coefficient $\beta = \sum_{i=1}^{3} \frac{(\bar{x}_s - \bar{x}_a)}{\bar{x}_s}$, where $\bar{x}_s$ and $\bar{x}_a$ represent the mean fitness of an herbivore or plant population in sympatric and allopatric combinations respectively. Positive values denote higher fitness on sympatric than on allopatric treatments (local adaptation); negative ones indicate the opposite (maladaptation) and values near zero indicate no differences in fitness between sympatric and allopatric combinations (no adaptation).

Results

There was significant differentiation among herbivore populations for all the variables measured but sex ratio (Table 1). In general, herbivores from Pedregal achieved both higher individual performance and population growth rate than herbivores from other populations (Table S1). In addition, plants eaten by herbivores from Pedregal experienced more damage and had less seeds than plants consumed by other herbivores (Table S1, S2). In contrast, herbivores from Tula performed less well in comparison with other populations. Specifically, these herbivores had low growth rate (Table S1) and, as a consequence, plants consumed by herbivores from Tula experienced low damage and higher fitness (Table S1). Interestingly, herbivores from Tula seem to induce higher tolerance on their host plants relative to the other herbivore populations (Table S1, S2).

Plant population also had a significant effect on herbivore individual performance (Table 1) suggesting variation in plant quality. Additionally, there was population differentiation in plant damage, seed production and tolerance (Table 1). In general, herbivores consuming plants from Tula had lower performance than on plants from other populations (Table S1, S2). Thus, plants from Tula achieved higher fitness and were more tolerant than the other plant populations (Table S1).

Patterns of Herbivore and Plant Local Adaptation

A significant interaction between herbivore and plant population was detected for all variables but herbivore population growth rate and plant seed production (Table 1). Thus, we tested for herbivore local adaptation using the variable of performance. As explained above, this variable was standardized for each herbivore population to eliminate differences among populations. After the standardization, a significant interaction between herbivore and plant population was again detected ($F_{9,\ 320} = 5.31$; $P < 0.0001$) thus allowing further contrasts. Separate one-way ANOVAs for each plant population indicated significant variation in performance among herbivore populations (all $F > 8.88$; all $P < 0.0001$). Post hoc contrasts showed that herbivores from Pedregal achieved higher performance than herbivores from Teotihuacan and Esperanza when grown on its native plants (Table 2, S2). On the contrary, herbivores from Teotihuacan and Esperanza had lower performance at their native habitats than herbivores from Pedregal (Table 2, S2) indicating a pattern of local maladaptation. These differences in performance between sympatric and each allopatric combination resulted in a continuum of local adaptation from locally adapted through not adapted to maladapted populations. Specifically, using our coefficient of local adaptation (β), Pedregal was the most locally adapted population (β = 1.66) followed by Tula (β = 0.58) while Esperanza and Teotihuacan were locally maladapted to their local hosts (β values of -0.94 and -10.85 respectively).

On the other hand, we did not detect a significant interaction between herbivore and plant population for plant seed production suggesting the absence of plant local adaptation (Table 1). That is, under the same environmental conditions, plants eaten by their native herbivores and foreign ones attained similar levels of seed production. Thus, we found no evidence of a biotic component promoting plant local adaptation. However, we detected a significant interaction for damage and tolerance to herbivory (Table 1). These results indicate that tolerance expression is influenced not only by the amount of damage plants experienced but also by the identity of the herbivore producing the damage.

Discussion

Overall, we found genetic differentiation among herbivore and plant populations in traits involved in the interaction. Additionally, there was variation among herbivore populations in their extent of local adaptation to their hosts. In contrast, we found no evidence of plant local adaptation to its natural specialist herbivore. Interestingly, the capacity to compensate for herbivore damage (tolerance) was higher in those plant populations where the native herbivores also showed higher levels of local adaptation (Fig. 1) suggesting that tolerance is an interaction trait whose expression may depend on the extent of local adaptation of the natural enemies (Jokela *et al*., 2000).

As predicted by the geographic mosaic theory of coevolution (Thompson, 2005), there was variation in the extent of herbivore local adaptation –from locally adapted through not adapted to maladapted. On the other hand, we found no evidence of plant local adaptation. The latter result is not unexpected given that plant performance is governed by the ability to cope with both biotic and abiotic local conditions (Biere & Verhoeven, 2008). Although plant local adaptation to abiotic factors, such as soil characteristics and climatic conditions, is well documented (Joshi *et al*., 2001; Macel *et al*., 2007) relatively little is known about the potential contribution of biotic factors, such as herbivores or pathogens, to plant local adaptation (Sork *et al*., 1993; Abdala-Roberts & Marquis 2007; Crémieux *et al*., 2008). Understanding the patterns of plant local adaptation requires a better integration of studies on plant adaptation to their abiotic and biotic environment (Biere & Verhoeven, 2008).

Which trait represents a good measure of fitness in local adaptation studies is not a trivial question. Using a single fitness measure without accounting for variation in life history traits and their relationships may provide a poor estimate of true fitness (see Laine, 2008). Here, we evaluated herbivore local adaptation using the variable of performance –which integrates survival with other important life history characters involved in the interaction with plants. Most studies on herbivore local adaptation have used survival as the only measure of fitness (Greischar & Koskella, 2007). Whether survival or performance are true estimates of *L. trilineata* fitness is difficult to say. However, the pattern of herbivore local adaptation shown here remain the same when using only survival (not shown), suggesting that this pattern of local adaptation may not be conditioned to specific estimates of fitness. Additionally, we estimated herbivore population growth rate and sex ratio. However, we only detected a significant herbivore by plant interaction for the sex ratio variable (Table 1). The absence of a significant interaction for growth rate could be explained because we controlled the initial herbivore density decreasing intraspecific competition among larvae. Because all larvae were randomly assigned to the plants, it is unlikely that the sex ratio was biased since the beginning of the experiment. Thus, the variation in sex ratio could be the result of differential survival between the sexes during the larval stage as found in other studies (Alstad, 1998).

It has been suggested that local plant genotypes may succumb less to abiotic stress than foreign genotypes. Thus, plants may present higher resistance and/or tolerance at their native site because of higher ability to capture limiting resources (White, 1984). Therefore, in a reciprocal transplant experiment, higher defenses of native genotypes would represent an evolved response to divergent selection promoted by abiotic rather than by biotic environmental conditions. However, we found that plant tolerance to herbivory was higher in those populations where the native herbivores showed higher levels of local adaptation (Fig. 1) suggesting that the expression of tolerance is may be partially determined by the biotic environment. In many plant-enemy interactions, the induction of plant responses after damage is triggered when the plant "senses" the presence of its enemies (Karban & Baldwin, 1997). Sensing is accomplished by one or more types of cellular receptors that are triggered by elicitor substances or other signals produced by the enemies. It is plausible that the plant benefits from rapidly responding through a compensatory response when the herbivore is locally adapted. Also, because the tolerance response may depend on the intensity of damage (Fornoni & Núñez-Farfán, 2000), and locally adapted insect populations imposed higher levels of leaf damage, this may have elicited a more intense compensatory response. If plants can recognize its native adapted natural enemies increasing their tolerance response, then this defense strategy could compensate for a potential higher amount of damage of natural enemies. Therefore, we concluded that the expression of plant tolerance results from the interaction between the genotype of the plant, the genotype of the herbivore and the environment in which the plant grows (an G × G × E interaction) (Restif & Koella, 2003). In this sense, while artificial defoliation allows the evaluation of fitness consequences of leaf area loss, natural damage will trigger a more complex induced response (tolerance) that deserve further examination.

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Table 1. Results from the ANOVAs and ANCOVAs for the traits measured to test for herbivore and plant local adaptation. *F*-values are shown along with statistical significance. Initial plant size was only included as a covariate in the analyses for plant damage and seed production.

	Sources of Variation			
Herbivore Traits	**Herbivore Population**	**Plant Population**	**Herbivore Population × Plant Population**	**Initial plant size**
Individual Performance	46.88***	9.71***	6.78***	-----
Population Growth Rate	4.14*	1.02	0.11	-----
Sex Ratio	1.72	1.15	18.31*	-----
Plant Traits				
Relative Damage	81.67***	5.92***	3.57***	0.05
Seed Production	8.57***	8.22***	1.15	64.18***
Tolerance to Herbivory	4.59*	11.32**	10.41**	-----

* P < 0.05; ** P < 0.005; *** P < 0.0005

Table 2. Local-foreign criterion for herbivore performance: differences between native and foreign herbivores within the same plant population. For each plant population, contrasts were used to ask whether mean performance of sympatric herbivores was significantly different from each allopatric herbivore population. Contrasts are made within rows, that is, the performance of the four herbivore populations were compared within the same plant population. Grey cells correspond to the sympatric combinations.

Plant Populations	**Herbivore Populations**			
	Differences in herbivore performance between the sympatric and each allopatric plant population			
	Pedregal	Teotihuacan	Tula	Esperanza
Pedregal	0.0361 ± 0.0044	0.0112 ± 0.0015 (**0.0002**)	0.0283 ± 0.0028 (0.3387)	0.0090 ± 0.0008 (< **0.0001**)
Teotihuacan	0.0453 ± 0.0071 (**0.0002**)	0.0052 ± 0.0007	0.0085 ± 0.0016 (0.9925)	0.0179 ± 0.0027 (0.4596)
Tula	0.0194 ± 0.0045 (0.7809)	0.0016 ± 0.0004 (0.2545)	0.0155 ± 0.0016	0.0166 ± 0.0023 (0.9935)
Esperanza	0.0320 ± 0.0059 (**0.0265**)	0.0104 ± 0.0012 (0.8292)	0.0179 ± 0.0029 (0.9816)	0.0153 ± 0.0018

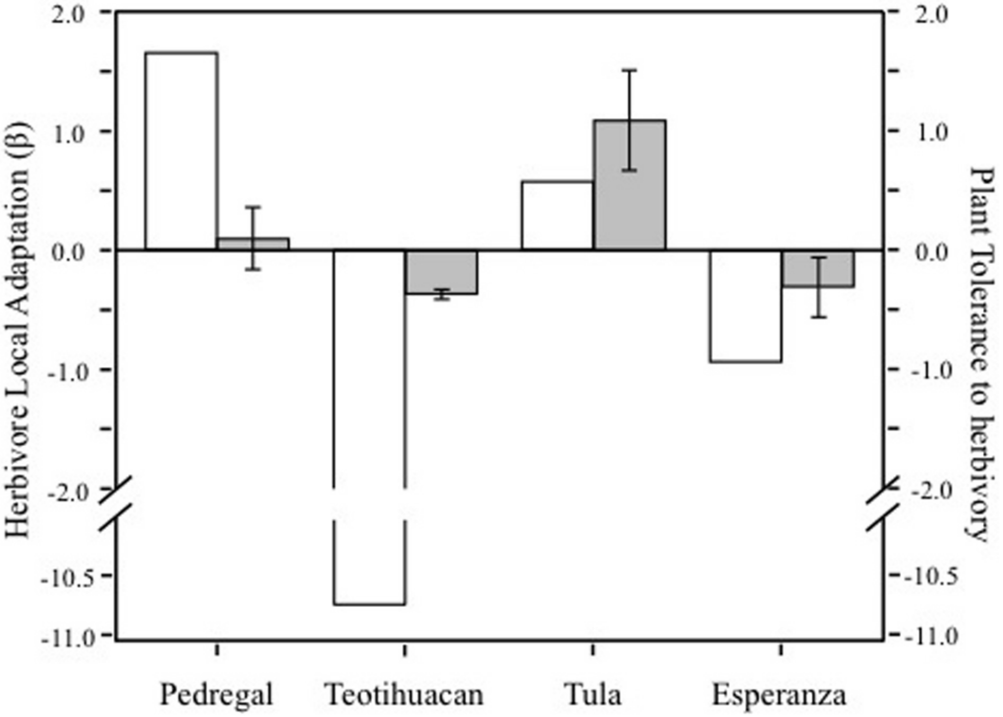

Figure 1. Variation in the extent of herbivore local adaptation (β) and the expression of plant tolerance to herbivore damage. Open and closed bars represent herbivore adaptation and plant tolerance respectively. Errors for herbivore adaptation not shown. Note that tolerance is higher in those populations where the native herbivores are more adapted to their host plants.

Supplementary Material

Table S1. Summary of descriptive statistics (means ± SE) for the herbivore and plant traits measured in the local adaptation experiments. Standard errors are shown within parenthesis. Superscripts indicate grouping according to a Tukey's comparison. Different letters indicate differences between populations ($\alpha < 0.05$).

	Herbivore Traits			**Plant Traits**		
By Herbivore Population	*Individual Performance*	*Population Growth Rate*	*Sex Ratio*	*Relative Damage*	*Fitness (seeds)*	*Tolerance to Herbivory*
Pedregal	0.034 ± 0.003 ^{A}	3.68 ± 0.79 ^{A}	1.17 ± 0.17 ^{A}	0.60 ± 0.03 ^{A}	691.90 ± 35.80 ^{B}	-0.17 ± 0.28 ^{B}
Teotihuacan	0.009 ± 0.001 ^{C}	2.68 ± 0.65 ^{AB}	0.98 ± 0.18 ^{A}	0.42 ± 0.02 ^{B}	770.23 ± 36.55 ^{AB}	0.02 ± 0.28 ^{B}
Tula	0.021 ± 0.002 ^{B}	0.60 ± 0.52 ^{B}	1.11 ± 0.16 ^{A}	0.15 ± 0.01 ^{C}	881.83 ± 31.57 ^{A}	0.76 ± 0.59 ^{A}
Esperanza	0.015 ± 0.001 ^{BC}	1.15 ± 0.78 ^{AB}	1.07 ± 0.16 ^{A}	0.37 ± 0.02 ^{B}	731.79 ± 37.05 ^{B}	-0.10 ± 0.27 ^{B}
By Plant Population						
Pedregal	0.026 ± 0.002 ^{AB}	1.75 ± 0.96 ^{A}	1.12 ± 0.16 ^{A}	0.42 ± 0.03 ^{A}	664.19 ± 31.43 ^{B}	0.10 ± 0.26 ^{AB}
Teotihuacan	0.028 ± 0.004 ^{A}	1.17 ± 0.62 ^{A}	1.13 ± 0.05 ^{A}	0.42 ± 0.04 ^{A}	686.63 ± 28.63 ^{B}	-0.37 ± 0.04 ^{B}
Tula	0.016 ± 0.002 ^{B}	2.47 ± 1.19 ^{A}	1.16 ± 0.24 ^{A}	0.31 ± 0.03 ^{A}	878.04 ± 42.16 ^{A}	1.09 ± 0.42 ^{A}
Esperanza	0.021 ± 0.003 ^{AB}	2.71 ± 0.84 ^{A}	0.93 ± 0.15 ^{A}	0.38 ± 0.03 ^{A}	846.90 ± 32.69 ^{A}	-0.31 ± 0.25 ^{B}

Table S2. Means (± SE) of traits measured in each herbivore-plant population combination treatment in the local adaptation study. The standard errors for the variables of herbivore population growth rate and sex ratio as well as plant tolerance to herbivory are not shown due to lack of replicates.

		Herbivore Traits			**Plant Traits**		
Herbivore Population	**Plant Population**	*Individual Performance*	*Population Growth Rate**	*Sex Ratio**	*Relative Damage*	*Fitness (seeds)*	*Tolerance to Herbivory**
Pedregal	Pedregal	0.036 ± 0.004	3.03	1.49	0.62 ± 0.03	523.42 ± 59.26	-0.43
Pedregal	Teotihuacan	0.045 ± 0.007	2.25	1.22	0.72 ± 0.03	640.17 ± 55.94	-0.30
Pedregal	Tula	0.019 ± 0.005	5.93	1.29	0.42 ± 0.06	885.92 ± 71.91	0.65
Pedregal	Esperanza	0.032 ± 0.006	3.51	0.7	0.62 ± 0.04	718.08 ± 61.49	-0.59
Teotihuacan	Pedregal	0.011 ± 0.001	3.77	1.23	0.54 ± 0.03	655.83 ± 50.14	0.04
Teotihuacan	Teotihuacan	0.005 ± 0.001	2.24	1.03	0.46 ± 0.04	662.33 ± 68.87	-0.38
Teotihuacan	Tula	0.002 ± 0.001	1.05	0.46	0.33 ± 0.05	881.75 ± 82.69	0.82
Teotihuacan	Esperanza	0.010 ± 0.001	3.65	1.2	0.34 ± 0.05	881.00 ± 65.16	-0.32
Tula	Pedregal	0.028 ± 0.003	0.01	0.71	0.21 ± 0.03	796.17 ± 56.85	0.80
Tula	Teotihuacan	0.009 ± 0.002	0.05	1.07	0.10 ± 0.02	745.08 ± 35.64	-0.48
Tula	Tula	0.016 ± 0.002	2.14	1.5	0.14 ± 0.03	948.92 ± 74.23	2.33
Tula	Esperanza	0.018 ± 0.003	0.19	1.17	0.14 ± 0.02	1037.17 ± 45.32	0.40
Esperanza	Pedregal	0.009 ± 0.001	0.22	1.04	0.33 ± 0.07	681.33 ± 64.82	0.07
Esperanza	Teotihuacan	0.018 ± 0.003	0.15	1.2	0.39 ± 0.04	698.92 ± 65.54	-0.30
Esperanza	Tula	0.017 ± 0.002	0.77	1.41	0.35 ± 0.05	795.58 ± 108.56	0.55
Esperanza	Esperanza	0.015 ± 0.002	3.47	0.65	0.42 ± 0.03	751.33 ± 49.43	-0.72

Capítulo 4

La selección dependiente de la frecuencia mantiene variación en los niveles de resistencia y tolerancia

Frequency-dependent selection maintains variation in host resistance and tolerance

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Keywords: defense polymorphisms, frequency-dependent selection, host-enemy interactions, resistance, tolerance

Summary

It has been suggested that natural enemies have the potential to maintain genetic variation in defensive traits by creating frequency dependent selection (FDS) on their hosts. The aims of this study were to evaluate (1) if resistance and tolerance are under FDS, and (2) if herbivore density –and thus, damage incidence– are mechanisms through which herbivores exert FDS on their hosts. To answer these questions, the frequency of resistant and tolerant hosts within experimental plots was manipulated. Three frequency treatments were used: 0.1, 0.5 and 0.9 of resistant hosts in plots where the alternative host was tolerant. At the end of the experiment, plant damage and fitness as well as final herbivore density were estimated. We found that resistance was under positive FDS, while tolerance was under negative FDS. Changes in herbivore density –and thus, in damage incidence– can account for these results. Overall, our results suggest that intermediate levels of resistance and tolerance can be maintain in natural populations because of negative FDS acting on tolerance.

Introduction

Understanding the maintenance of genetic variation in natural populations remains as a major question in evolutionary biology. In host-enemy interactions, hosts usually present genetic variation for both resistance (Fritz & Simms, 1992) and tolerance (Núñez-Farfán *et al*., 2007) mechanisms. Moreover, individual hosts commonly allocate resources to both defense mechanisms (Fornoni *et al*., 2004a; Leimu & Koricheva, 2006). Frequency dependent selection (FDS) and environmental heterogeneity are the most commonly invoked mechanisms to explain variation in host defenses (Frank, 1996). It has been proposed that natural enemies have the potential to maintain genetic variation among resistance traits by creating negative FDS on their hosts (Barrett, 1988). Over time, FDS results in time-lagged oscillations between coevolving host and enemy genotypes –which is the essence of the Red Queen Hypothesis (Lively, 1996). Negative FDS assumes that rare host genotypes could be favored because they are less likely to be attacked and thus, will have higher fitness when rare. As the rare host genotypes become common, enemies capable of infecting them will be favored and thus will increase in frequency reducing the fitness of common host genotypes (Antonovics & Ellstrand, 1984).

There are relatively few studies supporting enemy-driven FDS on their hosts (Dybdahl & Lively, 1998; Roy, 1998; Brunet & Mundt, 2000; Siemens & Roy, 2005), and besides a couple of studies, this hypothesis has been initially formulated and usually tested in plant-pathogen systems (but see Siemmens & Roy, 2005). Additionally, these studies have focused on resistance/susceptible polymorphisms without considering tolerance traits. To our knowledge, only one theoretical study has modeled how the frequency of both resistant and tolerant genotypes could affect the incidence of enemy-attack and thus, the fitness associated with each defense strategy (Roy & Kirchner, 2000). This model suggests that resistance and tolerance traits can have different evolutionary dynamics. On one hand, resistance traits could converge toward a polymorphic equilibrium because as resistant hosts become more frequent, the overall incidence of attack in the population decreases, reducing the advantage of being resistant (*i.e.* resistance is considered to be under negative FDS). On the other hand, tolerance traits could go to fixation because as tolerant hosts become more prevalent, the risk and overall incidence of attack increases for both resistant and tolerant hosts. That is, at higher incidence of attack tolerant hosts will have a fitness advantage over resistant hosts because they can compensate for loss area (*i.e.* tolerance is considered to be under positive FDS). To date, we are aware of no study that has manipulated the frequency of both resistant and tolerant hosts to test these assumptions.

The assumptions of the model described above will hold whenever resistance reduce the amount of damage and tolerance reduce the fitness consequences of this damage. However, changes in natural enemies´ density could modify both the assumptions and predictions of this model. It has been demonstrated that tolerance, unlike resistance, does not negatively affect natural enemy survival or performance (Espinosa & Fornoni, 2006). This diferential effect at the individual level could also change the natural enemies´ demography. Empirical evidence shows that host resistance reduces the abundance of natural enemies within a population (Underwood & Rausher, 2000; Thaler *et al.*, 2001). Thus, it seems reasonable to suppose that in those populations where the frequency of resistant hosts is high the enemy population size could decrease. On the other hand, because tolerance does not exert a negative effect upon enemy survival, it is expected that the population size of the natural enemies increases with the frequency of tolerant hosts within the population (Roy & Kirchner, 2000; Espinosa & Fornoni, 2006). As the enemy population size increases it is expected that the mean level of damage hosts experience increases as well. When natural enemies reach high densities (*i.e.* within a single seasons), it is probable that the advantage of rare resistant genotypes decreases because the probability of being found and attack could increase. In the same sense, the capacity to tolerate damage could decrease with an increment in damage levels (Fornoni & Núñez-Farfán, 2000; Fornoni *et al*., 2003; Hutha *et al*., 2003). Therefore, finding negative or positive FDS acting on host defenses could depend on the population demography of the natural enemies.

Here, we tested if host resistance and/or tolerance are under FDS in a plant-herbivore system (*Datura stramonium* - *Lema trilineata*). In addition, we evaluated if the incidence of damage and herbivore density are mechanisms through which natural enemies exert FDS on their hosts. To answer these questions, the frequency of plant genetic families that express relatively high resistant and low tolerance against those with low resistance and high tolerance was experimentally manipulated and exposed to an initial similar density of their natural herbivore.

Material and Methods

D. stramonium L. (Solanaceae) is the host of the folivorous leaf beetle *L. trilineata* (Olivier) (Chrysomelidae) in Central Mexico. All but the pupa stage of this herbivore occur on the leaf tissue of its host, where it can survive and reproduce for up to four generations per season (*pers. obs.*). Previous studies have shown that herbivore damage reduces *D. stramonium* fitness (Valverde *et al.*, 2001) while plant resistance has a negative effect on *L. trilineata* performance (Espinosa & Fornoni, 2006). Plant material used in this study was gathered from a population in Central Mexico (18°N, 99°W), for which the existence of additive genetic variation and genotypic selection acting on both resistance and tolerance to folivorous herbivores have previously been demonstrated under natural field conditions (Fornoni *et al.*, 2003, 2004b). From this data set, six genetic families (full-sibs) were selected according to its defense strategy: three families with high resistance and low tolerance and three families with low resistance and high tolerance. These two groups differ in resistance ($F_{1,\,23}$ = 12.78, P = 0.0016) and tolerance ($F_{1,\,21}$ = 5.48, P = 0.0292), but no variation within groups was detected for both defenses. Thus, families were considered replicates of these two contrasting defensive phenotypes representing a continuous expression of resistance and tolerance.

During the summer of 2008, seeds were germinated to obtain 60 plants per family (N = 360). Two weeks after germination, plants were individually transplanted to 4-liter pots, filled with potting soil, and placed in a greenhouse at the Instituto de Ecología (UNAM). Two months after transplant, plant size (height × number of leaves) was recorded and, afterwards, all plants were taken to a common garden. Three frequency treatments were used: 0.1, 0.5 and 0.9 of resistant genotypes where the alternative host was mostly tolerant. Ten plants of similar size were randomly placed inside mesh-cages (130 × 75 × 100 cm) according to the treatments. There were twelve replicates per treatment. All cages were randomly assigned to four blocks within the common garden. Simultaneously, around 500 larvae of *L. trilineata* were collected from the field and taken to the laboratory (25°C; 12:12 L:D) until adult emergence. When most of the plants started flowering, four pairs of virgin-adults were placed inside each cage and allowed to reproduce, oviposit and consume plants freely. This phenological stage corresponds to the moment at which *L. trilineata* starts consuming its host under natural conditions (*pers. obs.*). The experiment was conducted for two months, until the next generation of herbivores emerged. Because *D. stramonium* presents inbreeding depression (Núñez-Farfán *et al*., 1996), and variation in the extent of autonomous selfing due to herkogamy, all plants were manually out-crossed. This procedure avoids possible sources of variation in fitness among plants not related to our frequency treatments. At the end of the experiment, the following variables were estimated: plant relative damage and fitness as well as herbivore final population density. To estimate relative damage, leaves were first dried for 3 weeks at 25°C, then scanned (HP Scanjet G3110) to obtain digital images and finally measured using SigmaScan Pro 5 (SPSS, 1999). Leaf length was used to estimate the original area (leaf area = 0.329*leaf length^2; $r^2 = 0.98$; Núñez-Farfán & Dirzo, 1994). Relative damage per plant was then estimated as the difference in area. Plant fitness was estimated as the number of seeds produced. Finally, final number of herbivore adults was recorded per cage.

Statistical Analyses. All response variables were analyzed with an ANOVA. The model included the following factors: type of defense (resistant or tolerant), frequency, their interaction and block. Given the relatively high levels of damage experienced, around 25% of the plants did not produce any seeds, thus biasing the distribution of this variable. Therefore, plant fitness was analyzed using the CATMOD procedure of SAS 8.0 (SAS 1999) –which fits linear models to functions of categorical data. Final herbivore density was ln-transformed to improve normality, and the model for this variable included only the factors of frequency and block. The analyses for the variables of plant damage and herbivore density were performed in JMP 7.0 (SAS 2007).

Results

Overall, the mean level of damage experienced by plants was around 50%, which is relatively high given that under natural conditions *D. stramonium* usually presents between 10-60% of leaf damage (Valverde *et al*., 2001). As a consequence, mean plant fitness was relatively low (148.22 ± 8.57 seeds). Additionally, final herbivore density was on average 119.31 ± 16.75 adults per cage. On the other hand, there was no effect of the defense strategy on plant damage and fitness (Table 1). That is, plant damage and fitness does not appear to be dependent on whether an individual plant is resistant or tolerant. On the other hand, the frequency of resistant and tolerant plants within the experimental plots had a significant effect on all the variables measured (Table 1), indicating a strong context-dependent response of damage, plant fitness and herbivore density. Specifically, plant damage was higher at the lower frequency (0.1) of resistant plants (0.56 ± 0.02%) in comparison with the damage experienced at the intermediate (0.5) (0.46 ± 0.02%) and high frequency (0.9) (0.43 ± 0.02%) treatments. (Fig. 1a). Plant fitness was lower at the frequency of 0.1 of resistant plants (81.57 ± 9.77 seeds) but increased at the intermediate (0.5) and high (0.9) frequency of resistant plants (0.5: 157 ± 14.11 seeds; and 0.9: 205.21 ± 17.43 seeds respectively) (Fig. 1b). Interestingly, the pattern observed with plant fitness was opposite to the pattern of the herbivore final density. That is, herbivore density was high at the frequency of 0.1 of resistant plants (22.03 ± 3.85 growth rate) and decreased at the frequencies of 0.5 (13.63 ± 3.42) and 0.9 (9.08 ± 2.72) of resistant plants (Fig. 1c).

Finally, there was a significant interaction between defense and frequency for plant fitness (Table 1). Post hoc comparisons revealed that while the fitness of resistant plants increased with its frequency (*i.e.* positive frequency dependent selection) (Fig. 2a), the fitness of tolerant plants was higher at low frequency of tolerant plants (*i.e.* negative frequency dependent selection) (Fig. 2b).

Discussion

Here, using a plant-herbivore system, we found that resistance was under positive FDS, while host tolerance was under negative FDS, a pattern that is at odds with previous theoretical expectations (Barrett, 1988; Antonovics & Thrall, 1994; Lively, 1996; Roy & Kirchner, 2000; Restiff & Koella, 2004). Differences in herbivore density and damage incidence can help explain these results. Final herbivore density, and thus the amount of damage plants experienced, was higher when the frequency of resistant plants was low, and both variables decreased as the frequency of resistant plants within the experimental populations increased. This pattern suggests that host resistance reduced herbivore load in the context of this experiment, and that negative FDS acting on host tolerance can explain the maintenance of intermediate levels of tolerance found in natural populations. Overall, our results suggest that mixed defensive strategies can be maintain in natural populations because of negative FDS acting on tolerance.

Empirical studies have shown that host resistance reduces the abundance of natural enemies (Underwood & Rausher, 2000; Thaler *et al.*, 2001). Accordingly, we found that herbivore density –and thus, the mean amount of damage– was lower in those experimental populations where the frequency of resistant plants was high. This result indicates that resistance can also decrease the incidence of attack for both resistant and tolerant families. Thus, an advantage associated with commonness is expected among resistant genotypes whenever resistance reduces herbivore damage (Siemens & Roy, 2005). Whenever resistance reduces damage, it is expected that resistance will continue to spread through the host population. On the other hand, we also found that host tolerance was under negative FDS. That is, the fitness of tolerant plants was high when rare and decreased with its frequency. Given that when resistant genotypes were common –and thus, tolerant genotypes were rare–, the overall level of damage decreased within the population, it is plausible that tolerant genotypes achieve greater fitness advantage because they can completely compensate the relatively low amount of damage. However, as the frequency of tolerant genotypes increases –and thus, resistant plants become rare– the herbivore density and mean damage levels increases as well. Empirical studies indicate that tolerance capacity decreases with the amount of damage (Fornoni & Núñez-Farfán, 2000; Fornoni *et al*., 2003; Hutha *et al*., 2003). Thus, it is expected that tolerance benefit decreases with its frequency. Hence, the maximal benefit of tolerance would be attained at low to moderate levels of damage within the population (Garrido & Fornoni, *unpublished*, see Chapter 1). This dynamic could explain the presence of intermediate levels of tolerance within natural populations (Núñez-Farfán *et al*., 2007).

Theory predicts that resistance traits can not become fixed because as the frequency of resistant genotypes increases, the overall damage level will be too low that the benefit of being resistant will decrease (May & Anderson, 1983; Antonovics & Thrall, 1994). That is, whenever resitance carries a cost, herbivory can not be eliminated by natural selection for host resistance. Our results suggests that before resistance can eliminate damage, tolerant genotypes will gain fitness benefits that will allow them to increase in frequency. In other words, tolerant genotypes could invade a population eventhough resistant genotypes still express higher benefits than costs. As the frequency of tolerant hosts increases, the overall damage level increases as well. Because tolerance benefit decreases with damage any other resistant genotype capable of avoiding damage will now be favored and so on. Therefore, exclusive allocation of resources to tolerance and exclusive allocation to resistance are expected to be evolutionary unstable. Intermediate levels of resistance and tolerance can thus be maintained because of negative FDS acting on tolerance.

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Table 1. Results of the ANOVAs for the variables of plant relative damage and fitness and herbivore final density. Sample sizes were N = 360 for plant damage and fitness and N = 36 for herbivore final density. Values in bold were significant for a $P < 0.05$.

Variable	Source of Variation	d.f.	SS	F / χ^2	P
Plant relative damage	Defense	1	0.0006	0.0154	0.9014
	Frequency	2	0.3974	4.9468	**0.0076**
	Frequency × Defense	2	0.0732	0.9115	0.4029
	Block	3	3.3644	27.919	**< 0.0001**
	Error	351	14.0991		
Plant fitness	Defense	1	−	2.46	0.1166
	Frequency	2	−	63.75	**< 0.0001**
	Frequency × Defense	2	−	26.45	**< 0.0001**
	Block	3	−	46.64	**< 0.0001**
Herbivore final density	Frequency	2	66288.72	12.45	**0.0001**
	Block	3	207384.75	26.03	**< 0.0001**
	Error	30	79679.17		

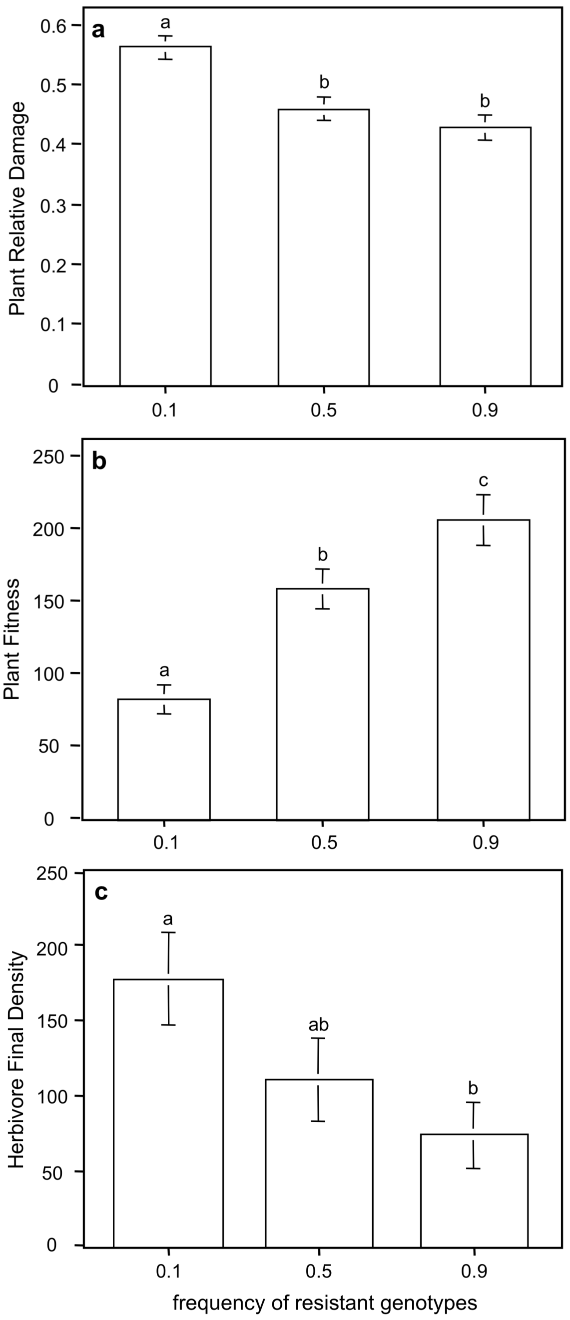

Figure 1. Effects of the frequency of resistant genotypes within the population on (**a**) plant damage, (**b**) plant fitness, and (**c**) herbivore final density. Bars labeled with different letters indicate statistically significant differences.

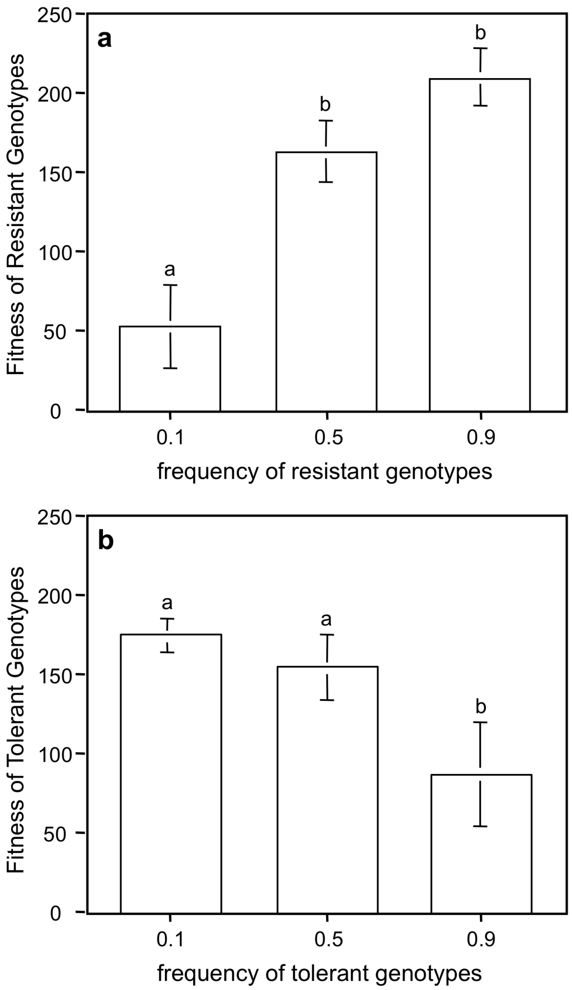

Figure 2. Fitness of (**a**) resistant and (**b**) tolerant genotypes as a function of its frequency. Host resistance and tolerance were under negative and positive frequency-dependent selection respectively. Bars labeled with different letters indicate statistically significant differences.

Capítulo 5

Variación en la colonización por micorrizas modifica la expresión de la tolerancia a la defoliación

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Variation in arbuscular mycorrhizal fungi colonization modifies the expression of tolerance to aboveground defoliation

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Summary

1. Plant association with arbuscular mycorrhizal fungi (AMF) has been considered as a factor increasing plant tolerance to herbivory. However, this positive effect could decrease with colonization density if the benefit:cost ratio of the AMF-plant association changes. We measured plant performance and tolerance to defoliation across a gradient of commercial AMF (*Glomus* sp.) inoculum concentration.
2. Six plant genetic families were grown under greenhouse conditions and subjected to five increasing levels of AMF inoculum concentration and to the presence/absence of 50% artificial damage following a full-factorial design.
3. AMF colonization increased linearly with inoculum concentration while foliar area, root mass, flowering phenology and seed production expressed non-linear functions. Plant genetic variation in the benefit function of AMF colonization was also detected. We show a negative interaction between AMF concentration and plant tolerance to defoliation.
4. *Synthesis*. The negative correlation between plant tolerance and AMF concentration suggests that defoliation can reduce AMF benefits and that natural variations in AMF can limit the evolution of optimum levels of tolerance. Moreover, genetic variation in the shape of the reaction norms to AMF in the presence/absence of defoliation suggests that plants may evolve in response to variation in densities of AMF and herbivores.

Introduction

Recently, we have gained an increasing appreciation of how multispecies interactions can act synergistically or antagonistically to alter the cological and evolutionary outcomes of interactions in ways that differ fundamentally from outcomes predicted by pairwise interactions (Strauss & Irwin 2004). In particular, aboveground-belowground feedbacks play a fundamental role in controlling the abundance and distribution of plants and their interactions with other community members (Van der Putten *et al.* 2001; Wardle *et al.* 2004). Soil biota contributing to feedbacks include arbuscular mycorrhizal fungi (AMF), which colonize the roots of a great diversity of vascular plants. AMF obtain photosynthates from plants while enhancing nutrient uptake by the host plant; AMF may also improve plant performance when plants are attacked by pathogens (reviewed in Borowicz 2001) or by insect herbivores (reviewed in Gange 2007).

The AMF-plant relationship, while often considered mutualistic, can entail costs. The amount of carbon allocated to AMF is estimated to range from 4 to 20% of a plant's total C budget (Smith & Read 1997). Scattered throughout the literature are examples of the conditionality of this relationship exemplified by a continuum of the effects of AMF colonization on hosts from positive, through null to negative (Francis & Read 1995; Johnson *et al.* 1997; Jones & Smith 2004). For any particular host plant−fungus combination, the whole gamut of the continuum may be expressed, depending on environmental conditions and genotypes involved (reviewed in Johnson *et al.* 1997). Moreover, it has been suggested that the benefit of a plant associating with fungal symbionts depends not only on the presence of AMF, but also on colonization density

(Gange & Ayres 1999). Thus, the plant performance continuum may reflect both the identity and the density of AMF colonization.

Feedbacks between above- and belowground interactions may occur when other interactors, like herbivores or pollinators, compete for plant resources with AMF. In the presence of herbivores, plants lose not only foliar area and water but also their carbon−fixing capacity through loss of photosynthetic tissue (Gange 2007). Because herbivores and AMF both extract energy from plants, albeit in different forms, they are likely to interact (Gehring & Whitham 1994), especially when some resources are limited (*e.g.* carbon, phosphorus, nitrogen, etc.). If AMF and defoliation creates a resource limiting environment for the plant, an increment in the density of AMF colonization would constrain the ability of the plant to reduce the negative effect of defoliation in terms of fitness (*i.e.* tolerance). In contrast, whenever AMF provides a surplus of resources for the host plant to decrease the costs of tissue lost by defoliation, a positive relation between the density of AMF colonization and the plant tolerance to defoliation would be expected (Borowicz 1997; Kula *et al*. 2005; Bennett *et al*. 2006; Bennett & Bever 2007). Given that the benefit gained by the host plant from the association with AMF can depend on colonization density (Gange & Ayres 1999), we specifically evaluated plant tolerance to aboveground defoliation across a gradient of AMF inoculum concentration.

In this study, we measured foliar area, root mass, flowering phenology, seed production, total colonization and arbuscules percentage across a range of AMF inoculum concentrations, and across plant genotypes in both the presence and absence of defoliation. In addition, we evaluated whether the expression of tolerance to defoliation (differences in seed production between damaged and undamaged full sib plants) varied along a gradient of inoculum concentration. Here, we show for the first time a negative interaction between AMF inoculum concentration and the expression of tolerance to aboveground defoliation, suggesting a negative below-aboveground interaction.

Material and Methods

Datura stramonium L. (Solanaceae) is a cosmopolitan summer annual species that inhabits disturbed areas and borders of cultivated fields (Weaver & Warwick 1984; Núñez-Farfán & Dirzo 1994). A complete description of this species can be found elsewhere (Fornoni & Núñez-Farfán 2000). Plant material used in this study was gathered from a population of *D. stramonium* in Central Mexico (18°N, 99°W). Based on a previous study that detected significant additive genetic variation in plant defenses (tolerance and resistance) to herbivory under natural conditions (Fornoni *et al*. 2003, 2004) we specifically choose six genetic families that differ in tolerance but not in resistance (see Espinosa & Fornoni 2006). To minimize environmental maternal effects, each family was self-pollinated under greenhouse conditions for two generations prior to conducting the experiment. In February 2007, seeds from each family were germinated in sterile vermiculite in a greenhouse at UC Davis (California, USA) to obtain 40 plants per family (N = 240). Background soil consisted of a 6:4 mixture of commercial potting soil (MetroMix, Sun Gro Horticulture Canada CM Ltd.) and a fine sandy loam collected from Yolo County (California, USA) steam sterilized twice. Concentration of Nitrogen and Phosphorus in soil were 0.76% and 0.05% respectively. Pots of 1200 ml each were filled with the background soil and inoculated with commercial mycorrhizal inoculum

(MycoApply® Endomycorrhizal granular inoculum containing spores of four *Glomus* species: *G. intraradices*, *G. mosseae*, *G. aggregatum* and *G. etinucatum*) obtained from Mycorrhizal Applications (Grants Pass, Oregon, USA) according to five inocula treatments.

Inocula treatments consisted of a gradient in the concentration of inoculum within the pot: 0 ml, 42 ml, 84 ml, 167 ml and 333 ml. That is, the proportion of sterile background soil and inoculum was manipulated without changing total soil volume. We used a gradient from zero through 333 ml of inoculum in 1.2 liter pots, which produced the maximum levels of root colonization in an earlier study (Bennett & Bever 2009). This gradient provided a range of root colonization from 0 to 31% (see Results). The amount of inoculum added to each pot is not likely to change soil structure significantly, given the highly porous background soil used in the experiment. Inoculum was mixed into the background soil to ensure maximum contact between roots and inoculum. Immediately after inoculation plants were transplanted. There were 8 plants per inoculum concentration per family. All pots were randomized following a complete block design within the greenhouse (25°C, 60% humidity and 12:12 L:D).

Three weeks after transplanting, half the plants (N = 4) in each inoculum treatment were subjected to weekly rounds of 50% leaf defoliation of each expanded new leaf using a hole-punch. In the field, plants are attacked by leaf beetles -which feed in a shot-hole pattern-, and can damage up to 80% of leaf area (Núñez-Farfán & Dirzo 1994). Thus, our defoliation treatment mimics natural damage patterns reasonably well, even though hole punches lack chemical cues provided by beetle saliva. Besides the apparently absence of alkaloid induction after damage in *D. stramonium* (Shonle & Bergelson 2000), there is a plethora of defense responses that are likely to be induced after herbivory damage. To reduce this source of variation, we choose artificial damage to ensure that all plants lose the same amount of leaf area. Thus, it is more likely that our artificial damage elicits tolerance responses triggered by alteration of source-sink relations through the loss of leaf area. The relationship between leaf length and leaf area (leaf area = 0.329*leaf length^2; $r^2 = 0.98$; Núñez-Farfán & Dirzo 1994) was used to estimate the absolute amount of area to be removed under the defoliation treatment. The experiment finished after 17 weeks, when all plants had stopped reproducing. During the whole experiment plants were not fertilized.

The following variables, involved in the interaction with AMF and damage, were measured or estimated: foliar area, root mass, days to the first flowering, seed production, total colonization, arbuscules percentage and tolerance to defoliation. Foliar area was estimated at week 12 just before plants started flowering. The same relationship between leaf length and area mentioned above was used to estimate total foliar area per plant. Following harvest, plant roots were washed free of soil dried at 35°C for three days and weighed using an electronic balance (OHAUS). To estimate total colonization, a 2 g sample of dried roots from each plant was hydrated in water overnight and stained with Trypan Blue. Colonization levels were assessed using the gridline intersection method (McGonigle *et al*. 1990). Approximately 120 intersections per slide were recorded to give a measure of percentage root length colonized. A site was considered colonized if hyphae, vesicles, arbuscules or spores were present. Arbuscules percentage was then estimated as the ratio of arbuscules between total AMF colonization, thus this variable represents a fraction of the total AMF infection. Non-AMF were detected in roots at levels below 3% (Bennett, *unpublished data*) corresponding to expected airborne greenhouse contamination levels. Finally, to estimate tolerance, seed production was first standardized per plant genotype to control for differences in vigor among families. Then, given that defoliation was experimentally imposed, we estimated tolerance as the difference in standardized seed production between damaged and undamaged replicates for each plant genotype (Strauss & Agrawal 1999). This way of estimating tolerance avoids possible sources of variation that are usually incurred when damage is imposed by natural herbivores (Tiffin & Inouye 2000; Lehtilä 2003). For instance, herbivore preference for particular host genotypes can generate differences in damage and thus in tolerance.

Statistical Analysis

The effect of a gradient in AMF inoculum concentration on the plant response variables was analyzed with an ANOVA. The model included the following predictors of performance: family, defoliation, inoculum and all the interactions between these factors. Inoculum concentration was considered as a continuous effect; thus, quadratic effects were included in the analyses. All other effects were considered fixed. Root mass was root-square transformed to improve normality. Days to flowering were analyzed as a survival analysis following the Cox regression model (Cox 1972). Because the distribution of the variables of seed production, total colonization and arbuscules percentage best fit a Poisson distribution, we used Generalized Linear Model (GLM) with the Log link function option. The analyses for these three variables were corrected for overdispersion. Because all these measurements were taken on the same replicates, a Bonferroni correction to maintain the overall experiment-wise error rate was performed.

Finally a regression analysis between inoculum concentration and plant tolerance to defoliation was performed. All analyses were performed in JMP 7.0 (SAS 2007).

Results

There was genetic variation in foliar area, flowering day, seed production and arbuscules percentage (Table 1). The defoliation treatment had a significant effect on all the variables measured except AMF colonization (Table 1). In particular, plants under the defoliation treatment had 30% more foliar area (98.7 ± 2.7 cm^2) than undamaged plants (68.4 ± 2.4 cm^2). This increase in foliar area was, at least in part, the result of more leaves being produced by defoliated plants relative to non-defoliated ones ($F_{1,\ 238} = 13.64$; $P = 0.0003$), suggesting compensation for foliar area lost. On the other hand, plants under the defoliation treatment had 24% less root mass (0.71 ± 0.02 gr.) than undamaged plants (0.93 ± 0.06 gr.). Damaged plants flowered on average 4 days later (88.3 ± 1.4 days after transplant) than undamaged plants (84.6 ± 1.3 days after transplant). Finally, damaged plants produced 30% fewer seeds (75.2 ± 5.5 seeds) than undamaged plants (106.8 ± 7.1 seeds).

Interestingly, there was variation among families in the effect that defoliation had on arbuscules percentage (Defoliation × Family interaction; Table 1). There was a positive linear relationship between inoculum concentration and AMF total colonization ($r = 0.50$; $P < 0.0001$). In general, mycorrhizal fungi increased plant performance. Specifically, there was a positive relationship between inoculum concentration and foliar area, root mass, seed production, and colonization but a negative one with flowering day (Table 1; Fig. 1). We also detected a significant quadratic effect of inoculum upon all variables measured but colonization (Table 1; Fig. 1). The quadratic decelerating function between inoculum concentration and plant performance indicates an optimum level of AMF concentration (167 ml of inoculum) that maximizes AMF benefit (Fig. 1). Importantly, significant genetic variation in plasticity among families in the linear and non-linear component of the relationship between inoculum concentration and plant performance was detected for all the variables measured but colonization (Inoculum and Inoculum^2 × Family interactions; Table 1). Moreover, a significant interaction between AMF and defoliation was detected for all variables but foliar area and arbuscules percentage (Inoculum and Inoculum^2 × Defoliation interactions; Table 1) indicating that plant performance was affected by both environmental factors. In general, defoliation reduced the effect that the fungi had on root mass, flowering day, seed production and total colonization (Fig. 2). In particular, plants under the defoliation treatment had, on average, less seeds across the whole gradient of inoculum in comparison with undamaged plants (Fig. 2c), suggesting that herbivore damage could diminish the beneficial effects of AMF on plant seed production. In addition, we found evidence of genetic variation in the linear and quadratic components of the norm of reaction for all variables but colonization (Inoculum and Inoculum^2 × Defoliation × Family interactions; Table 1; Fig. 3). Specifically, there were differences among genotypes in their performance response that are conditional on AMF inoculum concentration.

Because there were no differences in tolerance among families when averaged across AMF inoculum concentration levels ($F_{4,\,24} = 0.72$; $P = 0.6149$), at the end of the experiment, we estimated tolerance for each level of inoculum concentration pooling the data of all plant families. These estimations were used in a regression analysis including only the linear and quadratic components of the inoculum factor. We found that the AMF concentration gradient had a significant negative linear effect (r^2 = -0.40; $F_{1,\,27}$ = 5.89; P = 0.0222; Fig. 4) and a marginally significant quadratic effect ($F_{1,\,27}$ = 3.48; P = 0.0728) on tolerance level expressed by *D. stramonium*. This result indicates a negative interaction between AMF colonization and tolerance to defoliation (i.*e.* below-aboveground interaction).

Discussion

The results presented here showed a significant multi-species interaction between the plant response to AMF and the plant response to foliar damage. Specifically, defoliated plants had reduced benefits from the association with AMF. This below-aboveground interaction was expressed as a negative correlation between tolerance to defoliation and concentration of AMF inoculum. It is likely that the negative interaction arises because both AMF and herbivores consume resources from the host plant. In turn, natural variations in AMF densities may also limit the evolution of optimum levels of tolerance to defoliation and may account for the maintenance of genetic variation and the presence of intermediate levels of tolerance found within plant populations (Núñez-Farfán *et al.* 2007). At the same time, reduction in the benefits of the mutualism between AMF and the host plant through defoliation may also condition the evolutionary outcome of this interaction.

Mycorrhizal Fungi, Plant Performance and Defoliation

Gange and Ayres (1999) proposed a simple model predicting a general curvilinear relation between colonization density and plant benefit, where benefit is maximized at some intermediate value of colonization. Accordingly, we found a curvilinear relationship between a proxy of colonization density (AMF inoculum concentration) and plant performance. All plant characters but flowering day increased significantly reached a plateau (at an intermediate point) and then declined. An increase in plant performance due to AMF is the most common result reported in the literature whenever the presence/absence of AMF is manipulated (Smith & Read 1997). However, our study shows that positive associations between AMF and plant fitness may not be proportional and that at high colonization densities, mycorrhizas may also compete with plants for nutrients, immobilize N, affect root exudation and the rhizosphere micro flora, all of which could lead to reduced benefits (Johnson *et al*. 1997; Gange & Ayres 1999; Jones & Smith 2004). Our results indicate that benefits for the host plant from associating with AMF appear to be maximal at intermediate levels of inoculum (*i.e.* at an optimum density of mycorrhizal colonization). Moreover, we found genetic variation in the shape of the function response to AMF and defoliation (Fig. 3). Specifically, there was variation in the optimum level at which plant families achieved higher performance, probably because the benefit:cost ratio of the association with AMF changes nonlinearly; thus, this optimum point can evolve to changing densities of AMF and herbivores.

Unlike previous expectations, we found no evidence that mycorrhizal colonization provided an advantage for defoliated plants. In fact, AMF had little effect on plant performance in the presence of damage, suggesting that the presence of herbivores could limit the plant's ability to benefit from AMF. Our results are similar to those found by Gange and co-workers (2002) where AMF had no effect on plant biomass when insects were abundant, but a positive one when insects were reduced. In other words, mycorrhizal infection could be beneficial to host plants only when the herbivore load is reduced. Nevertheless, it is possible that the use of commercial AMF and manual defoliation may not accurately reflect true relationships between AMF and damage in the field. The commercial mix used in this study includes commonly found mycorrhizae and previous analyses (not shown) suggest that plants achieve similar fitness when growing with commercial and natural inoculum collected from the field ($\chi^2 = 0.03$; $P = 0.8691$). On the other hand, because plants were subjected to artificial damage we were not able to evaluate the full set of induced responses that would have occurred after natural herbivory. Thus, the question remains if the interaction found between AMF, plants and artificial damage is similar to that expected under natural conditions. So far, we have only shown that evaluating the effect of a gradient in mycorrhizal colonization can gives us novel insights about the AMF-plant-herbivore interaction. Given that tolerance responses differ among plant species and also depend on the intensity of damage (Fornoni & Núñez-Farfán 2000; Huhta *et al.* 2003), future studies should address this multispecies interaction in other plant systems and under different densities of herbivores.

Mycorrhizal Fungi and Tolerance to Defoliation

Here, we found that even high levels of AMF colonization did not reduce the negative effects of damage on plant fitness. Furthermore, it is likely that both AMF and defoliation reduced photosynthates from the host plant decreasing not only the benefits of AMF colonization but also the capacity for compensating damage. Because the experiment was conducted outside the growing season, we can not rule out the possibility that reduced light availability could have intensified the stress by loss of photosynthates due to AMF and defoliation. Additionally, tolerance to defoliation decreased linearly with increasing

AMF inoculum concentration suggesting that mycorrhizae join with herbivores to further limit the access to plant resources. This result is in accordance with the expectation that tolerance would be greater at rich-resource environments (Compensatory Continuum Hypothesis, see Wise & Abrahamson 2005). Thus, at low density of AMF colonization plants would be able to allocate a greater amount of the limiting resources for tolerance to aboveground defoliation.

It has recently been proposed that in order to fully understand the evolution of plant defenses, it is necessary to consider the role played by belowground biota (Van der Putten 2003). Our study provides evidence that the adaptive value of tolerance to defoliation could be the result of interactions between a plant's genetic background and variation in AMF colonization experienced by host plants. The findings of this study show that not only did AMF decrease tolerance to herbivory, but also that this association can potentially change the long-term dynamic of plant-herbivore interactions as a result of reduced tolerance levels in the presence of AMF. Whenever tolerance represents the only profitable strategy to cope with an increasing amount of damage (Jokela *et al*. 2000), AMF can reduce the adaptive value of tolerance through an ecological cost.

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Table 1. Results of the ANOVAs for the effects of genetic family, defoliation and inoculum gradient concentration on plant performance. Values followed by asterisks were significant after a Bonferroni correction (* $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$). A significant quadratic effect of inoculum indicates a non-linear relationship between the response variable and the gradient of inoculum concentration.

		Plant Response Variables				**Interaction Response Variables**	
Source of Variation	*df*	*Foliar Area*	*Root Mass*	*Flowering Day*	*Seed Production*	*Total Colonization*	*Arbuscules*
		F	*F*	χ^2	χ^2	χ^2	χ^2
Family	5	4.14 **	2.76 †	60.85 ***	32.96 ***	6.31	16.7 *
Defoliation	1	42.11 ***	18.37 ***	18.39 ***	25.26 ***	5.35 †	4.97
Defoliation × Family	5	1.03	1.1	7.05	14.59 †	3.84	16.49 *
Inoculum	1	51.84 ***	39.34 ***	27.21 ***	35.51 ***	61.17 ***	43.89 ***
Inoculum^2	1	71.29 ***	22.17 ***	47.18 ***	30.22 ***	0.34	8.71
Inoculum × Family	5	10.08 ***	8.53 ***	34.9 ***	31.13 ***	2.6	5.36
Inoculum^2 × Family	5	13.15 ***	3.42 *	56.28 ***	39.68 ***	1.42	10.49
Inoculum × Defoliation	1	2.19	16.03 ***	11.55 **	15.49 ***	0.62	2.54
Inoculum^2 × Defoliation	1	5.33 †	7.85 *	9.52 †	12.28 **	7.02 *	4.89
Inoculum × Defoliation × Family	5	4.44 ***	4.91 **	11.42 †	12.52 †	4.0	2.5
Inoculum^2 × Defoliation × Family	5	5.55 ***	1.56	15.14 *	33.67 ***	2.54	0.47
Block	3	14.82 *	4.21 *	22.93 ***	207.49 ***	2.17	0.68

† Values significant at a $P < 0.05$.

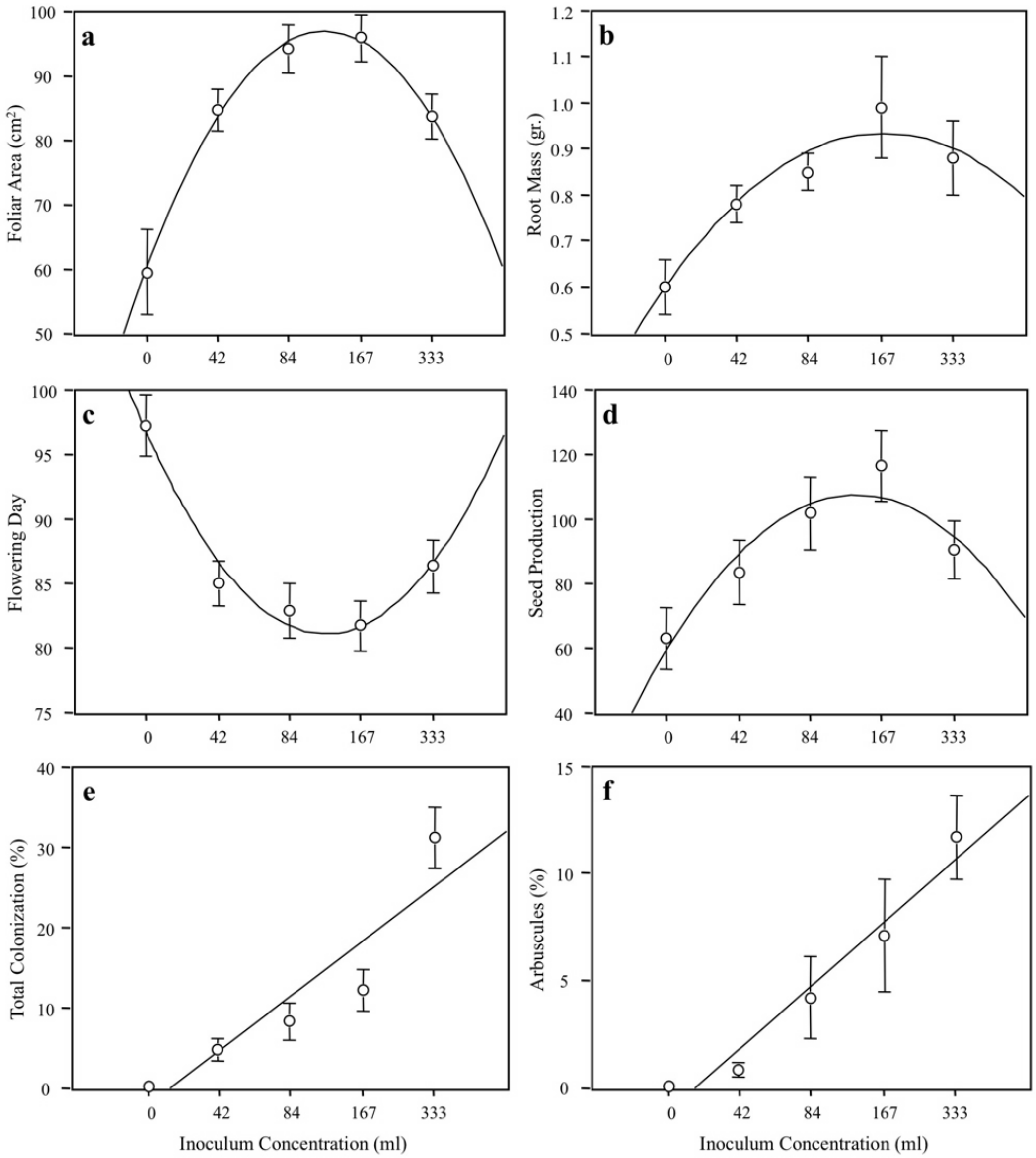

Figure 1. Linear and quadratic effect of AMF inoculum concentration on (**a**) foliar area, (**b**) root mass, (**c**) flowering day, (**d**) seed production, (**e**) total colonization percentage, and (**f**) arbuscules percentage of total colonization. In general, plants achieved their maximal performance at a concentration of 167 ml of inoculum.

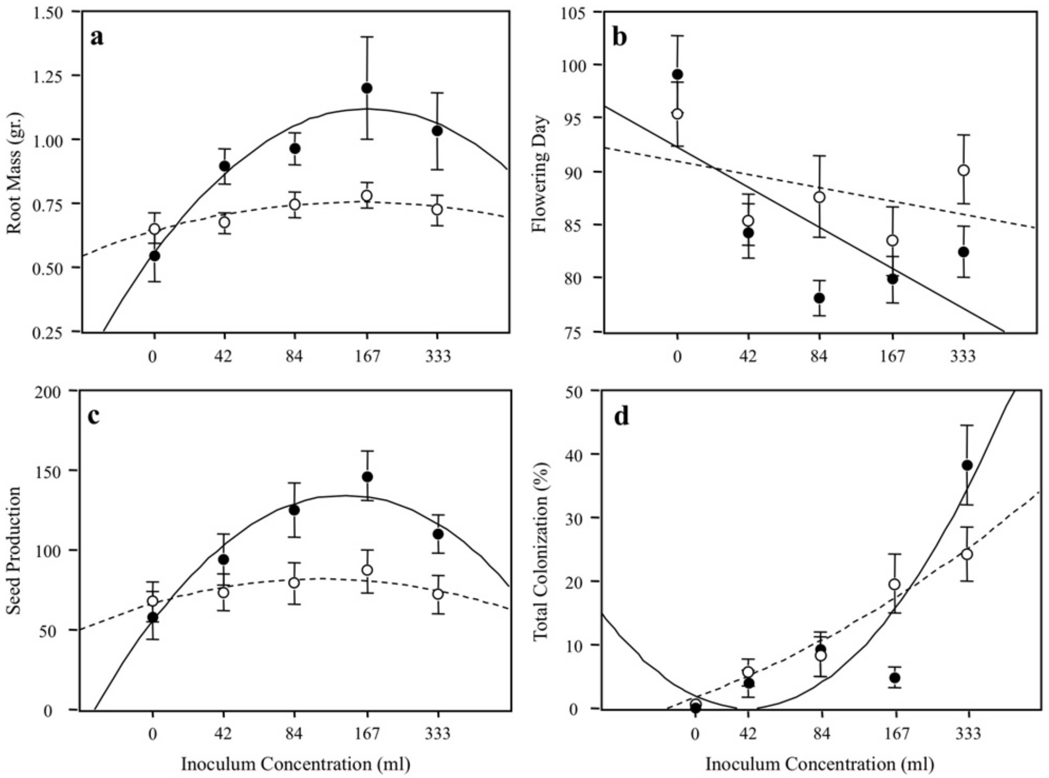

Figure 2. Differences between undamaged (continuous line) and damaged (dashed line) plants in (**a**) root mass, (**b**) flowering day, (**c**) seed production and (**d**) total colonization percentage along a fungal inoculum concentration gradient. In general, the positive relationship between inoculum and performance disappeared with defoliation.

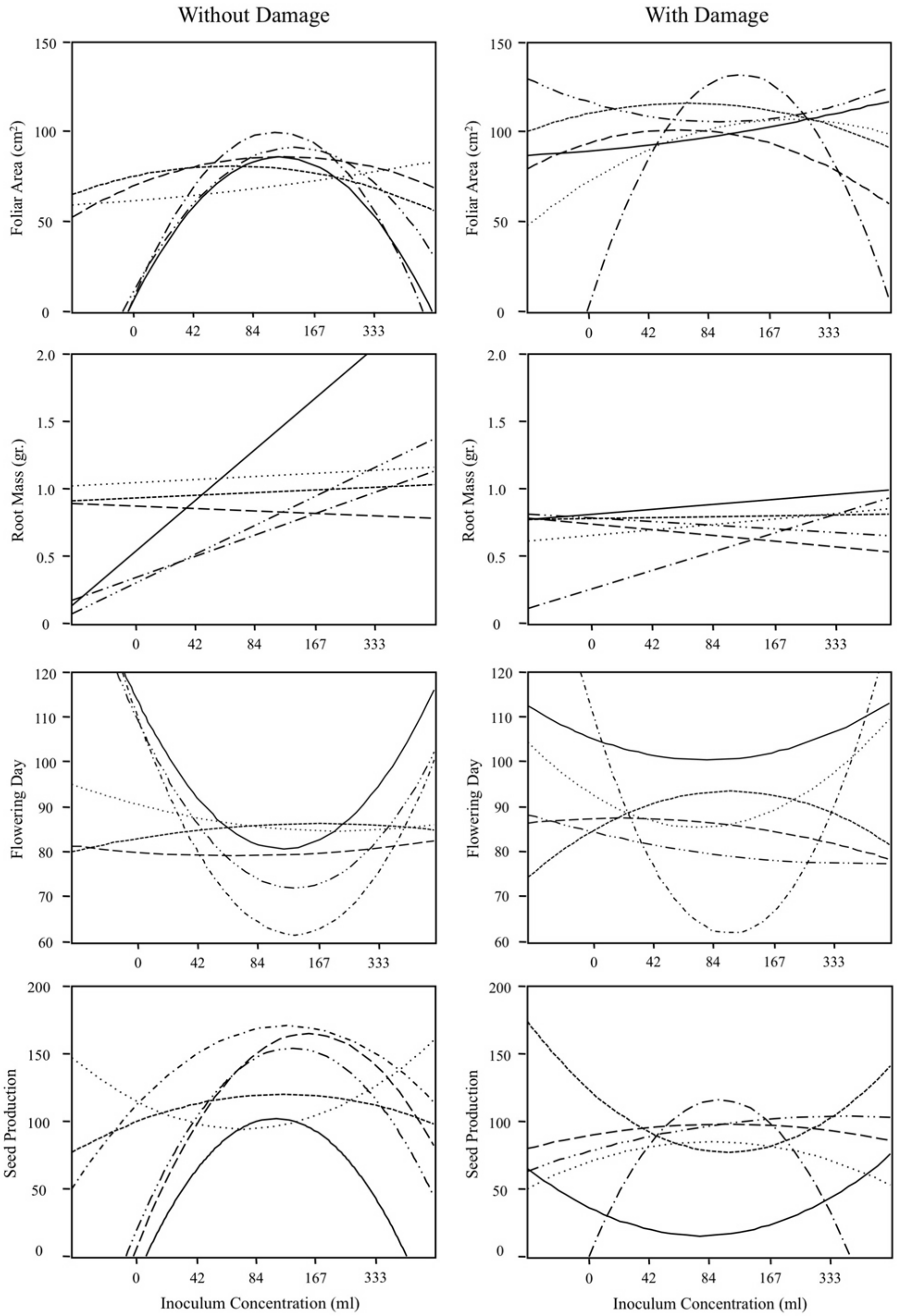

Figure 3. Genetic variation among plant families in plasticity for foliar area, root mass, flowering day and seed production along the AMF inoculum concentration gradient in the presence and absence of damage. Each line represents one family. Means and error bars were omitted.

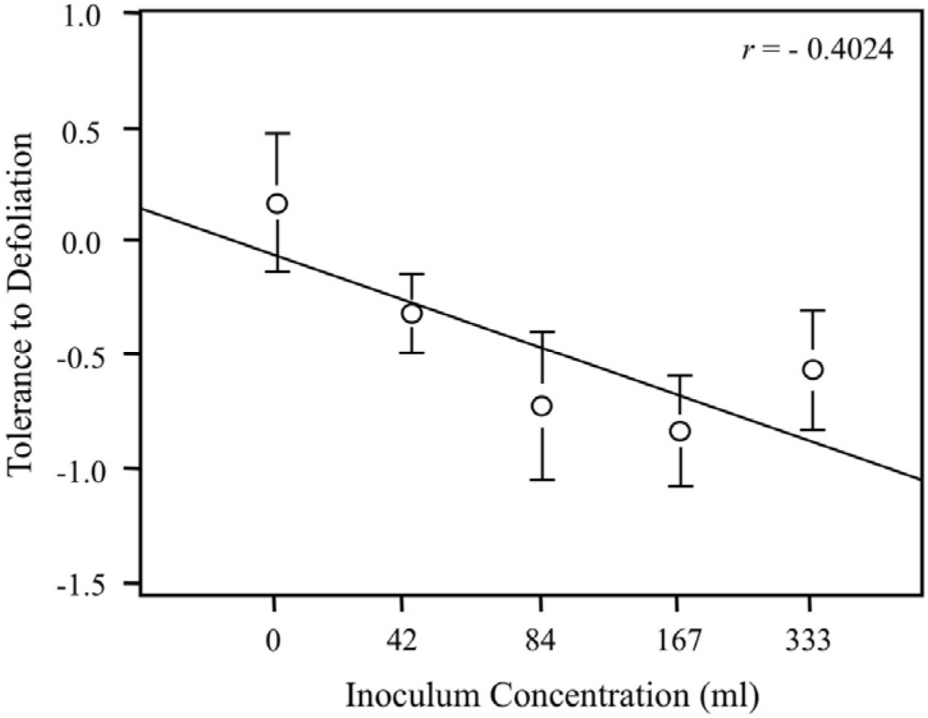

Figure 4. Tolerance to defoliation decreased linearly with inoculum concentration. Tolerance was calculated as the difference in standardized seed production between related damaged (D) and undamaged (U) plant families (D-U).

Discusión General

¿Por qué las plantas presentan niveles intermedios de resistencia y tolerancia? Responder esta pregunta representa uno de los grandes retos para la ecología evolutiva de las interacciones entre las plantas y sus enemigos naturales. En general, los resultados obtenidos en esta tesis indican que la estabilidad evolutiva de las estrategias de defensa mixtas podría ser el resultado de cambios en los valores adaptativos de ambas estrategias de defensa. Dichos cambios están mediados por el nivel de adaptación local de los enemigos naturales, la cantidad de daño que presenten las plantas y, la frecuencia de ambos genotipos defensivos dentro de las poblaciones. Es decir, cuando la adaptación de los enemigos naturales reduce la efectividad de la resistencia, la estrategia más factible sería la tolerancia. Por el contrario, cuando el beneficio de la tolerancia disminuya debido a incrementos en la cantidad de daño, la selección natural favorecerá a aquel mutante resistente que evite o disminuya el daño. La presencia de estrategias mixtas implicaría que los enemigos naturales responderían a la resistencia de las plantas a una menor tasa, adaptándose más lentamente a sus plantas huésped que si éstas se defendieran exclusivamente a través de la resistencia. En este sentido, la tolerancia reduciría la respuesta coevolutiva de los enemigos naturales y, el presentar estrategias mixtas podría representar una ventaja adicional para compensar la mayor tasa evolutiva de los enemigos naturales.

La teoría del mosaico geográfico coevolutivo (Thompson, 2005) predice variación geógrafica en el resultado de las interacciones. Como resultado de estas diferencias, la interacción podría (1) coevolucionar en algunas de las poblaciones, (2) afectar la evolución de sólo una de las especies interactuantes en otras poblaciones o, (3) no tener efecto alguno en el resto de las poblaciones. De igual forma, la presencia de estrategias mixtas o puras en algunas poblaciones podría ser resultado de las condiciones particulares bajo las cuales evolucionan las plantas y sus enemigos naturales. Es decir, a pesar de que la predicción general de este trabajo es la presencia de estrategias mixtas, estrategias puras de resistencia o de tolerancia podrían ser favorecidas bajo ciertos escenarios ecológicos-evolutivos.

La propuesta teórica planteada en el Capítulo 1 se basó en los siguientes supuestos: ambas estrategias de defensa tienen un efecto diferencial sobre la respuesta evolutiva de los enemigos naturales; la efectividad de la resistencia disminuye cuando los enemigos naturales se encuentran localmente adaptados y, el valor adaptativo de la tolerancia disminuye a medida que aumenta el nivel de daño (ver Capítulo 1). De cumplirse estos supuestos, se esperaría que la selección natural favoreciera a aquellos genotipos que presentan una estrategia de defensa mixta. Sin embargo, cuando alguno de estos supuestos no se cumpla, es razonable suponer que estrategias puras podrían ser favorecidas. Incluso, es razonable esperar que las plantas siempre presenten ambas estrategias y que, dependiendo del contexto en el que se desarrollen, cambie la asignación relativa a cada una de las defensas. De esta forma, estrategias mixtas sesgadas hacia la resistencia o hacia la tolerancia podrían ser favorecidas dependiendo de las circunstancias.

En el segundo capítulo de esta tesis se presenta la primera evidencia experimental sobre los efectos diferenciales que ambas estrategias de defensa tienen sobre los enemigos naturales (Espinosa & Fornoni, 2006). Mientras que la resistencia afecta negativamente la supervivencia y el desempeño de los enemigos naturales, la tolerancia no tiene efecto alguno. Es posible, sin embargo, que en otros sistemas la tolerancia afecte positivamente el desempeño de los enemigos naturales (Stinchcombe, 2002). No obstante, las predicciones de esta tesis podrían no verse afectadas aún cuando la tolerancia tuviera un efecto positivo. Es decir, la tolerancia seguiría favoreciendo un aumento en la densidad de herbívoros y, como resultado, los niveles de daño aumentarían disminuyendo el beneficio de la tolerancia (ver Capítulos 1 y 4). Por otro lado, ambas estrategias podrían tener un efecto negativo. En ese caso, debería evaluarse si la intensidad de los efectos son iguales. Si la tolerancia ejerciera un efecto negativo menos intenso que la resistencia, es posible que los niveles de daño se incrementen lentamente prolongando el beneficio de ser tolerante. De esta forma, podrían seleccionarse estrategias mixtas sesgadas hacia la tolerancia.

Evidencia empírica indica que tanto herbívoros como patógenos se adaptan rápidamente a sus plantas huésped, reduciendo así el éxito reproductivo de los genotipos resistentes más comunes dentro de la población (Chaboudez & Burdon, 1995; Ebert & Hamilton, 1996; Mopper & Strauss, 1998; Roy, 1998; Lively &

Dybdahl, 2000). En sistemas agrícolas y en poblaciones naturales se ha comprobado una reducción en la efectividad de varios insecticidas y transgénicos poco tiempo después de ser utilizados o introducidos en los cultivos, reflejando la velocidad a la que los enemigos naturales pueden sobrellevar la resistencia de las plantas (Gould, 1998; Ruasher, 2001; Vacher *et al*., 2003). En el Capítulo III se presenta evidencia de variación geográfica en los niveles de adaptación local de los herbívoros. Además, se muestra que la expresión de la tolerancia parece depender del nivel de adaptación local de los herbívoros. Es decir, en aquellas poblaciones donde los herbívoros se encuentran adaptados localmente, las plantas expresan mayores niveles de tolerancia. De esta forma, podría suponerse que bajo un escenario de adaptación local, una mayor expresión de la tolerancia sería favorecida. Por el contrario, en aquellas poblaciones donde la resistencia aún sea efectiva en reducir o evitar el daño se espera que la resistencia sea favorecida (Brunet & Mundt, 2000; Siemens and Roy 2005; Capítulo 4).

Modelos teóricos han propuesto que niveles intermedios de resistencia y tolerancia podrían ser favorecidos si ambas estrategias se encuentran bajo selección dependiente de la frecuencia (Roy & Kirchner, 2000; Restif & Koella, 2003). Los resultados presentados en el cuarto capítulo indican que las estrategias mixtas podrían ser favorecidas dado que la tolerancia se encuentra bajo selección dependiente de la frecuencia negativa. Este resultado contradice algunos de los supuestos de los modelos. En el experimento presentado en el capítulo 4, la resistencia redujo la densidad de herbívoros y la cantidad de daño. Por esta razón, los genotipos resistentes obtienen mayores beneficios que los tolerantes, lo que permite que aumenten en frecuencia (Brunet & Mundt, 2000; Siemens & Roy, 2005). Cuando el nivel de daño disminuye, aquellos genotipos tolerantes podrían invadir la población ya que pueden compensar completamente el daño. De esta forma, los genotipos tolerantes aumentarían en frecuencia. Sin embargo, dado que la tolerancia promueve un incremento en el crecimiento poblacional de los enemigos naturales (Roy & Kirchner, 2000; Espinosa & Fornoni, 2006) se espera que el beneficio de la tolerancia disminuya con la frecuencia de genotipos tolerantes dentro de la población. Es decir, se esperaría que la tolerancia estuviera bajo selección dependiente de la frecuencia negativa. En el Capítulo IV se presenta evidencia que apoya esta predicción.

Uno de los aspectos que no ha sido considerado en ninguno de los modelos desarrollados hasta el momento, es el posible efecto que tendría la presencia de un tercer interactuante en la expresión de las estrategias de defensa. Por ejemplo, el valor adaptativo de la tolerancia podría mantenerse cuando la presencia de un tercer nivel trófico (parásitos o parasitoides de los herbívoros) disminuya la cantidad de daño que reciben las plantas. Una revisón reciente, indica que la presencia de parasitoides disminuye la incidencia de daño sobre las plantas (Halaj & Wise, 2001). Incluso se ha reportado que las plantas pueden manipular el comportamiento del tercer nivel trófico para reducir la densidad de sus herbívoros y así evitar el daño (Gómez & Zamora, 1994; van Loon *et al.*, 2000; Fritzsche-Hoballah & Turlings, 2001). A pesar de que el sistema de estudio utilizado a lo largo de la tesis es propicio para evaluar si la presencia de parasitoides mantiene el beneficio de la tolerancia, aún se desconocen aspectos biológicos importantes que permitirían manipular su presencia.

En los últimos años se ha propuesto que para poder entender la evolución de las defensas de las plantas es necesario considerar el papel que juega la biota edáfica en la expresión de las defensas (Van der Putten, 2003). Específicamente, se ha propuesto que la presencia de micorrizas podría incrementar la capacidad de tolerar si éstas incrementan el acceso a recursos limitantes para la planta huésped (Borowicz, 1997; Kula *et al*., 2005; Bennett *et al*., 2006). Contrario a las predicciones, en el último capítulo de esta tesis, se muestra que la expresión de la tolerancia está correlacionada negativamente con la concentración de micorrizas en el suelo. Es decir, cuando las plantas son dañadas y además se encuentran infectadas por micorrizas, el costo por la pérdida de fotosintatos es muy alto, reduciendo los beneficios de la obtención de nutrientes proporcionados por las micorrizas. Este resultado sugiere que la expresión de la tolerancia es mayor en ambientes ricos en recursos como se plantea en el Modelo del Recurso Limitante (Wise & Abrahamson, 2005). De esta forma, cambios en la densidad de micorrizas en el suelo podrían limitar la evolución de niveles óptimos de tolerancia y explicar la presencia de niveles intermedios de tolerancia en condiciones naturales (Núñez-Farfán *et al*., 2007).

Los resultados de esta tesis ponen en duda algunos de los supuestos teóricos que han servido de base para modelar la evolución conjunta de la resistencia y la tolerancia. Por ejemplo, varios modelos han supuesto que la efectividad de la resistencia no cambia en el tiempo (Simms & Rausher, 1987; Fineblum & Rausher, 1995; Abrahamson & Weis, 1997; Tiffin, 2000; Fornoni *et al.*, 2004) o que el beneficio de ser tolerante aumenta con el nivel de daño que experimenta la planta (Roy & Kirchner, 2000). Pocos estudios teóricos han considerado cómo las respuestas coevolutivas de los enemigos naturales influyen en la expresión de las estrategias de defensa (Jokela *et al.*, 2000; Roy & Kirchner, 2000; Restif & Koella, 2003). En particular, ningún modelo considera simultáneamente el efecto que las defensa tienen sobre los enemigos naturales y cómo estos efectos influyen a su vez en la expresión de la resistencia y la tolerancia. Los nuevos modelos que se desarrollen durante los siguientes años tendrán el reto de incorporar nueva evidencia empírica a sus supuestos con el propósito de generar escenarios más realistas que nos permitan entender la evolución de las defensas en las plantas.

Perspectivas

En los últimos diez años, hemos comprendido un poco mejor cómo evolucionan las estrategias de defensa de las plantas. Sin embargo, aún prevalece la noción general de que la tolerancia no afectaría el proceso coevolutivo entre las plantas y sus enemigos naturales. En general, en esta tesis se presenta evidencia de que este supuesto no es necesariamente cierto. Por ejemplo, en el Capítulo III se muestra que la expresión de la tolerancia está influenciada no sólo por la cantidad de daño sino por la identidad del herbívoro que lo produce. Este resultado indica que la tolerancia podría estar determinada por interacciónes entre diferentes genotipos de plantas y herbívoros (G × G × E interactions). Interacciones a este nivel podrían ser el motor para que la tolerancia generara un proceso coevolutivo. Tener una idea más clara de cuáles son los mecanismos de tolerancia podría ayudarnos a entender cómo la tolerancia afectaría la respuesta coevolutiva de los enemigos naturales.

Si la expresión de la tolerancia representa una ventaja para sobrellevar las mayores tasas evolutivas de los enemigos naturales, deberíamos considerarla como una respuesta alternativa al uso desmesurado de insecticidas y pesticidas. Cada año se pierde aproximadamente el 30% de la producción agrícola en nuestro país a causa de diversas plagas (SAGARPA, 2009). La introducción de genotipos tolerantes a los cultivos podría reducir las pérdidas económicas y representar un ahorro en la compra de insecticidas y pesticidas. Sin embargo, aún es necesario un mayor conocimento sobre los genes que están involucrados en la expresión de la tolerancia. La búsqueda de estos genes implicaría también romper con el esquema de que la tolerancia es exclusivamente una respuesta plástica (Strauss & Agrawal, 1999) y de que podrían existir bases genéticas controlando la plasticidad fenotípica (Via *et al*., 1995).

Finalmente, el cambio climático global producido por la emisión de gases de invernadero podría incrementar la temperatura del planeta de 1 a 3.5°C en los próximos 50-100 años. Los efectos directos e indirectos de este proceso sobre los ecosistemas serán extremadamente complejos a través de tiempo y del espacio geográfico. Un meta-análsis reciente indica que la nitrificación del suelo y la productividad de las plantas han aumentado 64 y 19% respectivamente en los últimos veinte años (Rustad *et al*., 2001). Este incremento podría cambiar los patrones de daño ocasionados por insectos herbívoros, ya que la obtención de nitrógeno es fundamental para el desarrollo y la reproducción de los herbívoros (revisado en Awmack & Leather, 2002). De ser así, comprender cómo evolucionan conjuntamente la resistencia y la tolerancia a la herbivoría se convertiría en un tema de interés general.

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