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UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO

INSTITUTO DE ECOLOGIA

DOCTORADO EN CIENCIAS BIOMEDICAS

EVOLUCION DE LA DEFENSA EN PLANTAS CONTRA
ENEMIGOS NATURALES.

T E S I S

QUE PRESENTA:

JUAN ENRIQUE FORNONI AGNELLI

PARA OBTENER EL GRADO DE:

DOCTOR EN CIENCIAS BIOMEDICAS

TUTOR: DR. JUAN NUÑEZ-FARFAN

MEXICO, D. F.   SEPTIEMBRE, 2002

.......Para Gabi.......

TESIS CON
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CONTENIDO

RESUMEN

En este estudio se abordó el análisis teórico y experimental de la variación cuantitativa en rasgos de las plantas (resistencia y tolerancia) involucrados en la interacción con enemigos naturales (patógenos y herbívoros). En particular, la primera etapa del proyecto consistió en el desarrollo de un modelo de optimización simple (costo-beneficio) sobre la evolución de la defensa en plantas. El objetivo principal de este modelo fue el de proponer una hipótesis suceptible de poner a prueba que ofreciera mayor integración entre la teoría y la evidencia empírica existente. Así, el modelo presentado en el Capítulo I predice que la presencia de estrategias de defensa mixtas (apoyada por la evidencia empírica), podría ser más común que lo que se había planteado anteriormente. Los parámetros del modelo involucrados en la presencia de estrategias mixtas fueron la variación entre poblaciones en los costos y/o beneficios de ambas formas de defensa (tolerancia y resistencia), y la forma de la función de los costos y beneficios. La parte experimental del proyecto (presentado en los Capítulos II y III) confirmó la variación espacial (interpoblacional) en la magnitud del valor adaptativo de la tolerancia. Además los patrones de selección divergentes para la tolerancia entre poblaciones a nivel genético también apoyaron la hipótesis de que el resultado de la interacción entre plantas y enemigos naturales puede variar entre poblaciones como resultado de diferencias entre éstas en el valor adaptativo de las diferentes estrategias de defensa. La presencia de costos, variación genética heredable y selección divergente para la tolerancia apoyan uno de los supuestos principales del modelo propuesto. De acuerdo con las predicciones del modelo, estos resultados permiten explicar la existencia de estrategias mixtas, y la variación espacial en los patrones de asignación a la defensa en plantas.

ABSTRACT

In this study I theoretically and experimentally evaluated the adaptive value of quantitative variation in plant defensive traits (resistance and tolerance) against natural enemies (herbivores and pathogens). The first chapter of the thesis presented an optimization model of plant defense evolution. The main goal of the model is to propose a testable hypothesis that could better link empirical evidence with theory. Hence, the model predicts that the presence of mixed strategies may be more common than previous models proposed. The most important parameter behind the behavior of the model was the magnitude of costs and benefits of resistance and tolerance and the shape of the costs and benefits function for both strategies. The experimental chapters of the thesis support the assumption of spatial variation on the adaptive values of tolerance to herbivory in the annual *Datura stramonium*, provided the finding of divergent selection on tolerance between populations. Thus spatial variation in the outcome of the interaction between plants and natural enemies is expected. Overall, the presence of additive genetic variation, allocational costs, and divergent selection on tolerance partially validate the model proposed, and can help to understand spatial patterns of plant defense allocation.

INTRODUCCIÓN GENERAL

El estudio de las interacciones bióticas consiste básicamente en el análisis de la respuesta fenotípica de los individuos de una especies en relación a los fenotipos de los individuos de otras especies (Agrawal 2001). Históricamente, las interacciones entre plantas y animales han atraído la atención de los ecólogos evolutivos, debido probablemente, a que una proporción importante de las adaptaciones que hoy reconocemos en la naturaleza son rasgos involucrados en las interacciones bióticas (Darwin 1859). En este sentido, la *coevolución* (*sensu* Erhlich & Raven 1964) entre plantas y animales ha motivado el estudio del origen y las causas del mantenimiento de la diversidad biológica (Futuyma 1998; Kareiva 1999; Rausher 2001). En particular, las interacciones antagonistas (depredador-presa, planta-patógeno, planta-herbívoro) han sido los sistemas utilizados para desarrollar y evaluar la existencia del fenómeno de la coevolución como resultado de una "carrera armamentista" (van Valen 1970; Janzen 1980). En esta dinámica, tanto las víctimas como sus enemigos naturales responden de manera recíproca a la variación fenotípica de la especie con la que interactúan. Debido a que este tipo de interacción produce efectos negativos recíprocos entre las especies, la "carrera armamentista", es una metáfora que hace referencia al hecho que ambas especies interaccionan en un callejón donde la única salida es la evolución de respuestas evolutivas (defensas y contra-defensas) que reduzcan o eliminen los efectos negativos sobre la adecuación entre las especies interactuantes.

A partir del estudio de Erhlich & Raven (1964) sobre la coevolución entre las mariposas y sus plantas hospederas, se consideró durante aproximadamente 30 años que la única forma de respuesta evolutiva de las plantas a sus enemigos naturales eran las defensas químicas y físicas. La función principal de estos atributos defensivos es la de reducir o evitar el ataque por parte de los enemigos naturales (Strauss & Agrawal 1999). Así se definió a la resistencia como una forma de defensa cuyos componentes incluyen la presencia de (1) tricomas foliares y caulinares, (2) dureza de las hojas, (3) producción de compuestos secundarios como taninos,

alcaloides, glucosinolatos, etc (Rosenthal & Kotanen 1994). Estos componentes de la resistencia pueden ser inducidos por el ataque de los consumidores o presentarse de manera constitutiva antes de que el daño ocurra (Strauss & Agrawal 1999). Estudios experimentales indican que las poblaciones de plantas más atacadas presentan niveles de defensa constitutiva más elevados con respecto a aquellos de las defensas inducidas (Zangerl & Berenbaum 1990). En aquellos sistemas donde la magnitud y predecibilidad del ataque por enemigos naturales es elevada se espera que la importancia ecológica y evolutiva de las defensas constitutivas sea más alta que la de las defensas inducidas (Zangerl & Bazzaz 1992).

A finales de los años 80, una forma de respuesta de las plantas ante la presión de los enemigos naturales se integró al escenario evolutivo, la *tolerancia* (Painter 1958; Burdon 1987; Rosenthal & Kotanen 1994). Esta forma de defensa consiste en reducir el impacto negativo del daño en términos de adecuación después de que el daño ha ocurrido (Burdon 1987). Es decir, no evita el ataque, sino que reduce su efecto sobre las plantas. A pesar de que los mecanismos involucrados en la tolerancia de las plantas al consumo de sus enemigos naturales no se han dilucidado experimentalmente, varios estudios coinciden en que el mecanismo básico de la tolerancia es una respuesta relacionada directa o indirectamente con los procesos de crecimiento (Rosenthal & Kotanen 1994). La activación de meristemos secundarios, incremento en la actividad fotosintéticas de los tejidos no dañados, reasignación de recursos de partes de la planta que no han sido dañadas, movilización de recursos almacenados en estructuras de reserva, e incremento en la tasa de crecimiento son parte de los atributos de las plantas que se suponen participan en la respuesta de tolerancia frente al herbivorismo (Rossenthal & Kotanen 1994; Strauss & Agrawal 1999; Stowe et al. 2000). En la actualidad no existe evidencia que confirme la existencia de correlaciones genética entre estos atributos y la tolerancia (Stowe et al. 2000). Debido a que en principio el beneficio de ambas estrategias de defensa (resistencia y tolerancia) se consideró redundante, se propuso que ambas formas de defensa podrían funcionar como alternativas defensivas en los modelos de defensa en

plantas que se desarrollaron a partir de los años 90 (Fineblum & Rausher 1995; Abrahamson & Weis 1997; Mauricio et al. 1997).

La incorporación de la tolerancia como una estrategia defensiva alternativa a la resistencia cuestionó uno de los supuestos más importantes de la coevolución en las interacciones antagonistas: *efectos negativos recíprocos entre las especies interactuantes*. Debido a que la tolerancia atenúa los efectos negativos de los enemigos naturales sobre las plantas, esta forma de defensa reduce las oportunidades de que ocurra un proceso coevolutivo (Tiffin 2000; Cáp. I). Si las plantas pueden enfrentar la presión de los enemigos naturales sin involucrarse en un proceso coevolutivo a través de la tolerancia, no habría razones para esperar adaptación local entre las poblaciones de plantas en atributos de resistencia frente a la variación genética de sus enemigos naturales locales. La falta de evidencia en favor de la adaptación local de las plantas en rasgos de resistencia involucrados en la interacción con enemigos naturales (Linhart & Grant 1996; Schluter 2000), sugiere que esta hipótesis podría explicar la ausencia de un patrón de especialización local de las poblaciones de plantas. Sin embargo, la evidencia empírica indica que tanto la tolerancia como la resistencia pueden presentarse simultáneamente en un mismo individuo dentro de las poblaciones (Fineblum & Rausher 1995; Mauricio et al. 1997; Mauricio 2000; Pilson 2000). La pregunta que se deriva de la evidencia empírica es: *¿Por qué las plantas presentan más de una forma de defensa dentro de las poblaciones?* (Mauricio et al. 1997; Tiffin & Rausher 1999; de Jong & van der Meijden 2000; Tiffin 2000; Cáp. I).

Paralelamente al desarrollo de modelos teóricos sobre la evolución de la tolerancia y resistencia de las plantas contra sus enemigos naturales (Fineblume & Rausher 1995; Mauricio et al. 1997; Jokela et al. 2000; Tiffin 2000), comenzó a acumularse evidencia experimental sobre el valor adaptativo de la tolerancia y la resistencia y sobre las posibles restricciones para la evolución de las diferentes estrategias defensivas (Simms & Triplett 1994; Fineblum & Rausher 1995; Mauricio et al. 1997; Agrawal et al. 1999; Tiffin & Rausher 1999; Fornoni & Núñez-Farfán 2000; Mauricio 2000; Pilson 2000; Stinchcombe 2001; Valverde et al. 2001, 2002; ver Cap. III). Sin embargo, los estudios empíricos revelaron una fuerte inconsistencia con las

predicciones de los modelos teóricos (Mauricio 2000; Cáp. I). Por un lado, los modelos teóricos predicen inicialmente que la tolerancia y resistencia representan dos alternativas mutuamente excluyentes (Fineblum & Rausher 1995; Mauricio et al. 1997). Es decir, que dentro de las poblaciones los genotipos podrían ser o totalmente resistentes o totalmente tolerante, pero no serían favorecidos por selección natural aquellos genotipos parcialmente tolerantes y resistentes a la vez. Por otro lado, la evidencia experimental reveló que dentro de las poblaciones hay genotipos tolerantes y resistentes, y que la selección opera favoreciendo la "coexistencia" de ambas alternativas de defensa en los individuos (Mauricio et al. 1997; Pilson 2000). Basado en estos antecedentes el propósito general de la tesis fue incrementar el conocimiento sobre la evolución conjunta de las dos estrategias de defensa en plantas a través de una propuesta teórica complementada con evidencia experimental.

En el Capítulo I se presenta el análisis teórico y la propuesta de un modelo de optimización sobre la evolución de la resistencia y tolerancia en plantas contra sus enemigos naturales que incorpora supuestos más realistas a los utilizados por modelos anteriores. Debido a que una gran cantidad de estudios sugieren que ambas alternativas defensivas se presentan en los individuos de las poblaciones, uno de los objetivos principales del análisis teórico fue la evaluación de las condiciones para que la tolerancia y la resistencia sean favorecidas simultáneamente. Es decir, las condiciones para que la superficie adaptativa de la resistencia y tolerancia tuviera un solo pico adaptativo que correspondiera a valores intermedios de asignación de ambas estrategias defensivas.

El sistema de estudio elegido para la parte experimental fue la herbácea anual *Datura stramonium*. Esta especie mantiene una interacción estrecha con insectos herbívoros generalistas (*Sphenarium purpurascens*) y especialistas (*Lemma trilineata*, *Epitrix párvula, Manduca sexta*) (Núñez-Farfán 1991). *D. stramonium* se caracteriza por la presencia de alcaloides (scopolamina e hiosciamina) con función defensiva (Shonle & Bergelson 2000), y tricomas foliares (Valverde et al. 2001). Estudios recientes indican la ausencia de inducción en los niveles de resistencia (Shonle & Bergelson 2000). Las poblaciones naturales de *D. stramonium* en México experimentan niveles de

daño por herbívoros variable (10 – 50 %) pero regular entre generaciones (Núñez-Farfán 1991; Núñez-Farfán & Dirzo 1994; Núñez-Farfán et al. 1996; Valverde et al. 2001, 2002). Estas características hacen de esta especie un sistema apropiado para el estudio de la ecología evolutiva de la interacción con enemigos naturales.

En el Capítulo II (Fornoni & Núñez-Farfán 2000) se presenta un estudio experimental que evalúa la magnitud de la variación genética de la tolerancia en *D. stramonium*. La existencia de varianza genética aditiva para la tolerancia es un requisito indispensable para que esta estrategia defensiva pueda evolucionar. Asimismo, el estudio evalúa el papel que tienen varios caracteres reproductivos así como el crecimiento para explicar las diferencias en tolerancia entre las líneas endogámicas utilizadas. Debido a que el mecanismo responsable de la tolerancia no ha sido determinado completamente, se consideró necesario evaluar la respuesta reproductiva de las plantas a la defoliación como una aproximación al entendimiento del mecanismo asociado con la tolerancia de las plantas. Por último, el estudio considera la posible existencia de costos de asignación para la tolerancia a la defoliación artificial. La existencia de costos permite evaluar el valor adaptativo de la tolerancia considerando posibles restricciones a la evolución (límites a la asignación de las plantas a la defensa como resultado de limitación de recursos por el ambiente u otras funciones vitales).

En el Capítulo III se presenta la evidencia de un estudio experimental realizado en condiciones de campo, cuyo objetivo fue evaluar la variación entre poblaciones naturales en el valor adaptativo de la tolerancia en *Datura stramonium* para poner a prueba los supuestos del modelo (Cap. I). El estudio abarcó el análisis de la variación genética aditiva de la tolerancia en dos poblaciones del centro de México, y un análisis de selección natural que opera sobre la tolerancia en ambos sitios. Este análisis se basó en un diseño de transplantes recíprocos combinado con material proveniente de cruzas dialélicas. Por último, este estudio también evaluó la presencia y la variación en los costos y beneficios (en términos de adecuación) de la tolerancia. En conjunto este capítulo permitió validar parcialmente la propuesta teórica del Capítulo I.

Los Apéndices I y II presentan estudios experimentales complementarios al proyecto de tesis doctoral. Ambos estudios abordan la evaluación de supuestos y predicciones de la teoría para la resistencia al herbivorismo. El Apéndice I (Valverde et al. 2001) explora a través de un estudio de campo y un experimento en condiciones controladas el valor de los tricomas foliares como componente de la resistencia en *D. stramonium*. Este análisis incluyó el estudio de siete poblaciones del centro de México para analizar la variación geográfica en el valor de los tricomas como componentes de la resistencia al herbivorismo. El Apéndice II (Valverde et al. 2002) explora experimentalmente a través de un diseño de jardín común, la asociación entre la resistencia y un componente potencial de la tolerancia: el crecimiento. El mismo estudio describe los patrones de selección sobre ambos rasgos de las plantas en presencia y ausencia de los herbívoros.

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CAPÍTULO I

DINÁMICA EVOLUTIVA DE LAS ESTRATEGIAS DE DEFENSA DE LAS PLANTAS CONTRA SUS ENEMIGOS NATURALES: TOLERANCIA Y RESISTENCIA

Evolution of mixed strategies of plant defense allocation against natural enemies

Juan Fornoni,[1] Juan Núñez-Farfán,[1] Pedro Luis Valverde,[2] and Mark D. Rausher[3]

[1] Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, C.P. 04510, México Distrito Federal, México.

[2] Departamento de Biología, Universidad Autónoma Metropolitana, Apartado Postal 55-535, C.P. 09340, México Distrito Federal, México.

[3] Biology Department, Duke University, Durham, NC 27708, USA.

Corresponding author:

Juan Fornoni

Departamento de Ecología Evolutiva, Instituto de Ecología,

Universidad Nacional Autónoma de México,

Apartado Postal 70-275, C.P. 04510,

México Distrito Federal, México.

e-mail: jfornoni@miranda.ecologia.unam.mx

ABSTRACT

In this study we present a simple optimization model for the evolution defensive strategies (tolerance and resistance) of plants against their natural enemies. The model specifically evaluates the consequences of introducing (i) variable costs and benefits of tolerance and resistance and (ii) non-linear costs and benefits functions for tolerance and resistance. Incorporating these assumptions, the present model of plant defense predicts different evolutionary scenarios, not expected by previous work. Basically, the presence of an adaptive peak corresponding to intermediate levels of allocation to tolerance and resistance can arise when the shape parameter of the cost function is higher than the corresponding of the benefit function. The presence of two alternatives peaks of maximum tolerance and maximum resistance occurs only when benefits of tolerance and resistance interact less than additive. Finally, the presence of one peak of maximum resistance or maximum tolerance depends on the relative values of the magnitude of costs for tolerance and resistance. An important outcome of our model is that under a plausible set of conditions, variable costs of tolerance and resistance can represent an important aspect involved in the maintenance of intermediate levels of tolerance and resistance, and in favoring adaptive divergence in plant defensive strategies among populations. The model offers a framework for future theoretical and empirical work to understand spatial variation in levels of allocation to different defensive strategies.

INTRODUCTION

During the last decade, the understanding of plant-enemy interactions has benefited by the incorporation into models of an ubiquitous type of defense (i.e., tolerance). Tolerance has been defined as the ability of a plant genotype to reduce the negative effects of consumers (e.g., herbivores, pathogens) on plant fitness (Rosenthal & Kotanen 1994; Strauss & Agrawal 1999; Stowe *et al.* 2000). Unlike resistance (i.e., the ability of a plant to reduce the attack of natural enemies), tolerance is not considered to affect negatively the success of herbivore or pathogen populations (Rosenthal & Kotanen 1994; Fay et al. 1996; Tiffin 2000a; but see Stinchcombe 2002). Consequently, the evolution of tolerance can limit reciprocal coevolution between plants and their enemies, whereas the evolution of resistance prolongs such coevolutionary relationships (Rausher 2001).

The joint evolution of plant tolerance and resistance to natural enemies has attracted substantial theoretical attention over the last seven years (Rosenthal & Kotanen 1994; Fineblum & Rausher 1995; Strauss & Agrawal 1999; Mauricio 2000; Roy & Kirchner 2000; Tiffin 2000a; Stowe *et al.* 2000). Several experimental studies have tested some of the predictions made by these theoretical analyses (Simms & Triplett 1994; Fineblum & Rausher 1995; Mauricio *et al.* 1997; Stowe 1998; Agrawal *et al.* 1999; Tiffin & Rausher 1999; Fornoni & Núñez-Farfán 2000; Pilson 2000; Roy & Kirchner 2000; Stinchcombe 2002; Valverde et al. 2002). Results of these empirical investigations often do not support theoretical expectations (*see* Mauricio 2000; Bergelson *et al.* 2001). For instance, previous models for the joint evolution of tolerance and resistance have suggested that they represent two alternative evolutionary strategies of defense (Fineblum & Rausher 1995; Mauricio *et al.* 1997; Roy & Kirchner 2000; Valverde et al. 2002). Specifically, these models predict a fitness landscape with two adaptive peaks, one corresponding to complete tolerance and the other to complete resistance. The expectation of mutual exclusivity arises from the assumption that costs of tolerance and resistance are increasing linear functions of resource allocation and having both strategies would be redundant. Thus, natural

selection would not simultaneously favor an increase in tolerance and resistance (Simms & Triplett 1994).

Despite the reasonableness of this argument, there is little empirical evidence supporting the existence of two mutually exclusive peaks in the resistance-tolerance fitness landscape (e.g., Mauricio *et al*. 1997; Tiffin & Rausher 1999; Roy & Kirchner 2000). On the contrary, numerous studies have found the existence of intermediate levels of both tolerance and resistance in natural populations (Simms & Rausher 1987; Núñez-Farfán & Dirzo 1994; Fineblum & Rausher 1995; Mauricio *et al*. 1997; Tiffin & Rausher 1999; Fornoni & Núñez-Farfán 2000; Juenger & Bergelson 2000; Pilson 2000; Shonle & Bergelson 2000). These results indicate that the maintenance of intermediate levels of both tolerance and resistance are favored by natural selection. Because these results are at odds with published theoretical expectations, we have explored additional models for the joint evolution of tolerance and resistance. We show here that the prediction on the existence of two alternative peaks in the selective surface arises out from some specific assumptions that have been made about the nature of costs and benefits of these characters. In addition, we show that alteration of these assumptions in ways consistent with empirical information about the nature of costs and benefits leads to predict that intermediate levels of both tolerance and resistance may often be favored at evolutionary equilibrium.

EMPIRICAL BACKGROUND

A substantial body of empirical evidence has accumulated regarding the nature of costs and benefits functions of plant defensive characters. This information not only indicates that some assumptions made by previous models were not completely correct, but also suggest in some cases how those assumptions should be modified. Because this information motivates the way in which we relaxed these assumptions in our model, we first discuss the implications of this evidence.

The shape of cost and benefit functions

The scarcity of empirical evidence regarding the shape of cost and benefit functions has led previous models to assume that these functions are linear (Fineblum & Rausher 1995; Mauricio *et al.* 1997; Roy & Kirchner 2000; Tiffin 2000a; Weis & Hochberg 2000). However, recent studies have revealed that non-linear cost and benefit functions may be more common than previously expected (Skogsmyr & Fagerström 1992; Mauricio *et al.* 1997; Tiffin & Rausher 1999; Bergelson *et al.* 2001). For example, Skogsmyr & Fagerström (1992) showed that the cost function of resistance (estimated as reduced growth) can vary from being almost linear to being a concave upward function of resistance allocation. Bergelson *et al.* (2001), in a recent review, similarly found costs and benefits to be commonly non-linear. Although fewer studies have attempted to estimate the shape of the costs function for tolerance to herbivory, non-linearity appears also to be the case for this type of cost (Mauricio et al. 1997; Tiffin and Rausher 1999; Pilson 2000). The evidence thus suggests that the consequences of nonlinearity of cost and benefit functions merit examination. We evaluated this, by using qualitatively similar form of cost functions like that presented by Skogsmyr and Fagerström (1992) for which a clear mechanistic basis was developed. In our case, we simplified the number of parameters associated with the form of the cost function while maintaining a qualitatively similar shape in order to make the analysis tractable.

Relative values of maximum costs of tolerance and resistance

A second simplifying assumption made in previous analyses is that the costs of maximal resistance and maximal tolerance are equal. However, several sources of evidence call into question the appropriateness of this assumption. First, the only empirical study that has estimated allocation costs of tolerance and resistance simultaneously revealed that these costs differ (Pilson 2000). Second, two studies have demonstrated experimentally that costs of resistance or tolerance are higher when resources are more limited (Bergelson 1994; Hochwender *et al.* 2000). Because tolerance and resistance are frequently genetically uncorrelated (Simms & Triplett

1994; Mauricio *et al.* 1997; Tiffin & Rausher 1999; de Jong & van der Meijden 2000; Stinchcombe 2002) and are thus controlled by different genes and physiological processes, it seems likely that their costs will be affected by different environmental resources. Moreover, since such resources are likely to vary independently, it is also likely that costs of tolerance and resistance will differ, and that the relative costs of these two types of defense will vary along environmental gradients (reviewed in Herms & Mattson 1992). By relaxing the assumption that costs of resistance and tolerance are equal, we are able to explore the consequences of environmental variation on the magnitude of costs of tolerance and resistance.

THE MODEL

The standard approach to modeling the evolution of defense is to assume that each additional unit of resource investment in defense increases both the benefits and costs of resistance (Simms & Rausher 1987; Fineblum & Rausher 1995; Mauricio *et al.* 1997; Jokela *et al.* 2000; Tiffin 2000a). This phenomenon is typically modeled by an equation of the form

$$W_i = W_0 + B(R_i, T_i) - C(R_i, T_i) \qquad (1)$$

where W_i is the fitness of a plant of genotype i, W_0 corresponds to the fitness of a plant with no tolerance and no resistance, T_i and R_i are the amounts of resources allocated to tolerance and resistance, respectively, by genotype i, $B(R_i, T_i)$ is the increase in fitness associated with the benefits of resistance and tolerance, and $C(R_i, T_i)$ is the decrease in fitness due to costs of tolerance and resistance.

Equation (1) represents a two-dimensional fitness surface, where fitness is a function of allocation to tolerance and resistance. The shape of that surface will depend on the detailed form of the functions $B(R_i, T_i)$ and $C(R_i, T_i)$. Because the model developed by Mauricio *et al.* (1997) is representative of previous assumptions about the nature of these functions, and thus serves as a basis of comparison with our more

general model, we begin our analysis by describing their assumptions regarding these two functions:

(1) *Unit costs of allocation to resistance or tolerance are constant, i.e.* costs increase linearly with allocation to resistance or tolerance. This assumption is represented by the following linear relationships between costs and allocation for resistance and tolerance respectively, i.e., $C(\gamma_1 R_{max}, 0) = \gamma_1 C(R_{max}, 0)$, and $C(0, \gamma_2 T_{max}) = \gamma_2 C(0, T_{max})$. Here, R_{max} and T_{max} represent the lowest allocations to resistance and tolerance, respectively, that result in the maximum benefit, and γ_1 and γ_2 each vary between 0 and 1 and represent the proportions of the maximal allocations realized. Although values of γ_1 and γ_2 greater than 1 are possible, such values would be characterized by no additional fitness benefit but by additional costs. Net fitness corresponding to such allocations would thus be lower than net fitness with allocations corresponding to R_{max} and T_{max} , and thus need not be considered.

(2) *The interaction between costs of tolerance and resistance is additive, i.e.,* $C(R,T) = C(R,0) + C(0,T)$.

(3) *Benefits of tolerance, and of resistance, when present alone increase linearly with allocation to tolerance or resistance.* This assumption can be stated as $B(\gamma_1 R_{max}, 0) = \gamma_1 B(R_{max}, 0)$ for the benefit of resistance, and $B(0, \gamma_2 T_{max}) = \gamma_2 B(0, T_{max})$ for the benefit of tolerance. This assumption indicates that benefits of resistance and tolerance increase linearly up to R_{max} and T_{max}, when maximum benefit is attained. Further allocation will show a diminishing fitness return. Hence, the model considers the parameter space for which any benefit of allocation to defense is expected.

(4) *Benefits and costs per unit allocation were equal for resistance and tolerance.* This assumption implies that $T_{max} = R_{max}$ and that $C_T = C_R$.

(5) *The combined benefits of resistance and tolerance are less than additive.* This assumption implies that for an individual plant being completely tolerant or resistant results in a greater benefit return than having a mixed strategy of partial tolerance and resistance. In Mauricio *et al.*'s model, a multiplicative interaction between benefits of resistance and tolerance was employed to produce this kind of sub-additive

relationship:

$$B(R,T) = B(\gamma_1 R_{max}, \gamma_2 T_{max})$$
$$= B_{max}[1 - (1 - \gamma_1)(1 - \gamma_2)].$$

Combining all these assumptions into equation (1) results in the following equation to estimate the expected fitness of a given genotype (W_i) as:

$$W_i = W_0 + B_{max}[1 - (1 - \gamma_1)(1 - \gamma_2)] - C_{max}(\gamma_1 + \gamma_2). \tag{2}$$

Equation (2) produces a fitness surface with two adaptive peaks, isolated by a valley of suboptimal combinations of partially resistant and tolerant genotypes. One of these peaks corresponds to the fitness of a completely resistant genotype and the other to the fitness of a completely tolerant genotype (Fig 1).

ANALYSIS AND RESULTS

Two series of analyses were carried out. The first series consisted in relaxing sequentially assumption (1), (3), (4), and (5). For clarity each assumption was relaxed individually, leaving the other assumptions unchanged, in order to evaluate its specific effect on the adaptive surface. We maintained the assumption that costs of tolerance and resistance interact additively because there are no evidences to suspect this condition is not satisfied in natural populations. The second series of analyses examined the effects of relaxing more than one assumption simultaneously to visualize their combined effect on the adaptive landscape.

Non-linear functions for costs and benefits . We let the cost functions for tolerance and resistance deviate from linearity by employing the following general expressions for the cost functions:

$$C(\gamma_1 R_{max}, 0) = \gamma_1^a C(R_{max}, 0)$$

and

$$C(0, \gamma_2 T_{max}) = \gamma_2^a C(0, T_{max}),$$

where a is a shape parameter that describes how costs of defense increase per unit allocation. For simplicity we assume that a is the same for tolerance and resistance. Because we consider $0 \leq \gamma \leq 1$ to be the parameter space for which any benefit of allocation to defense can be obtained, when $a > 1$, marginal costs will increase more than linearly with allocation (concave upward cost function). We believe this possibility will be more likely to occur under resource limiting conditions if resources diverted to defense compromise the allocation to other fitness-correlated traits. The condition where $a < 1$ was also evaluated.

The same approach was used to obtain non-linear benefit functions for tolerance and resistance. In this case, the shape parameter is represented by b. General benefit functions for tolerance and resistance can thus be written as:

$$B(\gamma_1 R_{max}, 0) = \gamma_1^b B(R_{max}, 0)$$

and

$$B(0, \gamma_2 T_{max}) = \gamma_2^b B(0, T_{max}).$$

Again, we assume for simplicity that b is the same for tolerance and resistance. Although for values of $b \leq 1$ this shape parameter has been interpreted as the effectiveness of defense (Fagerström *et al.* 1987; Simms & Rausher 1987; Jokela *et al.* 2000), we also evaluated the condition were $b > 1$. Introducing these assumptions into equation (2) yields:

$$W_i = W_0 + B_{max}[1 - (1 - \gamma_1^b)(1 - \gamma_2^b)] - C_{max}(\gamma_1^a + \gamma_2^a). \tag{3}$$

The fitness surface corresponding to equation (3) was generated for a number of different values of the parameters a and b, by calculating W_i for each combination of (γ_1, γ_2), where γ_1 and γ_2 took values between 0 and 1, and using increments of 0.1. Equation (3) was evaluated for several values of a and b between 0.2 and 2 (see Frank 1994). The other parameter values were $B_{max} = 1$ and $C_{max} = 0.7$.

Allowing cost and benefit functions to be non-linear produced, in some cases, adaptive surfaces that are qualitatively different from that predicted by previous models (Fineblum & Rausher 1995; Mauricio *et al.* 1997). While some parameter

combinations do produce a fitness surface with two adaptive peaks, one corresponding to complete tolerance, the other to complete resistance (Fig 2A), other parameter values yield fitness surfaces with only one adaptive peak corresponding to intermediate values of both tolerance and resistance (Fig 2B). Moreover, the evolutionary scenarios depicted by Fig. 2A and 2B seem to depend in a regular way on the relative values of the shape parameters a and b. In all cases we examined, when $a > b$ the fitness function for tolerance and resistance present one adaptive peak of intermediate tolerance and resistance (i.e., a mixed strategy of defense) (Fig 3A). Conversely, when $a \le b$ the fitness function presents two peaks corresponding to either maximum or minimum tolerance and viceversa for resistance (Fig 3B). When $a > b$, the height of the adaptive peak (W_{max}) increases as a function of the positive difference between a and b (Fig 4A). Conversely, when $a < b$, the height of the adaptive peak decreases as the negative difference between a and b increases (Fig 4B). A negative difference between a and b will correspond to a situation where there are little scope for any fitness return per unit of defense allocation. According to the results (Fig. 3, Equation (3)), subsequent analyses were carried out using values of a and b of 0.5, 1 and 2 as representative of the linear and non-linear functions for costs and benefits.

Different maximal costs for tolerance and resistance. We examined the effect of allowing maximal costs of tolerance and resistance to differ. We did this by incorporating separate maximal costs of resistance and tolerance, C_R and C_T respectively, into equation (2) to yield

$$W_i = W_0 + B_{max}[1 - (1 - \gamma_1)(1 - \gamma_2)] - C_R\gamma_1 - C_T\gamma_2. \qquad (4)$$

Equation (4) was numerically resolved 200 times for every combination of C_R and C_T values between 0 and 2 at 0.1 intervals, and letting $B_{max} = 1$. Values of γ's were the same as in previous analyses. Numerical exploration of the (C_R, C_T) space indicates that the conditions for the existence of two alternative adaptive peaks depend on the

relative values of C_R and C_T with respect to B_{max} (Fig. 5). For instance, when C_R and C_T are both less than B_{max}, any combination of C_R and C_T results in an adaptive surface with two alternatives peaks (lower left region in Fig. 5). When $C_R < B_{max} < C_T$ one peak of maximum resistance is predicted (upper left region in Fig. 5), whereas when $C_T < B_{max} < C_R$ one peak of maximum tolerance is predicted (lower right region in Fig. 5). As expected, having C_R and C_T higher than B_{max} is maladaptive and neither tolerance nor resistance is favored (upper right region in Fig. 5). These results indicate that increasing the maximum cost (or cost per unit allocation) of one type of defense lowers the height of the adaptive peak corresponding to that defense, until the peak disappears when the maximum cost exceeds the maximum benefit. As long as costs of tolerance and resistance are linear, the adaptive surface retains two peaks even if the relative costs of the two types of defense change.

Additive and greater than additive benefit interaction. The interaction between benefits of resistance and tolerance had been considered previously to be less than additive. To relax this assumption and allow the possibility of additive or greater-than-additive benefits, we modeled the total benefit associated with resistance and tolerance as

$$B(R,T) = B_{max}(\gamma_1 + \gamma_2 + k\gamma_1\gamma_2),$$

where $0 \geq k \geq 1$. The situation $k = 0$ corresponds to an additive interaction between tolerance and resistance benefits, while $k > 0$ corresponds to a synergistic (greater-than-additive) interaction. When $k < 0$ correspond to a less than additive interaction between benefits (Mauricio *et al.* 1997). A similar rationale was previously followed to evaluate the interaction between benefits of allocation of resources to resistance against different natural enemies (Hougen-Eiztman & Rausher 1994). Substituting our modification into equation (2) yields:

$$W_i = W_0 + B_{max}(\gamma_1 + \gamma_2 + k\gamma_1\gamma_2) - C_{max}(\gamma_1 + \gamma_2). \tag{5}$$

When benefits are additive or more than additive (i.e., $k \geq 0$), the fitness surface has a single peak corresponding to a combination of maximum tolerance and resistance, as long as the maximum benefit, B_{max}, exceeds the maximal costs, C_{max}.

Relaxing Multiple Assumptions. In the second series of analyses, assumptions (1), (3), (4) and (5) were relaxed simultaneously by incorporating equations (3), (4), and (5), into equation (2), yielding:

$$W_i = W_0 + B_{max}(\gamma_1{}^b + \gamma_2{}^b + k\gamma_1{}^b\gamma_2{}^b) - C_R\gamma_1{}^a - C_T\gamma_2{}^a. \tag{6}$$

Equation (6) was simulated for each of the three types of benefits interaction (additive, subadditive, and greater than additive), and combinations of values of a (i.e., 0.5, 1, 2) and b (i.e., 0.5, 1, 2), yielding a total of 27 C_R-C_T phase spaces. Each C_R-C_T phase space was evaluated for a range of values of C_R and C_T between 0 and 2 at 0.1 intervals, letting $B_{max} = 1$ following the same rationale as for the evaluation of Equation (4). Numerical exploration of 5400 adaptive surfaces was conducted to determine the location of maximum within the surface. The results of this analysis indicated that three qualitatively different adaptive surfaces can occur depending on the type of benefit interaction, the relation between the shape parameters and relative values of costs of tolerance and resistance (Table 1, Fig. 6).

This analysis revealed that the presence of adaptive surfaces with one peak of maximum tolerance or resistance depends on the relative values of the corresponding cost for each strategy. When costs of tolerance are higher than those of resistance a peak of maximum or almost maximum resistance is expected (Fig 6A, B, C, D). The reverse will occur when costs of resistance are higher than those of tolerance. The presence of an adaptive surface with two alternative peaks of maximum tolerance and resistance occurs when benefits interact less than additive (Fig 6A, B). The size of the area corresponding this type of adaptive surface in Fig 6B depends on the relative values of a and b. When the positive difference between a and b increase the opportunities for the existence of adaptive surfaces with two alternatives peaks is

proportionately reduced. Conversely, when $a = b$, the area below B_{max} will correspond to adaptive surfaces with two alternative peaks as that predicted in Fig 6A.

Finally, the presence of adaptive surfaces with one peak corresponding to intermediate levels of tolerance and resistance appears when the parameter that describe the shape of the cost functions (i.e., a) is greater than the parameter that describe the shape of the benefit function (i.e., b) (Fig 6B, D). In this scenario an intermediate peak will represent a state of evolutionary equilibrium.

Within these combinations of costs and benefit functions whether the peak is bias toward more allocation to tolerance than resistance depends on the relative values of costs for both strategies. For example, when costs of tolerance are lower than those of resistance (i.e., $C_T < C_R$) the intermediate peak will be biased towards more allocation to tolerance than resistance (Fig 6D). The reverse is expected when costs of resistance are lower than costs of tolerance.

DISCUSSION

Our results indicate that several different outcomes of the joint evolution of tolerance and resistance are possible depending on the type of interaction between benefits of tolerance and resistance, the relative magnitudes of costs and the shapes of the cost and benefit functions. In particular, three generalizations can be drawn from our analysis:

(1) *In order for fitness surfaces to have two alternative peaks, the combined benefits of tolerance and resistance must be less than additive* (i.e., $k < 0$). Mauricio et al. (1997) demonstrated that two peaks *can* occur if $k < 0$. Our analysis extends this result and demonstrate that two peaks *cannot* occur if $k > 0$. The general explanation for this result is that multiple peaks can arise only if resistance and tolerance provide redundant benefits, which is possible only if increasing one of these types of defense has little effect on fitness when the other provides substantial benefits. Two out of three studies that have evaluated the joint pattern of selection on tolerance and

resistance have found significant correlational selection between tolerance and resistance (Tiffin and Rausher 1999; Pilson 2000). However, while the Tiffin and Rausher (1999) study support the existence of an adaptive landscape with two adaptive peaks, the other report an adaptive surface with one intermediate peak of tolerance and resistance (Pilson 2000). Interestingly, these studies indicate that benefits of tolerance and resistance can interact either subadditively or greater than additively. On the other hand, Mauricio et al. (1997) did not find significant correlational selection between tolerance and resistance and concluded that benefits interact additively. Given that few studies have evaluated the shape of the fitness landscape for tolerance and resistance, further studies are needed before assuming a specific kind of interaction between benefits of tolerance and resistance.

(2) *In general, in cases in which there is one fitness peak, whether that peak corresponds to maximal or almost maximal resistance, or to maximal or almost maximal tolerance depends on the relative costs of tolerance and resistance, i.e. on the relative values of* C_R *and* C_T. The former occurs when $C_R < C_T$, the latter when $C_R > C_T$. This result makes intuitive sense in that it indicates that selection will favor the cheaper of two redundant defenses.

Previous models suggested that the evolution of tolerance and resistance within populations may be determined by the initial frequencies of tolerant and resistant genotypes (Mauricio *et al.* 1997; Roy & Kirchner 2000). This expectation depends on the existence of two peaks in the fitness surface, a condition that, according to our model, will arise only under certain conditions. Under a different scenario, the evolutionary outcome will depend not on initial levels of tolerance and resistance, but on their relative costs. Furthermore, this result suggests that differences among populations in the relative magnitudes of tolerance and resistance may be caused by environmental differences that influence the relative magnitudes of the respective costs. Although a recent review at the species level pointed out the wide range of natural variation in the magnitude of costs of plant resistance (6-73% of fitness loss in the absence of herbivory; Strauss et al. 2002) no study have examined

the natural variation in the magnitude of costs of defense among natural populations (Cap. III). Even though proportionately few studies evaluated costs of tolerance some found significant allocational costs (Simms and Triplett 1994; Tiffin and Rausher 1999; Pilson 2000; Koskela et al. 2002; Stinchcombe 2002; Cap. III) while others not (Mauricio et al. 1997; Agrawal et al. 1999; Fornoni and Núñez-Farfán 2000).

Our model predicts that in those plant populations where levels of resource limitation increase allocational costs of resistance more than those of tolerance, selection is expected to favor a strategy of tolerance rather than resistance. It remains to be determined, however, how frequently this type of variation in relative costs actually contributes to variation among populations in defensive strategies. Also, within populations, spatial variation in the availability of resources can generate selective heterogeneity at a local scale maintaining genetic variation in ecologically important traits (Stratton 1994). Specifically, it is expected that costs of resistance will increase under light limitation when plants defend themselves by producing carbon-based secondary chemicals. On the other hand, under nutrient limitation costs related to the production of nitrogen-based secondary chemicals are expected to increase. It is less clear what to expect regarding the costs of tolerance since mechanisms underlying this strategy are unkonwn (Rosenthal & Kotanen 1994; Stowe *et al.* 2000; Tiffin 2000b). A profitable approach to addressing this issue would be to determine how costs of tolerance and resistance change across specific resource-availability gradients. An important outcome of our model is that under a plausible set of conditions variable costs of tolerance and resistance can represent an important aspect involved in the maintenance of intermediate levels of tolerance and resistance, and in favoring adaptive divergence in plant defensive strategies among populations.

(3) *A mixed strategy generally can only occur when the shape parameter of the costs function is higher than that of the benefit functions (i.e., a > b).* Recent studies for several plant species indicated that the shape of cost and benefit functions are commonly non-linear (Mauricio & Rausher 1997; Tiffin and Rausher 1999; Pilson 2000; Bergelson *et al.* 2001; Stinchcombe 2002). Based on the recognition that a cost of

defense represents redirection of resources away from some other fitness-enhancing functions, under resource limitation cost functions are expected to increase more than linearly with allocation to defense (Jokela et al. 2000). This argument suggests that within the range of habitats a given species occupies, non-linear cost functions may be more common in areas with higher level of resource limitation. To the extent that this expectation is realized, our model predicts that a mixed defense strategy consisting of partial allocation to both resistance and tolerance should be more common than previously noted (Fineblum & Rausher 1995; de Jong and van der Meijden 2000; Jokela et al. 2000).

Future empirical work should be directed toward the examination of the effect of the environment on the magnitude and shape of cost functions for different alternatives plant strategies of defense to improve our understanding of spatial and temporal patterns of plant defense allocation.

ACKNOWLEDGEMENTS

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Table 1. Results of simulations using Equation (6) under different set of parameter combinations. Asterisk indicate the occurrence of a given fitness surface.

Benefit interaction	Relative concavity between costs and benefits	Type of fitness surfaces		
		One peak of max. (R) or max (T)	Two alternative peaks of max. (R) and (T)	One intermediate peak of partial levels of (R) and (T)
$k < 0$	$a<b$	*	*	
	$a=b$	*	*	
	$a>b$	*	*	*
$k = 0$	$a<b$	*		
	$a=b$	*		
	$a>b$			*
$k > 0$	$a<b$	*		
	$a=b$	*		*
	$a>b$			*

Figure Legends

Figure 1. Fitness surfaces for resistance (γ_1) and tolerance (γ_2) using Equation (2). Values of the parameters are $W_0 = 0.1$, $B_{max} = 1$, $C_{max} = 0.7$.

Figure 2. **A.** Fitness surface produced using Equation (3) for $a = 1$ (linear cost function) and $b = 1$. **B.** Fitness surface for $a = 2$ (concave upward cost function) and $b = 1$. The positive signs (+) indicate the location of the adaptive peaks within the surface. The negative sign (–) indicate the region of the lowest fitness values within the fitness surface. Values of the parameters are $W_0 = 0.1$, $B_{max} = 1$, $C_{max} = 0.7$. Number of contours where adjusted to facilitate a clear visualization of the shape of the fitness surface.

Figure 3. **A.** Fitness function for resistance using Equation (3) for several values of the shape parameter b (between 1 and 2 at 0.2 intervals: left panel, and between 0.2 and 1 at 0.2 intervals: right panel) leaving a fixed at 2. **B.** Fitness function for resistance using Equation (3) for several values of the shape parameter a (between 1 and 2 at 0.2 intervals: left panel, and between 0.2 and 1 at 0.2 intervals: right panel) leaving b fixed at 2. For all panels $T = 0.3$

Figure 4. Relationship between the magnitude of the adaptive peak (W_{max}) and the difference between the shape parameters a and b using Equation (3) when $a > b$ (**A**), and when $a < b$ (**B**). Values of the other parameters are $W_0 = 0.1$, $B_{max} = 1$, $C_{max} = 0.7$, $T = 0.3$.

Figure 5. Four regions in a (C_R, C_T)-space with different implications for the evolution of tolerance and resistance. Letting $W_0 = 0.1$, $B_{max} = 1$, when $0 \geq C_R \leq 2$ and $0 \geq C_T \leq 2$, Equation (4) predicts that the lower left region corresponds to an evolutionary scenario with two alternative peaks of maximum tolerance and resistance (i.e., when $C_R, C_T < B_{max}$). The upper left region corresponds to an evolutionary scenario with one optimum peak of maximum resistance (i.e., when $C_R < B_{max} < C_T$). The lower

right region corresponds to an evolutionary scenario with one optimum peak of maximum tolerance (i.e., when $C_T < B_{max} < C_R$). The upper right region corresponds to an evolutionary scenario with no adaptive peak (i.e., when $C_T, C_R > B_{max}$).

Figure 6. Hypothetical evolutionary scenarios for the evolution of tolerance and resistance predicted by Equation (6). "(R)" and "(T)" (grey regions) indicates pure strategies of resistance or tolerance are favored, respectively. "(R,T)" (dark region) indicates the region in which two peaks are present in the adaptive landscape, one corresponding to complete resistance, the other corresponding to complete tolerance. A. C_R, C_T-space when $a \leq b$, and $k < 0$. B. C_R, C_T-space when $a > b$, and $k < 0$. The white region (upper right corner) corresponds to a mixed strategy of intermediate equivalent values of R and T (i.e., $\gamma_1 = \gamma_2$). The darkest region represents the area in which two alternative peaks of maximum R and T are expected. C. C_R, C_T-space when $a \leq b$ and $k \geq 0$ produce adaptive surface of only one peak corresponding to maximum T or R. D. C_R, C_T-space when $a > b$ and $k \geq 0$. The region above the diagonal line corresponds to a mixed strategy biased toward more allocation to R than T (i.e., $\gamma_1 > \gamma_2$). Below the diagonal line there is a region where a mixed strategy biased toward more allocation to T than R is expected (i.e., $\gamma_2 > \gamma_1$). The combination when $a = b$ and $k > 0$ also produce a fitness surface with intermediates peaks of tolerance and resistance. For all panels, $W_0 = 0.1$, and $B_{max} = 1$.
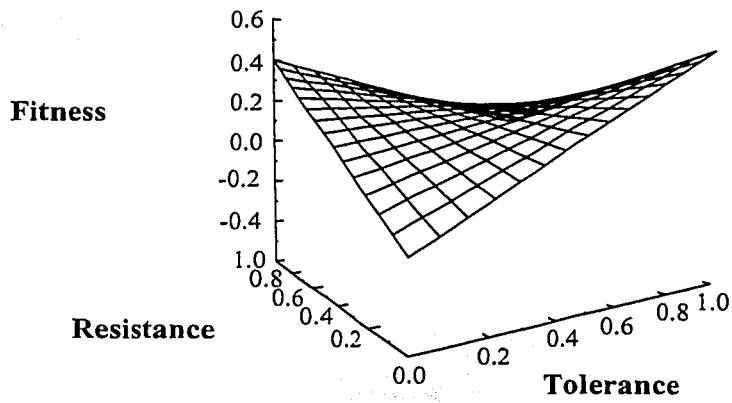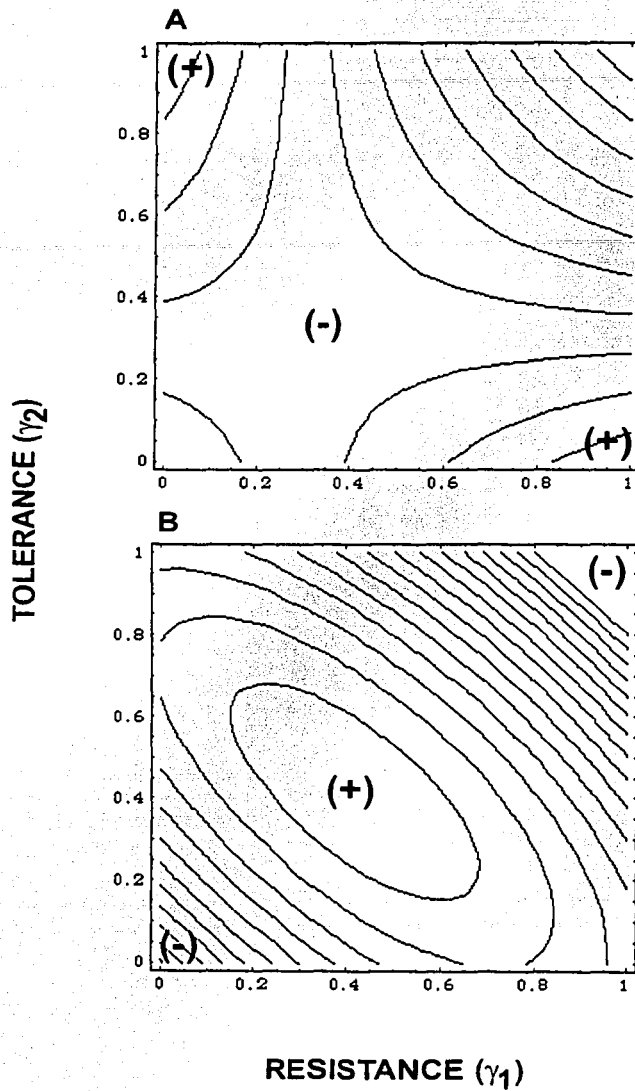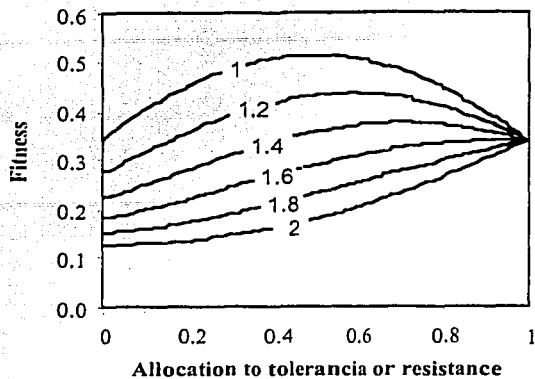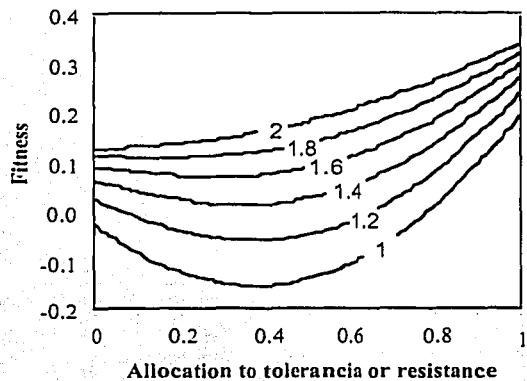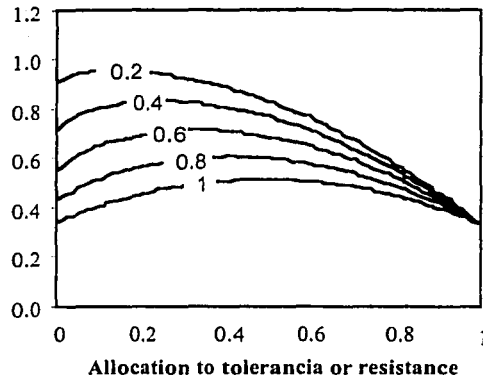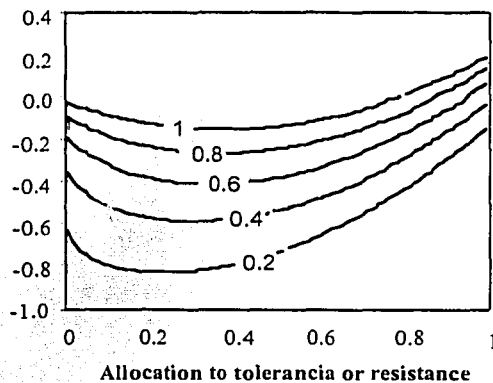
Figure 1.

RESISTANCE (γ_1)

Figure 2

Figure 3.

Figure 4.

Costs of Resistance (C_R)

Figure 5.

Costs of Resistance (C_R)

Costs of Tolerance (C_T)
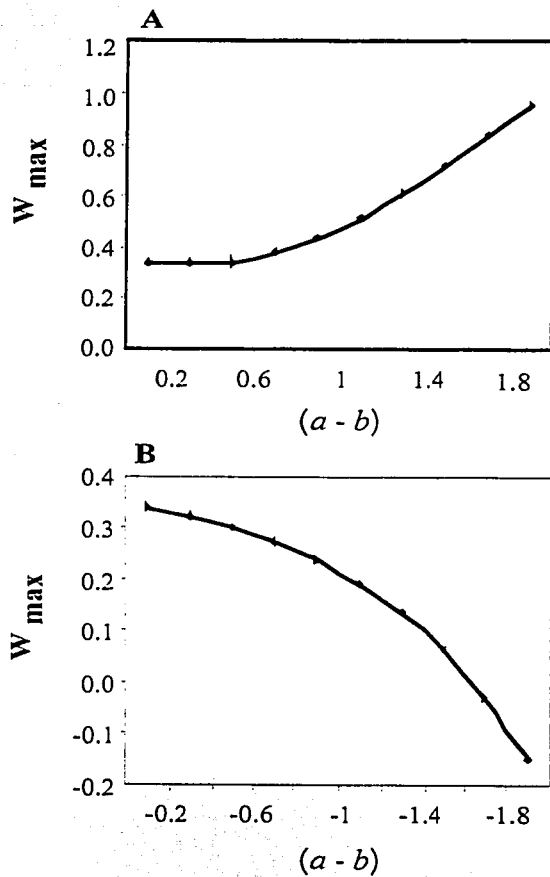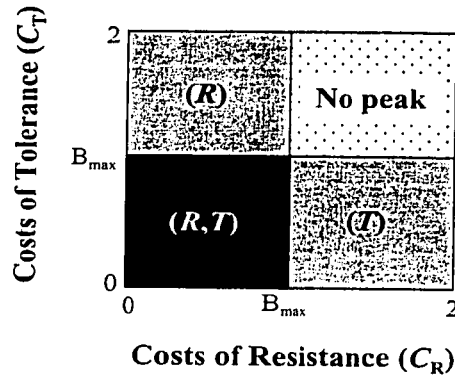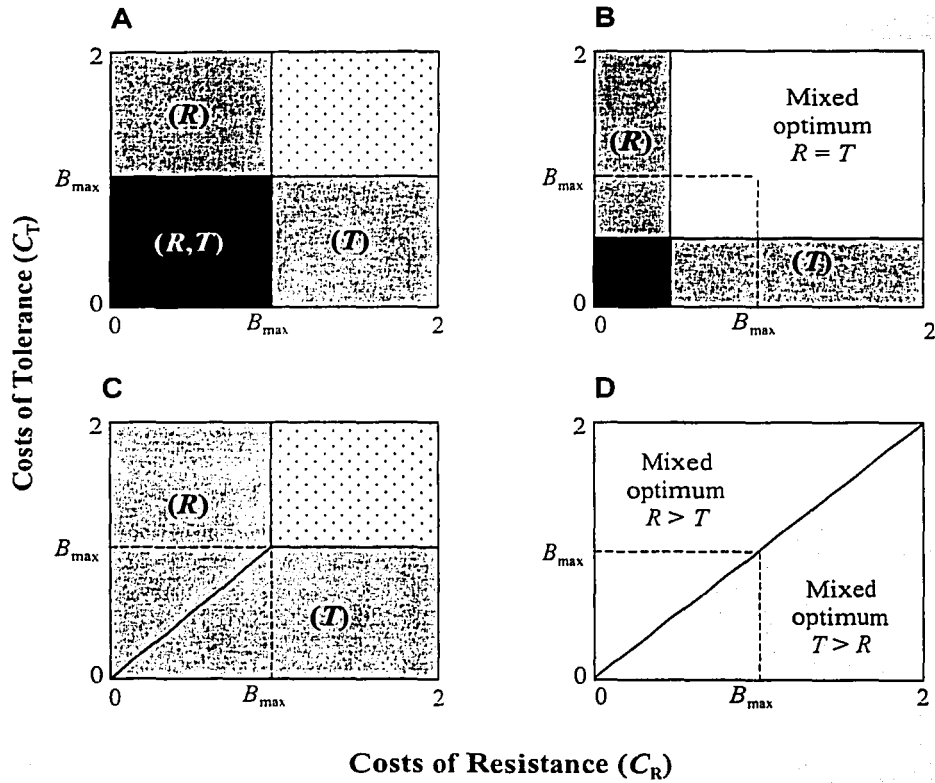
Figure 6.

CAPÍTULO II

ECOLOGÍA EVOLUTIVA DE *Datura stramonium*: VARIACIÓN

GENÉTICA Y COSTOS PARA LA TOLERANCIA A LA

DEFOLIACIÓN

30

EVOLUTIONARY ECOLOGY OF *DATURA STRAMONIUM*: GENETIC VARIATION AND COSTS FOR TOLERANCE TO DEFOLIATION

Juan Fornoni[1,2,3] and Juan Núñez-Farfán[1,4,5]

[1]*Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, C.P. 04510 México, Distrito Federal, Mexico*
[2]*Universidad Nacional de Córdoba, Casilla de Correo 345, 5000 Córdoba, Argentina*
[3]*E-mail: jfornoni@miranda.ecologia.unam.mx*
[4]*E-mail: farfan@servidor.unam.mx*

Abstract.—The incorporation of plant tolerance after damage as a new alternative to cope with herbivory, as opposed to resistance, opened new avenues for our understanding of coevolution between plants and herbivores. Although genetic variation on tolerance to defoliation has been detected in some species, few studies have been undertaken with nonagricultural species. In this study, we explore in the annual weed *Datura stramonium* the existence of genetic variation for tolerance and fitness costs of tolerance. To determine which fitness-related trait was responsible for possible differences in tolerance, growth rate, total flower and fruit production, and the number of seeds per fruit were recorded. Inbred line replicates of *D. stramonium* from a population of Mexico City were exposed to four defoliation levels (0%, 10%, 30%, and 70%). Our results from a greenhouse experiment using controlled genetic material (inbred lines) indicated that significant genetic variation for tolerance was detected across defoliation environments. Defoliation reduced plant fitness from 15% to 25% in the highest levels of defoliation. Differences on tolerance among inbred lines were accounted by a differential reduction in the proportion of matured fruits across defoliation levels (up to 20%). Within defoliation levels, significant genetic variation in plant fitness suggests that tolerance could be selected. The correlation between fitness values of inbred lines in two environments (with and without damage) was positive ($r_g = 0.77$), but not significant, suggesting absence of fitness costs for tolerance. The finding of genetic variation on tolerance might be either due to differences among inbred lines in their capability to overcome foliar damage through compensation or due to costs incurred by inducing secondary metabolites. Our results indicate the potential for norms of reaction to be selected under a gradient of herbivory pressure and highlights the importance of dissecting induced from compensatory responses when searching for potential causes of genetic variation on tolerance.

Key words.—Costs, *Datura stramonium*, genotype × environment interactions, herbivory, phenotypic plasticity, tolerance.

The large variation in plant damage among populations, individuals, and years encountered in plant-herbivore systems (reviewed in Marquis 1992) suggests that the intensity of herbivory might be highly heterogeneous in nature. Plants can prevent or compensate for tissue damage by means of resistance or tolerance, respectively. Whereas resistance is usually measured as the reciprocal of the amount of damage experienced by plants (Rausher 1996; but see Mauricio 1998), tolerance has been defined as the capacity of a plant to buffer the negative effects of herbivory on fitness through growth and reproduction (Burdon 1987). However, this definition of tolerance does not consider the fact that the amount of damage a plant withstands is usually environment dependent. Considering that the intensity of herbivory is variable, one may expect a given genotype, or its progeny, to experience a range of herbivory pressures. In an evolutionary context, tolerance should instead be viewed as a fitness norm of reaction of genotypes under a gradient of herbivory pressure (Mauricio et al. 1997). The analysis of plant responses using phenotypic trajectories under different levels of herbivory may offer valuable insights on both evolutionary constraints and on its potential consequences on the evolution of plant defenses. However, the existence of genotype-by-environment (G × E) interaction has rarely been assessed in evo-

lutionary studies of plant-herbivore interactions (Edmunds and Alstad 1978; but see Via 1984; Strauss 1990; Bergelson 1994; Schlichting and Pigliucci 1998).

If tolerance has inherent costs, a negative genetic correlation in fitness between damaged and undamaged plants of a given plant genotype might be expected (Simms and Triplet 1994), provided that plants with high levels of tolerance will pay fitness costs for being resistant (i.e., avoid herbivore attack). Thus, tolerance evolution can be constrained either by its associated costs and/or by costs of resistance (Fineblum and Rausher 1995). Despite the vast literature on resistance (Fritz and Simms 1992), few studies have attempted to assess the amount of genetic variation for tolerance (Simms and Triplett 1994; Fineblum and Rausher 1995; Mauricio et al. 1997; Agrawal et al. 1999). The constraints on the evolution of plant defense characters deserve further study.

Previous studies analyzing tolerance among genotypes within a population have found significant genetic variation (Simms and Triplett 1994; Fineblum and Rausher 1995; Mauricio et al. 1997; Agrawal et al. 1999). Nevertheless, only the study of Simms and Triplett (1994) has detected costs for tolerance to a pathogen species in *Ipomoea purpurea*. The present study was aimed at experimentally evaluating in the annual weed *D. stramonium*, the existence of (1) genetic variation for tolerance to defoliation; (2) genetic variation in fitness (number of seeds per plant) and four fitness related traits (growth rate, total flower and fruit production, number

[5] Present address: Department of Ecology and Evolutionary Biology, University of Connecticut, Torrey Life Science Building, 75 North Eagleville Road, Storrs, Connecticut 06269-3043.

of seeds per fruit); and (3) fitness costs of tolerance to defoliation. In addition, we attempted to determine which of the fitness-related characters measured was responsible for possible differences on tolerance among inbred lines within a population on central Mexico.

MATERIALS AND METHODS

Plant System and Study Site

Datura stramonium L. (Solanaceae, jimsonweed) is a cosmopolitan summer annual species. This herb inhabits disturbed areas and borders of cultivated fields (Weaver and Warwick 1984; Núñez-Farfán 1991). The amount of leaf tissue damaged by herbivores ranges from 0% to 90% of the plant's leaf area (Núñez-Farfán and Dirzo 1994). The plant material used in this study was collected from a population located in the Pedregal of San Angel Ecological Preserve in southern Mexico City. A complete description of the species and study site can be found elsewhere (Núñez-Farfán and Dirzo 1994; Núñez-Farfán 1995 and references therein).

Genetic Material

We created six partially inbred lines of *D. stramonium* by randomly selecting seeds from a pool of genotypes belonging to a population studied in 1989 in the Pedregal of San Angel Ecological Preserve (Núñez-Farfán and Dirzo 1994; Núñez-Farfán et al. 1996). During two years (1989, 1992), seeds from the original families were germinated and resulting plants automatically self-fertilized in common-garden conditions to diminish environmental maternal effects and to increase genetic identity within each family (self-sibs; Geber 1990; Mauricio et al. 1997).

Experimental Design and Protocol

For each inbred line, 60 seeds from the first five fruits were selected and subjected to 12-h temperature changes (5°C to 20°C) in darkness for dormancy breakdown during four days. To stimulate germination, part of the seed coat was removed near to the area where the radicule emerges; the seeds were then allowed to imbibe water for 48 h. All seeds were placed over filter paper in petri dishes in controlled environmental chambers (Conviron 630, Winnipeg, Manitoba, Canada), with a 12:12 L:D photoperiod, 28°C (day) and 22°C (night) temperatures, and constant humidity (85%; Reisman-Berman et al. 1991). The first 18 germinated seeds of each inbred line were planted in plastic pots (3 L) filled with a commercial homogenized soil. Filter paper was placed at the bottom of each pot to prevent soil losses during periodic watering. Seedlings were transplanted when the first two true leaves emerged. Although approximately 50% of the initial sample of seeds germinated, seed mass of seedlings planted covered the range of seed mass present in the initial seed pool (Kolmogorov-Smirnov test, $D_{max} = 0.01$, $P > 0.05$; Sokal and Rohlf 1995). We established an experimental population in the greenhouse using a completely randomized design with six inbred lines and four defoliation levels. One hundred fifteen individual plants (6 inbred lines × 4 defoliation levels × 4–6 plant replicates per combination) were maintained under the same soil, water, and light conditions throughout

the experiment. Each plant of each line was randomly assigned to one of four defoliation levels: 0%, 10%, 30%, or 70% of leaf area removed. Defoliation range selected falls within the natural range of herbivory that *D. stramonium* plants experience in this population (see fig. 1 in Núñez-Farfán and Dirzo 1994). Plants were randomly rearranged every 30 days during the first three months to avoid environmental heterogeneity within the greenhouse (Potvin 1993).

Treatment Application

To analyze fitness differences among inbred lines, leaf area removal was carried out at the same phenostage for all plants (see Coleman et al. 1994). This procedure allowed us to compare reproductive output among plants by assessing environmental (defoliation) and genetic, inbred line effects on tolerance without confounding effects due to developmental variation among plants (but see Rice and Bazzaz 1989). Because flower initiation is sequential, leaf area removal for each plant was performed when the first flower at the fourth branching order was in anthesis. At this time, three characters related to plant size were measured: total leaf area, total number of leaves, and plant height. The relationship between leaf length and leaf area for *D. stramonium* (leaf area = 0.329[leaf length]^2, $r^2 = 0.98$; Núñez-Farfán and Dirzo 1994), was used to estimate total leaf area and the amount of leaf area to remove under each defoliation level. The results of a multivariate analysis of variance (MANOVA) for total leaf area, total number of leaves, and plant height revealed that there were no differences in size among genotypes at the time they reached the predetermined phenostage ($F_{Wilk's \lambda} = 1.33$, df = 15,304, $P > 0.1$). This result indicated that the criterion chosen for leaf area removal time was appropriate to avoid size variation in subsequent analyses.

Artificial defoliation was carried out by making 27-mm^2 holes in each leaf with a paperpunch. Leaf discs were removed without damaging the main and largest lateral leaf veins. This procedure allowed us to extract a given percentage of leaf area relative to leaf number and size variations within and among plants. Leaf area removal of plants assigned to the highest defoliation level of 70% was carried out in two extractions of 35% each. The second extraction was performed 15 days after the first defoliation, because this is the amount of time herbivores take to consume almost all the leaves of a plant (J. Núñez-Farfán, pers. obs.).

Before imposing defoliation, we examined whether seed mass was correlated with seedling (days to emergence) and adult (leaf number at 60 days, the time of first opened flower, and total number of flowers) characters in the same plant material used for the experiment. None of the correlations was statistically significant ($P > 0.05$ in all cases, correlation coefficients ranged from $r = -0.20$ to $r = 0.06$, n = 167; J. Fornoni and J. Núñez-Farfán, unpubl. data). Thus, seed mass was not considered as covariable in the following analyses of tolerance to defoliation (but see Schmitt et al. 1992).

Characters Measured

Absolute maternal fitness (number of seeds per plant) and four related traits were estimated: growth rate following leaf

area removal, total flower production, total fruit production, and number of seeds per fruit (i.e., seed set). Growth rate was calculated as the number of leaves produced per plant from the time it was defoliated (0) until the end of the experiment (1), divided by the number of leaves at defoliation (0) ([number of leaves$_1$ − number of leaves$_0$]/number of leaves$_0$; Fineblum and Rausher 1995).

Individual plant variation in herkogamy (spatial variation between stigma-anthers position) can generate differences in fitness in predominantly self-fertilizing plants due to differential opportunity for selfing among plants (Webb and Lloyd 1986). Although *D. stramonium* is highly self-compatible (91.8%; Núñez-Farfán et al. 1996) and fertilization of ovules is not limited by pollinators visitation, an effect of the herkogamy level on the plant's outcrossing rate has been reported (Motten and Antonovics 1992). Because pollinators were absent in the greenhouse and seed set resulted from natural self-fertilization, herkogamy was recorded for each flower to determine its relationship with number of seeds per fruit. Regression analysis showed that herkogamy explains only 1% of the variance in number of seeds per fruit ($r = 0.013$; $F = 1.4$; $P < 0.05$; $n = 476$; J. Fornoni and J. Núñez-Farfán, unpubl. data). Thus, differences in fitness can not be attributed to differences in herkogamy.

Data Analysis

Data analysis integrated previous approaches for the analysis of norms of reaction and standard quantitative genetics analysis for full-sib experiments (Fry 1992; Sultan and Bazzaz 1993a,b; Simms and Triplett 1994; Falconer and Mackay 1996; Mauricio et al. 1997). Variables were transformed to ensure normality assumptions. Growth rate was square-root transformed and total fruit production was log transformed. The Bartlett test was used to assess homogeneity of variances (Sokal and Rohlf 1995). Univariate mixed-model analyses of variance (ANOVA, Type III SS) were performed using the multivariate general linear hypothesis (MGLH) module of SYSTAT (vers. 5.2.1). For each of the five characters, inbred line was considered as a random effect and defoliation level as the fixed effect. The F-ratio for the random effect and the interaction effect term were calculated using the model error MS as denominator. The interaction's MS was used as denominator for the fixed effect to calculate the F-value according to Scheffe's model (Fry 1992; Simms and Triplett 1994). Following these estimations of F-values, a significant interaction would indicate potential genetic differences among lines in their response to the defoliation gradient (i.e., differences in fitness among inbred line across defoliation levels). Significant defoliation treatment effect would indicate whether defoliation has negative or positive effects upon plant fitness across the defoliation gradient. Unplanned comparisons among defoliation levels and inbred lines were determined using the Student Newman-Keuls test (SNK) for multiple comparisons (Underwood 1997). The probability of Type I error was controlled using the Bonferroni adjustment procedure (Day and Quinn 1989).

Because tolerance is operationally estimated as the slope in the relationship between fitness and damage (Mauricio et al. 1997), an analysis of covariance (ANCOVA, Type III SS)

TABLE 1. Analysis of covariance of relative fitness of inbred *Datura stramonium* lines subject to defoliation. Sums of squares Type III were used.

Source of variation	df	SS	F	P
Inbred line	5	0.940	7.61	<0.0001
Defoliation	1	28.472	1153.15	<0.0001
Inbred line × defoliation	5	0.710	5.75	<0.001
Error	102	2.518		

was performed (MGLH module, SYSTAT 5.2.1) to decompose the inbred line × defoliation interaction derived from the two-way mixed ANOVA. Relative fitness was estimated by dividing the total number of seeds per plant by the maximum total number of seeds per plant. Relative fitness was considered as the response variable, defoliation as the covariate, and inbred line as the fixed factor. Thus, a significant inbred line × defoliation interaction will indicate truly genetic differences on tolerance among inbred lines within the population (i.e., differences on overall reaction norm slopes for fitness among inbred lines; Simms and Triplett 1994; Mauricio et al. 1997). Because the use of full-sibs does not allow an accurate estimation of additive genetic variance component, a conservative estimation of genetic variance among inbred lines was preferred. Thus, when the inbred line effect resulted significant in the two-way mixed-model ANOVA (following Scheffe's model), separate one-way ANOVAs (model II; module MGLH, SYSTAT 5.2.1) were performed to assess genetic variation within defoliation levels. This procedure was performed using the model variable = constant + line to see whether the significant line effect detected in the two-way mixed ANOVAs indicate consistent genetic differences (Sultan and Bazzaz 1993a).

A two-way mixed ANOVA (module MGLH, SYSTAT 5.2.1) was performed to detect a potential reproductive "trade-off" between damaged and undamaged plants of the same inbred line (i.e., fitness costs of tolerance to herbivory). Here, F-ratio for the inbred line effect was obtained by dividing the inbred line MS by inbred line × defoliation MS (Hocking's model in Fry 1992). This test assesses whether the fitness of an inbred line expressed in different defoliation environments is correlated (see Fry 1992). A significant inbred line effect test for the presence of a significant genetic correlation ($r_g > 0$) of character states between environments (i.e., no costs of tolerance). In contrast, if the interaction effect is statistically significant, costs of tolerance may exist. The magnitude of such costs should be detected as a correlation of family means of character states between environments (i.e., defoliation levels). If fitness of undamaged plants is negatively correlated with fitness of damaged plants ($r_g < 0$), fitness costs for tolerance to defoliation may exist (Simms and Triplett 1994; Strauss and Agrawal 1999).

RESULTS

Genetic Variation for Tolerance

The results of an ANCOVA indicated that inbred lines differed in their response after damage provided the significant inbred line × damage level interaction (Table 1). This means that inbred lines differed for the slope of the rela-
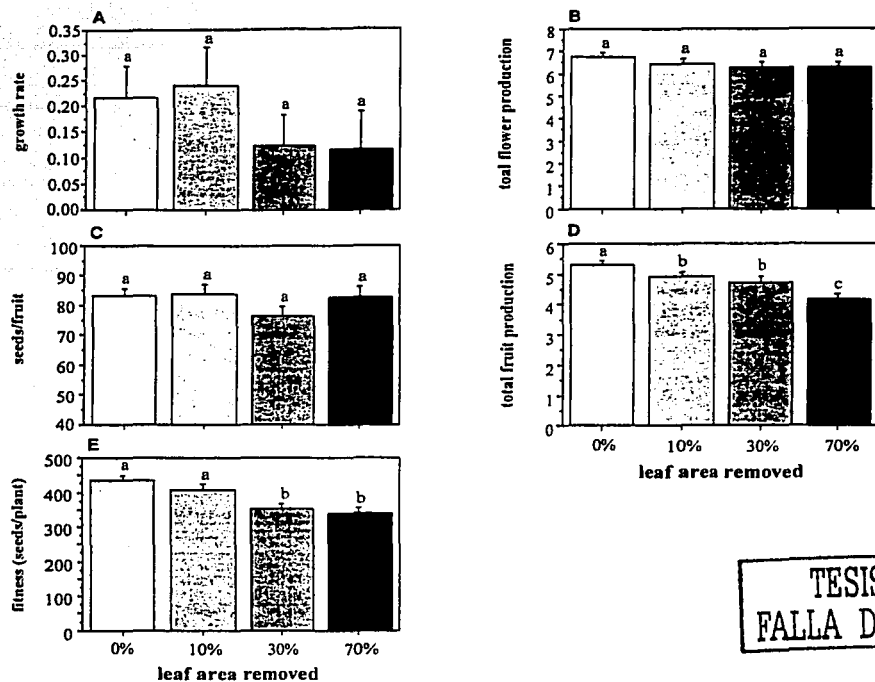
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FIG. 1. Means (+1 SE) for five fitness components of *Datura stramonium* under a defoliation gradient. (A) Growth rate; (B) total flower production; (C) seeds per fruit; (D) total fruit production; (E) fitness (seeds per plant). Different letters between pairs of defoliation treatments indicate significant differences at *P* < 0.006 following Student-Newman-Keuls tests with the Bonferroni correction. Data were graphed using untransformed values.

tionship between fitness and damage (i.e., tolerance). Thus, genetic variation for tolerance was detected within the studied population (Table 1). Nonlinear terms were not included because in a previous analysis the second-order interactions were not statistically significant.

Defoliation Effect on Fitness and Four Related Traits

No differences among defoliation levels in growth rate, total flower production, and number of seeds per fruit were found (Fig. 1A, B, C). Significant differences were found between the control and the other defoliation levels in total fruit production (SNK; *P* < 0.05; Fig. 1D). No differences in total fruit production were observed between intermediate levels of defoliation (SNK; *P* > 0.05; Fig. 1D), whereas significant differences were found between both intermediate defoliation levels (10–30%) and the highest defoliation treatment (70%; SNK; *P* < 0.05; Fig. 1D). On average, all genotypes of *D. stramonium* showed a significant reduction in absolute maternal fitness (number of seeds per plant; Fig. 1E), indicating fitness costs of defoliation. Thus, tolerance differences were due to the effect of defoliation on the proportion of flowers that matured fruits (i.e., fruit set), because there were no differences in total flower production. Indi-

vidual plants with 30% of their leaf area removed had a fitness reduction of 15%, whereas in those with 70% of leaf area removed, fitness losses approached 25% (Fig. 1E). Plants in the control treatment produced approximately 20% more seeds than the two highest intensity defoliation treatments (SNK; *P* < 0.05 for each paired comparison; Fig. 1E). However, no differences in fitness between the control plants and the lowest defoliation level (10%) were observed (SNK; *P* > 0.05), suggesting that either the analysis was not able to detect small fitness differences or that plants can tolerate low levels of damage without a significant fitness reduction (Fig. 1E).

Responses of inbred lines across the defoliation gradient accounted for a significant amount of variance in fitness and in the three reproductive traits (total flower production, total fruit production, and number of seeds per fruit). Significant inbred line effects were found for total flower and fruit production, seeds per fruit, and fitness, whereas no differences in growth rate were evident (Table 2). Similarly, all characters but growth rate showed statistically significant inbred line × defoliation interactions in the mixed models (Table 2, Fig. 2), indicating that inbred lines also differed in their phenotypic trajectories across the defoliation gradient.

TABLE 2. Two-way mixed-model analyses of variance (Scheffé's model) for five fitness-related traits in six inbred lines of *Datura stramonium* under four levels of defoliation. Sums of squares Type III were used.

Variable	Source of variation	df	SS	F	P
Growth rate	inbred line	5	1.541	1.85	0.111
	defoliation	3	0.526	1.46[1]	0.264
	inbred line × defoliation	15	1.796	0.72	0.759
	error	90	14.991		
Total flower production	inbred line	5	120.268	14.66	<0.001
	defoliation	3	71.467	1.80[1]	0.190
	inbred line × defoliation	15	198.310	8.06	<0.001
	error	90	147.611		
Total fruit production	inbred line	5	76.574	13.87	<0.001
	defoliation	3	77.284	3.69[1]	0.036
	inbred line × defoliation	15	104.557	6.29	<0.001
	error	90	99.717		
Number of seeds per fruit	inbred line	5	11120.883	6.66	<0.001
	defoliation	3	10693.528	2.25[1]	0.124
	inbred line × defoliation	15	23706.890	4.73	<0.001
	error	90	30050.095		
Fitness (seeds per plant)	inbred line	5	386729.039	8.84	<0.001
	defoliation	3	610250.039	4.93[1]	0.014
	inbred line × defoliation	15	618338.885	4.71	<0.001
	error	90	786913.274		

[1] $F_{\text{defoliation}} = MS_{\text{defoliation}}/MS_{\text{inbred line} \times \text{defoliation}}$; details in the Materials and Methods section.
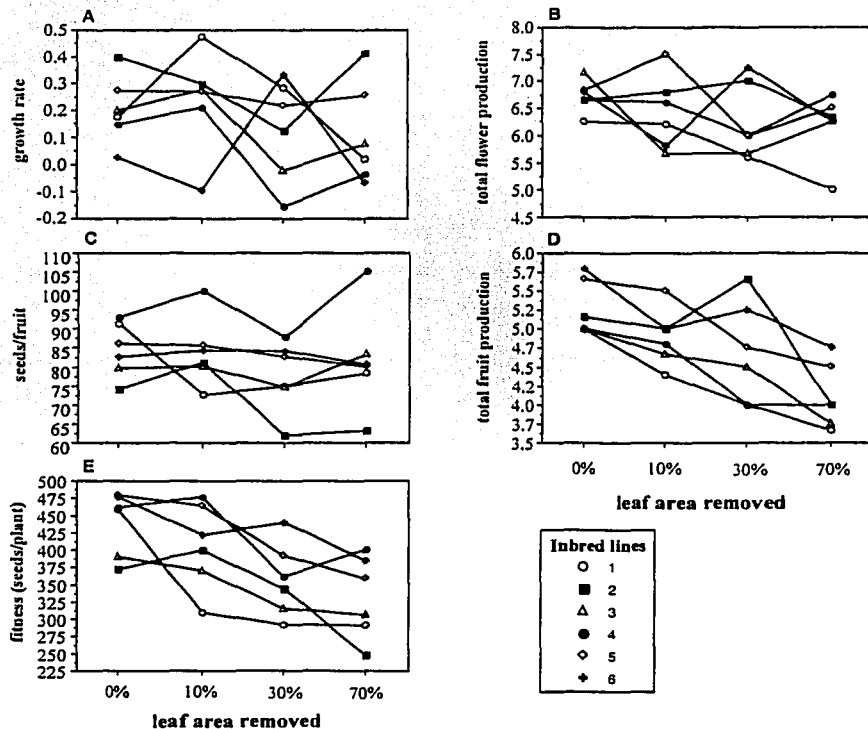
FIG. 2. Norms of reaction of six inbred lines of *Datura stramonium* under a defoliation gradient. (A) Growth rate; (B) total flower production; (C) seeds per fruit; (D) total fruit production; (E) fitness (seeds per plant). Data were graphed using untransformed values. Bars indicate ± 1 SE.

TABLE 3. F-values from one-way analyses of variance of five fitness-related traits in *Datura stramonium* within each of four defoliation levels. Numbers in parentheses correspond to sample size within each defoliation level.

	Defoliation level			
	0% (30)	10% (31)	30% (28)	70% (24)
Variable			F	
Growth rate	0.73ns	0.90ns	1.91ns	0.94ns
Total flower production	0.59ns	2.79*	1.27ns	1.07ns
Total fruit production	1.07ns	0.94ns	2.25ns	1.31ns
Number of seeds per fruit	1.30ns	1.41ns	1.87ns	3.23*
Total seeds per plant (fitness)	3.43*	3.35*	4.32*	3.41*

* $P < 0.05$; ns, nonsignificant.

Genetic Variation on Reproductive Traits

Although the initial analyses indicated a significant inbred line effect for total flower and fruit production, number of seeds per fruit, and fitness (Table 2), one-way ANOVAs within defoliation levels (environments) revealed that only plant fitness showed statistically significant differences among inbred lines. Thus, only absolute maternal plant fitness showed consistent genetic variation among inbred lines within environments (Table 3). Significant genetic variation was also detected for seed set on the 70% defoliation level and for total flower production in the 10% defoliation level. However, given the sample sizes, the absence of differences among lines must be taken with caution.

Fitness Costs of Tolerance

The inbred line effect tested under Hocking's F estimation was not significant (SS = 1.451, $F_{5.5} = 1.83$; $P > 0.05$; $n = 54$), indicating no significant covariance in plant fitness between the control and the higher defoliation level. Although the inbred line × defoliation interaction was significant (SS = 0.789, $F_{5.43}$, = 5.42; $P < 0.001$, $n = 54$), it was not as strong as needed to detect a negative correlation between the control and the higher defoliation level ($r_g = 0.77$, $P > 0.05$, $n = 6$). Power analysis indicated that a sample size of 17 inbred lines would be necessary to detect a significant correlation of that magnitude (Cohen 1977); however, a positive correlation does not support the presence of costs for tolerance. Even when the overall model was significant ($F = 6.29$, $P < 0.00001$, $n = 54$), and with a strong power test ($1 - \beta = 0.99$ holding α at 0.05; SAS Institute 1995), the results showed no evidence of constraints on the expression of fitness between the control and the higher defoliation level.

DISCUSSION

The results for *D. stramonium* indicated the presence of significant genetic variation on tolerance. In addition, no evidence of fitness costs for tolerance was detected. Variation in tolerance among inbred lines was mainly due to differences in the proportion of matured fruits, because differences in total fitness were more related to total fruit production than to total flower production or number of seeds per fruit. The results of this study showed that leaf damage produced a

reduction in total plant fitness, which supports previous observations in the field (Núñez-Farfán and Dirzo 1994).

Although genetic variation on tolerance to defoliation have been found in several recent studies (Fineblum and Rausher 1995; Mauricio et al. 1997; Stowe 1998; Agrawal et al. 1999), few studies measured the negative effects of damage in terms of fitness (see referencs in Strauss and Agrawal 1999). As in *D. stramonium*, it has been found that fruit production accounted for differences on tolerance in *I. purpurea* and *Brassica rapa* (Fineblum and Rausher 1995; Stowe 1998).

Several factors could account for the existence of genetic variation in tolerance found in *D. stramonium*. For instance, if different lines allocate different amounts of resources toward induced resistance after defoliation, differences on tolerance might be a consequence of a different amount of resources devoted to increase resistance after damage rather than to diminish fitness costs of defoliation through a compensatory response (i.e., tolerance). In a recent study with a congeneric species, *D. wrightii*, induction of sticky glandular trichomes has been found after foliar damage (Elle et al. 1999). However, in *D. stramonium* a positive correlation between early versus late foliar damage under natural conditions has been detected ($r^2 = 0.04$, $P < 0.0001$, $n = 338$), suggesting no induction of defense (P. L. Valverde and J. Núñez-Farfán, unpubl. data). Thus, only after further evidence supports the absence of defense induction in *D. stramonium* can this hypothesis be ruled out. Nevertheless, our results highlight the importance of dissecting induced from compensatory responses when searching for potential causes of variation in tolerance.

In contrast, genetic variation on tolerance may be related to many growth traits involved in the response of plants after natural or experimental defoliation (Strauss and Agrawal 1999). For instance, tolerance to herbivores can be promoted physiologically if damaged plants can reallocate resources to undamaged branches and stimulate their further growth (Bilbrough and Richards 1993) or increase the metabolic activity of remaining photosynthetic tissues (Whitham et al. 1991; but see Kimberly and Reekie 1995). Morphologically, tolerance after damage may result in the activation of secondary meristems (Coughenour 1985), if resources and meristems are not limited (see Geber 1990). In *D. stramonium*, resource limitation seems to explain the negative effect of defoliation on fitness. First, damage reduces leaf area, and thus resources, suggesting a higher metabolic activity of the remaining tissues given the absence of differences in growth rate (measured as relative leaf production after damage) between damaged and undamaged plants. Second, in *D. stramonium* the production of new branches, leaves, and flowers is sequential and the damage by herbivores is concentrated in leaf tissue rather than on meristems. Thus, the negative effect on fruit production but not on flower production indicated that defoliation exerted a significant negative effect on the proportion of flowers that matured to fruit. In summary, our results for *D. stramonium* indicate that the pattern of fitness decrement after damage was a consequence of resource limitation (i.e., defoliation), expressed as a reduction in the proportion of flowers that matured fruits. Given the great morphological and developmental limitation of this species, compensation, if any, seems to be expressed at the leaf level.

The absence of differences in growth rate between damaged and undamaged plants found in other species (Bilbrough and Richards 1993; Rosenthal and Welter 1995; this study) is in agreement with the hypothesis that tolerance in *D. stramonium* may be expressed as an increment of metabolic activity in remaining photosynthetic tissues or as an increment in leaf size rather than on the production of new leaves (Whitham et al. 1991; but see Kimberly and Reekie 1995). Thus, genetic variation on physiological traits related with tolerance might be expected, but additional studies are needed.

Ecological Genetics of Tolerance

The apparent absence of an across-environment negative relationship in fitness indicated no fitness costs of tolerance in *D. stramonium*, because its expression seems not to be constrained under low versus high levels of damage (see Simms 1992; Simms and Tripplet 1994). Similar studies did not detect costs of tolerance in *Arabidopsis thaliana* and *Raphanus raphanistrum* (Mauricio et al. 1997; Agrawal et al. 1999). If there were no costs of tolerance, it may imply that traits related to tolerance to defoliation might be beneficial for plants even when herbivores are scarce or absent, if such traits constitute a buffer for plants from other environmental stresses (Rosenthal and Kotanen 1994). If so, other factors than herbivory may select for high levels of tolerance in natural populations.

However, an upper limit to the beneficial effects of tolerance is expected in environments with strong herbivore pressure. This limit may be expressed as an allocational adjustment among reproductive components to buffer the negative effects of leaf damage (allocational costs; Simms 1992). In the absence of costs, natural selection will increase levels of tolerance within populations and will reduce genetic variation in tolerance. However, the presence of genetic variation on tolerance may be maintained if a trade-off with other traits constrains the effect of natural selection on tolerance. If, for instance, an ecological trade-off between defense and pollination exists, genetic variation in defensive traits could be maintained in the absence of allocation costs (Simms 1992; Strauss 1997).

Regarding the cost-benefit model for the evolution of tolerance and resistance (Fineblum and Rausher 1995), costs of tolerance might be obscured by equal or greater costs of resistance (Simms and Triplett 1994). Thus, in our case, undetected costs of tolerance may be a consequence of resistance costs, although at present there is no evidence regarding this issue in *D. stramonium*. In contrast, if costs are expressed only when damage is present, undamaged genotype replicates will not pay any cost for being tolerant and no costs would be detected. If the mechanisms related with tolerance to herbivores also buffer fitness decrements caused by other stresses (i.e., fire, physical damage, competition), costs may be more difficult to detect when plants suffer no defoliation.

Previous studies have addressed the lack of significant genetic variation within plant populations as a potential bias for the detection of costs of defense to herbivores and pathogens (Simms and Triplett 1994). Despite the presence of genetic variation on tolerance in *D. stramonium*, the lack of a genetic trade-off between low versus high levels of damage

may result from the small number of lines used in our experiment (i.e., low power test; but see Results). In addition, another potential source of bias of our results is the fact that the expression of costs is environment dependent. In that case, the water and light regime that plants experienced in the greenhouse might have been sufficient to attenuate fitness costs of tolerance. However, because the life cycle of *D. stramonium* occurs during the rainy season, in open disturbed sites, at least a probable excess of water and/or light should not be responsible for the apparent absence of costs for tolerance. Finally, the use of inbred lines following two generations of selfing could also bias the experiment toward finding a positive correlation between damaged and undamaged plants, if homozygous individuals for slightly deleterious alleles appeared during the selfing generation (Roff 1992). However, for the same population, no inbreeding depression on fruit production, plant survival, and growth characters have been detected in *D. stramonium* (Núñez-Farfán et al. 1996). Finally, although inbreeding depression was found for number of seeds per fruit in the same population (Núñez-Farfán et al. 1996), the nonsignificant effect of the defoliation environment on seeds per fruit (cf. Table 2) and the absence of genetic variation within defoliation environments for seeds per fruit (cf. Table 3) indicate that inbreeding depression might not be responsible for undetected cost of tolerance.

When viewing tolerance as the norm of reaction for fitness along a defoliation intensity gradient (Mauricio et al. 1997), the evolution of the response might be retarded because of significant phenotypic plasticity. The evolutionary importance of phenotypic plasticity relies on the existence of (1) G × E interactions (i.e., genetic variation for phenotypic plasticity); and (2) genetic variation within the environment (Via et al. 1995). These two conditions would lead natural selection to optimize the overall slope of the reaction norms along a defoliation gradient. Our results demonstrated significant G × E interactions, indicating different genotypic responses to foliar damage. Also, genetic variation in fitness within environments was found. Both results suggest the potential for norms of reaction to evolve. Although the existence of a significant interaction between inbred line and defoliation in many reproductive characters indicate the existence of genetic variation on norms of reaction, the magnitude was not as strong as needed for the detection of a significant negative correlation on character states between environments (i.e., defoliation level). The presence of a negative correlation in fitness between defoliation levels would constraint the evolution of genetically differentiated phenotypes, provided the existence of fitness costs. Thus, in our case the evolution of a plastic plant response after damage may be favored (Schlichting and Pigliucci 1998). The absence of costs for tolerance may have resulted from selection favoring plastic responses to defoliation, if the intensity of damage in natural populations is highly variable within and among generations.

The use of a norm of reaction perspective in the analysis of tolerance would provide additional information regarding the shape of the response after damage. For instance, it has been found that damage can result in a positive rather than a negative effect on plant fitness, but this response depends

on characteristics of the environment (low competition, high nutrients; see Whitham et al. 1991). Experimental studies with more than one treatment of defoliation can incur an additional problem because the interaction term in mixed-model ANOVAs will not indicate truly genetic differences among genotypes for the slope of the relationship between fitness and damage. In this case, a significant, nonlinear fit in the relationship between fitness and damage would indicate that the operational use of the slope for the mentioned relationship could not properly describe the pattern of response. Additional studies should consider this approach to test the linearity assumption of the response after damage used on previous studies, particularly if tolerance is measured under natural conditions. Furthermore, if tolerance diminished fitness differences between damaged and undamaged plants of *D. stramonium*, the amount of phenotypic plasticity for fitness-related traits may expresses an adaptive component of phenotypic plasticity. Because phenotypic plasticity can mask differences in fitness among genetic lines along a defoliation gradient, an upper limit to detect genetic differences on tolerance might be expected.

ACKNOWLEDGMENTS

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CAPÍTULO III

ECOLOGÍA EVOLUTIVA DE *Datura stramonium*: VARIACIÓN GENÉTICA ADITIVA, COSTOS, SELECCIÓN NATURAL Y ADAPTACIÓN LOCAL PARA LA TOLERANCIA AL HERBIVORISMO

1

Environment-dependent costs and benefits: a test of variation among populations in the adaptive value of tolerance to herbivory in *Datura stramonium*

Juan Fornoni

Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, C.P. 04510, México Distrito Federal, México. jfornoni@miranda.ecologia.unam.mx

Running head: environment-dependent selection on tolerance to herbivory

ABSTRACT

The study of adaptive genetic variation in the wild remains one of the major goals of evolutionary biology. In this study I explore for two natural populations of the annual *Datura stramonium* the causes of phenotypic divergence in plant defensive traits against natural herbivory following a reciprocal transplant experiment using paternal half-sibs families. The analyses indicated the presence of significant additive genetic variation on tolerance to herbivory . Two-way ANOVAs revealed that one of the populations (i.e., Ticumán) was locally adapted and genetically differentiated for being less tolerant to herbivory than the other population (i.e., Sto. Domingo). Ticumán population was 24% less tolerant than Sto. Domingo. Analysis of allocational costs of tolerance indicated that at Ticumán costs of tolerance were higher than at Sto. Domingo. The pattern of contrasting selection on tolerance indicated that while negative selection was detected on Ticumán, positive selection was detected in Sto. Domingo. This study shows that environment-dependent selection on tolerance can lead to adaptive differentiation among natural populations of *D. stramonium*. The pattern of contrasting selection on tolerance was further supported by the presence of different magnitude of costs between sites. Overall, the results support the expectation that environment-dependent costs of tolerance can generate differences among populations in the evolutionary outcome of plant-herbivore interactions.

INTRODUCTION

The study of adaptive genetic variation in the wild remains one of the major goals of evolutionary biology (Fisher, 1958; Endler 1986; Mosseau et al. 2000). Accordingly, understanding character evolution requires the analysis of selection under different abiotic and biotic environmental conditions given that populations often experience different selective environments (Wade and Kalisz 1990). Adaptive differentiation among populations requires the simultaneous occurrence of two conditions: (1) heritable variation in traits that enhance fitness, and (2) differential pattern of selection among populations on these traits. If both conditions are meet, absent any other force opposing natural selection (i.e., genetic drift, gene flow), local adaptation is expected.

Plant characters involved in plant-herbivore interactions are particularly suitable traits for studies of adaptive variation in the wild because the phenotypic expression of defensive plant traits is affected not only by the ecological dynamics of natural enemies but also by the biotic and/or abiotic environmental conditions (Mashinski and Whitham 1989; Herms and Mattson 1992; Rosenthal and Kotanen 1994; Abrahamson and Weis 1997; Stowe et al. 2000). In addition, significant heritable variation within populations and differential selection on defensive plant traits among populations have been reported (Berenbaum and Zangerl 1992; Benkman 1999; Tiffin and Rausher 1999; Fornoni and Núñez-Farfán 2000; Goméz and Zamora 2000; Valverde et al. 2001, 2002). However, disproportionately few studies have detected local adaptation in ecologically important plant traits (van Tienderen and van der Toorn 1991; Linhart and Grant 1996). Furthermore, few if any have evaluated the presence of local adaptation in plant traits involved in the interaction with natural enemies.

Plants can respond to herbivore damage either avoiding attack (i.e., being resistant) or diminishing the negative effects of damage on fitness through a compensatory response (i.e., being tolerant) (van der Meijden et al. 1988; Fineblum and Rausher 1995). Given that significant amounts of genetic variation as well as intermediate levels of tolerance has been found in some plant species, the analysis of

the genetic constraints on tolerance has received increasing attention (Fineblum and Rausher 1995; Mauricio et al. 1997; Tiffin and Rausher 1999; Fornoni and Núñez-Farfán 2000; Roy and Kirchner 2000; Tiffin 2000).

One such a constraint is an allocational cost (fitness costs) (*sensu* Simms 1992). If a tolerant genotype have higher fitness when herbivores are present but low fitness when herbivores are absent, an allocational cost may exist (Simms and Triplett 1994; Mauricio 1998; Agrawal et al. 1999; Tiffin and Rausher 1999). Although recent studies showed the existence of this type of cost in natural populations (Simms and Triplett 1994; Tiffin and Rausher 1999; Stichcombe 2001), there are no data to determine whether variable costs of tolerance among populations is a widespread phenomenon (but see Stinchcombe 2002).

Previous theoretical studies predict that tolerance and resistance are mutually redundant exclusive strategies of defense under the assumption of equal fixed costs of plant defensive strategies within populations (Fineblum and Rausher 1995; Mauricio et al. 1997; Tiffin 2000). However, a recent model demonstrated that if unit costs of tolerance are environmentally-dependent, this dependent variation can generate different tolerance-fitness functions among populations (Cap. I), and hence differences in the adaptive value of tolerance among populations. In a recent study using plant genotypes from one population, Stinchcombe (2001) combined the presence/absence of different species of herbivores that feed upon *Ipomoea hederacea*, and demonstrated that selection and costs of tolerance can vary in a context-dependent fashion. However, still remains unanswered whether the environment-dependent expression of benefits and costs for tolerance can lead to adaptive differentiation in this defensive strategy among populations. Thus, the evolutionary dynamics of tolerance should be addressed in more than one population. Given that studies of natural selection and quantitative genetics of tolerance have been conducted commonly within a single population (Mauricio et al. 1997; Agrawal et al. 1999; Tiffin and Rausher 1999; Fornoni and Núñez-Farfán 2000; Stinchcombe 2002), there is no evidence regarding how frequent environment-dependent selection occurs and if it is an important component of the evolution of tolerance among populations.

If an environmental component alters the cost/benefit ratio of allocating resources to tolerance, we hypothesized that selection on tolerance will act different on a given set of genotypes between environments. Absent other evolutionary processes opposing selection, local adaptation to tolerate damage by herbivores is expected provided the existence of additive genetic variation within populations. This hypothesis assumes that any environmental factor that alter the unit costs of allocation to tolerance can potentially generate differences among plant populations in the evolutionary response to herbivory, and thus generate fitness variation among environments.

In this study we explore the evolutionary consequences of environment-dependent selection on tolerance to herbivory in two populations of the annual *Datura stramonium*, following a reciprocal transplant design using paternal half-sib families. In *D. stramonium*, significant genetic variation on tolerance to defoliation has been detected, although no evidence of costs was found under greenhouse conditions (Fornoni and Núñez-Farfán 2000). In order to estimate selection acting on tolerance, we first determined the existence of additive genetic variation for tolerance to herbivory in the experimental populations. Second, we asked: (1) Does the pattern of selection on tolerance and associated costs vary among populations?, If so, (2) Are populations locally adapted to tolerate herbivory damage?

MATERIALS AND METHODS

Plant Species and Study Sites

Datura stramonium L. (Solanaceae) is an annual herb distributed in North America. In Mexico occurs on disturbed areas in a wide variety of habitats, ranging from temperate forests, to dry tropical forests and xerophytic shrublands. Across these plant communities *D. stramonium* experiences significant levels of foliar damage ranging from 10 to 50% of the total leaf area (Valverde 2001). Among the main folivore insects that consumes foliage of this species are specialist insects (i.e., *Epitrix* sp., *Lema trilineata*: Coleoptera: Chrysomelidae), and generalist insect (i.e., *Sphenarium*

purpurascens: Orthoptera: Acrididae, and *Manduca* sp.: Sphingidae) (Núñez-Farfán and Dirzo 1994; Núñez-Farfán et al. 1996; Valverde et al. 2001).

From a previous survey in several natural populations of *D. stramonium* in Central Mexico (Valverde 2001), two populations separated about 37 km were selected for our reciprocal transplant experiment. However they differ greatly in altitude above sea level. One population (hereafter Ticumán, 18° 45'N and 99° 07'W, 961 masl) inhabits a tropical dry forest with a mean annual precipitation of 802.1 mm and a mean annual temperature of 23.1°C. The other population (hereafter Sto. Domingo, 19° 00'N and 99° 03'W, 2050 masl) is in a pine-oak forest with a mean annual precipitation of 1463.2 mm and a mean annual temperature of 19.9°C. Between these populations significant differences on the average proportion of leaf area damaged by herbivorous insects has been detected ($P < 0.01$; $\overline{X}_{Ticumán} = 0.109 \pm 0.004$, and $\overline{X}_{Sto.\ Domingo} = 0.441 \pm 0.014$, respectively) (Valverde et al. 2001). In both populations positive directional selection on resistance to folivorous insects was detected (Valverde 2001), indicating that herbivory might constitute a selective pressure favoring plant defense against natural enemies. In addition, a subsequent common garden experiment revealed the existence of genetic differentiation in resistance against herbivorous insects between the populations of Ticumán and Sto. Domingo (Valverde et al. 2002). In both locations experimental plots were settled in cultivated areas at less than 1 km from the nearest natural population of *D. stramonium*, were the parental seeds for the breeding design were collected.

Experimental Design

Experimental plants of our experiment were obtained following a North Carolina I cross-breeding design to obtain paternal half-sibs progenies (Falconer and Mackay 1996; Lynch and Walsh 1998). Controlled crosses were performed between January and May 1999 obtaining 25 and 23 paternal half-sibs families for the populations of Ticumán and Sto. Domingo, respectively. Each sire plant was used as pollen source for mating with two randomly selected dam plants from its corresponding population. Experimental seeds (half-sibs) were germinated in the greenhouse

following the protocol proposed by Fornoni and Núñez-Farfán (2000). When the first two true leaves appeared, plants were reciprocally transplanted to the experimental plots (July 1999). Number of sibs per dam ranged between 4 to 12 plants for a total of 1199 plants. Because of the low number of replicates in fourteen paternal families, germinated seeds were only planted at the site of origin of each family. Thus our experimental design was not completely replicated at the sire level. During two days in late July 1999, seedlings were randomly planted 1m apart from each other in a regular grid within each experimental plot, in a completely randomized design. Weeds were removed every two weeks to avoid interference with the experimental plants. One week after the first transplant, less than 3% of the original plants were replaced because of mortality. After the second planting no mortality was observed within the experimental plots.

During four days at the beginning of November 1999 (when plants ended their growing season), half the leaves of each individual plant were used to obtain the proportion of leaf area consumed by folivorous insects. These leaves were randomly selected after collecting all the leaves of the plants. For each leaf, the total area damaged was calculated using an Image Analysis System (WinDIAS-Basic, Delta-T Devices Ltd., Cambridge, England) to obtain the average proportion of leaf area damaged for each individual plant. At the same time, all fruits of the plants were collected and the total number of seeds per plant was counted to obtain an estimate of maternal fitness.

Tolerance Estimates

Tolerance was estimated as the norm of reaction of fitness across a damage intensity gradient (Abrahamson and Weis 1997; Mauricio et al. 1997; Tiffin and Rausher 1999; Fornoni and Núñez Farfán 2000). For each paternal half-sib family, tolerance was estimated as the slope of a regression of fitness against proportion of leaf area damaged by herbivores (Mauricio et al. 1997; Tiffin and Rausher 1999). Since nonlinear effects were not detected in previous ANOVAs, tolerance was estimated using only linear regression coefficients.

Additive Genetic Variation on Tolerance

Before statistical analyses, the proportion of leaf area damaged by herbivores was transformed as arcsine $\sqrt{p}$, (where p is the proportion of damage of a plant). For the analysis of genetic variation on tolerance, maternal fitness was relativized for each experimental site as $\frac{w_i}{\overline{w}}$, where w_i correspond to the fitness of each individual plants, and $\overline{w}$ correspond to the mean fitness value at each transplant site. The existence of additive genetic variation in tolerance to herbivory was evaluated by means of nested ANCOVAs (Type III SS) in each site, for each population, using PROC GLM in SAS (SAS, 1999). According to the operational definition of tolerance as a norm of reaction of fitness on damage (Abrahamson and Weis 1997), log transformed relative fitness was entered as the dependent variable and the proportion of damage was entered as a covariate in the analysis. Because for this analysis we did not estimate any quantitative genetic parameter, relative fitness was transformed to ensure normality (Mitchell-Olds and Shaw 1987). Sire and Dams nested within Sire were considered random factors. A significant Damage × Sire interaction would indicate the existence of additive genetic variation for tolerance (i.e., differences among paternal half-sib families in the slope of the relationship between fitness and proportion of leaf area damaged). The RANDOM option of SAS was used to perform appropriate F estimates. Only when the interaction Damage × Dam (Sire) was significant, the F-test for the interaction Damage × Sire was corrected using the MS of Damage × Dam (Sire) as denominator of the F-test (see Appendix B in Hougen-Eitzman and Rausher 1994; Tiffin and Rausher 1999).

In order to avoid possible bias of our estimates of tolerance due to differential effects of plant size on tolerance among paternal families, we calculated the relative rank of tolerance for each paternal family with and without the possible effect of size on the estimates of tolerance (Tiffin and Rausher 1999). For this analysis relative fitness was regressed against plant size to obtain residuals of relative fitness. Estimates of tolerance using relative fitness and residual relative fitness (without

potential size effects) was used to perform a Spearman rank correlation between both estimates of tolerance for each population at each experimental site. These analyses were performed using JMP statistical package (SAS 1995). These correlations were all highly significant and positive (average pairwise correlation coefficient was 0.80, (range 0.70 – 0.86, $P < .005$), indicating that plant size did not alter differentially our estimates of tolerance among paternal families. Thus the final analyses of genetic variation on tolerance were performed without considering plant size. In addition, parallel analyses revealed no evidence of nonlinear effects of herbivory on plant fitness (Damage^2 terms from the ANOVAs were all not significant), or evidence that nonlinear effects of herbivory on fitness differ among paternal families (Damage^2 × sire from the ANOVAs were all not significant). These indicate that using the slope of the regression between fitness and damage is appropriate for this study, and that variation in resistance may not bias the analysis of genetic variation in tolerance (Stinchcombe and Rausher 2002).

Testing Local Adaptation on Tolerance

Using a reciprocal transplant experiment, a local adaptation hypothesis predicts that populations in their site of origin outperform alien populations in terms of fitness (Clausen and Hiesey 1958; van Tienderen and van der Toorn 1991; Schimtt 1999; Núñez-Farfán and Schlichting, 2001), a result not expected under random differentiation. Statistically this prediction can be supported if a significant Site × Population interaction is detected in a two-way ANOVA using paternal mean fitness as the response variable. This implies that at least one population changes its average fitness in relation to the other population in a given site. However, if the pattern of fitness differences among populations does not mach the local adaptation hypothesis, a significant Site × Population interaction will not necessarily indicate that populations are locally adapted. In order to construct an adaptive hypothesis of population differentiation on tolerance genetic differences on tolerance between populations across sites should be detected first. This was accomplished performing the same two-way ANOVA as for fitness. Second, a significant relationship between

paternal mean fitness and tolerance within site should be in agreement with average values of tolerance at each site. A positive and/or stepper slope for the relationship should correspond to the site with higher average levels of tolerance. Conversely, a negative and/or less stepper slope between fitness and tolerance should correspond to the site with lower levels of tolerance. This analysis will anticipate the presence of differential selection on tolerance between sites, a necessary condition for adaptive divergence between populations. To answer this point an ANCOVA was performed using paternal mean relative fitness (within site) as dependent variable. Site and Population were entered as fixed factors and standardized tolerance (across site) as covariate. Tolerance was standardized across site as $\left(\dfrac{x_i - \overline{x}}{stdx}\right)$, where x_i corresponds to family values of tolerance, $\overline{x}$ corresponds to the mean value of tolerance (across site), and $stdx$ represent the standard deviation of tolerance. This analysis specifically evaluated the presence of differences between sites in the relationship between fitness and tolerance. A significant Site × Tolerance interaction will indicate the existence of potential differences between sites in selection acting on tolerance. Our expectation was that if selection acted differently upon tolerance between sites, populations could be locally adapted to tolerate herbivory.

Natural Selection on Tolerance

The value of tolerance for each paternal half-sib family was used to determine, at the population level, how is natural selection acting on tolerance to herbivory. The analyses were carried out using non- transformed relative fitness per site and standardized tolerance. Since tolerance can only be estimated at the genotypic level, the analysis is equivalent to that proposed by Rausher (1992). In this analysis, partial regression coefficients can be used to estimate selection gradients (Lande and Arnold 1983; Rausher 1992). Directional selection on tolerance was estimated using linear regressions of relative fitness on standardized tolerance, and stabilizing and disruptive selection was estimated using linear and quadratic terms in the regression analysis (Lande and Arnold 1983). Selection gradients were estimated using the

program FREE-STAT (version 1.10) (Mitchel-Olds 1989). Jackknife procedures were performed to estimate standard errors and probabilities for each selection gradient (Mitchel-Olds and Shaw 1987). Between and within site differences in selection gradients were evaluated comparing the slopes in separated ANCOVAs (Tolerance × Site and Tolerance × Population interaction, respectively) (Mitchel-Olds and Shaw 1987; Dudley 1996; Zar 1999; Caruso 2000). At both between and within site levels, linear and quadratic models were evaluated. These analyses were performed using the GLM module in SAS (SAS 1999).

Costs of tolerance

Allocational costs to tolerance at each site were estimated using the relationship between relative fitness and damage for each sire family. Using the Y-intercept value of each family as an estimate of fitness in the absence of herbivory, a significant negative covariance between fitness at zero damage and tolerance (slope of the regression between fitness and damage) will indicate the presence of costs for tolerance (Mauricio et al. 1997; Tiffin and Rausher 1999; Stinchcombe 2001). However, this analysis produces a bias in the estimation of costs due to non-independence between the Y-intercept (fitness in the absence of herbivory) and the slope (tolerance). Thus, a correction for the bias on the sample covariance was performed to estimate the true covariance (Appendix B in Mauricio et al. 1997). Standard errors for the estimated corrected covariance were obtained by means of a jackknife procedure (Sokal and Rohlf 1995). Because this analysis was used to determine if the true covariance differ significantly from zero, a one-tailed t statistic was used to calculate 95% confidence intervals. The presence of non-linear costs functions was also evaluated.

RESULTS

Additive Genetic Variation for Tolerance

The analyses indicated the presence of significant additive genetic variation for tolerance to herbivory in both sites (Table 1). In Ticumán, significant additive genetic

variation for tolerance was detected for the native population (i.e., Ticumán), while only among maternal parents differences for tolerance was detected for the alien population (i.e., Sto. Domingo) provided the significant Damage × Dam (Sire) interaction (Table 1A). In Sto. Domingo, a significant Damage × Sire interaction was detected in the ANCOVAs only for the native population (Table 1B).

Local adaptation and genetic differentiation on tolerance
Two-way ANOVA for fitness and tolerance detected significant Site, Population and Site × Population interaction effects (Table 2). In Ticumán plants produced more seeds than at Sto. Domingo (Fig. 1A; Table 2). The Ticumán population in its native site outperformed Sto. Domingo population (Fig. 1A). In contrast, no differences in relative fitness were detected between populations in the site of Sto. Domingo (Fig. 1A). The significant interaction Site × Population indicated that the performance of populations differed between sites indicated local adaptation (Table 2). The results indicate that only the population of Ticumán is locally adapted.

For each site, mean damage was $\bar{X}_{Ticumán} = 0.2305 \pm 0.1281$ and $\bar{X}_{Sto.\ Domingo} = 0.2381 \pm 0.2200$. An ANOVA revealed no differences between sites in the average level of foliar damage ($F_{1,1127} = 0.44$, $P = 0.5055$). Within both sites, the Ticumán population was significantly less damaged than Sto. Domingo population ($F_{1,1127} = 22.48$, $P < 0.0001$). Despite the absence of differences in average level of damage between sites, significant differences on tolerance were detected (Table 2). The ANOVA indicated that at the site of Ticumán plants were 16 % less tolerant to foliar damage than at Sto. Domingo (Fig. 1B; Table 3). Although the population of Ticumán was on average less tolerant to foliar damage than Sto. Domingo population (Fig. 1; Table 2), only within the site of Ticumán significant differences in tolerance between populations were detected. In this site the population of Ticumán was 24 % less tolerant than the population of Sto. Domingo (Fig. 1). The differences in tolerance between sites for the population of Ticumán accounted for the significant Site × Population interaction in the ANOVA (Fig. 1; Table 2). In Ticumán damage was mainly caused by the specialist beetle *Epitrix parvula*, while in Sto. Domingo damage

was mainly caused by the generalist grasshopper *Sphenarium purpurascens* (Valverde 2001).

Costs of tolerance

Allocation to tolerance against herbivory represents a fitness cost for plants of *D. stramonium*. In the site of Ticumán, observed product-moment correlation coefficient between tolerance and fitness (at zero damage) was –0.96, which correspond to a covariance of –0.0128. The corrected correlation and covariance between tolerance and fitness in the absence of herbivory was $r = -0.49$ ($P = 0.0007$) and -0.0067, with a 95% confidence interval of 0.0059, significantly different from zero at $P < 0.05$. At this site no evidence of non-linear costs of tolerance were detected. In the site of Sto. Domingo no evidence of costs of tolerance were detected. Even though observed product-moment correlation coefficient between tolerance and fitness (at zero damage) was –0.77, which correspond to a covariance of –0.0025, the corrected covariance was 0.0004. The presence of a positive covariance in Sto. Domingo suggests either the absence of costs at this site or our inability to detected small values of costs of tolerance. Besides both possible causes of undetected costs at Sto. Domingo, the results support the expectation of differential allocational costs of tolerance between sites.

The same analysis was performed using only the populations at their native site were genetic variation was previously detected. This analysis was carried out to evaluate whether pooling all genotypes in each site altered the expression of genetic costs of tolerance. In both transplant site the native population express significant costs of tolerance. Observed covariances for Ticumán and Sto. Domingo were –0.0156 and –0.0034. Corrected covariance and confidence interval at 95% were –0.0062 ± 0.0001, and -0.0017 ± 0.0009 for Ticumán and Sto. Domingo, respectively. Again, this pattern supports our previous result that costs at Ticumán were higher than at Sto. Domingo since confidence intervals show no overlap between corrected covariances.

Patterns of selection on Tolerance

Results from ANCOVA (c.f. Table 3) revealed a significant interaction Tolerance × Site indicating that the relationship between tolerance and fitness differed between sites (Table 2). This means that the adaptive value of tolerance varies between sites. This analysis was performed first the total available number of paternal families, and second, using only those paternal families that were present at both experimental plots. Predicted values of both analyses were correlated using a Spearman rank correlation test to evaluate if the absence of complete replication at the sire level qualitatively affect the results of the analysis. This procedure indicated that predicted values of both analysis were highly positively correlated ($r_{Spearman} = 0.97$, $P < 0.0001$), thus no bias in the final analysis was present due to the absence of complete replication at the sire level.

Partial regression analyses within sites indicated that the pattern of selection on tolerance was different between sites (Table 4). While positive directional selection was detected in Sto. Domingo, negative directional selection was detected in Ticumán (Fig. 2). Paired contrasts of linear selection gradients between sites for both populations also support our finding of contrasting selection on tolerance (Table 4). The patterns of selection on tolerance were consistent with site differences on mean values of tolerance. For example, the site of Sto. Domingo, where tolerance was positively selected, had the highest mean value of tolerance while the site of Ticumán, where tolerance was negatively selected, had the lowest mean value of tolerance. Within site, only in Ticumán significant differences between populations were detected in the intensity of selection on tolerance (Table 4). In this site natural selection was significantly more intense on the alien (i.e., Sto. Domingo) than on the native (i.e., Ticumán) population. In both sites, no significant nonlinear selection gradients were detected. However, the absence of significant nonlinear effects in the selection analysis must be taken with caution given the low number of paternal families available for these analyses.

DISCUSSION

The results of this study show that environment-dependent selection on tolerance can lead to adaptive differentiation between two natural populations of *Datura stramonium*. The pattern of contrasting selection on tolerance is further supported by the finding of differential costs of tolerance between sites altering the adaptive value of this trait. At the site were tolerance was negatively selected, higher costs of tolerance were detected, while at the site were tolerance was positively selected, costs of tolerance were lower. Despite the presence of contrasting selection on tolerance between sites, only for one of the populations we found evidence of local adaptation for lower levels of tolerance to herbivory. At this site the native population (Ticumán) expressed higher fitness and genetic differentiation for being less tolerant than the alien population (Sto. Domingo). Overall, the results validate the hypothesis that an environmentally dependent effect upon costs of tolerance can alter the adaptive value of this trait (Cap. I). Thus, our results support the theoretical expectation that environment-dependent costs of plant defense traits can generate differences among populations in the evolutionary outcome of plant-herbivore interactions.

Environment-dependent adaptive value of tolerance to herbivory
Variation among populations in the adaptive value of plant responses to natural enemies can result from variation in patterns of selection on defensive traits induced by abiotic and/or biotic environmental factors (Stinchcombe and Rausher 2001). Recent studies shown that the presence/absence of natural enemies (i.e., putative selective agent), either naturally or experimentally manipulated, can alter the pattern of selection on defensive plant traits (Mauricio and Rausher 1997; Benkman 1999; Gomez and Zamora 2000; Stinchcombe 2001). Theoretical and experimental work indicated that population abundance and composition of natural enemies assemblages can also condition the adaptive value of plant tolerance to herbivory (Tiffin and Rausher 1999; Stinchcombe 2001). Other less direct sources of variation that can also affect the adaptive value of tolerance among populations is the

availability of pollinators (Paige 1999). However, the importance of this source of variation among populations is expected to induce changes in the adaptive value of tolerance whenever plant reproductive success depends on the availability of pollen vectors. Besides the relatively scarce evidence addressing direct and indirect biotic environmental induced changes in the adaptive value of tolerance, more studies support the hypothesis that variation in physical factors like nutrients, water and light can alter the ability of plants to compensate herbivory damage through fitness compensation (Maschinski and Whitham 1989; Herms and Mattson 1992; Juenger and Bergelson 1997; Stowe et al. 2000).

In *D. stramonium* we favor the explanation that a physical environmental factor may be involved in observed variation between populations in the adaptive value of tolerance. First, given *D. stramonium* is a predominantly self-fertilizing plant (Motten and Antonovics 1992; Núñez-Farfán et al. 1996) any variation in the availability of pollinators is not expected to constrain plant response to herbivory nor maternal fitness. Second, between experimental sites, no differences in levels of foliar damage were detected, suggesting similar herbivore load, thus variations on levels of damage may not be related to variations in the adaptive value of tolerance (but see Tiffin and Rausher 1999). Previous studies showed that equivalent fitness benefits of tolerance and resistance were expressed under levels of damage around 10% of defoliation (Fornoni and Núñez-Farfán 2000; Valverde et al. 2002: Appendix II). The results of our experiment in turn, suggest that under higher levels of defoliation (more than 20% of defoliation) redundancy between tolerance and resistance may disappear because of differential environmental effects upon costs and benefits on each defensive strategy. Third, although we did not manipulate the presence/absence of species of herbivorous insects, available data for *Ipomoea purpurea* indicate that tolerance to different species of folivore insects were positively genetically correlated (Tiffin and Rausher 1999). Since the same type of herbivores (i.e., folivores) cause foliar damage in *D. stramonium* in our experiment, there are no reasons to expect variation in fitness benefits of tolerance to different species of folivore insects.

However, further studies are necessary to confirm if tolerance to the same type of damage is a general plant response (but see Stinchcombe 2001).

Natural history of *D. stramonium* indicates that this species is always present in open and disturbed areas where no light limitation and intense competition are expected (Núñez-Farfán 1991). With respect to potential abiotic factors related to variation among population in the adaptive value of tolerance, our experimental setup precludes the possible effects of variation in light and nutrient availability since the experimental plots were located in open and fertilized cultivated fields. Thus other physical environmental factors like water availability may be responsible for variation in the adaptive value of tolerance between populations of *D. stramonium*.

Local adaptation for tolerance to herbivory

When searching for local adaptation, it is generally assumed that populations are close to an adaptive peak, although this may not be necessary the case. Besides natural selection, other evolutionary processes (i.e., genetic drift, gene flow) can move populations from the nearest adaptive peak (Wright 1988; Thompson 1994; Gomulkiewicz et al. 2000). For example, genetic drift can decouple populations from the ecological conditions under which previous adaptations functioned, reducing the overall success of the population (Wright 1932). Similarly, gene flow can reduce the performance of a population by introducing genetic variation from populations exposed to different selection regimes (Gomulkiewicz et al. 2000). Our results showed that at the temperate forest site (Sto. Domingo) both the native and alien population attained lower fitness than at the dry tropical forest (Ticumán). Also, in this site the native population did not showed evidence of local adaptation (i.e., higher fitness than the alien population) nor genetic differentiation on the average level of tolerance. This pattern suggests that undetected local adaptation for the population from the temperate site may have resulted from a lower intensity of natural selection upon genetic variation as compared to other evolutionary processes (i.e., genetic drift, gene flow) or that a recent event of colonization may be responsible for the absence of fitness differences at the temperate forest site. The presence of additive

genetic variation for tolerance suggests that, if any, genetic drift may have been not reduced the variation in tolerance at the temperate forest site. Both asymmetrical gene flow and/or a recent colonization event of the temperate forest site may account for the absence of local adaptation on tolerance. This possibility is further supported by the finding of higher intensity of selection acting on tolerance at this site, and the absence of differences between populations on the strength of selection.

In the dry tropical forest where the native population showed evidence of local adaptation, the intensity of selection on tolerance was significantly higher for the alien population than the native population. Theoretically, it is expected that the intensity of selection on a trait will diminish over time as the amount of genetic variation is eroded (Falconer and Mackay 1996). The presence of additive genetic variation and directional selection acting on tolerance suggest either lack of sufficient time for natural selection to erode genetic variation on tolerance, or that some opposing force may be maintaining the variation in this defensive strategy (i.e., ecological trade-offs).

Regarding the geographic mosaic theory of coevolution, recent theoretical work indicated that selection mosaics and the pattern of gene flow among populations can strongly affect the coevolutionary trajectories of populations (Thompson 1994; Gomulkiewicz et al. 2000). The pattern of genetic differentiation and contrasting selection on tolerance suggest the possible existence of hot and cold spots in the spatial distribution of the coevolutionary outcome of the interactions between *D. stramonium* and it insects herbivores. For instance, the opportunities for a coevolutionary response of plants against their natural enemies (i.e., a response mediate through resistance) may be higher in those populations were tolerance is selected against. This scenario may be that expressed at the site of Ticumán. Conversely, in the site of Sto. Domingo were natural selection favor increasing levels of tolerance, the coevolutionary response of plants may be attenuated, possibly representing a cold spot. These evidences revealed the importance of spatial variation in the selective environment experienced by populations in shaping the selection mosaic. Finally, the detection of asymmetrical local adaptation, strongly suggest the

possible role of the pattern of gene flow among population as a source of variation in the evolutionary outcome of species interactions (Thompson 1994).

Conclusions

The present study experimentally demonstrated that an important component of the variation in the phenotypic expression of tolerance among natural populations of *D. stramonium* is an environment-dependent effect upon costs and benefits of this strategy of defense against natural enemies. Our results support the hypothesis that environment-dependent costs and benefits of plant defense strategies (tolerance and resistance) can lead to different evolutionary outcomes for the interaction between plants and herbivores (Cap. I). This in turn, suggest that spatial variation in the tolerance-resistance fitness surface of *D. stramonium* is expected (Cáp. I), and should be addressed in future studies. In those populations where tolerance is selected against, plants would respond through a resistance strategy of defense. Conversely, if tolerance is favored by natural selection, the evolution of resistance traits could be constrained if redundancy between tolerance and resistance diminish the overall benefits of traits conferring resistance against natural enemies. Previous studies are in agreement with our expectation since the dry tropical forest population has higher average resistance than the temperate forest population both in the field and under common environmental conditions (Valverde et al. 2001, 2002). Before further generalization relating the expression of different strategies of defense with particular ecological conditions can be proposed, limiting abiotic/biotic resources for tolerance and resistance must be determined as well as the mechanisms through which plant tolerate herbivory damage.

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Table 1. ANCOVA for detecting additive genetic variation on tolerance for each population in both sites. Relative fitness per site was entered as the response variable, and damage as covariable.

A. Site Ticumán

Population	Source of variation	df	Type III SS	F	P
Sto. Domingo	Sire [a]	18	0.4789	0.95	0.5371
	Dams (Sire)	19	0.5417	2.71	0.0004
	Damage	1	0.0029	0.28	0.5954
	Damage × Sire [b]	18	0.2310	1.22	0.2511
	Damage × Dams (Sire)	19	0.4919	2.46	0.0012
	error	161	1.6947		
Ticumán	Sire [c]	24	1.2597	1.59	0.1246
	Dams (Sire)	25	0.8357	1.77	0.0140
	Damage	1	0.0247	1.31	0.2534
	Damage × Sire [d]	24	0.9579	2.11	0.0002
	Damage × Dams (Sire)	25	0.7080	1.20	0.0606
	error	342	6.4554		

[a] F value is Satterthwaite approximation, df = 18, 19.51.

[b] $MS_{Damage \times Sire} / MS_{Damage \times Dams (Sire)}$.

[c] F value is Satterthwaite approximation, df = 24, 25.91.

[d] $MS_{Damage \times Sire} / MS_{error}$.

Table 1. Continued.

B. Site Sto. Domingo

Population	Source of variation	df	Type III SS	F	P
Sto. Domingo	Sire [a]	22	0.9355	2.67	0.0100
	Dams (Sire)	23	0.3663	1.02	0.4404
	Damage	1	0.0007	0.05	0.8235
	Damage × Sire [b]	22	0.6389	1.86	0.0129
	Damage × Dams (Sire)	23	0.4969	1.38	0.1184
	error	244	3.8098		
Ticumán	Sire [c]	15	0.1484	0.55	0.8768
	Dams (Sire)	16	0.2728	0.60	0.8767
	Damage	1	0.0146	0.52	0.4734
	Damage × Sire [b]	15	0.1962	0.46	0.9549
	Damage × Dams (Sire)	16	0.2580	0.57	0.9011
	error	123	3.4804		

[a] F value is Satterthwaite approximation, df = 22, 24.64.

[b] $MS_{Damage \times Sire} / MS_{error}$.

[c] F value is Satterthwaite approximation, df = 15, 20.35.

Table 2. Results of two-way ANOVAs for fitness (seeds/plant), and tolerance for two natural populations of *D. stramonium* grown in a reciprocal transplant experiment. Values of tolerance were obtained from the regression of fitness on damage for each paternal family. Site and Population were considered fixed factors. Type III sums of square were used.

Character	Source	df	MS	F	P
Fitness	Site	1	27.759	163.78	0.0001
	Pop.	1	2.330	13.74	0.0004
	Site × Pop.	1	1.444	8.51	0.0046
	Error	79	13.389		
Tolerance	Site	1	0.028	23.18	0.0001
	Pop.	1	0.006	5.49	0.0215
	Site × Pop.	1	0.013	11.29	0.0012
	Error	79	0.095		

Table 3. ANCOVA of relative fitness in *Datura stramonium*. Site and Population were considered fixed effects, and standardized tolerance was entered as the covariate (see *Data Analysis*). A significant Tolerance × Site interaction indicates significant differential selection on tolerance between the two sites. Sum of square type III were used.

Source of variation	df	SS	F	P
Site	1	0.3666	1.60	0.2091
Population	1	0.0819	0.35	0.5509
Site × Population	1	0.2068	0.90	0.3443
Tolerance	1	0.0783	0.34	0.5599
Tolerance × Site	1	6.4585	28.28	0.0001
Tolerance × Population	1	0.8899	3.89	0.0520
Tolerance × Population × Site	1	0.4664	2.04	0.1571
Error	73	17.1257		

Table 4. Linear selection gradients for tolerance to herbivory of two populations of *Datura stramonium* following a reciprocal transplant experiment. Comparisons of selection gradients between and within sites following ANCOVA are indicated (paired *t*-tests). Standard errors and probabilities of selection gradients correspond to jackknifed estimates. All quadratic terms were not significant. $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$

Population	Site Ticumán β (SE)	Site Sto. Domingo β (SE)	$t_{between\ sites}$
Sto. Domingo	-0.9485***	12.7146*	3.20**
	(0.1376)	(5.1137)	
Ticumán	-0.2288*	9.9939**	2.35*
	(0.0828)	(2.9673)	
$t_{between\ pop(site)}$	3.20**	0.45	
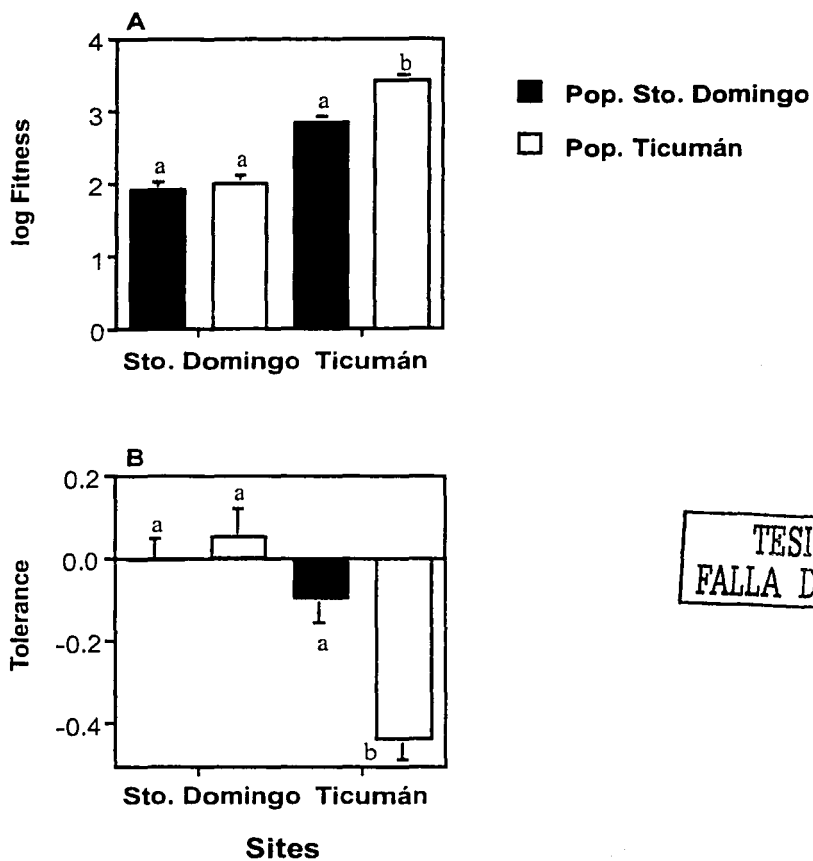
Figure 1. Mean + 1SE of **(A)** log fitness (seeds/plant) and **(B)** tolerance to herbivory of two populations of *Datura stramonium*. Different letters indicate significant differences between populations within site at $P < 0.01$.
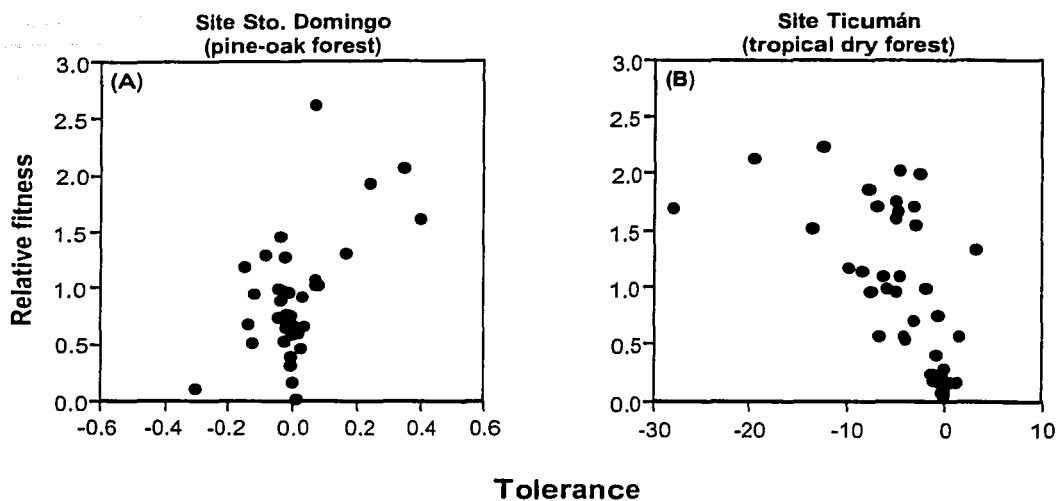
Figure 2. Scatterplot for relative fitness and standardized tolerance for two experimental populations of *Datura stramonium* grown at two sites; (A) Ticumán, and (B) Sto. Domingo.

DISCUSIÓN GENERAL

Con respecto a la pregunta inicial de este estudio *¿Por qué las plantas presentan más de una forma de defensa contra sus enemigos naturales dentro de las poblaciones?*, los resultados obtenidos revelaron que la variación en la magnitud de los costos de las diferentes formas de defensa (tolerancia y resistencia), así como la no linealidad en la forma de la función de los costos y beneficios en términos de adecuación pueden explicar la presencia de más de una forma de defensa (i.e., estrategias mixtas) dentro de las poblaciones de plantas (Capítulo I). A pesar que, la evidencia empírica sugiere que podría existir una relación entre la magnitud del costo de la defensa y la disponibilidad de recursos (Stowe et al. 2000), no hay estudios sobre la posible relación entre la forma de las funciones de costo y beneficio y el ambiente físico o biótico. Ambas relaciones permitirían ligar posibles escenarios ecológicos-evolutivos.

El modelo desarrollado asume que la abundancia de herbívoros es constante y lo suficientemente importante como para ejercer efectos negativos sobre la adecuación de las plantas. Sin embargo, si la abundancia de enemigos naturales puede condicionar la magnitud y forma de las funciones de costo y beneficio de cualquiera de las estrategias defensivas, ésta debería considerarse formalmente en estudios posteriores. Actualmente, se ha evaluado de manera teórica como la cantidad de daño que experimentan las plantas afecta la forma de la función del costo (Tiffin & Rausher 1999), y la magnitud y forma de la función de beneficios (Simms & Rausher 1987; Abrahamson & Weis 1997).

El Capítulo II resalta la importancia de la cantidad de daño que experimentan las plantas como determinante del potencial adaptativo de la tolerancia. Es decir, el nivel de daño a partir del cual la planta no es capaz de compensar al menos parcialmente el costo del herbivorismo. Los resultados experimentales en condiciones de invernadero permitieron determinar el umbral aproximado a partir del cual las plantas (*Datura stramonium*) expresan una reducción en la adecuación como resultado del daño foliar. Este umbral (10-30% de daño foliar) fue confirmado por estudios en condiciones experimentales de un jardín común (Apéndice II) donde las plantas

fueron capaces de compensar completamente niveles de daño promedio de 12%, y en donde tanto la resistencia como un componente potencial de la tolerancia (el crecimiento compensatorio) resultaron igualmente favorecidos por la selección natural. Otro aspecto importante de este estudio fue la determinación de la existencia de variación genética para la tolerancia al daño foliar, y la ausencia aparente de costos en condiciones controladas sin limitación de recursos (Fornoni & Núñez-Farfán 2000).

El Capítulo III permitió validar uno de los supuestos del modelo: *los costos de cualquiera de las estrategias de defensa pueden variar entre poblaciones.* Los resultados del experimento de transplantes recíprocos utilizando material proveniente de cruzas controladas, permitieron determinar no sólo la variación en los costos de la tolerancia, sino que esta diferenciación es consistente con las diferencias en los patrones selectivos observados entre poblaciones. La tolerancia resultó no favorecida (selección negativa) en la población de la Selva Baja Caducifolia, mientras que fue positivamente seleccionada en la población del Bosque de pinos y encinos. La presencia de selección negativa y la magnitud más elevada del costo en la Selva Baja es consistente con la expectativa de que si el costos de asignar recursos a la tolerancia supera al beneficio, ésta no debería ser seleccionada. Por lo tanto, la detección de selección negativa confirma esta predicción, y resalta que el costo de la tolerancia en términos de adecuación puede representar una proporción importante de recursos para las plantas. Aún cuando la tolerancia podría ser seleccionada por otros agentes además de los enemigos naturales, el experimento de transplantes recíprocos eliminó cualquier otro posible agente de selección sobre la tolerancia. Es decir que no es completamente posible descartar el hecho de que en condiciones naturales otros agentes pudieran seleccionar la tolerancia (por ejemplo la competencia, Aaerssen 1995). Si esto ocurriese los patrones de selección observados sobre la tolerancia podrían verse parcial o totalmente alterados por presiones selectivas encontradas entre rasgos correlacionados, o por sinergismo o interferencia entre agentes de selección que actúen sobre la tolerancia. En este sentido, podría no ser tan directa la relación entre los patrones de selección y la magnitud del costo de la tolerancia. Hasta

el momento pocos estudios han intentado evaluar el potencial como agente selectivo sobre la tolerancia de otros factores del ambiente. En un experimento con *Ipomoea purpurea* se encontró que la competencia no ejerció ninguna presión selectiva sobre la tolerancia de las plantas al herbivorismo (Tiffin, datos no publicados).

El diseño de transplantes recíprocos utilizado en este estudio permitió determinar la existencia de un componente ambiental significativo asociado a la variación en la magnitud del costo de la tolerancia. La evidencia de selección contrastante entre sitios confirma que el resultado de la interacción entre *D. stramonium* y sus insectos herbívoros puede variar espacialmente. De esta manera, el proceso coevolutivo entre las plantas y sus enemigos naturales podría depender del valor adaptativo relativo de ambas formas de defensa (resistencia y tolerancia) como función del ambiente. En conjunto, los resultados apoyan la existencia de selección en mosaico y variación espacial en la distribución de "hot spots" donde el proceso coevolutivo operaría con mayor intensidad (Thompson 1994).

Perspectivas

La evidencia acumulada en los últimos 35 años a permitido constatar que contrariamente a lo que la teoría coevolutiva predice, las plantas parecen no expresar un patron de coadaptación a nivel de las poblaciones locales con sus enemigos naturales (Futuyma 1983; Seger 1992). Si consideramos al proceso coevolutivo como un resultado dinámico de evolución recíproca entre especies a través de los rasgos involucrados en la interacción entre ellas, a nivel de las poblaciones locales se espera al menos cierto grado de acoplamiento entre las especies. De esta manera, el proceso coevolutivo tendería a favorecer la divergencia de caracteres y en su escenario más extremo la especiación (Thompson 1994). A nivel microevolutivo la teoría predice que los mismos patrones filogenéticos de especialización recíproca que se observan a niveles taxonómicos superiores deberían observarse a nivel intraespecifico.

Actualmente la mayoría de los reportes de adaptación local en plantas se refieren a factores del ambiente físico (metales pesados, temperatura, altitud, etc.) (Linhart & Grant 1996; Schluter 2001; ver Berenbaum & Zangerl 1998). A pesar de que

existe en la actualidad una gran cantidad de evidencia en favor de la hipótesis de que los enemigos naturales de las plantas rápidamente se adaptan localmente a sus hospederos (Futuyma & Moreno 1988; Mopper & Strauss 1996; Zandt & Mopper 1998; Schluter 2001), es muy contrastante la escaza evidencia de plantas adaptadas localmente a sus enemigos naturales (Futuyma 1983; Linhart & Grant 1996; Schlutter 2001). Esta asimetría en el resultado evolutivo de la interacción permite cuestionarse la generalidad de una de las predicciones fundamentales de la teoría coevolutiva para las interacciones tróficas (antagonistas): *"la carrera armamentista entre las especies interactuantes resulta en una escalada de adaptaciones y contra-adaptaciones que producen pares o grupos de especies adaptados localmente al ambiente genético de la(s) especie(s) con la(s) que interactúan"* - Esta predicción se deriva del supuesto de que la tasa de respuesta coevolutiva recíproca de las especies es similar (van Valen 1973). Es decir, la tasa evolutiva de cambio en los rasgos involucrados en la interacción es equivalente. Sin embargo, las plantas parecen tener un "retraso aparente" en su respuesta a los enemigos naturales a escala microevolutiva, lo que se expresa como la ausencia de adaptación local en rasgos "supuestamente" involucrados en el proceso coevolutivo. Este retraso aparente ha sido atribuido al hecho de que en la mayoría de los sistemas naturales el tiempo generacional de las plantas es mucho mayor que el de los enemigos naturales. Aunque esta hipótesis ha sido planteada anteriormente para explicar la coexistencia en las interacciones antagonistas (ver Krebs & Davies 1993 para ejemplos en animales), un gran número de modelos coevolutivos, poblacionales y genéticos, asumen por simplicidad que las especies interactuantes tienen generaciones anuales (Levin & Udovic 1977; Schaffer & Rosenzweig 1978; Lawton & McNeill 1979; Frank 1993). Si la asimetría en el tiempo generacional permite explicar porqué los enemigos naturales se adaptan localmente a sus poblaciones de hospederos (Levin 1983), mientras que no ocurre lo mismo con las plantas, entonces el proceso no operaría de manera recíproca. La consecuencia más importante de este fenómeno sería la inestabilidad del proceso coevolutivo debido a que los rasgos involucrados en la interacción evolucionarían a tasas diferentes. Si aceptamos que la asimetría en el tiempo generacional entre plantas y enemigos

naturales es un hecho común, cómo podrían las plantas compensar este efecto para mantener tasas evolutivas similares a las de sus enemigos naturales.

En la actualidad existen dos líneas de evidencia que podrían aportar información para resolver la inconsistencia a nivel microevolutivo en la dinámica del proceso coevolutivo: (1) la existencia de interacciones no competitivas (mutualismos) entre las plantas y el tercer nivel trófico (Marquis 1994; Abrahamson y Weis 1997), y (2) la posibilidad de que las plantas desarrollen estrategias de defensa mixtas que incluyan tanto la defensa a través de la resistencia como la tolerancia (de Jong & van der Meijden 2000; Tiffin 2000; Capítulo I).

La hipótesis del tercer nivel trófico. A partir del artículo de Lawton & McNeill (1979) se propuso la hipótesis de que los depredadores de los enemigos naturales de las plantas actuaban como reguladores de las poblaciones de insectos herbívoros contribuyendo al mantenimiento de las poblaciones de plantas (Caughley & Lawton 198, pág. 138). Recientemente Godfray (1995) propuso un modelo donde la estabilidad del sistema planta-herbívoro-depredador depende del costos para la planta de producir un compuesto volátil que sirva como señal para los enemigos naturales de los herbívoros. La presencia de costos fisiológicos y genéticos en la producción de compuestos volátiles en plantas es todavía un supuesto con relativamente poca evidencia empírica. Sin embargo existen actualmente evidencia sobre el beneficio para las plantas de producir compuestos volátiles como señal para los depredadores de los insectos herbívoros (DeMoraes et al. 1998). Considero que no hay suficiente evidencia para descartar la importancia de las interacciones a más de un nivel trófico como factor condicionante del éxito de las poblaciones de plantas (ver Lill et al. 2002).

La hipótesis de las estrategias de defensa mixtas. Durante los últimos 10 años los estudios sobre la evolución de las estrategias de defensa de las plantas han sugerido la posibilidad de que ambas estrategias (tolerancia y resistencia) podrían ser favorecidas dentro de las poblaciones (Capítulo I). Por un lado, se ha propuesto que

el valor adaptativo de una estrategia de defensa combinada resulta del hecho que las plantas normalmente son comidas por un grupo diverso de enemigos naturales (de Jong & van der Meijden 2000). Algunos de ellos son especialista mientras que otros son más generalistas en su dieta. Si asumimos que es común que los enemigos especialistas no sean negativamente afectados por los mecanismos de resistencia (Marak et al. 2000), la única respuesta que puede ofrecer una planta frente a este grupo de enemigos naturales es la tolerancia (de Jong & van der Meijden 2000). Por otro lado, la evidencia experimental indica que los generalistas normalmente son afectados por las defensas constitutivas e inducidas de las plantas (i.e., estrategia de resistencia) (Dirzo & Harper 1982; Marquis 1992; Rausher 1996; Apéndice I: Valverde et al. 2001; Apéndice II: Valverde et al. 2002). Por lo tanto ambas formas de defensa serían favorecidas cuando la presión de los enemigos naturales está compuesta de especies especialistas y generalistas simultáneamente.

Por otro lado se ha planteado que la selección favorecerá la evolución de estrategias mixtas cuando: (1) la concavidad asociada a la función que describe los costos de las diferentes estrategias defensivas sea mayor que la de la función de beneficios; y/o (2) cuando los costos asociados con cada estrategia son de magnitudes diferentes (Tiffin 2000; Capítulo I). Aún cuando la disponibilidad de recursos puede afectar negativamente la asignación a la defensa (Bergelson 1994; Hoechwender et al. 2000), pocos estudios han determinado la magnitud de los costos de cada forma de defensa, y la forma de la función de costos para ambas estrategias de defensa simultáneamente (Pilson 2000) ante situaciones de limitación de recursos.

Una o ambas de las hipótesis propuestas podrían favorecer la evolución de estrategias de defensa mixtas en las plantas. Si las defensas mixtas es un fenómeno común en la respuesta de las plantas ante la presión de los enemigos naturales, entonces es necesario incorporar la tolerancia a los modelos coevolutivos. Por definición la tolerancia no afecta negativamente a los enemigos naturales (Rosenthal & Kotanen 1994). Es decir, ellos no perciben ninguna respuesta por parte de la planta. Sin embargo, la planta a través de la tolerancia reduce el efecto negativo que los enemigos naturales ejercen sobre ellas (Rausher 2001). Retomando el problema de la

ausencia de adaptación local de las poblaciones de plantas a sus enemigos naturales, ¿podría una estrategia mixta explicar este patrón? En los párrafos siguientes se plantea la importancia que la integración de la tolerancia pudiera tener en los modelos coevolutivos como mecanismo para reducir la asimetría en el tiempo generacional entre plantas y enemigos naturales.

Históricamente, la asimetría en el tiempo generacional ha sido resuelta en los modelos teóricos simplemente asumiendo que las especies interactuantes tienen tiempos generacional iguales (Levin & Udovic 1977; Schaffer & Rosenzweig 1978; Lawton & McNeill 1979). La incorporación de la tolerancia permitiría modelar interacciones coevolutivas con tiempos generacionales diferentes combinados con tasas de respuestas evolutivas similares, haciendo a los modelos más realistas. En este sentido, la tolerancia permitiría a las plantas reducir: (1) la presión de selección acumulada de un consumidor con ciclo de vida más corto que el de la planta, y (2) la presión de las plantas sobre las poblaciones de enemigos naturales retardando el proceso coevolutivo. La tasa evolutiva de plantas y enemigos naturales puede asumirse que es similar en los rasgos involucrados en un proceso coevolutivo. La predicción más simple plantearía que los ciclos de coevolución ocurrirían a intervalos más grandes de lo que se ha planteado de manera teórica. El tamaño de los ciclos dependería de la capacidad de tolerancia de las plantas. Si las plantas fueran totalmente tolerantes no habría ningún beneficio en ser resistentes. Por lo tanto, el proceso coevolutivo operará en la medida que exista un beneficio asociado con la asignación de recursos a la resistencia. Cuanto menos tolerante sea un especie/población de planta habrá mayores oportunidades para que ocurra un proceso coevolutivo entre esta y sus enemigos naturales. En este modelo el aparente retraso en el proceso de adaptación de las plantas a sus enemigos naturales sólo reflejaría el hecho de que la expectativa de adaptación local (en rasgos de resistencia involucrados en la interacción) por parte de las plantas no necesariamente se debe cumplir cuando éstas son capaces de tolerar el daño por los enemigos naturales. Si esta hipótesis es cierta deberíamos esperar que las especies de plantas cuyo tiempo generacional es considerablemente grande con respecto al de sus enemigos naturales

fueran más tolerantes. Obviamente, los árboles se encuentran entre las especies de plantas con mayor diferencia generacional con respecto a sus enemigos naturales. Sin embargo, la evidencia experimental sobre la tolerancia en especies perennes es escasa para fundamentar alguna generalización.

Estudios recientes sugieren que la capacidad de tolerancia de los árboles podría ser mayor de lo que se ha considerado, debido a: (1) la mayor cantidad de modulos estructurales que potencialmente pueden incrementar la capacidad de compensación, (2) la mayor predecibilidad del daño dada por su mayor longevidad, (3) la mayor capacidad de pagar el costo del daño en más de una estación reproductiva, (4) su mayor concentración de biomasa en órganos nos foliares (aproximadamente 1 – 4 % de la biomasa total corresponde a tejido foliar) (Hauhioja & Koricheva 2000). Debido a que el estudio de las defensas en especies de plantas herbáceas y arbóreas ha recibido escasa atención, no es posible generalizar con respecto al nivel de tolerancia y resistencia entre estos grupos.

Si las plantas no hubiesen podido contrarrestar la presión del herbivorismo "el mundo no sería verde" (*sensu* Lawton & McNeill 1979). Los patrones macroevolutivos entre plantas y herbívoros apoyan de manera más consistente la predicción sobre especialización en rasgos involucrados en la interacción (Ehrhlich & Raven 1964; Mitter et al. 1991; Becerra 1997; Termonia et al. 2001). Sin embargo es muy escasa la evidencia a escala microevolutiva sobre adaptación recíproca entre plantas y herbívoros (Futuyma 1983; Berenbaum & Zangerl 1998). La Teoría del Mosaico Geográfico en Coevolución también ofrece una explicación complementaria a la ausencia de un patrón a escala de las poblaciones dado por el flujo de genes entre poblaciones sujetas a presiones selectivas diferentes (Thompson 1994). La hipótesis sobre la importancia de la tolerancia en la dinámica coevolutiva entre plantas y enemigos naturales a nivel de las poblaciones podría explicar parcialmente la inconsistencia entre los patrones macro y microevolutivos entre plantas y herbívoros, considerando que el valor adaptativo de la tolerancia puede ser alterado por las condiciones ambientales (Cap. III). Es decir que la ausencia o disminución en el valor adaptativo de la variación genética en atributos de resistencia podría no solo deberse

al flujo génico entre poblaciones con diferentes regímenes de selección, sino también a poblaciones con diferencias en el valor adaptativo de la tolerancia.

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APÉNDICE I

VARIACIÓN ENTRE POBLACIONES NATURALES DE *Datura stramonium* EN EL VALOR DE LOS TRICOMAS FOLIARES COMO COMPONENTES DE LA RESISTENCIA CONTRA INSECTOS HERBÍVOROS

Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium*

P. L. VALVERDE,*† J. FORNONI* & J. NÚÑEZ-FARFÁN*‡

*Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70–275, México 04510, Distrito Federal, Mexico
†Departamento de Biología, Universidad Autónoma Metropolitana, Unidad Iztapalapa, Apartado Postal 55–535, México 09340, Distrito Federal, Mexico
‡Department of Ecology and Evolutionary Biology, University of Connecticut, Torrey Life Science Building, Storrs, CT, USA

Abstract

This study assessed the role of leaf trichome density as a component of resistance to herbivores, in six populations of *Datura stramonium*. Phenotypic selection on plant resistance was estimated for each population. A common garden experiment was carried out to determine if population differences in leaf trichome density are genetically based. Among population differences in leaf trichome density, relative resistance and fitness were found. Leaf trichome density was strongly positively correlated to resistance across populations. In 5 out of 6 populations, trichome density was related to resistance, and positive directional selection on resistance to herbivores was detected in three populations. Differences among populations in mean leaf trichome density in the common garden suggest genetic differentiation for this character in *Datura stramonium*. The results are considered in the light of the adaptive role of leaf trichomes as a component of defence to herbivores, and variable selection among populations.

Introduction

Leaf trichome density is considered a mechanism of defence in plants to prevent or diminish damage by herbivores (Levin, 1973; Johnson, 1975; Rodriguez *et al.*, 1984; Juniper & Southwood, 1986; Marquis, 1992). Evidence from wild and cultivated species gives support to this ecological role (Duffey, 1986; Jeffree, 1986; David & Easwaramoorthy, 1988; Woodman & Fernandes, 1991; Bernays & Chapman, 1994; Peter *et al.*, 1995; Romeis *et al.*, 1999). However, although many studies have found significant selection on resistance against natural herbivores (Berenbaum *et al.*, 1986; Rausher & Simms, 1989; Marquis, 1992; Núñez-Farfán & Dirzo, 1994; Núñez-Farfán *et al.*, 1996; Mauricio *et al.*, 1997) few, if any, have examined whether patterns of selection on defensive traits vary among populations of the same

species. In this study we evaluated natural variation in both the defensive role of leaf trichome density as a component of resistance against natural herbivores, and the importance of resistance as a fitness-enhancing character among populations of the annual *Datura stramonium* L. The study of natural variation is important in guiding experiments to assess causality, and to estimate the strength of selection in the wild (Sinervo, 2000).

Besides defence, leaf trichomes may serve other physiological functions, hence selection on the antiherbivory role of leaf trichome density can either be constrained or synergistically favoured by selection imposed by other environmental stresses (Bell, 1997; Roy *et al.*, 1999). For instance, if leaf trichome density is correlated with other leaf characteristics, selection on those traits can produce changes in trichome density (Björkman & Anderson, 1990; Roy *et al.*, 1999) without being the target of selection. In addition, as for other plant traits, phenotypic variation in leaf trichome density may have both genetic and environmental (and their interaction) causal factors (Falconer & Mackay, 1996). Within-population genetic variation in leaf trichome density will lead to an

evolutionary change provided the trait is under selection. However, environmentally determined phenotypic variation in leaf trichomes (i.e. phenotypic plasticity; Ågren & Schemske, 1994; Schlichting & Pigliucci, 1998), may limit response to selection.

Within-population variability in resistance (i.e. the ability of a plant to avoid herbivore attack; Simms & Triplett, 1994; Rausher, 1996) has been documented in *Datura stramonium*, and this variability is related to plant fitness (Núñez-Farfán & Dirzo, 1994; Núñez-Farfán *et al.*, 1996; Fornoni & Núñez-Farfán, 2000). Although trichome density functions as a defensive trait against herbivory inother solanaceous species (Thurston, 1970; Duffey & Isman, 1981; Kennedy & Sorenson, 1985; Goffreda *et al.*, 1988; Wilkens *et al.*, 1996; van Dam & Hare, 1998a,b; Elle *et al.*, 1999), it is not known if leaf trichome density is associated with resistance, or if this relationship varies across populations of *Datura stramonium*.

The present study aimed specifically to address the following questions: (1) is individual variation in leaf trichome density related to resistance to herbivores? and if so (2) is resistance selectively favoured within populations? Given that populations of *Datura stramonium* occur in a broad variety of plant communities (Núñez-Farfán, 1991), phenotypic differences among populations in leaf trichome density might be both environmentally and genetically based. Thus, we also asked if (3) the relationship between leaf trichome density and resistance differs across populations of *Datura stramonium*, and (4) to what extent phenotypic differences are the result of phenotypic plasticity or genetic differentiation among populations. Under the null hypothesis that variation in leaf trichome density in *Datura stramonium* occurs for other reasons (e.g. temperature regulation), it is not expected to be related to resistance to herbivores.

Materials and methods

Plant species

Datura stramonium (Solanaceae) is a cosmopolitan annual weed occurring in a wide variety of plant communities in Mexico and North America (Avery *et al.*, 1959; Weaver & Warwick, 1984). Leaves of this species are eaten by at least two specialist herbivorous insects, *Epitrix parvula* (Coleoptera: Chrysomelidae) (Núñez-Farfán & Dirzo, 1994) and *Lema trilineata* (Coleoptera: Chrysomelidae) (Kogan & Goeden, 1970a,b; Kirkpatrick & Bazzaz, 1979; Peterson & Dively, 1981; Núñez-Farfán, 1995). Other herbivores have also been recorded to consume this species: *Sphenarium purpurascens* (Orthoptera: Acrididae) and two lepidopterans (*Manduca* sp., Sphingidae, and one unidentified species of Noctuidae) (Núñez-Farfán & Dirzo, 1994; Núñez-Farfán *et al.*, 1996). Complete description of the plant and insect species can be found elsewhere (Núñez-Farfán, 1991).

Data collection

In six populations of *Datura stramonium* from the central part of Mexico (four States) all natural growing individual plants (16–46) were marked and, at reproduction, foliar damage produced by herbivorous insects and the number of mature fruits were recorded. The six populations occurred in different plant communities: one in a tropical dry forest, two in pine–oak forests, and three in xerophytic shrub communities (Table 1). Distances between pairs of populations ranged from 20 to 300 km.

In each population the following data were taken for each individual plant: (1) total number of branches, (2) total number of fruits, and (3) average seed number per fruit (seed-set), from a sample of 10 fruits per plant.

Relative resistance, trichome density and fitness

A large random sample (mean = 31.62 standard error = 0.66) of fully expanded leaves was collected from each individual plant and measured with a leaf-area meter (Delta-T Devices, Cambridge, UK) to obtain standing leaf area (i.e. remnant undamaged leaf area). For each plant, relative damage was obtained by dividing consumed leaf area (CLA_i) by total leaf area (TLA_i). Original total leaf area was estimated using a regression analysis of leaf area as a function of leaf length following Núñez-Farfán & Dirzo (1994). Since leaf shape (hence, leaf area) varied slightly among populations, four different equations were applied to estimate original total leaf area (R^2 ranging from 0.964 to 0.987, $P < 0.001$,

Table 1 Environmental characteristics of six populations of *Datura stramonium* in central Mexico.

Number and locality of each population (State)	Habitat	Geographical coordinates*	Altitude above sea level (masl)	Mean annual precipitation (mm)*	Mean annual temperature (°C)*
I. Ticumán (Morelos)	tropical dry forest	18°N, 99°W	961	802.1	23.1
II. Santo Domingo (Morelos)	pine-oak forest	18°N, 99°W	2050	1463.2	19.9
III. Zirahuén (Michoacán)	pine-oak forest	19°N, 101°W	2174	1400.6	16.4
IV. Teotihuacan (State of México)	xerophytic shrub	19°N, 98°W	2294	559.6	14.8
V. Patria Nueva (Hidalgo)	xerophytic shrub	20°N, 99°W	1745	360.5	18.4
VI. Actopan (Hidalgo)	xerophytic shrub	20°N, 98°W	1990	458.5	16.9

*Data taken from García (1988). Means were calculated from 9 to 53 years of climatic records.

$n = 30–120$). Relative resistance to herbivores was estimated for each plant as $1 - (CLA_i/TLA_i)$ following previous studies (Berenbaum *et al.*, 1986; Fritz & Price, 1988; Simms & Rausher, 1989; Núñez-Farfán & Dirzo, 1994; Núñez-Farfán *et al.*, 1996; Tiffin & Rausher, 1999). For statistical analyses, resistance was arcsine-transformed to normalize its error distribution (Sokal & Rohlf, 1995).

Trichome density was measured as the total number of trichomes within an area of 2.5 mm^2 on the basal central area of the adaxial side of the leaf (see Mauricio *et al.*, 1997), using a dissecting microscope. This sampled area of the leaf gives a good estimate of the whole-leaf average trichome density: for 30 randomly chosen leaves from 15 different plants, trichome density in 2.5 mm^2 was highly correlated with the average trichome density of nine other 2.5-mm^2 areas within the same leaf ($R^2 = 0.81$, $F_{1.13} = 60.1$, $P < 0.0001$). Thus, in each population, average trichome density of 16–20 plants was calculated on a sample of 10 randomly chosen fully expanded mature leaves for each plant. For statistical analyses, trichome density was square-root transformed to normalize its error distribution (Sokal & Rohlf, 1995).

Maternal plant fitness was estimated as the average seed-set per fruit times total fruit number per plant. Because absolute maternal fitness varies with plant size (Núñez-Farfán, 1991), the analysis of fitness as a function of plant resistance to herbivores was made in each population using the residuals from the regression analysis (see Sinervo, 2000) of absolute maternal fitness (total seed number per plant) on plant size, as estimated by the total number of branches. Total number of seeds and branches were square-root transformed before statistical analyses (Sokal & Rohlf, 1995). Hereafter, residuals for maternal fitness will be referred to simply as fitness.

Statistical analyses

The effect of trichome density on plant resistance to herbivores among and within populations was analysed using covariance analysis (ANCOVA), under the null hypothesis that trichome density, the covariate, is not a plant resistance component. In the same way, the effect of leaf area on trichome density was analysed (see Roy *et al.*, 1999). Differences in average values among populations in plant resistance and trichome density were obtained through Tukey–Kramer HSD tests (Sokal & Rohlf, 1995). The relationship between fitness and plant resistance, within and among populations, was analysed by means of ANCOVA, where plant resistance was the covariate. The relationship between average resistance and average trichome density per population was assessed by means of a Spearman rank correlation (R_S) (Sokal & Rohlf, 1995, p. 598). The analyses were carried out using the JMP® statistical package (SAS Institute, 1995).

In order to estimate phenotypic directional selection gradients (β_i) for each population, linear regression analysis of individual fitness (w_i) as a function of the standardized resistance to herbivores ($X = 0$ and $s^2 = 1$) were performed (Lande & Arnold, 1983; Mitchell-Olds & Shaw, 1987; Nagy, 1997). Selection coefficients and standard errors were estimated using FREE-STAT (version 1.10; Mitchell-Olds, 1989). Jackknife estimates of the standard errors of the selection coefficients were also obtained (FREE-STAT). The Jackknife procedure permits approximate *t*-tests of significance which are robust to deviations from normality and to heterogeneity of residual variances (Mitchell-Olds, 1989). We estimated the selection coefficients only on resistance to herbivores following the reasoning that leaf trichome density is a putative component of resistance and correlated with it (Mauricio *et al.*, 1997; van Dam & Hare, 1998b; Elle *et al.*, 1999). Data of this study indicated no relationship between trichome density and fitness. Then a covariance analysis was performed to assess if trichome density is related to resistance (see Results). Because trichome density is correlated with resistance in 5 of 6 populations, this validates our criterion for not including trichome density in the selection analyses given the lack of independence between both traits (Mitchell-Olds & Shaw, 1987).

Population differentiation in trichome density

To determine possible genetic differences among populations in leaf trichome density, natural progenies (*sensu* Lawrence, 1984; hereafter families) from three populations were collected and grown in a common garden. Plants of a given family were derived from a single fruit and related as half- or full-sibs. Given the size of individual plants and the number of populations sampled in the field, three randomly selected populations were grown in the common garden due to space limitations. The three populations grown were: Population I, 15 families and 133 plants; Population II, 14 families and 118 plants; Population III, 10 families and 81 plants (cf. Table 1). Total sample size was 332. The common garden (59 × 13 m) was located in an area within the Pedregal de San Ángel Ecological Preserve (National Autonomous University of Mexico; UNAM) where *Datura stramonium* grows naturally (Núñez-Farfán & Dirzo, 1994). The seeds were germinated in the greenhouse (protocol in Fornoni & Núñez-Farfán, 2000), and then transplanted to the common garden under a complete randomized design once the first pair of true leaves appeared. Plants were spaced 1 m apart in a regular grid. When plants reached maturity (reproduction), trichome density was estimated for all plants, following the same methodology employed for field collected plants (see above). A nested-analysis of variance was performed to test differences due to population and family (within population) (Sokal & Rohlf, 1995), using the JMP® statistical package (SAS Institute, 1995).

Table 2 Mean leaf trichome density (trichomes × mm^{-2}) per plant (SE), plant resistance to herbivores (1 − relative damage) (±1 SE) and regression analysis of relative resistance (y) on trichome density (x) in six populations of *Datura stramonium* from central Mexico. Different letters for each character indicate significant among-population differences at $P < 0.01$ (see Materials and methods).

Population	Trichome density	n	Relative resistance	n	Model Regression model	R^2	d.f.	F
I	8.911 (0.517) b	24	0.891 (0.004) d	30	$y = 0.849 + 0.004x$	0.24	1, 22	7.298*
II	3.348 (0.346) a	20	0.559 (0.014) a	30	$y = 0.446 + 0.028x$	0.34	1, 18	9.290**
III	3.041 (0.262) a	20	0.515 (0.014) a	46	$y = 0.433 - 0.034x$	0.02	1, 18	0.372 ns
IV	6.929 (0.674) b	19	0.635 (0.029) b	30	$y = 0.433 + 0.034x$	0.42	1, 17	12.747**
V	15.429 (0.503) c	18	0.816 (0.013) c	18	$y = 0.612 + 0.013x$	0.25	1, 16	5.491*
VI	12.376 (0.550) c	16	0.797 (0.006) c	16	$y = 0.495 + 0.057x - 0.002x^2$	0.56	2, 13	8.329**

*$P < 0.05$; **$P < 0.01$; ns, not significant.

Results

Trichome density and resistance to herbivores

Populations experienced different average levels of damage (10–50% of total leaf area) (Table 2). In each population, all individual plants had some degree of foliar damage. In all populations, leaf damage was caused mainly by tobacco flea beetles (*Epitrix* spp., Coleoptera: Chysomelidae). Trichome density varied from 3.041 to 15.429 trichomes × mm^{-2} (Table 2). ANCOVA detected statistically significant differences among populations in plant resistance to herbivores, and a significant effect of trichome density on plant resistance (Tables 2 and 3a). Furthermore, the significant trichome density × population interaction indicated

that the slope for the relationship between trichome density and resistance varied among populations (Table 3a). In contrast, differences among populations in trichome density were not related with leaf area (Table 3b). In five out of six populations, a significant relationship between trichome density and plant resistance was detected and the explained variance (R^2) ranged from 0.50 to 0.68. Populations I, II, IV and V showed positive relationships, whereas population VI had a concave downward relationship between leaf trichome density and resistance (Table 2). Multiple comparisons also showed differences in trichome density and resistance among populations (Table 2). Finally, population mean resistance and trichome density were highly positively correlated across populations (Fig. 1, $R_S = 0.83$, $n = 6$, $P = 0.0416$).

Table 3 Analyses of covariance for plant resistance to (a) herbivores, (b) trichome density (trichome × mm^{-2}) and (c) fitness in *Datura stramonium*. All F-ratios were based on type-III sums of squares.

Trait	Source of variation	d.f.	SS	F
(a) Resistance	Population	5	0.208	7.67***
	Trichome density	1	0.062	11.57**
	Pop. × Trichom.	5	0.166	6.14***
	Error	105	0.570	
	R^2		0.868***	
(b) Trichome density	Population	5	9.319	12.61***
	Leaf area	1	0.006	0.04 ns
	Pop. × Leaf area	5	1.630	2.20 ns
	Error	105	15.512	
	R^2		0.832***	
(c) Fitness	Population	5	9823.375	4.90***
	Resistance	1	2979.598	7.44**
	Pop. × Resist.	5	7575.756	3.78**
	Error	123	49219.970	
	R^2		0.490***	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant.
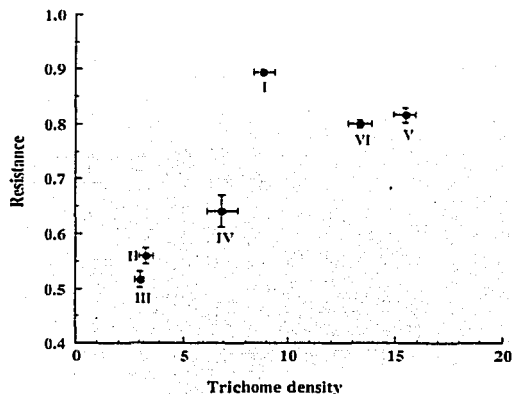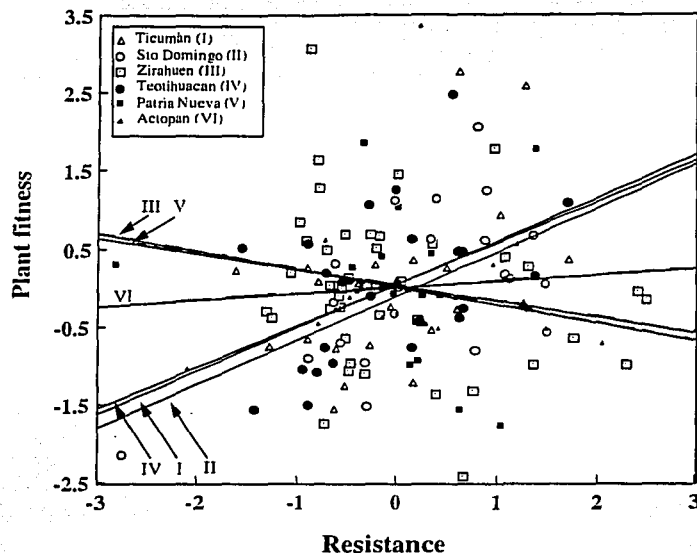

Fig. 1 Relationship between population mean resistance to herbivorous insects (1 − relative damage) and mean trichome density (trichome × mm^{-2}) in *Datura stramonium* ($R_S = 0.83$, $n = 6$, $P < 0.041$). Labels correspond to populations listed in Table 1. Bars indicate (±1 SE).

Fig. 2 Relationship between plant fitness and resistance to herbivores (standardized) in six populations of *Datura stramonium* (see Materials and methods, and Table 3). Significant selection (continuous line) and nonsignificant selection (dashed line) linear fit are indicated. Arrows indicate population number.

Phenotypic selection of resistance across populations

ANCOVA revealed significant differences among populations in fitness (Table 3c). Also, resistance to herbivores had a significant effect on plant fitness. The significant interaction between plant resistance and population on fitness indicates that the slope of the relationship between fitness and plant resistance differed among populations (Table 3c and Fig. 2). These results suggest differences among populations in the effectiveness of resistance against herbivorous insects, and imply that a similar amount of damage had different consequences on plant fitness among populations.

Significant directional selection coefficients were detected in three out of six populations. In Populations I, II and IV, resistance was positively favoured, indicating that higher fitness was attained by those plants with higher levels of resistance (Table 4 and Fig. 2). After Jackknife procedure, selection coefficients for these populations remained significant (Table 4). In contrast, no selection on resistance was detected in populations III, V and VI (Table 4 and Fig. 2).

Population differentiation in trichome density

Nested ANOVA revealed significant differences among populations in leaf trichome density, whereas no significant differences among families within population

were found (Table 5). Plants from population I had a significant higher mean leaf trichome density than plants from populations II and III, which did not differ from each other (Fig. 3). Thus, differences found in the field were maintained in a common garden suggesting genetic differentiation between populations for this character. However, the same experiment revealed the plastic nature of trichome density: the novel environment represented by the common garden had distinctive effects on the plants of the different populations (i.e. they tended to converge phenotypically; cf. Fig. 3).

Table 4 Directional selection coefficients (β) and standard errors (SE) of resistance to insect damage in six populations of *Datura stramonium*. R^2 for the lineal models and Jackknife estimates for the significant selection coefficients are provided. Sample sizes correspond to those of Table 2.

Population	β (SE)	R^2	Jackknife estimates	
			SE	$|t|$
I	0.553 (0.222)*	0.213	0.261	2.121*
II	0.564 (0.185)**	0.328	0.163	3.470**
III	−0.229 (0.051) ns	0.051	–	–
IV	0.529 (0.229)*	0.211	0.221	2.390*
V	−0.201 (0.270) ns	0.036	–	–
VI	0.081 (0.267) ns	0.007	–	–

*$P < 0.05$; **$P < 0.01$; ns, not significant.

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Table 5 Nested ANOVA of leaf trichome density (trichome × mm⁻²) for families of three populations of *Datura stramonium* in a common garden (see Materials and methods).

Source of variation	d.f.	SS	F
Population	2	2.483	9.02***
Families (Population)	36	4.954	1.21 ns
Error	293	33.134	

***$P < 0.001$; ns, not significant.

Discussion

Significant among-population variation in both leaf trichome density and plant resistance to herbivores coupled with the association of trichome density with resistance in most populations of *Datura stramonium*, support the expectation of a defensive role of trichomes within populations. In addition, trichome density affected plant fitness through its association with plant resistance. However, the effectiveness of leaf trichome density varied among populations. Directional selection of phenotypes with higher resistance to herbivores was significant in only three populations of *Datura stramonium*. Thus, these results support the adaptive hypothesis of trichome density as a defensive trait against herbivory. Even though leaf trichome density is a phenotypically plastic character, our evidence indicated significant population differences in trichome density, highlighting the potential for genetic differences among populations on this defensive trait.

Leaf trichome density is regarded as a component of plant defence against herbivores (Levin, 1973; Johnson, 1975; Marquis, 1991, 1992; Kennedy & Barbour, 1992; Ågren & Schemske, 1993; Rausher, 1996; Mauricio & Rausher, 1997; van Dam & Hare, 1998a,b; Elle *et al.*, 1999). However, few studies have estimated phenotypic selection on resistance to herbivores in different populations of the same species. Relevant to this goal, the present results demonstrated that (1) trichome density is a component of plant resistance to herbivores in most populations of *Datura stramonium* sampled (cf. Tables 2, 3a), and (2) selection for higher resistance to herbivores occurred in some populations (Table 4). These results are in agreement with the finding of selection on resistance to herbivores in other populations of *Datura stramonium* (Núñez-Farfán & Dirzo, 1994; Núñez-Farfán *et al.*, 1996). Selection is expected to vary spatially and temporally in plant–animal interactions, and this constitutes the raw material of the coevolutionary process (Thompson, 1999). Yet the experimental study of adaptation makes necessary, first, the analysis of natural populations (see Sinervo, 2000) to identify potential coevolutionary hotspots (Thompson, 1999).

The relative effectiveness of trichome density as a defensive trait differed among populations (significant population × trichome density interaction; cf. Table 3a). In addition, the result that resistance may or may not be
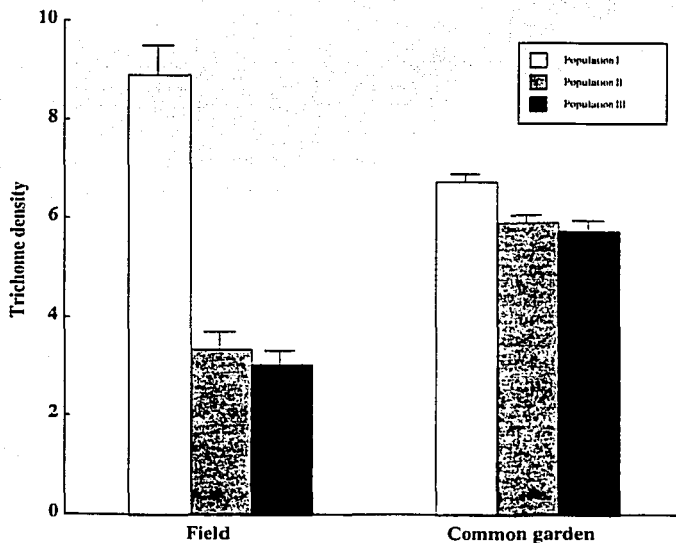
Fig. 3 Average values of leaf trichome density (trichome × mm⁻²) in the field and in the common garden of three populations of *Datura stramomiun*. Bars represent mean (+1 SE).

selectively advantageous in a given population is reflected in the interaction between population and resistance (Table 3c and Fig. 2). In fact, for three populations no evidence of selection on resistance was detected suggesting that natural levels of damage did not exert significant negative effects on individual plant fitness, and that other factors besides trichome density might determine resistance. Recently, it has been proposed that compensation after damage constitutes an alternative strategy of plant defence besides resistance (Maschinski & Whitham, 1989; Belsky *et al.*, 1993; Simms & Triplett, 1994; Fineblum & Rausher, 1995; Mauricio *et al.*, 1997). If some populations of *D. stramonium* compensate following damage, selection on resistance might not be expected (Herms & Mattson, 1992). A recent study in *D. stramonium* indicates that this species can compensate for foliar damage (Fornoni & Núñez-Farfán, 2000); however, it is not possible to establish, at present, if those populations do not have selection on resistance.

Although resistance could be the best strategy under certain environments, lack of genetic variation brought about by genetic drift (i.e. founder effects) will constrain selection. Similarly, phenotypic plasticity in defensive traits may limit selection despite the presence of genetic variation is present in certain environments (see Núñez-Farfán & Dirzo, 1994; Fornoni & Núñez-Farfán, 2000). Also, the capacity of plants to produce inducible defences (see Zangerl & Berenbaum, 1990; Underwood *et al.*, 2000) once damage has occurred may prevent the detection of selection on resistance. We did not examine whether there are inducible defences in *Datura stramonium*, or if genetic variation for induction occurs in natural populations.

It must be stressed that population differences in leaf trichome density may occur even if it is not a component of plant resistance. For instance, trichome number might be positively or negatively selected in different stressful environments because it is correlated with other characters (e.g. leaf size; see Roy *et al.*, 1999). However, if trichome density were not a resistance component in *Datura stramonium*, no relationship between trichomes and resistance would be expected either among or within populations. In this study, the results for *Datura stramonium* show that variation in trichome density is independent on leaf size (cf. Table 3b). Furthermore, no relationship between leaf size and trichome density was found for two populations (I and III) of this species in the greenhouse (P. L. Valverde, unpublished data). Leaf trichomes have been proposed to reduce water loss in water-limited environments (Turner & Kramer, 1980; Fitter & Hay, 1987). Still, this does not exclude the possibility that leaf trichomes function as a component of plant resistance to herbivores (Woodman & Fernandes, 1991). The present data support leaf trichome density as a component of resistance regardless of selection imposed by other environmental factors.

Response to selection within populations is expected only if part of the phenotypic variation in leaf trichome density is genetic in origin (Falconer & Mackay, 1996). Leaf trichome density is a highly variable plant character (Ågren & Schemske, 1994; Roy *et al.*, 1999), and evidence of environmental induction (e.g. phenotypic plasticity) has been documented (Sharma & Dunn, 1969; Conklin, 1976; Wilkens *et al.*, 1996; Elle *et al.*, 1999). Several studies have detected heritable variation for leaf trichomes (van Dam & Hare, 1998a; Elle *et al.*, 1999; van Dam *et al.*, 1999). In *Datura stramonium*, the common garden experiment revealed the plastic nature of leaf trichome density since the population averages tended to converge (i.e. their change was in opposite directions; cf. Fig. 3). Yet, the populations analysed maintained their differences, suggesting genetic differentiation. The analysis did not reveal within-population differences among families and thus no potential for selection to change genetic frequencies at loci determining leaf trichomes. However, the common garden experiment involved only a small number of families per population. Thus, genetic variation for trichome density may exist in natural populations of *Datura stramonium* but a quantitative genetics study of this character in natural conditions is needed.

Selection on traits involved in plant–animal interactions is not expected to act in the same magnitude and direction across populations of a species. Due to the relevance of the environment in modulating genetic variation, the study of natural variation is important to estimate selection in characters of putative adaptive value and in guiding experiments aimed to establish causality (Mousseau, 2000; Sinervo, 2000). As the present results show, the analysis of variation in defensive traits in only one population might result in misleading conclusions when evolutionary inferences are made above the level of populations (Thompson, 1994, 1999).

Acknowledgments

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APÉNDICE II

EL CRECIMIENTO COMPENSATORIO Y LA RESISTENCIA COMO ALTERNATIVAS DEFENSIVAS CONTRA ENEMIGOS NATURALES EN *Datura stramonium*

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Evolutionary ecology of *Datura stramonium*: equivalent plant fitness benefits of compensation by growth and resistance against herbivory

Pedro L. Valverde[*][†], Juan Fornoni[*] & Juan Núñez-Farfán[*]

[*]Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, México 04510, Distrito Federal, México.
[†]Departamento de Biología, Universidad Autónoma Metropolitana, Unidad Iztapalapa, Apartado Postal 55-535, México 09340, Distrito Federal, México)

Correspondence: P.L. Valverde, Departamento de Biología, Universidad Autónoma Metropolitana, Unidad Iztapalapa, Apartado Postal 55-535, México 09340, Distrito Federal, México.
e-mail: plvp@xanum.uam.mx

Running headline: selection on growth and resistance to herbivory

Abstract

This study evaluated how natural selection act upon two proposed alternatives of defence (growth and resistance) against natural enemies in a common garden experiment using genetic material (selfed-sibs) from three populations of the annual plant *Datura stramonium*. Genetic and phenotypic correlations were used to search for a negative association between both alternatives of defence. Finally, the presence/absence of natural enemies was manipulated to evaluate the selective value of growth as a response against herbivory. Results indicated the presence of genetic variation for growth and resistance (1 – relative damage), while only population differentiation for resistance was detected. No correlation between growth and resistance was detected either at the phenotypic or the genetic level. Selection analysis revealed the presence of equal fitness benefits of growth and resistance among populations. The presence/absence of natural herbivores revealed that herbivory did not alter the pattern of selection on growth. The results indicate that both strategies of defence can evolve simultaneously within populations of *Datura stramonium*.

Introduction

The hypothesis that plants respond against natural enemies either allocating resources to defensive resistance traits (i.e., secondary chemicals, trichomes, spines, etc.) or compensating damage through a growth related response has been amply accepted (van der Meijden *et al.*, 1988; Herms & Mattson, 1992; Belsky *et al.*, 1993; Strauss & Agrawal, 1999). Provided the presence of allocational costs associated with both alternatives of defence, a negative correlation between them is expected (van der Meijden *et al.*, 1988; Fineblum & Rausher, 1995). However, the assumption that both alternatives of defence are negatively correlated has not been strongly supported with experimental data. Although some studies have intended to validate this assumption (Bilbrough & Richards, 1993; Rosenthal & Welter, 1995; Stowe *et al.*, 2000), few studies detected a negative phenotypic or genetic correlation between resistance and estimates of compensation to herbivory damage (Coley, 1986; Han & Lincoln, 1994; Stowe, 1998). Hence, the generality of this assumption has been repeatedly questioned (Rosenthal & Welter, 1995; Strauss & Agrawal, 1999; de Jong & van der Meijden, 2000; Purrington, 2000; Stowe *et al.*, 2000). Empirical evidence suggest that the stable coexistence of both strategies of defence within populations may be more common than previously noted (Mauricio *et al.*, 1997; de Jong & van der Meijden, 2000), and the conditions for the presence of multiple strategies against natural enemies may need re-evaluation (Tiffin, 2000; de Jong & van der Meijden, 2000). Simultaneous analyses of selection patterns on components of a compensatory response and resistance against herbivory are needed. Despite the development of evolutionary models of plant defence allocation (Coley *et al.*, 1985; Simms & Rausher, 1987; Fagerström *et al.*, 1987; Fineblum & Rausher, 1995; Tiffin, 2000; de Jong & van der Meijden, 2000), and many studies that detected natural selection acting on components of resistance against herbivory (Rausher & Simms, 1989; Simms & Rausher, 1989; Fritz & Simms, 1992; Núñez-Farfán & Dirzo, 1994; Mauricio & Rausher, 1997; Tiffin & Rausher, 1999; Gomez & Zamora, 2000; Marak *et al.*, 2000; Shonle & Bergelson, 2000; Bergelson *et al.*, 2001; Stinchcombe & Rausher, 2001; Valverde *et al.*, 2001), few if any, have examined the joint selection

pattern on compensation and resistance. Moreover, although both strategies could be beneficial to plants against natural enemies, it is necessary to see if the pattern of selection on defence changes when enemies are absent (Mauricio & Rausher 1997). If other factors besides herbivory can select for compensatory ability (Aarssen & Irwin, 1991; Belsky *et al.*, 1993; Rosenthal & Kotanen, 1994; Aarssen, 1995) the pattern of selection on traits related to compensation could not be affected when enemies are absent. This effect can generate variation in levels of compensation not related to herbivory.

Similarly, at least three factors can generate variation in the levels of resistance not necessarily accounted for by observed patterns and intensity of selection (Conover & Schultz, 1995). First, spatial and temporal variation among plant populations in composition of herbivores community can produce inter-population variation in average levels of damage not related to different selection pressure across environments (Coley & Aide, 1991; Fritz & Simms, 1992). Second, the phenotypic expression of resistance can be environmentally modified, producing variation among populations as a result of phenotypic plasticity rather than genetic differentiation (Fritz, 1990; Marquis, 1992). Third, if plants can completely compensate for damage imposed by natural enemies, and redundant benefits can be gained through each strategy of defence, no relationship between the level of damage and fitness should be expected (Abrahamson & Weis, 1997; de Jong & van der Meijden, 2000).

In this study the annual herb *Datura stramonium* was used as a model system to evaluate the joint pattern of selection on plant traits involved in the interaction with herbivores (i.e. growth and resistance), and the presence of a negative association between them (van der Meijden *et al.*, 1988). In this species, significant directional and stabilizing selection on resistance has been detected in natural and experimental populations (Núñez-Farfán & Dirzo, 1994; Núñez-Farfán *et al.*, 1996; Shonle & Bergelson, 2000; Valverde *et al.*, 2001). Also, significant genetic differentiation and variation among populations in the relative value of a physical component of resistance (i.e., trichome density) was detected (Valverde *et al.*, 2001).

Parallel greenhouse experiments indicated that this species compensate levels of foliar damage of approximately 10% of total leaf area, indicating a benefit of compensation under relatively low levels of damage (Fornoni & Nuñez-Farfán, 2000). These results suggest that under certain levels of damage both alternatives of defence (resistance and compensation) could offer equivalent fitness benefits. Being an annual, this species can not store resources that can be subsequently directed to restore primary biomass lost to herbivores. Moreover, because the ability to activate secondary meristems for regrowth is absent, compensation for foliar damage can only be accomplished increasing the total leaf area, leaf longevity, and/or the metabolic activity of remaining photosynthetic tissues (Fornoni & Núñez-Farfán, 2000). Since *D. stramonium* is a colonizing annual weed characterized by high rates of growth (Weaver & Warwick 1984; Benner & Bazzaz 1987), we focused on increase in total leaf area as a putative trait related with compensation of herbivory damage (van der Meijden *et al.*, 1988; Rosenthal & Kotanen 1994; Strauss & Agrawal 1999; Stowe *et al.* 2000). Provided previous field studies found significant phenotypic differences among populations in levels of resistance to herbivory (Valverde *et al.*, 2001), this study addressed the following questions: 1) Are differences in resistance among populations in the field maintained under the same herbivory regime in a common garden? 2) Do populations differ in growth (increase in total leaf area) in a common garden experiment? 3) Is there a negative correlation between growth and resistance? If so, 4) How does natural selection act upon resistance and growth when plants are grown under the same herbivory load? Finally, 5) to what extent does selection on plant growth change when herbivores are absent?

Materials and methods

Plant-herbivore system

The annual weed *Datura stramonium* (Solanaceae) occurs in a wide variety of plant communities in North America (Avery *et al.*, 1959; Weaver & Warwick, 1984). In Mexico, this herbaceous plant inhabits open, cultivated and disturbed sites where it

attains on average 1 m in height (Núñez-Farfán, 1991). Some species of specialist and generalist herbivorous insects are closely associated with *D. stramonium*. The most important herbivorous insects are *Lema trilineata* and *Epitrix* spp. (Coleoptera: Chrysomelidae) (Valverde *et al.*, 2001). Both specialist insects are present throughout most of the geographic range of *D. stramonium* and they can remove up to 90% of the plant total leaf area (Núñez-Farfán & Dirzo, 1994). Leaf damage produced by *Epitrix* spp. is present in most populations of *D. stramonium* and consists of small holes in the leaf blades. Also, generalist insects feed upon *D. stramonium* in Central Mexico, including *Sphenarium purpurascens* (Orthoptera: Acrididae) and two lepidopterans (*Manduca* spp.: Sphingidae, and one unidentified species of Noctuidae; Núñez-Farfán & Dirzo, 1994). *S. purpurascens* is very abundant in Central Mexico (including the Pedregal de San Angel, our study site) and it may cause severe leaf damage on plants of this species (Núñez-Farfán & Dirzo, 1994). Defence induction in *Datura stramonium* has not been detected (Shonle & Bergelson, 2000), and resistance appears to be constitutive.

Plant material
Seeds of *D. stramonium* for the common garden experiment were collected in three populations in Central Mexico. Populations from Sto. Domingo (19°00'N and 99°03'W) and Zirahuén (19°27'N and 101°44'W) inhabit pine-oak forests and have an average annual precipitation (temperature) of 1463.2 (19.9°C) and 1400.6 mm (16.4°C), respectively. The Ticumán population (18°45'N and 99°07'W) inhabits a tropical dry forest with an average annual precipitation and temperature of 802.1 mm and 23.1°C, respectively. The populations of Sto. Domingo and Zirahuén are geographically isolated ($\approx$ 400 km apart), while Sto. Domingo and Ticumán are $\approx$ 30 km apart, but differ in altitude by about 900 m. In a previous study, the natural levels of leaf damage were higher in Sto. Domingo and Zirahuén than in Ticumán (Valverde, 2001; Valverde *et al.*, 2001). In contrast, average trichome density was higher for Ticumán than in the two other populations (Valverde *et al.*, 2001).

Study site

The study was carried out in the Pedregal de San Angel Ecological Reserve
(19°20'02"N and 99°08'26"W), located within the campus of the National
Autonomous University of Mexico (UNAM) in southern Mexico City. The climate at
this site is seasonal with an annual mean precipitation of 800 mm, and annual mean
temperature of 15.5° C. The rainy season occurs in the summer (May-September). *D.
stramonium* grows naturally in the study site and both generalist and specialist
herbivorous insects associated to this species are present. The vegetation is a
xerophytic shrubland on rocky volcanic hills.

Experiment

In October 1996, 20 randomly selected individuals were chosen from each
population. From each individual, 50 seeds of a single fruit were selected. *D.
stramonium* is a hermaphroditic species with a high level of self-compatibility
(91.8%: Núñez-Farfán *et al.*, 1996) and low rates of outcrossing (1.3 to 18.7%: Motten
& Antonovics, 1992). Thus, seeds within a fruit are most likely related as full-sibs
(hereafter families).

On July 20, 1997, all seeds were sown in plastic pots (4 litres) filled with a
commercial homogenised soil, in a greenhouse. On 18 August, a total of 684
seedlings were planted into 10 litres plastic pots within the experimental plot
(common garden: 59 × 13 m) in the Pedregal de San Angel Ecological Reserve. This
procedure allowed us to reduce any possible micro-environmental variation within
the experimental plot. Because some families did not germinate, seedlings from 39
maternal families (Sto. Domingo, $n = 14$; Ticumán, $n = 15$; Zirahuén, $n = 10$) were
planted into the common garden.An average of 17.5 individual plants per family
was planted (range 11-20). Seedlings were transplanted following a completely
randomised design. This was aimed at reducing possible spatial variation in insect
abundance within the experimental plot. Plants were spaced 1 m apart in a regular
grid. Periodically, natural weeds were removed to eliminate interference with
other plants. Half of the plants of each family was allocated randomly to the

insecticide treatment (herbivores absent) and the other half to the control treatment (herbivores present). In the insecticide treatment plants were treated with two applications of a systemic carbofuran insecticide (FURADAN®, FMC) at one-month intervals at a concentration of $1.25 \text{ ml} \times \text{l}^{-1}$ (insecticide treatment). The first application of insecticide occurred five days after transplanting. In the control treatment, plants were supplied with water at the same volume (500 ml) as the insecticide treatment plants. In a pilot experiment in the greenhouse, no significant differences were found between plants treated with insecticide and untreated plants of *D. stramonium* for growth ($F_{1,46} = 0.409$, $P = 0.5255$), number of fruits ($F_{1,46} = 0.007$, $P = 0.9322$) and total number of seeds ($F_{1,46} = 0.061$, $P = 0.8056$).

For each plant, we estimated the total leaf area 10 (t_0) and 50 (t_1) days after transplanting to obtain a measure of growth which was calculated as $[(\ln \text{leaf area}_1 - \ln \text{leaf area}_0)/(t_1 - t_0)]$ (Pearcy *et al.*, 1989). For this estimation, leaf area measures were taken from the initial vegetative stage characterised by rapid growth, to the reproductive stage of fruit initiation. Leaf area for each leaf was estimated measuring leaf length and using a regression model (leaf area $= 0.329 \cdot$ (leaf length)^2, $r^2 = 0.987$, $n = 120$, $P < 0.001$; Núñez-Farfán & Dirzo, 1994). Leaf length was measured using a digital calliper to the nearest 0.01 mm.

On November 10, 1997 (three months after the start of the experiment) we harvested all leaves of each plant to measure leaf damage. For each plant, relative leaf damage for all leaves was measured directly with a Color Windows Image Analysis System (WinDIAS-Basic, Delta-T Devices Ltd., Cambridge, England). For a given plant, relative resistance was defined as (1 – relative damage) (Núñez-Farfán & Dirzo, 1994; Núñez-Farfán *et al.*, 1996; Valverde *et al.*, 2001). This estimate of resistance represents the reciprocal of the amount of damage experienced by plants during a fixed period of time (90 days). Considering that larger plants could experience more damage because they represent a high quality patch for natural herbivores, our estimate of resistance may be biased since larger plants would appeared as being relatively less resistant than smaller plants. Both the phenotypic and family level correlations between plant size and damage were not significant ($r = -0.064$, $r = 0.0343$, respectively). Thus our estimation of resistance was not biased

by size differences among plants and families. All fruits were collected for each plant, and we counted the total number of seeds per plant as an estimate of individual plant fitness.

Data analysis

We first evaluated the presence of population differentiation and genetic variation within populations for resistance by means of nested ANOVAs. The analysis for resistance was only performed in the control treatment (herbivores present). Population was entered as a fixed factor and family nested within populations was considered a random factor. Resistance was arc sine transformed to ensure normality. Second, we evaluated population differentiation, genetic variation and treatment effect on growth by means of a nested ANCOVA, including both herbivory treatments. For this analysis population and the presence/absence of herbivores were considered as fixed factors, and family nested within populations was considered a random factor. Type III sums of squares were used. Third, an ANOVA for each population was performed in order to detect genetic variation on resistance (control treatment), and growth (control and insecticide treatment). In order to search for the existence of a negative relationship between growth and resistance for each population, phenotypic and broad sense genetic correlations were performed only in the control treatment (herbivores present) using the Pearson product-moment correlation coefficient. The presence of allocational costs of resistance was evaluated through broad sense genetic correlation between family mean values of fitness (insecticide treatment) against resistance (control treatment) (Simms & Rausher, 1987; Simms, 1992). This procedure was performed for each population.

Selection analyses. Estimation of selection gradients in each treatment for each population was performed to determine the magnitude and direction of natural selection on growth. Because resistance can only be estimated in the presence of herbivores, selection analyses for each population were performed using the control treatment plants. For these analyses resistance and growth were

standardized to a mean of 0 and a standard deviation of 1. Relative fitness (w_i) of a given plant was estimated as $w_i = W_i / \overline{W}$, where W_i is the total number of seeds per plant i, and $\overline{W}$ is the average for this character for all plants used in our experiment. Selection gradients were estimated using multiple regression analysis (Lande & Arnold, 1983). In order to estimate directional (β_i) selection gradients, partial multiple linear regressions were conducted on relative fitness (w_i) as a function of standardised growth and resistance (Lande & Arnold, 1983; Mitchell-Olds & Shaw, 1987). In the absence of herbivores only selection on growth was estimated. Selection gradients and their standard errors were estimated using FREE-STAT (version 1.10, Mitchell-Olds, 1989). Jackknife estimates of the standard errors of the significant selection gradients were also obtained. The Jackknife procedure permits to approximate t-tests of significance, which are robust to deviations from normality and to heterogeneity of residual variances (Mitchell-Olds, 1989). Because the two independent variables (growth and resistance) were standardized, selection gradient estimates are comparable in units of standard deviations of untransformed variables (Lande & Arnold, 1983; Nagy, 1997). All selection analyses were performed for both individual phenotypes (i.e., phenotypic selection; Lande & Arnold, 1983) and family means (i.e., genetic selection; Rausher, 1992). Selection on family means was performed to avoid biases in the estimation of selection gradients caused by environmentally induced correlation between fitness and the examined traits. In the absence of environmental correlation between fitness and traits similarity between phenotypic and genetic analyses of selection is expected (Rausher, 1992).

Comparison of selection patterns within treatments. To test whether selection on growth and resistance differ among populations in the presence of herbivores, we performed an ANCOVA for relative fitness. In this analysis growth and resistance were entered as covariates and population was considered a fixed factor. A significant growth × population and/or resistance × population interaction would indicate that the patterns of selection on growth and/or resistance differ among populations. Similarly, in the absence of herbivores, ANCOVA was performed to

compare selection gradients among populations for growth. For each treatment separately, subsequent ANCOVAs were performed to compare pairs of populations for which significant selection gradients were detected. All analyses were performed at the phenotypic and genetic level.

Comparison of selection patterns on growth across treatments. In order to test if the presence/absence of herbivores altered the relationship between fitness and growth, an ANCOVA was performed in which fitness was the response variable, growth was considered as a covariate and treatment and population were considered fixed factors. A significant Population × Treatment interaction will indicate that the presence/absence of herbivores differentially altered the fitness response of populations. A significant Growth × Treatment interaction will indicate that the presence/absence of herbivores altered the selection pattern on growth. All the analyses were performed at the individual and family level. Type III sums of squares were used. All analyses were performed using the JMP® statistical package (SAS Institute, 1995).

Results

At the end of the experiment plants attained an average height of 24.64 ± 7.65 cm, and an average total leaf area of 117.94 ± 80.72 cm². Herbivores consumed on average 11.67 % (SE = 5.76) of the total leaf area. Leaf damage ranged from 1.06 to 47.80 % of total leaf area. *Lema trilineata, Epitrix parvula, Sphenarium purpurascens* and lepidopterans were the main herbivores responsible for leaf damage in our experiment. Plant growth was higher when herbivores were present as compared with the insecticide treatment (Table 1 and 2B). In general, the absence of herbivory increased plant fitness ($F_{1,603} = 9.59$, $P < 0.01$). Higher average plant fitness (15% more) was attained in the absence of herbivores than in their presence (Table 1). Both in the presence and absence of herbivores no differences in growth were detected among populations (Table 1 and 2B). In the presence of herbivores, significant differences among populations for resistance were detected (Table 1 and

2A). The Ticumán population had the highest relative resistance than the Zirahuén and Sto. Domingo populations (Table 1).

Genetic variation on growth and resistance

Significant genetic variation among families for growth and resistance to herbivory was present in our experiment (Table 2). Specifically, in the control treatment (herbivores present), analysis of variance for each population revealed the presence of significant genetic variation for growth ($F_{14,115} = 1.80$, $P < 0.05$) and resistance ($F_{14,115} = 1.89$, $P < 0.05$) for the Population of Ticumán. Similarly, significant genetic variation on growth was detected for the population of Sto. Domingo ($F_{13,106} = 4.18$, $P < 0.0001$), although no genetic variation was detected for resistance ($F_{13,106} = 1.27$, n.s.). For the population of Zirahuén we did not detect significant genetic variation on growth ($F_{9,72} = 0.83$, n.s.) nor resistance ($F_{9,71} = 1.08$, n.s.). In the insecticide treatment (herbivores absent), significant genetic variation on growth was detected for the population of Sto. Domingo ($F_{13,107} = 2.08$, $P < 0.05$), while no variation was detected for the two other populations (Ticumán: $F_{14,122} = 1.50$, n.s.; Zirahuén: $F_{9,82} = 1.31$, n.s.).

Correlation between traits and treatments

We found no significant phenotypic correlation between resistance and growth for any of the populations ($0.001 < r < 0.026$, $P > 0.07$). Similarly, at the family level, the broad-sense genetic correlation between growth and resistance were not significant ($0.014 < r < 0.160$, $P > 0.1$). Family mean correlations between fitness in the absence of herbivores and resistance were performed to explore the existence of allocational costs of resistance. Again, we did not detect any significant correlation that would indicate the existence of costs of resistance ($-0.29 < r < -0.17$, $P > 0.29$).

Natural selection on resistance and growth

Selection analysis on resistance at both phenotypic (individual plants) and genetic (family means) levels revealed differences on the selection patterns among populations in the presence of herbivores (control treatment). Significant

directional selection for higher resistance was detected in the phenotypic- and family-level analyses for the population of Ticumán (Table 3 and Fig. 1C). In contrast, no significant selection on resistance to herbivores was detected at any level, phenotypic or genetic, for Sto. Domingo and Zirahuén (Table 3, Fig. 1A and 1B). At phenotypic and genetic (family) levels, selection for higher growth was found for Sto. Domingo and Zirahuén but not for Ticumán (Table 3 and Fig. 2). The presence of significant selection patterns on growth for the population of Zirahuén (Fig. 2B) should be taken with caution provided the statistically absence of genetic variation within this population. In addition, significant phenotypic correlational selection between growth and resistance was detected only for the population of Sto. Domingo ($\gamma = 0.267$, $P < 0.01$).

In the presence of herbivores, at the genetic level an ANCOVA supported the existence of differences in selection gradients for growth and resistance among populations (Growth × Population interaction: $F_{2,27} = 4.03$, $P < 0.05$, and Resistance × Population interaction: $F_{2,27} = 6.55$, $P < 0.01$). Thus, the significant Growth × Population interaction resulted from the absence of significant selection on growth for the population of Ticumán (Table 3). Further ANCOVA revealed no significant differences in selection gradients for growth between Sto. Domingo and Zirahuén ($F_{1,20} = 0.77$, n.s.) (Table 3). Similarly, the significant Resistance × Population interaction was due to the absence of selection on resistance for the populations of Sto. Domingo and Zirahuén (Table 3). No paired comparisons between selection gradients for resistance were performed because significant selection was detected only in the population of Ticumán (Table 3).

In the absence of herbivores, positive phenotypic selection on growth was detected for all three populations (Table 3, Fig. 2). For this trait, an ANCOVA revealed significant differences in selection gradients among populations (growth × population interaction: $F_{2,345} = 5.73$, $P < 0.01$) (Table 3). A comparison of the magnitude of the phenotypic selection gradients between populations indicated that Ticumán had a higher slope and differed significantly from Sto. Domingo ($F_{1,255} = 7.33$, $P < 0.01$) and Zirahuén ($F_{1,226} = 5.76$, $P < 0.05$ (Table 3, Fig. 2). No difference in

the magnitude of the selection gradients was found between Sto. Domingo and Zirahuén ($F_{1,209}$ = 0.01, n.s., Table 3, Fig. 2A, B). At the genetic level, positive directional selection on family means was detected only for Ticumán and Zirahuén (Table 3). At this level, an ANCOVA detected marginal differences in genotypic selection gradients among populations (growth × population interaction: $F_{2,33}$ = 3.18, P = 0.054) (Table 3). Paired comparison between the two significant selection gradients for growth indicated the absence of differences between the populations of Ticumán and Zirahuén ($F_{1,21}$ = 0.37, n.s.) (Table 3, Fig. 2B, C).

The fitness response of populations differed to the presence/absence of herbivores independently of variation in growth (Table 4). A significant effect of growth on fitness was detected both at the phenotypic and family levels (Table 4), supporting the results of the selection analyses presented above. The presence/absence of natural herbivores altered the effect of growth on fitness as indicated by the significant Growth × Treatment interaction at the phenotypic level (Table 4). At the family level, the interaction Growth × Treatment was marginally significant (Table 4). At both levels of analysis, the effect of growth on fitness changed depending on the population and environment (presence/absence of herbivores) (significant Growth × Population × Treatment interaction) (Table 4).

Discussion

This study demonstrates significant differences among populations of *D. stramonium* in resistance to herbivorous insects but not in growth, and significant genetic variation in both traits was detected. The maintenance of differences among populations in a common garden environment was consistent with previous results found in the field (Valverde *et al.*, 2001). The absence of a negative correlation between growth and resistance indicated that both traits can evolve independently within populations. Natural selection acted differently among populations on each defensive trait in the presence of herbivores. In general, our results support the expectation that selection favour the evolution of the strategy for which genetic variation was detected. Both alternatives of defence offered equivalent fitness

benefits against herbivory damage . The presence/absence of herbivores differentially affected plant fitness, growth and the relationship between them. Finally, our results indicated that multiple defence strategies can evolve in *D. stramonium*.

To the extent that plants have to deal with a limited pool of resources, a negative correlation between growth and resistance has been expected (van der Meijden *et al.*, 1988; Fineblum & Rausher, 1995; Tiffin, 2000). However, little evidence support this assumption (Coley, 1986; Han & Lincoln, 1994; Stowe, 1998) suggesting that the conditions under which a negative correlation occurs may be more restrictive than previously thought (de Jong & van der Meijden 2000). In fact, several explanations have been offered recently to account for the apparent absence of a negative association between growth and resistance.

First, the absence of a compromise between growth and resistance suggest that each of these traits may be limited by different type of resources. The synthesis of the precursor of condensed tannins (phenylpropanoids) competes directly with the synthesis of proteins, hence with growth, because share the same precursor, phenylalanine (Haukioja *et al.*, 1998). However, other products of secondary metabolism like terpenoids and hydrolizable tannins do not directly compete with growth (Haukioja *et al.*, 1998). Thus the opportunities for a trade-off between growth and resistance may hold for certain types of secondary compounds only.

Second, growth compensation and resistance may not necessarily be alternatives (de Jong & van der Meijden, 2000) when specialist herbivores overcome plant chemical defences. While growth compensation reduces the negative effect of specialist herbivores, resistance avoids the attack of generalist herbivores (de Jong & van der Meijden, 2000; Marak *et al.*, 2000). Although in our experiment plants were eaten by both specialists (i.e., *Lemma trilineata* and *Epitrix parvula*) and generalist herbivores (i.e., *Sphenaruim purpurascens*), no data are available to suggest the effect of plant resistance upon specialists herbivores. However, for some plant-herbivore systems, specialist consumers have circumvented plant resistance mechanisms (DaCosta & Jones, 1971; Metcalf, 1986; de Jong & van der

Meijden, 2000; Marak *et al.*, 2000). Thus further information is needed before generalizing this hypothesis.

Third, if plants differ in their ability to obtain soil nutrients and/or water, individuals that capture more resource will be able to allocate higher levels of resource to resistance and growth than those that capture smaller amounts of resources (Mole, 1994). Although in our experiment all plants were exposed to the same abiotic conditions, differences among individuals and families in rate of resource capture could account for the absence of a negative correlation between growth and resistance.

Finally, the degree of resource limitation or levels of herbivore damage may constrain the presence of a trade-off (Roff, 1992; Mole, 1994). For instance, if plants can obtain enough resources for the demands of growth and resistance, no trade-off is expected. In our experiment, levels of damage were comparatively lower than in the field and experimental records (Núñez-Farfán & Dirzo, 1994; Fornoni & Núñez-Farfán, 2000; Valverde *et al.*, 2001), suggesting that plants possibly experienced less stressful conditions than in their native habitats. It is expected that under strong resource limitation or herbivory damage the opportunities for the existence of a negative correlation between growth and resistance would be higher.

Growth and resistance as alternative strategies to cope with herbivory

Empirical and theoretical evidence indicates that increments in plant growth can be associated with a compensatory response to herbivory (Belsky *et al.*, 1993; Rosenthal & Kotanen, 1994; Strauss & Agrawal, 1999). Some studies have shown that fast growing plants are better able to compensate leaf damage than slower growing plants (van der Meijden *et al.*, 1988; Jing & Coley, 1990). Hence, growth and resistance have been considered alternative strategies of plants against herbivory (van der Meijden *et al.*, 1988; Herms & Mattson, 1992), although little empirical evidence has confirmed such contention in natural systems (van der Meijden *et al.*, 1988; Fineblum & Rausher, 1995; Mauricio *et al.*, 1997; Pilson, 2000). In our experiment, plant populations responded to the same herbivory pressure through two different alternatives of defence. When herbivores were present, growth was

positively selected in plants from Sto. Domingo and Zirahuén, whereas resistance was selected in plants from Ticumán. Provided average maternal fitness did not differ among populations and that the magnitude and direction of significant selection gradients for resistance and growth (herbivores present) were similar, our results support the hypothesis that plants can respond to herbivory either avoiding damage (i.e., being resistant) or compensating (i.e., being tolerant). Thus both alternatives of defence offered equivalent fitness benefits against herbivory (van der Meijden *et al.*, 1988; Simms & Triplett, 1994; Fineblum & Rausher, 1995). However, equality of fitness benefits offered by each alternative of defence may change as average levels of damage and/or resource limitation differentially affect unit costs of allocation to each strategy (Tiffin, 2000).

In natural systems, the plant's ability to compensate leaf area lost due to herbivory has direct advantages (Herms & Mattson, 1992; Strauss & Agrawal, 1999; Tiffin, 2000), although the adaptive role of compensation as a selective response to herbivory has been debated extensively (Aarssen & Irwin, 1991; Belsky *et al.*, 1993; Aarssen, 1995; de Jong & van der Meijden, 2000). If fitness compensation for leaf damage were selectively imposed by factors other than herbivory, only the comparison of selection patterns between plants subjected to herbivores and plants without herbivores can reveal whether traits related to compensation are part of an adaptive response against herbivory or not (Rausher, 1996). This rationale assume that compensation of herbivory damage impose allocational costs in the absence of herbivores. Although we detected a significant Growth × Treatment interaction, patterns of selection on growth for the population where growth was selected in the presence of herbivores (i.e., Sto. Domingo and Zirahuen) was not altered in the absence of herbivores. Thus our results do not support the hypothesis that herbivores are the principal selective agent directing the evolution of compensation by growth in *D. stramonium* (but see Mauricio, 2000). A possible explanation for undetected differences in selection on growth between treatments may be that costs related to compensatory growth may be harder to detect under low levels of damage. Although some studies indicated that costs of resistance and compensation increase under high stressful conditions (Herms & Mattson, 1992; Bergelson, 1994;

Hochwender *et al.*, 2000), still further evidence is needed to determine the relationship between resource limitation and the magnitude of costs.

Summarizing, our results add a new piece of evidence supporting: (1) the absence of a negative association between growth and resistance against herbivory, and (2) the hypothesis that both strategies of defence can represent alternatives against herbivory damage. Thus, both strategies of defence can evolve simultaneously within populations. Yet, the conditions that favours the evolution of multiple defensive strategies within plant populations remains to be investigated.

Acknowledgements

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Table 1. Means for growth (absolute increment in leaf area [cm^2] during 40 days), resistance (only measured in the presence of herbivory) and fitness (total number of seeds per plant) in the presence and absence of herbivores for three populations of *Datura stramonium*. Standard errors are given in parenthesis.

Populations	Herbivores present			Herbivores absent	
	Growth	Resistance	Fitness	Growth	Fitness
Sto. Domingo	104.24 (44.92)	0.863 (0.005)	540.58 (39.13)	96.04 (63.78)	562.01 (45.49)
Zirahuén	130.39 (72.24)	0.877 (0.006)	538.4 (45.91)	111.23 (80.26)	526.50 (55.38)
Ticumán	97.55 (42.33)	0.895 (0.005)	544.46 (33.46)	90.35 (54.29)	775.12 (103.17)
Average	105.26 (28.65)	0.879 (0.003)	541.61 (22.24)	97.83 (37.50)	637.77 (46.24)

Table 2. Nested analyses of variance of resistance to herbivores (A) and growth (B) in plants representing 39 families from three populations of *Datura stramonium*.

Character	Source of variation	df	SS	F	P
A. Resistance	Population	2	0.1329	6.96	0.0027
	Fam (Pop)	36	0.3458	1.48	0.0417
	Error	296	1.9136		
B. Growth	Population	2	0.0024	1.30	0.2834
	Fam (Pop)	36	0.0333	2.17	0.0110
	Treatment	1	0.0026	6.21	0.0173
	Pop × Treatment	2	0.0002	0.51	0.6028
	Fam (Pop) × Treatment	36	0.0152	1.25	0.1479
	Error	608	0.2053		

Table 3. Directional (β_i) selection gradients on resistance to herbivores and growth for three populations of *Datura stramonium* in the presence and absence of herbivores. Standard errors are given in parentheses. After jackknife analyses, selection estimates remained significant. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n.s $P > 0.05$.
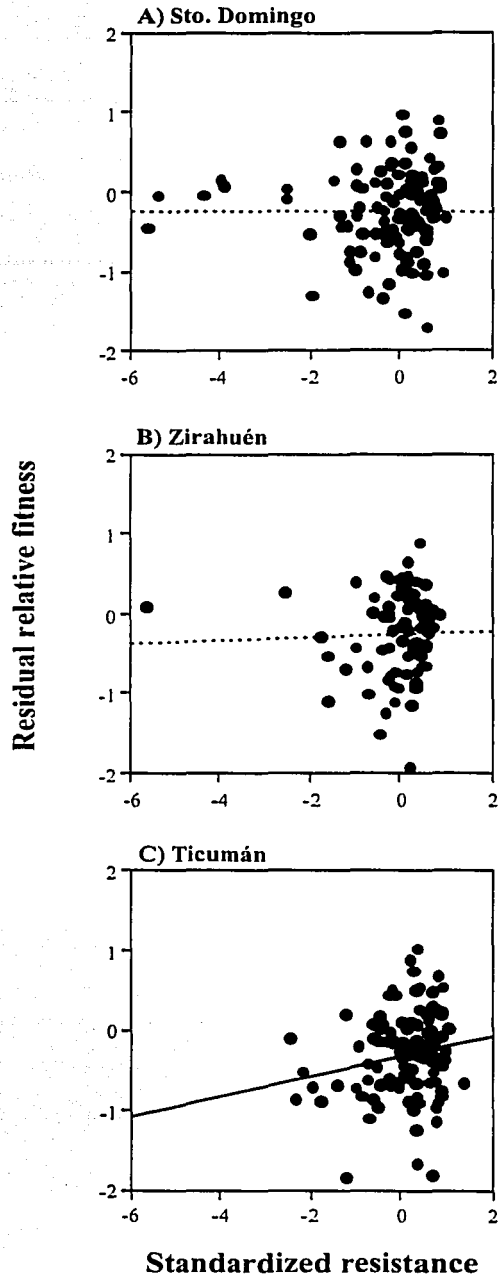
Populations	Characters	Herbivores present		Herbivores absent	
		β phenotypic	β family means	β phenotypic	β family means
Sto. Domingo	Growth	0.265*** (0.057)	0.166*** (0.045)	0.355*** (0.069)	0.014 n.s (0.173)
	Resistance	0.068 n.s (0.051)	-0.173 n.s (0.103)	-------	-------
Zirahuén	Growth	0.310** (0.075)	0.341* (0.196)	0.317** (0.088)	1.666** (0.469)
	Resistance	0.048 n.s (0.071)	0.255 n.s (0.179)	-------	-------
Ticumán	Growth	0.108 n.s (0.061)	-0.141 n.s (0.099)	0.668** (0.175)	0.983* (0.390)
	Resistance	0.219** (0.069)	0.290* (0.114)	-------	-------

Table 4. Analysis of covariance of the effects of growth, herbivory treatment and difference among three populations on relative fitness in plants of *Datura stramonium* grown in a common garden. Analysis based on individual values (phenotypic analysis) and family means values (family means analysis).
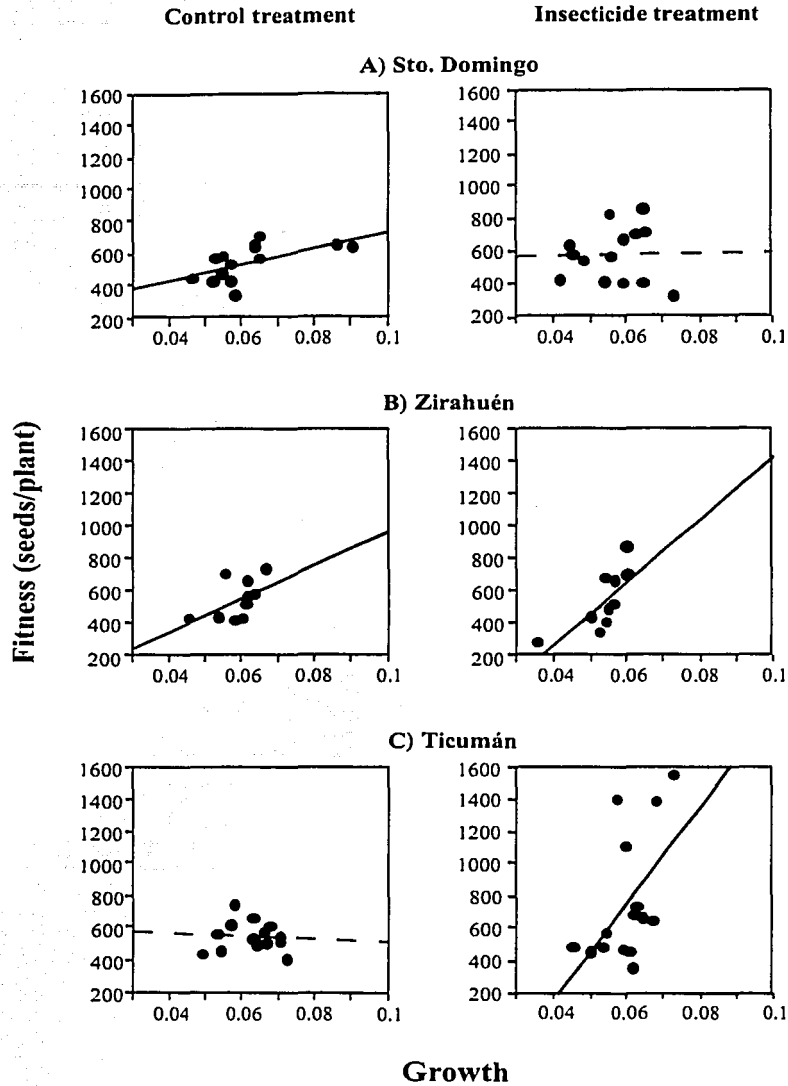
Source of variation	Phenotypic analysis				Family means analysis			
	df	SS	F	P	df	SS	F	P
Population	2	2.34	1.02	0.3594	2	0.411	1.98	0.1452
Treatment	1	5.47	4.79	0.0290	1	0.277	2.67	0.1066
Pop. × Treat.	2	11.28	4.93	0.0074	2	0.722	3.49	0.0362
Growth	1	84.82	74.22	0.0001	1	1.263	12.19	0.0009
Growth × Pop.	2	4.71	2.06	0.1281	2	0.511	2.46	0.0925
Growth × Treat.	1	10.09	8.83	0.0031	1	0.381	3.67	0.0594
Growth × Pop. × Treat.	2	16.79	7.35	0.0007	2	0.881	4.25	0.0182
Error	670	765.61			66	6.835		

Figure 1. Relationship between residual relative fitness (after removing growth effect) and standardized resistance to herbivorous insects for three populations of *Datura stramonium* in the presence of herbivores at the phenotypic level (see Table 3). Significant (—) and non significant (---) selection gradients are indicated.

Figure 2. Linear relationship between mean family value of fitness (total number of seeds per plant) and growth for three populations of *Datura stramonium* in the presence and absence of herbivores (see Table 3). Significant (—) and non significant (---) selection gradients are indicated.

A) Sto. Domingo

B) Zirahuén

C) Ticumán

Control treatment

Insecticide treatment

Fitness (seeds/plant)

Growth

AGRADECIMIENTOS

Quiero agradecer muy especialmente a Juan Núñez-Farfán por la libertad, confianza y amistad que en todo momento estimuló mi desarrollo intelectual y profesional. A los miembros de mi comité tutorial, César Domínguez y Wilhem Hansberg por su paciencia y buena disposición a colaborar y aportar ideas que mejoraron el proyecto y los manuscritos.

La claridad de Mark Rausher en sus ideas sirvieron como un punto de referencia invaluable para desarrollar los aspectos teóricos del proyecto. Las críticas constructivas de Carlos Cordero, Rodolfo Dirzo, Jose Luis Osorno, Victor Parra, Carlos Montaña y Roxana Torres fueron de gran ayuda para mejorar el manuscrito final de la tesis. Las discusiones y revisiones de mis escritos de Anurag Agrawal, Raúl Alcalá, Carl Schlichting, Luis Eguiarte, Nestor Mariano, Mauricio Quesada, Fernando Rosas, Daniel Hare, Cristina Martinez, Raúl Cueva, Mike Gilchrist, Karina Boege, Joel Kniskern, Lalo Morales, Germán Avila, John Stinchcombe, Pedro Luis Valverde, Stephen Weller, Sara Good, Thomas Whitham, y Mark Rausher fueron de gran ayuda para ordenar y transmitir mis ideas con claridad. A todos ellos.....GRACIAS.

Quiero agradecer a todos los amigos del Instituto, en particular de los laboratorios de Genética Ecológica y Evolución e Interacciones Bióticas muchos de los cuales me ayudaron en el trabajo de campo y en el invernadero. A Don Toribio Hernández y Santiago Oliveros por prestarme parte de sus terrenos de cultivo (Ticumán y Sto. Domingo) para realizar los experimentos de campo. A Eduardo Cuevas y su familia por prestarme su casa en Ticumán durante la temporada de campo de 1999. Jesus Vargas y Rafael Torres participaron activamente en el proyecto colaborando tanto en el campo como en el invernadero y en el laboratorio.

Quiero agradecer de la manera más sincera a la UNAM a través del Instituto de Ecología, y a la RLB (95-P5), la Secretaría de Intercambio Académico, la Universidad Nacional de Córdoba (Argentina), CONACyT (25662-N), PAPIT (IN222801), la Secretaría de Relaciones Exteriores del Gobierno de México y la