Induced twining in *Ipomoea purpurea* (L.) Roth.: response threshold and induction by volatiles and snail damage

Inducción de trepado en *Ipomoea purpurea* (L.) Roth.: umbral de la respuesta e inducción por volátiles y por daño por caracoles

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ABSTRACT

Twining in some Convolvulaceae can be induced by leaf damage and jasmonic acid application. This induced response is believed to reduce the likelihood of future leaf damage and it is limited by drought. This response has been detected in the Convolvulaceae family using artificial damage. The mechanisms and ecological implications of this response are still unknown. In this study was tested if the induced twining requires a threshold level and if it is induced by volatiles and/or by snail damage. Three separated greenhouse experiments were conducted in order to test the induced twining in *Ipomoea purpurea* (Convolvulaceae) by applying different levels of artificial damage (0, 1, 5, 10 perforations to the leaves), volatiles (ground leaves) and natural damage by snails. Plants receiving the two higher damage levels twined faster than the individuals exposed to the two lower damage levels. Plants exposed to grounded leaves (volatiles) twined faster than control plants. Finally, twining was induced by snail damage more than in undamaged plants. Most growth traits did not change in any treatment. Twining in *I. pupurea* can be induced by artificial or natural damage, and also by volatiles emitted by damaged leaves of neighbor plants, making the induced twining an ecologically relevant response.

Keywords: Induced twining, *Ipomoea purpurea*, Convolvulaceae, induced responses, herbivory.

RESUMEN

El trepado en algunas Convolvulaceae puede ser inducido por el daño foliar y por la aplicación de ácido jasmónico. Esta respuesta inducida se cree que reduce la probabilidad de daño futuro a las hojas y está limitada por la sequía. Se ha detectado esta respuesta en la familia Convolvulaceae usando daño artificial. Los mecanismos y las implicancias ecológicas de esta respuesta aún se desconocen. En este trabajo, se evaluó si la inducción de trepado requiere un nivel umbral de daño y si se puede inducir por volátiles y/o por daño por caracoles. Se realizaron tres experimentos independientes donde se registró la inducción de trepado en *Ipomoea purpurea* (Convolvulaceae) aplicando diferentes niveles de daño (0, 1, 5, 10 perforaciones a las hojas), volátiles (hojas molidas) y daño natural por caracoles. Las plantas expuestas a los dos más altos niveles de daño artificial se enroscaron más rápido que las expuestas a los dos niveles menores de daño. Las plantas expuestas a hojas molidas (volátiles) se enroscaron más rápido que las plantas control. Finalmente, el trepado se indujo con el daño por caracoles, siendo más rápido que en las plantas no dañadas. La mayoría de los rasgos de crecimiento no se alteraron en ningún tratamiento. El trepado en *I. purpurea* puede ser inducido por daño artificial o natural, como también por volátiles emitididos por hojas dañadas de plantas vecinas, convirtiendo la inducción de trepado en una respuesta inducida ecológicamente relevante.

Palabras clave: Inducción de trepado, *Ipomoea purpurea*, Convolvulaceae, respuestas inducidas, herbivoría.

INTRODUCTION

Herbivory is one of the most important interactions in nature, where plants can be negatively affected in their physiology, growth and survival (Gurevitch *et al.* 2006). Plants respond to herbivory in several ways that may reduce further damage (Karban & Baldwin 1997). Attacked plants often exhibit rapid responses in terms of chemical and physical defenses, which may deter feeding (Agrawal 2000). Nevertheless, other defensive strategies used by plants include escaping or avoiding herbivores in time and/or space (Karban & Baldwin 1997, Gianoli & Molina-Montenegro 2005, Atala & Gianoli 2008).

It has been showed that plants from Convolvulaceae family can induce twining after mechanical damage to leaves (see Gianoli & Molina-Montenegro 2005, Atala & Gianoli 2008). Nevertheless, in *Ipomoea purpurea*, a Convolvulaceae, twining is also induced by jasmonic acid application on leaves surface (Atala & Gianoli 2008). Previous studies have compared the twining rate of undamaged vs. damaged plants; it is less known, however, about the damage threshold that needs to be reached for the induced twining to be triggered. Damage threshold has been described for other induced responses to herbivory (Gianoli & Niemeyer 1997, Underwood 2000), but it is usually associated to the cost of such responses rather than the necessary thresholds (Agrawal 2005).

Jasmonate (MeJA) is a methyl-ester of Jasmonic Acid (JA) and it is a common volatile emitted after leaf damage (Staswick 1992, Albrecht *et al.* 1993, Reinbothe *et al.* 1994). JA, a non-volatile form, can induce the accumulation of many defense-related molecules such as protease inhibitor, ethylene, and alkaloids (Karban & Baldwin 1997). Volatiles emitted by a plant after leaf damage such as MeJA can be perceived by a neighbor plant, where they can trigger induced responses without actual damage (Baldwin *et al.* 2006). Artificial damage to the leaves and chemical induction under controlled conditions are useful tools for assessing induced responses. However, the application of natural damage (e.g., exposition to herbivores) could be more relevant to understanding the twining in an ecological context (Agrawal *et al.* 1999, Lehtilä & Boalt 2004).

Ipomoea purpurea (L.) Roth. is a Convolvulaceous climbing plant present in Chile (Matthei 1995), whose twining has been shown to be induced by mechanical (leaf damage) and/or chemical (jasmonic acid application) stimulus without an evident cost -at least- in growth (Atala & Gianoli 2008). On the other hand, previous studies have shown that induced responses can result in a fitness cost (Agrawal *et al.* 1999, Baldwin & Hamilton 2000), but there is also evidence of costless responses (Karban 1993, Agrawal & Karban 1999). Jasmonate application is known to induce tendril coiling in *Bryonia dioica*, a non-Convolvulaceous species (Falkenstein *et al.* 1991, Weiler *et al.* 1993, Blechert *et al.* 1999). It is not known, however, if volatiles emitted by wounded leaves of other plants can induce twining. Previous studies tested only direct application of an aqueous solution of jasmonic acid to the leaf surface (Atala & Gianoli 2008). Thus, *I. purpurea* is a suitable target species to test the effects of natural herbivory, volatiles and damage threshold on twining, and asses the ecological relevance of the induced twining.

In the present study it was hypothesized that for *I. purpurea*: 1) There should be no damage threshold for the induced twining, as no cost are reported for this response, 2) Volatile presence should induce twining, and, 3) Natural damage by snails should also induce twining, similarly to the effect of artificial damage. These hypotheses were tested by conducting three separated experiment were twining rate of *I. purpurea* individuals subjected to different artificial damage levels, volatiles and natural damage.

MATERIAL AND METHODS

Approximately 200 seeds of *Ipomoea purpurea* were put in warm water for 24h to induce germination. Afterwards, they were put in Petri dishes on top of moistened paper towels until germination. After appearances of radicule plants were transplanted to plastic trays filled with commercial soil. Each tray contained 96 sockets of 200 ml. The trays were put in a greenhouse located at Universidad de Concepción, Campus Los Ángeles, Chile (37°28′ S, 72°21′ W). Two weeks-old seedlings, with 2 fully expanded leaves, were put in plastic bags filled with 1.5 l of the same soil of the trays. Plants were randomly separated in three groups for three different experiments. 80 plants were assigned to the damage threshold experiment, 36 for the volatiles experiment, and 24 for the natural damage experiment (see below). Plants were watered daily to field capacity. The experiments were conducted in the summer, from December 2011 to February 2012. For all three experiments, when plants had 5-6 true leaves (8-9 weeks-old) an artificial support was put to the left of the shoot, barely in contact with the plant and registered the twining time as the time for the completion of one full turn around the support (360°-turn). Before the beginning of the experiments we measured apex height, plant size, and basal stem diameter were measured. All plants twined after 24 h, moment at which apex height, stem diameter, plant size, and internode length were measured. Apex height increase and diameter increase were calculated from initial and final values. Growth rate was calculated as: (final size-initial size) / time. There were no statistical differences in the initial values of measured traits between plants of different treatments of the same experiments (ANOVA, $p > 0.05$, data not shown). Morphological traits were measured using a digital caliper (0,001 resolution; Mitutoyo Corp., Japan.)

DAMAGE THRESHOLD

When plants were ready to begin the experiment (see above), we separated 80 individuals in 4 groups of 20 plant each: Control (0 perforations) = no damage, 1 = one perforation in one leaf, 5 = five perforations in five leaves (one per leaf), 10 = two perforations in five leaves (two per leaf). The perforations were 6 mm-diameter circles made with a paper punch. In the field, snail damage to the leaves of *I. purpurea* occurs and is commonly circular in shape (personal observation). The statistical differences in twining percentage at different times between treatments were analyzed using a Wilcoxon survival test and a proportion test (Atala & Gianoli 2008, 2011) included in STATISTICA 7 software (StatSoft Inc., USA). The effect of different damage levels on growth traits was analyzed using a one-way ANOVA for all traits except for Apex height increase and Diameter increase were a Kruscal-Wallis test was used. Internode length was transformed with Ln ((x) +1) and Growth rate was Ln-transformed in order to normalize the data. Damage levels were based on field observation of natural damage and previously tested damage levels for the induced twining.

VOLATILES

To determine the effect of volatiles on the induced twining we covered plants with 2000 ml plastic bottles. Bottles were transparent and had 4 small holes to avoid over-heating. We randomly assigned 36 plants to two treatments (18 plants per treatment); volatiles treatment consisted in plants inside the bottles with the addition of 2 g of grounded leaves (from other plants) inside (at ground-level). Control plants were put inside the bottles in the same conditions, but without grounded leaves. The statistical differences in twining percentage at different times between control and volatiles-exposed plants were analyzed using a Wilcoxon survival test and a proportion test (see above). The differences in growth traits between treatments were analyzed using a t-test for all traits except for Apex height increase and Diameter increase were a Mann-Whitney test was used. Internode length was transformed with Ln ((x) +1) and Growth rate was Ln-transformed in order to normalize the data.

NATURAL DAMAGE

In order to know the effect of natural damage on induced twining 24 plants were used and were randomly assigned to two treatments: control and natural damage (12 plants per treatment). All plants were covered with a white mesh cone 40 cm tall and 25 cm wide at the base. Plants in the natural damage treatment were exposed to herbivory by two adult snails (*Helix aspersa* Müller). All snails were of similar size, were visually healthy and were left for 48 h without food previous to the initiation of the experiment. Control plants were not exposed to any damage. Previous to this experiment, it was checked snails actually ate the plant in the same experimental setup. The statistical differences in twining percentage at different times between control and snail-damaged plants were analyzed using a Wilcoxon survival test and a proportion test (see above). The differences in growth traits between treatments were analyzed using a t-test for all traits. Internode length was transformed with Ln and Growth rate and Diameter increase were root-transformed to normalize the data.

RESULTS

DAMAGE THRESHOLD

Artificial damage induced twining in *Ipomoea purpurea* (Fig. 1a), particularly the two higher damage levels. There were differences in twining time between treatments (Wilcoxon test, p=0.0052). The proportion of twining plants did not differ between plants damaged with one hole and control (undamaged) plants (Fig. 1a, proportion test p>0.05). Damaging with 5 perforations rapidly induced twining and 8 h after the damage was applied there were no differences between 5 and 10 perforations in twining percentage. At 8 h, however, there were significant differences between the two lower (0 and 1) and the two higher (5 and 10) damage levels (proportions test, p<0.05). After 24 h all plants were twining around the support. There were no statistical differences in growth traits between damage treatments (Table I, ANOVA/ Kruscal-Wallis test, p>0.05).

VOLATILES

Volatiles present in grounded leaves of *Ipomoea purpurea* induced twining in undamaged plants (Fig. 1b). Overall, there were no differences in twining time between groups (Wilcoxon test, p>0.05). However, statistical differences in the percentage of twining plants between control and volatile-exposed plants were found after 10 h of the beginning of the experiment (Fig. 1b, proportion test, p<0.05). After 24 h all plants from both treatments were twining. Control plants had a higher stem diameter increase compared to plant exposed to volatiles (Table II, t-test, p<0.05). There were no differences in other grow-related parameters (Table II, t-test/Mann-Whitney test, p>0.05).

NATURAL DAMAGE

Natural leaf damage by snails induced twining in *Ipomoea purpurea* (Fig. 1c). There were differences in twining time between damaged and undamaged plants (Wilcoxon test, p=0.029). Statistical differences in the % of twining plants between control and damaged plants were also found after 6 h of the beginning of the experiment (Fig. 1c, proportion test p<0.05). At 8 h, the differences disappeared, and all plants were twining after 12 h (Fig 1c). Plants damaged by snails had a larger apex height increase compared to non-damaged plants (Table III, t-test, p<0.05). There were no differences in other grow-related parameters (Table III, t-test, p>0.05).

TABLE I. Average ± SE of growth traits in *Ipomoea purpurea* individuals exposed to artificial damage (0, 1, 5, or 10 perforations to the leaves). The effect of the artificial damage on plant traits was analyzed using a one-way ANOVA except for Apex height increase and Diameter increase were a Kruscal-Wallis test was used. *n* = 20 plants per treatment.

TABLA I. Promedio ± ES de parámetros de crecimiento en individuos de *Ipomoea purpurea* expuestos a daño artificial (0, 1, 5 ó 10 perforaciones a la hoja). El efecto del daño artificial sobre los rasgos de las plantas se analizó usando un ANDEVA de una vía, excepto para incremento en la altura del ápice e incremento en diámetro donde se usó el test de Kruscal-Wallis. *n* = 20 plantas por tratamiento.

TRAT	FINAL ÁPEX HEIGHt (cm)	APEX HEIGHT INCREASE (cm)	FINAL STEM DIAMETER (mm)	DIAMETER INCREASE (mm)	FINAL PLANT size (cm)	INTERNODE LENGHT (cm) *	GROWTH RATE (cm/h)*
0	17.99±0.67	4.19±0.78	3.29±0.09	0.08±0.03	25.36±0.88	3.87±0.49	0.23±0.03
1	18.07±0.71	4.94±0.92	3.31±0.10	0.03±0.01	24.66±0.73	3.39±0.36	0.24±0.03
5	17.23±0.57	3.29±0.76	3.20±0.07	0.06±0.01	25.96±0.57	3.57±0.28	0.28±0.02
10	16.95±0.79	3.68±0.80	3.13±0.08	0.10±0.03	23.65±0.60	3.03±0.30	0.23±0.02
F/ χ^2	0.6444	1.7374	0.9895	5.8655	1.9868	0.6824	1.0399
p	0.5890	0.6287	0.4027	0.1183	0.1236	0.5057	0.3802

*Internode length was transformed with Ln ((x) +1) and Growth rate was Ln-transformed in order to normalize the data./ El largo del entrenudo se transformó con Ln ((x) + 1) y tasa de crecimiento se transformó con Ln para normalizar los datos.

TABLE II. Average ± SE of growth traits in *Ipomoea purpurea* individuals exposed (volatiles) or not exposed (control) to grounded leaves. The effect of volatiles on plant traits was analyzed using a t-test except for Apex height increase and Diameter increase were a Mann-Whitney test was used. Different letters represent statistically significant differences (t-test, p<0.05). *n* = 18 plants per treatment.

TABLA II. Promedio ± ES de parámetros de crecimiento en individuos de *Ipomoea purpurea* expuestos (volátiles) o no expuestos (control) a hojas molidas. El efecto de los volátiles sobre los rasgos de las plantas se analizó con un test de t, excepto para Apex height increase (incremento en la altura del ápice) y Diameter increase (incremento en diámetro) donde se usó un test de Mann-Whitney. Letras distintas representan diferencias estadísticamente significativas (test de t, p<0,05). *n* = 18 plantas por tratamiento.

TRAT	FINAL ÁPEX HEIGHT (cm)	APEX HEIGHT INCREASE (cm)	FINAL STEM DIAMETER (mm)	DIAMETER INCREASE (mm)	FINAL PLANT SIZE (cm)	INTERNODE LENGHT (cm) *	GROWTH RATE (cm/h)*
Control	17.68±0.78	3.49±0.65	3.14±0.07	0.11±0.03[a]	22.17±0.55	3.73±0.42	0.19±0.02
volatiles	17.38±0.45	3.03±0.58	3.08±0.07	0.04±0.02[b]	21.62±0.46	2.87±0.32	0.24±0.03
t / U	0.332	0.536	0.579	5.4410	0.5886	1.565	-1.186
p	0.742	0.5955	0.5667	0.0197	0.4483	0.1269	0.2439

*Internode length was transformed with Ln ((x) +1) and Growth rate was Ln-transformed in order to normalize the data./ El largo del entrenudo se transformó con Ln ((x) + 1) y tasa de crecimiento se transformó con Ln para normalizar los datos.

TABLE III. Average ± SE of growth traits in *Ipomoea purpurea* individuals exposed (damage) or not exposed (control) to snail damage. The effect of the artificial damage on plant traits was analyzed using a t-test. Different letters represent statistically significant differences (t-test, p<0.05). *n* = 12 plants per treatment.

TABLA III. Promedio ± ES de parámetros de crecimiento en individuos de *Ipomoea purpurea* expuestos (daño) o no expuestos (control) al daño por caracoles. El efecto del daño por caracoles sobre los rasgos de las plantas se analizó con un test de t. Letras distintas representan diferencias estadísticamente significativas (test de t, p<0,05). *n* = 12 plantas por tratamiento.

TRAT	FINAL APEX HEIGHT (cm)	APEX HEIGHT INCREASE (cm)	FINAL STEM DIAMETER (mm)	DIAMETER INCREASE (mm) *	FINAL PLANT SIZE (cm)	INTERNODE LENGHT (cm) *	GROWTH RATE (cm/h) *
CONTROL	13.78±0.38[a]	1.20±0.32[a]	2.69±0.08	0.10±0.02	19.40±0.58	1.78±0.25	0.61±0.16
DAMAGE	15.90±0.55[b]	3.36±0.56[b]	2.50±0.08	0.13±0.04	18.13±0.61	1.93±0.28	0.72±0.11
t	4.035	3.357	-1.680	-0.040	-1.510	0.519	0.802
P	0.0006	0.0028	0.1072	0.9683	0.1452	0.6091	0.4312

*Internode length was transformed with Ln. Growth rate and diameter increase were root-transformed./ El largo de entrenudo se transformó con Ln. La tasa de crecimiento y el aumento en diámetro se transformaron con la función raíz cuadrada.

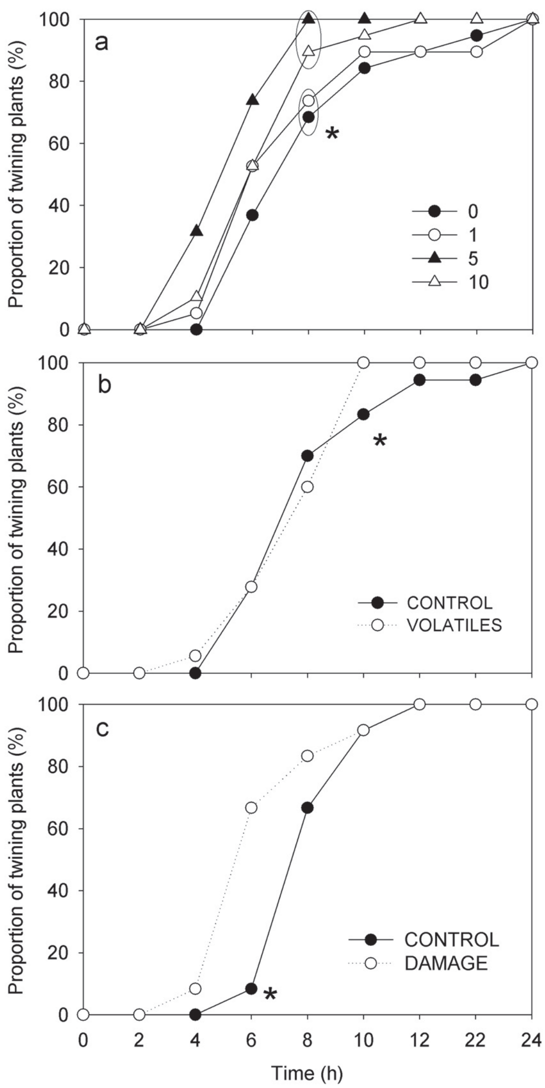

Figure 1. Proportion of *Ipomoea purpurea* individuals twining around an artificial support at different times. a = plants exposed to different artificial damage levels (0, 1, 5 and 10 perforations to the leaves), n = 20 plants per treatment. b = plants exposed (volatiles) and not-exposed (control) to grounded leaves, n = 18 plants per treatment. c = plants exposed (damage) and not-exposed (control) to natural damage by snails, n = 12 plants per treatment. Asterisk indicate statistical differences between the proportion of twinning plants at a given time (proportion test, p<0.05).

Figura 1. Proporción de individuos de *Ipomoea purpurea* enroscándose alrededor de un soporte artificial a diferentes tiempos. a = plantas expuestas a diferentes niveles de daño artificial (0, 1, 5 y 10 perforaciones a las hojas), n = 20 plantas por tratamiento. b = plantas expuestas (volátiles) y no expuestas (control) a hojas molidas, n = 18 plantas por tratamiento. c = plantas expuestas (damage) y no expuestas (control) a daño natural por caracoles, n = 12 plantas por tratamiento. Los asteriscos indican diferencias estadísticamente significativas entre tratamientos en la proporción de plantas enroscadas a un determinado tiempo (test de proporciones, p<0,05).

DISCUSSION

The induced twining has been described previously for Convolvulaceae species including *I. purpurea* (Gianoli & Molina-Montenegro 2005, Atala & Gianoli 2008, Atala & Gianoli 2009a). The ecological significance of this induced response, however, is not completely clear and up to day has been mainly speculated. Here is reported for the first time that the response shows a threshold level and that can be induced by volatiles and natural damage by snails. In natural conditions, *I. purpurea* individuals can be exposed to snail damage (personal observation) or volatile emissions from neighbor plants and the induced twining could help reduce further damage to apical portions of the plant (Gianoli & Molina-Montenegro 2005, Atala & Gianoli 2008). It has been suggested that natural damage is qualitatively and quantitatively different from artificial damage (Agrawal *et al.* 1999, Lehtilä & Boalt 2004). Results show that artificial and natural damage can induce twining in *I. purpurea*, giving the response an ecological relevance that could impact natural populations, reducing further leaf damage and, therefore, increasing fitness in the presence of herbivores.

I. purpurea grows very fast and only a few differences in growth were manifest between damaged and undamaged plants or between plants exposed or not exposed to volatiles. Moreover, in 24 h plants from all treatments were twining around the support. This species was used a model plant for reasons exposed early. For perennial, more slow-growing species like *Convolvulus arvensis*, the induce twining could have greater ecological consequences because the differences in growth and twining percentage could be greater between damaged and undamaged plants, or between plants exposed and not-exposed to volatiles (see Atala & Gianoli 2008). Additionally, *I. purpurea* plants have been shown to exhibit a certain degree of tolerance to damage (Atala & Gianoli 2009b) and further damage may be needed to impact growth.

The climbing habit is considered a key innovation in plant evolution as showed by a greater diversity in angiosperm clades with climbing species compared to closely related clades without them (Gianoli 2004). Climbing plants can access higher canopy levels (i.e. light) with small investment in support tissues (Darwin 1875, Putz & Holbrook 1991). They usually have high specific hydraulic conductance (Ks) due to large xylem vessels and xylem area compared to self standing plants (Isnard & Silk 2009, Jiménez-Castillo & Lusk 2013), resulting in higher growth rates compared to non-climbing plants (see Den Dubbelden & Verburg 1996). It has also been reported that climbing onto certain supports can confer associational resistance to herbivory (González-Teuber & Gianoli 2008). The induced twining could be yet another advantage of climbing plants. The current knowledge about this induced response suggests that it is an ecologically relevant response that helps climbing plants escape further herbivory to leaves, especially by ground dwelling herbivores (Atala & Gianoli 2008). Moreover, this response is induced, at least in *I. purpurea*, by the presence of volatiles that can be emitted by neighbor plants when inflicted by leaf damage. Previous studies have showed that drought can limit this response (Atala & Gianoli 2009a, Atala *et al.* 2011) and that jasmonic acid application can induce it (Atala & Gianoli 2008). We propose a model that summarizes the current information about the induced twining in *I. purpurea* (Fig. 2). The induced twining is induced by leaf damage and by volatiles emitted by wounded leaves. The response reduces further damage, especially by ground-dwelling herbivores, increasing plant fitness. Drought, on the other hand, limits this response and also negatively impact plant fitness. Some questions regarding the induced twining still remain: 1. if this is a widespread response or is only present in the Convolvulaceae and 2. the physiological, cellular and/or molecular mechanisms behind the increased twining rate after leaf damage. Preliminary studies have detected induced twining in *Elytropus chilensis*, a climbing plant belonging to the Apocynaceae (E. Gianoli, unpublished data). On the other hand, mechanistic explanations for the induced twining are not available to the date, but rapid water movements or cellular elongation are likely candidates. Further knowledge of the mechanism and ecological and environmental factors involved in the induced twining could have applications in other climbing plants of economical importance such as *Ipomoea batatas* (sweet potato) and *Convolvulus arvensis* (field bindweed), both belonging to the Convolvulaceae.

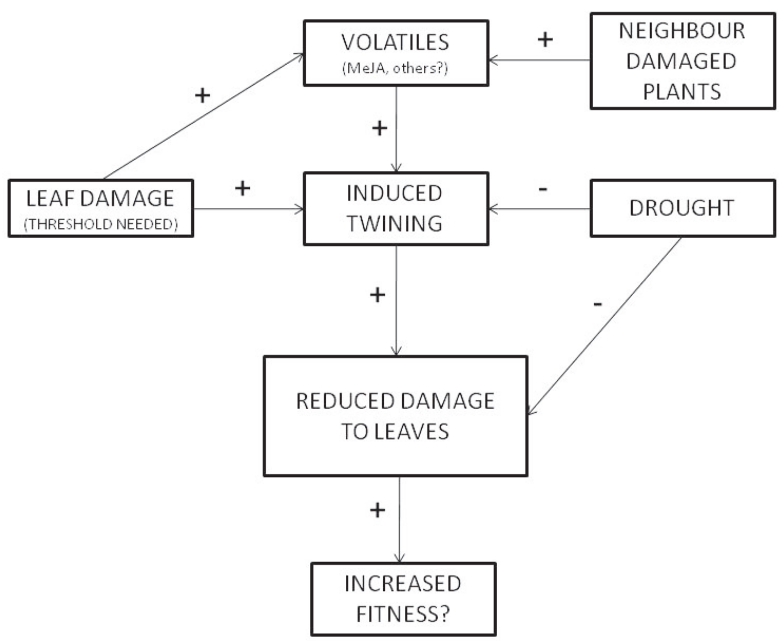

Figure 2. Model summarizing the current evidence and hypothesis about the induced twining in *Ipomoea purpurea*. + = induction/increase, - = reduction/inhibition.

Figura 2. Modelo que resume la evidencia actual e hipótesis sobre la inducción de trepado en *Ipomoea purpurea*. + = inducción/aumento, - = reducción/inhibition.

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