



SENSORIALIDAD VIBRATORIA EN TABACO: RESPUESTAS QUÍMICAS Y EFECTOS SOBRE LA PRODUCCIÓN FOLIAR

Tesis

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A mis padres, por su cariño y apoyo constante e
incondicional

BIOGRAFÍA



Nací en Cochabamba, la capital gastronómica de Bolivia, el año 1991. Desde muy pequeño tuve un gran interés en ser científico, sin siquiera saber en ese entonces las labores que uno desarrollaba. A los 17 años ingresé a la carrera de Biología en la Universidad Mayor de San Simón, donde obtuve mi título el año 2014. Mi tesis de grado fue evaluar la selección natural en rasgos morfológicos en mi insecto favorito *Alchisme grossa*. El año 2011 pude conocer el área de

la Ecología Química y gracias a LANBIO tuve la oportunidad de tener cursos tanto en Bolivia como en Chile; además, pude realizar dos pasantías en el Laboratorio de Química Ecológica de la Universidad de Chile: la primera el año 2012 y la segunda el año 2014. En estas pasantías pude trabajar en interacciones planta-insecto y en interacciones sociales en insectos. Estas experiencias me motivaron a continuar mi formación académica, a través del programa de Magíster en Ciencias Biológicas en la Universidad de Chile. Quiero continuar mi formación con estudios de doctorado y especializarme en temáticas de sociabilidad, biología evolutiva, comportamiento e interacciones para convertirme en un excelente investigador y docente.

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RESUMEN

La capacidad de percibir y responder a los estímulos físicos, tales como las señales vibratoriales (SV), es fundamental para la supervivencia de los seres vivos. El rol de los sentidos y la comunicación animal han sido ampliamente estudiados; sin embargo, en las plantas esta área de investigación se encuentra en una etapa temprana de desarrollo y se necesitan estudios más completos para comprender la ecología de las SV en las plantas, y especialmente para evaluar su importancia adaptativa. Estudiamos si las plantas de tabaco (*Nicotiana tabacum*) pueden responder químicamente a SV producidas por orugas de la polilla de la papa (*Phthorimaea operculella*), si la respuesta varía entre orugas y SV, y si esta respuesta implica costos en términos de producción foliar (número de hojas) para la planta. Las plantas de tabaco fueron expuestas a una oruga o al *playback* (reproducción) de SV de una oruga y luego se cuantificaron las defensas químicas inducidas (alcaloides) mediante cromatografía de gases acoplada a espectrometría de masas (GC-MS). Los niveles de nicotina fueron similares en las plantas de los tratamientos oruga y *playback* y en ambas más altos que en las plantas control. Esto indica que, al menos para el tabaco, el estímulo vibratorio de una oruga alimentándose es suficiente para producir una respuesta química similar a la provocada por toda la oruga. La producción foliar no difirió entre los tratamientos, probablemente porque las plantas no tenían un recurso limitante en las condiciones de crianza. Este estudio incrementa la comprensión actual sobre la comunicación y las capacidades sensoriales de las plantas, enfatizando el valor adaptativo de las SV para estos

organismos y aumenta la comprensión de la comunicación vibracional entre insectos y plantas.

ABSTRACT

The ability to perceive and respond to physical stimuli, such as vibrational signals (VS), is critical for the survival of living beings. The role of senses and communication in animals has been widely studied; however, in plants this research area is at an early stage of development and more comprehensive studies are needed to understand the ecology of VS in plants, and especially to evaluate their adaptive importance. We studied whether tobacco plants (*Nicotiana tabacum*) can chemically respond to VS produced by caterpillars of the potato moth (*Phthorimaea operculella*), whether the response varies between feeding caterpillars and only VS, and whether this response involves costs in terms of leaf production (number of leaves) for the plant. Tobacco plants were exposed to either a caterpillar or to the playback of the VS produced by a caterpillar and then chemical induced defenses (alkaloids) were quantified by gas chromatography – mass spectrometry (GC-MS). Nicotine levels were similar in plants of the caterpillar and playback treatments and in both higher than in control plants. This indicates that at least for tobacco, the vibrational stimulus of a chewing caterpillar is sufficient to produce a chemical response similar to that provoked by the whole caterpillar. Leaf production did not differ between treatments, probably because plants did not have a limiting resource in the rearing conditions. This study adds to the current understanding of the communication and sensory abilities of plants, by emphasizing the adaptive value of VS to these organisms and adds to the understanding of insect-plant vibrational communication.

INTRODUCTION

The ability to perceive and respond to physical stimuli is critical for the survival of living beings (Telewski 2006; Gagliano 2015). Communication through vibrations has been shown in many species (Cocroft 2010; Gagliano *et al.* 2012). In evolutionary terms, this process is advantageous since vibrations contain information not only about the sender, but also about the environment (Gagliano *et al.* 2012). The importance of vibrational signals (VS), those signals of vibrational nature that are substrate-borne (i.e., plant tissues, soil, etc.) rather than air-borne, is reflected in the fact that almost all organisms have evolved a mechanism or organ for the perception of these type of mechanical signals (Cocroft *et al.* 2014). Given the widespread occurrence of VS, it is expected that plants or other sessile organisms can benefit from the perception of these type of signals (Telewski 2006; Gagliano *et al.* 2012) and from the production of responses related to the nature of the stimulus.

Plant behavior has been defined as “a response to an event or environmental change during the lifetime of an individual” (Karban 2008). Under this definition, plants exhibit a great diversity of behaviors and responses to various stimuli (Karban 2008, 2015; Trewavas 2014), the most studied being those that are related to the attack by pathogens and herbivores (Hartmann 2004; Meiners 2015). Herbivory, mostly by insects, can induce the synthesis of secondary metabolites by the plant which act as chemical defenses that restrict herbivore success (Karban 2008; Mithofer & Boland 2012).

The role of senses and communication in animals has been widely studied; however, in plants this research area is at an early stage of development (Gagliano *et al.* 2012; Appel & Cocroft 2014; Gagliano 2015; Karban 2015). Although in recent years there have been great advances in the understanding of chemical communication in plants (Runyon *et al.* 2006; Meinwald & Eisner 2008; van Dam 2014), more comprehensive studies are needed to understand the ecology of VS in plants, and especially to evaluate their adaptive importance (Gagliano *et al.* 2012). Recently, Appel & Cocroft (2014) demonstrated that plants of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) can perceive and respond (in terms of chemical defenses) to VS of caterpillars that feed on them. Furthermore, they found that plants are also able to distinguish the type or origin of the signal perceived, and to respond based on the ecological context where it occurs.

The interactions of *Nicotiana* plants with different organisms have been studied from different approaches, particularly from the perspective of semiochemical compounds involved in the interactions (Dewey & Xie 2013). Thus, a great variety of secondary metabolites have been characterized in *Nicotiana*, among them alkaloids, whose concentration increases in the plant (induced chemical defenses) after being damaged by herbivorous insects (Baldwin 1988; Ohnmeiss *et al.* 1997; Shi *et al.* 2006). In the case of tobacco (*Nicotiana tabacum* L.), plants respond to herbivory by increasing the concentration of mainly four alkaloids: nicotine, nor nicotine, anabasine and anatabine, which act as chemical defenses against herbivores (Steppuhn *et al.* 2004; Dewey & Xie

2013). These compounds are produced in the roots of the plant and are then transported to the leaves through the xylem (Baldwin 1989; Baldwin *et al.* 1990; Karban 2008). However, the production of chemical defenses is costly for plants, particularly in terms of performance (Redman *et al.* 2001; Koricheva 2002) since plants have limited resources to invest in processes such as growth, reproduction or defense, where allocation of resources to one process occurs at the expense of the others (Baldwin *et al.* 1990; Redman *et al.* 2001).

The costs in terms of performance caused by the production of chemical defenses induced by VS produced by herbivorous insects have not been evaluated. They are of critical importance to explore the behavior of plants and their adaptive value, which would ultimately allow to better understand their biology and evolutionary ecology. In this work, we aim to: i) determine if tobacco plants (*N. tabacum*) can chemically respond to VS produced by caterpillars of the potato tuber moth (*Phthorimaea operculella* Zeller), ii) if the response varies between feeding caterpillars and only VS, and iii) if this response involves costs in terms of leaf production (number of leaves) as a proxy for performance for the plant.

MATERIALS AND METHODS

Plants and insects rearing

Burley tobacco seeds were germinated in peat (Kekkilä; Vantaa, Finland) and transplanted as seven-week-old seedlings into pots (14 cm tall × 17 cm diameter) with a mix containing potting soil (Armony; Pudahuel, Chile), sand (Armony; Pudahuel, Chile) and peat in a 4:1:1 proportion. Plants were grown under metal halide lamps (36 W) at 25 ± 2 °C with a 16:8 h L:D photoperiod. Plants were watered twice weekly, one day with water and the other with an aqueous fertilizer solution (Phostrogen Plant Food, Santiago, Chile; <http://www.bayergarden.co.uk/Products/p/Phostrogen-All-Purpose-Plant-Food>).

Phthorimaea operculella is a cosmopolitan pest of Solanaceae crops and weeds (Rondon 2010). Although it is primarily a pest of potato it can also be found in other Solanaceae species such as tobacco, which is considered its secondary host-plant (Varela & Bernays 1988; Rondon 2010; Rivera & Burrack 2012). *P. operculella* caterpillars are leaf-miners with usually light brown body with a characteristic brown head (Rondon 2010). They feed on the leaves leaving the epidermal areas on the upper and lower leaf surfaces intact (Rondon 2010). Potato tuber moths and caterpillars were reared at 25 ± 2 °C on tobacco plants grown in pots as described above. Individuals to start the colony were collected in Curacaví (Metropolitan Region, Chile 33.49° S – 71.02° W; 185 m above sea level) in March 2016 and reared under laboratory

conditions ever since. *P. operculella* caterpillars were fed tobacco leaves *ad libitum* and adults were fed with filter paper strips soaked with 10 % aqueous honey solution every two days (Golizadeh *et al.* 2014).

Vibration recordings and playback experiments

To record vibrations produced by a feeding caterpillar, one fourth-instar *P. operculella* caterpillar was positioned over the leaf of an 11-week old vegetative tobacco plant inside a clip cage (N = 20 caterpillar + plant combinations). Vibrations were recorded at 21.5 ± 0.8 °C with a laser Doppler vibrometer (Polytec CLV-2534). The laser was positioned at less than 1 cm from where the caterpillar was feeding.

To reproduce the caterpillar feeding vibrations, we used an actuator (Samsung LRA) coupled with an accelerometer (VibraMetrics 9001A) supported on a leaf by a peg covered with EVA rubber to avoid plant damage. Before playback experiments, the frequency response and amplitude of each setup was characterized, and a digital filter was designed to compensate for the response (Appel & Cocroft 2014; Cocroft *et al.* 2014). Also, the playback stimuli were filtered using MATLAB to yield playbacks that closely matched the temporal and spectral properties of the original recordings (Appel & Cocroft 2014, Cocroft *et al.* 2014).

The playback design was based on the mean feeding behavior of *P. operculella* caterpillars. They showed a feeding event duration of 9.70 ± 1.35 min (mean \pm sd) with a frequency of 2.48 ± 0.26 feeding events per hour (DTB, unpublished data) and a

pause (no feeding) duration of 15.98 ± 1.85 min between feeding events. In the playback experiments, caterpillar vibrations were played back to 11-week old naive tobacco plants, using the setup described above on the fifth leaf of the plant (counted from the plant apex). Playback stimuli consisted of 1 min of the original feeding vibrations recordings repeated 10 times (i.e., 10 min of stimuli) followed by a 16-min silence pause. This basic 26 min pattern was repeated eight times, thus lasting for 208 min. In each replicate ($N = 18$), we used a different recorded chewing vibration.

Plant chemical defenses

Tobacco chemical defenses (i.e., alkaloids) were extracted based on a modification of the protocol by Saitoh *et al.* (1985). The fifth tobacco leaf (counting from the apex) was cut, immediately freeze-dried with liquid nitrogen and pulverized in a porcelain mortar. The fifth leaf was selected because it exhibits greater induced alkaloidal responses (Baldwin 1989, 1999). The powdered leaf was transferred to a glass vial and weighed to estimate the amount of leaf extracted. Five mL of methanol were added to the vial and shaken at 600 rpm for 30 min. After that, the methanolic extract was filtered through filter paper (Whatman No. 1) and transferred into a Florence flask where it was evaporated to dryness using a rotary evaporator at 40 °C. After adding 5 mL of chloroform to the Florence flask, it was immersed in a 25 °C ultrasound bath for 10 min. Then, the extract was transferred into a separatory funnel and 5 mL of 5 % hydrochloric acid was added, thoroughly mixed and the organic phase collected and discarded; the acidic extract was washed twice with 5 mL of chloroform. After that, 5

mL of 29 % ammonia aqueous solution was added and the basic solution was extracted twice with 5 mL of chloroform. The organic phases were collected in a vial and then evaporated to dryness under a nitrogen flow.

The alkaloidal extract was re-dissolved in 70 μ L of chloroform and 30 μ L of internal standard solution (0.25 mM docosane in chloroform). Then, 1 μ L of the re-dissolved extracts was injected in a Shimadzu model GCMS-QP 2010 Ultra gas chromatograph (Shimadzu, Kyoto, Japan) equipped with an Rtx-5MS Crossbond 5% diphenyl 95% dimethyl polysiloxane capillary GC column (30 m length, 0.25 mm I.D., 0.25 μ m film thickness) (Restek, Bellefonte, PA, USA) and used in the splitless mode. Helium was used as the carrier gas at 50 ml/min. The mass spectrometer used electron impact (EI) ionization mode (70 eV) with an emission current of 250 μ A. The temperatures of the injection port, ion source and transfer line were set at 250 $^{\circ}$ C, 250 $^{\circ}$ C and 280 $^{\circ}$ C, respectively. The GC oven was programmed to remain at 30 $^{\circ}$ C for 3 min, then to increase to 230 $^{\circ}$ C at a rate of 18 $^{\circ}$ C / min and finally to remain at 230 $^{\circ}$ C for 5 min. Retention indexes were calculated based on chromatograms obtained from the periodic injection of a standard alkane mixture. Compounds were identified based on comparisons of their retention index and mass spectrum with those in the NIST14 database and with authentic standards.

To quantify the four main alkaloids (nicotine, nornicotine, anabasine and anatabine) present in tobacco, four calibration curves were constructed (one for each alkaloid) with five concentrations ranging from 0.0224 to 2 mg/mL and containing the same

amount of internal standard described above. Calibration curve were constructed using the concentration of standard versus the peak area ratio of each compound to the internal standard (Yuan *et al.* 2018). The concentration of each alkaloid expressed as $\mu\text{g} / \text{g}$ of fresh sample was determined using the calibration curves.

Experimental treatments

To compare how plants respond to experimental treatments, alkaloids of the fifth leaf counted from the apex of control plants ($N = 19$) were first extracted and quantified to detect the constitutive concentration of alkaloids (Kaplan *et al.* 2008). To determine the chemical response of tobacco to herbivory by *P. operculella* caterpillars, a 4th instar caterpillar was positioned on the fifth leaf of an undamaged plant, concealed inside a clip cage ($N = 18$), and observed every 10 min until it started feeding (i.e., started to build a feeding mine). After that, the caterpillar was allowed to eat *ad-libitum* for 3.5 hours; at that moment, it was carefully removed from the plant and the plant was left in the rearing environment described above. On the fifth day after caterpillar damage, the damaged leaf was cut, and alkaloids were extracted and quantified as described above.

Considering the treatment described on the “Vibration recordings and playback experiments” section and the two described treatments in this section, a total of three treatments were evaluated: 1) constitutive, 2) caterpillar and 3) playback. All plants were 11 weeks-old when they were subjected to any of the treatments.

Leaf production

The proxy used for performance was the number of leaves each plant produced five weeks after the fifth leaf was cut for the quantitation of alkaloids (i.e., when plants were 16 weeks-old; Kleiman & Aarssen *et al.* 2007).

Statistical analyses

The concentration of each alkaloid (nicotine, nor nicotine, anabasine and anatabine) and the leaf production were compared between treatments using General Linear Models (GLM) on R 3.4.4 (R Core Team, 2014).

RESULTS

The linear coefficient of determination (R^2) for the calibrations curves were over 0.94 (nicotine: 0.94, nornicotine: 0.96, anabasine: 0.98 and anatabine: 0.95). The most abundant alkaloid in all treatments was nicotine, followed by anatabine, nornicotine and anabasine (Table 1).

Nicotine levels significantly differed between treatments (Table 2), being 2.9 times higher in plants of the caterpillar treatment and 2.8 times higher in plants of the playback treatment with respect to the constitutive level. The concentration of nicotine from plants of playback and caterpillar treatments presented similar changes and both differed significantly from those of the constitutive treatment (Table 2, Figure 1i). Nornicotine, anabasine and anatabine concentrations did not differ significantly between treatments (Table 2, Figures 1ii, 1iii and 1iv).

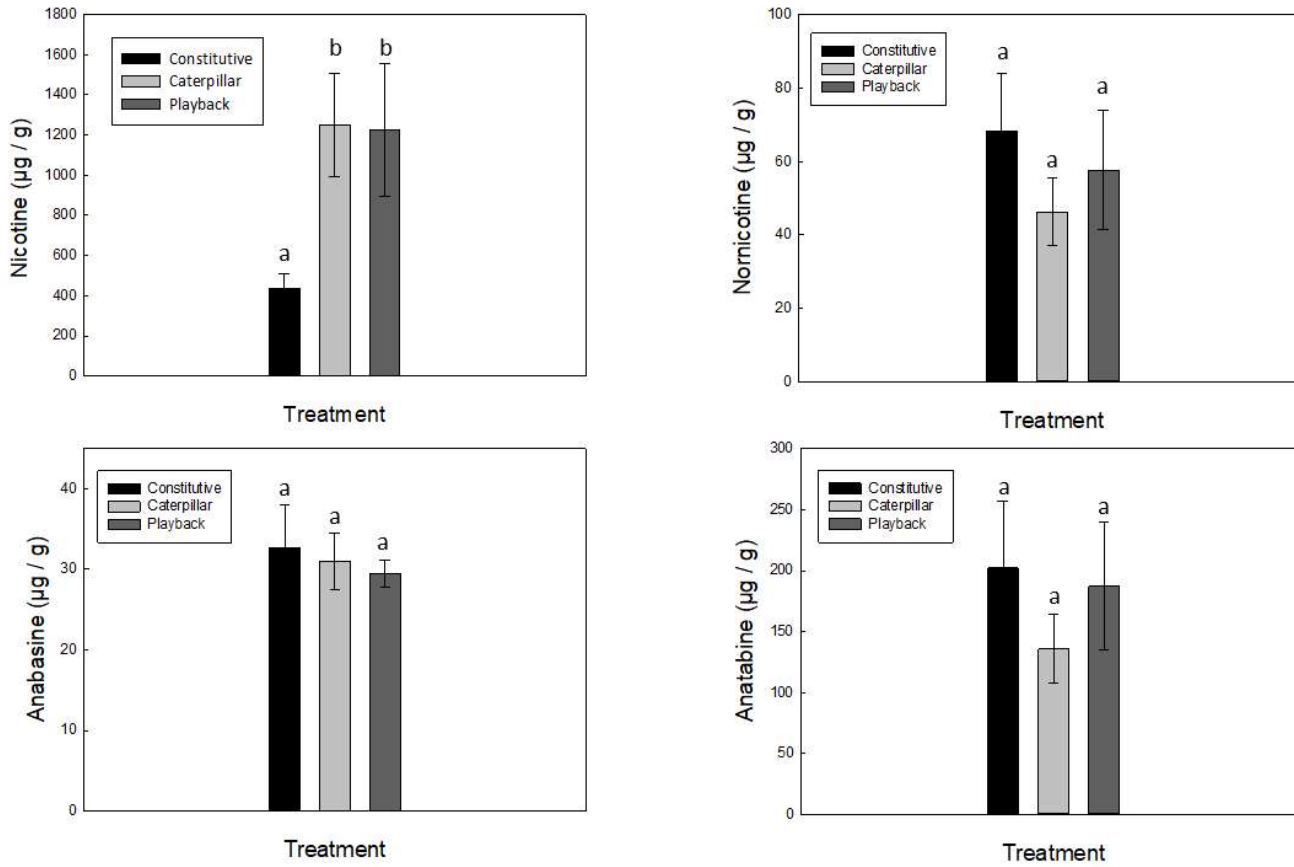
Table 1. Concentration of alkaloids expressed as $\mu\text{g/g}$ of fresh sample (mean \pm SE) for each treatment.

Treatment	Concentration of alkaloid ($\mu\text{g} / \text{g}$ fresh sample)	Sample number (<i>N</i>)
a) Nicotine		
Constitutive	435.5 \pm 73.7	19
Caterpillar	1249.7 \pm 256.3	18
Playback	1125.1 \pm 328.9	18
b) Nornicotine		
Constitutive	68.2 \pm 16.0	19
Caterpillar	46.3 \pm 9.1	18
Playback	57.7 \pm 16.4	18
c) Anabasine		
Constitutive	32.6 \pm 5.4	19
Caterpillar	31.0 \pm 3.5	18
Playback	29.5 \pm 1.6	18
d) Anatabine		
Constitutive	202.2 \pm 54.8	19
Caterpillar	135.7 \pm 28.1	18
Playback	187.3 \pm 52.5	18

Table 2. Results for the Generalized Linear Model for the concentrations of alkaloids expressed as $\mu\text{g/g}$ of fresh sample (AIC= Akaike information criterion).

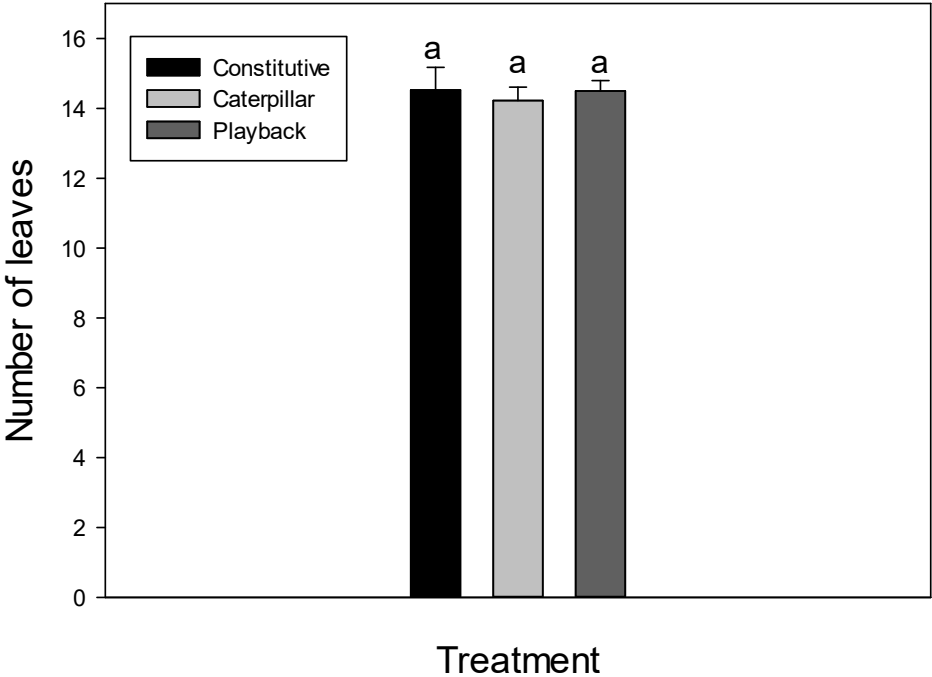
	Estimate	SE	t-value	P-value
a) Nicotine. ANOVA: $F = 3.7783$; $P = 0.02937$; AIC: 924.0				
Constitutive vs. Caterpillar	814.13	338.5	2.4	0.019
Constitutive vs. Playback	-789.6	338.5	-2.3	0.024
Caterpillar vs. Playback	-24.56	343.0	-0.1	0.943
b) Nornicotine. ANOVA: $F = 0.5807$; $P = 0.5632$; AIC: 602.3				
Constitutive vs. Caterpillar	-22.0	20.4	-1.1	0.286
Constitutive vs. Playback	10.5	20.4	0.5	0.609
Caterpillar vs. Playback	11.5	20.4	0.6	0.577
c) Anabasine. ANOVA: $F = 3.4066$; $P = 0.4087$; AIC: 460.8				
Constitutive vs. Caterpillar	-1.6	5.5	-0.3	0.767
Constitutive vs. Playback	13.2	5.5	2.4	0.203
Caterpillar vs. Playback	-11.5	5.5	-2.1	0.410
d) Anatabine. ANOVA: $F = 0.5477$; $P = 0.5816$; AIC: 744.7				
Constitutive vs. Caterpillar	-66.5	66.3	-1.0	0.321
Constitutive vs. Playback	14.9	66.3	0.2	0.823
Caterpillar vs. Playback	51.6	67.2	0.8	0.446

Figure 1. Concentration of each alkaloid expressed as $\mu\text{g} / \text{g}$ of fresh sample (mean \pm SE) for each treatment. Different letters indicate significant differences between treatments at $P < 0.05$. **i)** Nicotine, **ii)** Nornicotine, **iii)** Anabasine, **iv)** Anatabine. Note the different scales used for concentrations.



The number of leaves did not significantly differ between treatments (ANOVA: $F = 0.1254$; $P = 0.8824$; AIC: 238.9): it was 14.5 ± 0.6 (mean \pm SE) in the constitutive treatment, 14.2 ± 0.4 in the caterpillar treatment and 14.5 ± 0.3 in the playback treatment (Figure 2).

Figure 2. Number of leaves (mean \pm SE) on tobacco plants of each treatment.



DISCUSSION

The ability of plants to detect VS/sounds and the capacity to exhibit behavioral responses towards them has been only recently recognized (Gagliano 2013; Trewavas 2014; Karban 2015), particularly regarding behavioral responses to ecologically important VS/sounds for plants. For example, root tips of *Zea mays* bend towards a sound source of 220 Hz (Gagliano *et al.* 2012), roots of *Pisum sativum* can locate a water source by sensing the vibrations generated by moving water (Gagliano *et al.* 2017), plants from several species release pollen from anthers in response to the buzz (VS) produced by pollinators (De Luca & Vallejo-Marin 2013) and *A. thaliana* can perceive and chemically respond to VS of caterpillars that feed on them (Appel & Coccoft 2014). In this study, tobacco plants in the caterpillar and playback treatments increased their nicotine levels, indicating that they can chemically and behaviorally respond to the attack by *P. operculella* caterpillars and also to their feeding vibrations.

Furthermore, the chemical response (in terms of nicotine increase) of plants attacked by a 4th instar caterpillar did not differ from that of plants subjected to the playback of the VS of feeding caterpillars. This indicates that at least for tobacco, the vibrational stimulus of a chewing caterpillar is sufficient to produce a chemical response similar to that provoked by the whole caterpillar, in spite of the latter producing tissular damage and an oral secretion that can also trigger chemical plant responses (Basu *et al.* 2018). Additionally, considering the definition of plant communication given by Karban (2008) and Gagliano (2013) as the “transfer of cues from one individual to another without

any assumptions about intent or benefit for the emitter or receiver”, this study supports insect-plant communication based on vibrational cues.

Caterpillars of *P. operculella* induced greater defenses in tobacco than caterpillars of other species. In this study, nicotine increased 2.9 times their normal levels in undamaged plants; this chemical response is higher than that generated by caterpillars of *Helicoverpa zea* which increased nicotine levels by approximately 1.3 times in damaged plants with respect to undamaged plants (Musser *et al.* 2006), and by *Manduca sexta*, a tobacco specialist, which increased nicotine levels by 1.4 times with respect to undamaged plants (Kaplan *et al.* 2008). The differences in the induced nicotine levels could possibly be attributed to the feeding habit of the species: while *H. zea* and *M. sexta* feed on the whole plant tissue, *P. operculella* is a leaf-miner that feeds on the plant mesophyll, inside a feeding mine (Varela & Bernays 1988). The differences could also be caused by the oral secretions produced by each caterpillar species: for example, the oral secretions of *M. sexta* can attenuate the induced nicotine response of plants (McCloud & Baldwin 1997; Kahl *et al.* 2000). The oral secretions of *P. operculella* and their effects on tobacco plants have not been studied. Although our results suggest that its oral secretions could not attenuate the induced nicotine response of tobacco, further studies on this topic should be pursued to evaluate this hypothesis. It would also be interesting to compare the nicotine response of tobacco plants towards the VS of a specialist or a chewing herbivore (such as *M. sexta*) and a non-specialist or leaf miner (such as *P. operculella*), especially considering that plants

seem able to recognize and respond accordingly to different VS contexts (Appel & Cocroft 2014).

The exact mechanism through which plants detect VS is unknown. Recent studies have suggested that trichomes could act as acoustic antennae (Liu *et al.* 2017). Considering that tobacco plants present a high density of trichomes (Cui *et al.* 2011; Rivera & Burrack 2012), its ability to detect VS by means of trichomes is likely; however, further studies should be pursued to identify the mechanism by which tobacco plants detect VS.

The effects of ecologically relevant VS on plants, such as that produced by herbivory, has only been studied previously in *A. thaliana* (Appel & Cocroft 2014). That work demonstrated that VS of feeding insects elicited chemical defenses and that plants discriminated between different types of vibrations: chewing insects, insect courtship song and wind. However, the authors did not evaluate the performance effects or consequences of VS on plants. Agrawal (1998) evaluated the fitness consequence of induced chemical defenses in *Raphanus sativus*, considering the number of flowers as a proxy of male fitness and the number of seeds multiplied by the mass of seeds as a proxy of female fitness; he found that induced plants showed higher male and female fitness than control plants. Other studies found a reduction of seed production in *N. attenuata* when their defenses were induced with methyl jasmonate and herbivores were absent (Baldwin 1998), a reduction in male fitness (pollen grains) and a delayed first flower production in *R. raphanistrum* plants attacked by caterpillars of *P. rapae*

(Agrawal *et al.* 1999), a reduction in number of seeds in *Solanum lycopersicum* plants treated with jasmonic acid (Redman *et al.* 2001), and overall fitness costs imposed by the production of antiherbivore defenses revealed by a meta-analysis by Koricheva (2002). In the present study, we did not detect differences in terms of leaf production (as a proxy of performance) between chemically-induced plants (caterpillar and playback treatments) and control plants (constitutive treatment), similarly to what was observed by Baldwin (1989), who did not find evidence of compensatory growth (in terms of biomass) between damaged and undamaged plants of *Nicotiana sylvestris*. The lack of differences in leaf production in our system may be explained in terms of the hypothesis that states that a cost in performance is resource-based and defenses use a fitness-limiting resource (Baldwin 1999); the plants used in our experiments did not have a limiting resource (i.e. nitrogen) since they were fertilized once a week. Hence, to evaluate the possible performance consequences of induced defenses in tobacco exposed to VS, plants that have a limiting resource should be exposed to VS. Additionally, other proxies of fitness, such as number of flowers produced or age at first flower production, should be considered.

In this work, and in the work by Appel & Coccoft (2014), it was shown that treating plants with VS could be an appropriate method to disassociate leaf loss and chemical defenses to further our understanding on the performance costs of induced chemical defenses in plants; this method complements the application of jasmonates currently used to induce plant defenses without removing leaves or generating physical damage

on the plant (Redman *et al.* 2001). Also, vibrational methods such as the one used in this study could have potential applications in agriculture, especially as an alternative to the application of pesticides and as a method to enhance the plant's own chemical defenses.

Thus, the present study adds to our current understanding of the communication and sensory abilities of plants by emphasizing the ecological role of VS to these organisms. The study also adds to our understanding of insect-plant vibrational communication while highlighting the need for further studies revealing how widespread this type of communication is among plants (Mishra *et al.* 2016; Schöner *et al.* 2016; Gagliano *et al.* 2017).

CONCLUSIONS

The potato tuber worm (*Phthorimaea operculella*) can induce chemical defenses in tobacco, either by directly feeding on the plant or through the vibrational signals it produces while feeding.

Tobacco plants can perceive vibrational signals generated by feeding caterpillars and respond to them by increasing their chemical defenses, adding another dimension to our understanding of plant sensorial and behavioral responses and of insect-plant communication.

There were no differences in terms of leaf production (as a proxy of performance) between chemically-induced plants (caterpillar and playback treatments) and control plants (constitutive treatment), probably because plants were not resource-limited.

The tobacco-potato tuber worm system could constitute an excellent model for evaluating and for furthering our understanding of plant behavior, insect-plant communication and performance consequences of induced defenses, especially considering that tobacco is one of the best chemically studied plants and that both tobacco and *P. operculella* are cosmopolitan species, thus allowing this system to be studied globally.

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