Research Article

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Pyrethroid resistance is associated with a *kdr*-type mutation (L1014F) in the potato tuber moth *Tecia solanivora*

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Abstract

BACKGROUND: The Guatemalan potato tuber moth, *Tecia solanivora*, has been the most important pest species in Hispanico-American potato fields since its first record on potatoes in 1956 in Guatemala. This insect pest has been spreading to other parts of the world, including the Canary Islands in Europe. Tuber moth control relies heavily on the use of insecticides, including pyrethroids. Here, we assessed the likelihood of control failures and performed concentration–response bioassays in five Colombian strains of *T. solanivora* to evaluate their susceptibilities to the pyrethroid permethrin.

RESULTS: Evidence of control failures was observed in four strains tested, which exhibited moderate resistance levels (i.e. ranging from 5.4- to 24.4-fold). However, no spatial dependence was observed between the permethrin LC_{50} values and the geographic distances among the tuber moth strains. In order to evaluate whether permethrin resistance was mediated by potential mutations in the *para*-type sodium channels of *T. solanivora*, the IIS4–IIS6 region of the *para* gene was PCR amplified and sequenced from the five strains tested. As demonstrated across a range of different arthropod species that exhibited knockdown resistance (*kdr*), we observed a single point substitution (L1014F) at high frequencies in the *para* gene of all four resistant strains.

CONCLUSION: This is the first identification of a target-site-alteration-based resistance in the Guatemalan potato tuber moth *T. solanivora*, which is widespread and exhibits high frequencies among geographically distant strains, indicating that pyrethroids are probably becoming ineffective for the control of this pest species.

Keywords: Guatemalan potato tuber moth; potato pests; permethrin; *kdr* resistance; voltage-gated sodium channels

1 INTRODUCTION

The potato tuber moth *Tecia solanivora* (Povolny 1973) (Lepidoptera: Gelechiidae) is an invasive pest species native to Guatemala that has successfully colonised potato crops in Hispanic America (e.g. Central and South American countries including Venezuela, Colombia and Ecuador), besides the Spanish Canary Islands, causing significant losses in this crop.[1–6] This invasive pest species has great capacity to adapt to warmer regions under a scenario of climate change, which has turned *T. solanivora* into a threat that can reach new regions in the world.[7,8] Recently, it was reported for the first time in southern Mexico,[9] which raises invasion risks for several potato-growing regions of the United States, such as the south-west and the east coast.[10]

Adults of *T. solanivora* females lay eggs on the soil surface near the base of the potato stems. Newly hatched larvae burrow into the ground and feed on tubers, completely destroying the attacked tuber or facilitating the entry of pathogens.[8] In the Hispano-American countries, *T. solanivora* is a major potato pest capable of damaging up to 100% of the potato tubers.[1–4] Although some management strategies have been tested to control this insect pest in Hispanic America (e.g. pheromones, *Bt* plants, as well as some biological and cultural strategies), potato tuber moth control remains largely dependent on insecticide use.[4,8] However, in most of these Hispano-American countries, insecticide applications take place following a prescheduled calendar, which leads to insecticide overuse and potentially triggers a series of problems such as insecticide resistance and environmental contamination. The scenario threatens future control attempts in Hispano-American countries, where only a small number of registered insecticides are allowed to control the tuber moth,

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Table 1. Time, origin, place of collection and host type where the Colombian strains of *T. solanivora* were sampled

Month and year	County	Geographic coordinates	Host type (potato variety)
December 2012	Gualmatán	0° 55′ 5.9″ N, 77° 34′ 32.6″ W	Capiro
December 2012	Ospina Nariño	01° 3′ 15.85″ N, 77° 34′ 49.9″ W	Capiro
May 2013	Guaitarilla	01° 6′ 35.43″ N, 77° 33′ 24.64″ W	Capiro
January 2013	Iles	0° 59′ 3.23″ N, 77° 30′ 27.7″ W	Capiro
January 2013	Siachoque[a]		

[a] Pupae were obtained from the Entomological Laboratory of Corpoica in the county of Siachoque (Boyacá, Colombia).

including malathion during potato seed storage and organophosphates (e.g. chlorpyrifos, acephate, profenofos), permethrin and ultimately chlorantraniliprole against adults of *T. solanivora* in potato fields.[11] In Venezuela and Ecuador, chlorpyrifos, profenofos, triclorphon and fipronil are the insecticides used to secure suitable control levels.[12]

The invasive success of species such as *T. solanivora* generally depends on factors favouring its rapid adaptation and spread through different geographical areas.[13] Such factors are related not only to the environment but also to the genetic basis of traits such as insecticide resistance that may favour invasion success.[13,14] Despite its economic importance and potential risks associated with insecticide overuse, studies of insecticide resistance and resistance mechanisms among populations of the potato tuber moth *T. solanivora* have been wholly neglected. Moreover, genetic differentiation among Colombian populations from geographically distant and separated regions, as recently reported,[6] may have serious implications for the species management. Furthermore, the continuous transport of potato tubers between different regions can lead to genetic homogenisation between the existing populations, further complicating the control by insecticide use of this invasive pest if insecticide resistance prevails.

For these reasons, the present investigation was performed to assess the levels of pyrethroid resistance among field-collected populations of the tuber moth *T. solanivora*. We also reported the cloning and sequencing of a 470 bp fragment (domains IIS4 to IIS6) of the *para* gene from the studied tuber moth populations and the identification of a single mutation that has previously been reported to confer reduced target-site sensitivity to pyrethroids in several other arthropod pest species.[15–19]

2 MATERIALS AND METHODS

2.1 Insect populations and rearing

Insects were collected in potato fields of four counties (i.e. Gualmatán, Guaitarilla, Iles and Ospina Nariño) from the Colombian Department of Nariño during the years 2012 and 2013 (Table 1). Furthermore, insects of a laboratory strain were obtained from the Entomological Laboratory of Corpoica in the county of Siachoque (Boyacá) in Central Colombia (Table 1). The Siachoque strain is a field-collected population that has been reared for over 12 generations in an insecticide-free environment, but its susceptibility to pyrethroid was never tested before.

T. solanivora-infested potato tubers of all five populations were maintained under controlled conditions (a temperature of 14 ± 2 °C and a relative humidity of 70 ± 5%) at the Entomological Laboratory of the University of Nariño (Pasto, Nariño, Colombia; latitude 1° 14′ 1.858″ N, longitude 77° 17′ 35.871″ W, altitude 2488 m). Each population was established with a minimum of 400 individuals, which assured representative genetic variability.

The infested potato tubers were placed into transparent plastic containers (370 mL) with side windows (3 cm × 3 cm) covered with pieces of organza veil. Absorbent paper covered the bottom of each container to remove excess moisture and was changed daily. The larvae reached the prepupa stage approximately a month after oviposition, when they left the potato tuber to pupate in the absorbent paper. Using the characteristics of abdominal sternites described elsewhere,[20] we conducted pupal sex determination. Groups of 10 pupae (four males and six females) were placed in other plastic containers until adult emergence. Once the adults emerged, they were fed with water and honey syrup (10%). Black crepe paper was placed at the container bottom for female oviposition. Circular pieces of black crepe paper containing approximately 20 eggs were cut and placed in plastic containers provided with non-infested potato tubers and containing absorbent paper at their bottom. Tuber perforations were made with a sterile pin to facilitate neonate entry into the tubers. The populations were kept under laboratory conditions (i.e. 14 ± 2 °C and 70 ± 5% RH) and in an insecticide-free environment up to the sixth generation, when concentration–mortality bioassays were performed, and then up to the 12th generation, when molecular characterisation of the IIS4–IIS6 region of the *para*-orthologue gene was performed.

2.2 Concentration–mortality bioassays

Concentration–mortality bioassays were performed using a commercial formulation of the pyrethroid insecticide permethrin (384 g AI L^{-1}; DuPont, Bogotá, Colombia) dissolved in distilled water and Tween 80 (0.5%). Non-infested potato tubers were submerged into the insecticide solutions (calculated as mg AI L^{-1}) for 1 min and left to dry at room temperature for 24 h. Seven insecticide concentrations (ranging from 0.01 to 10000 mg AI L^{-1}) were used in these bioassays. In the control treatment, the potato tubers were immersed into distilled water with 0.5% Tween 80. The insecticide-treated potato tubers were fixed with silicone (avoiding any movement of the tubers) into plastic containers that subsequently received ten adults of the potato tuber moth *T. solanivora* (5–7 days old). Water and honey syrup (10%) were offered *ad libitum*. Ten replicates, each with ten adults, were used for each insecticide concentration. The mortality levels were assessed after 48 h of exposure, and insects were counted as dead if they were unable to move the length of their body when prodded with a fine-hair brush.

2.3 Spatial dependence of insecticide resistance

The distance between the sampling sites of each insect strain was determined using geographic coordinates determined by a global

Table 2. Oligonucleotide primers used to sequence the IIS4–IIS6 domain of the *T. solanivora* sodium channel. All primers are shown 5′ to 3′. Degenerate bases are represented using standard IUB codes: R = A + G; Y = C + T; M = A + C and N = A + C + G + T

Primer name	Sequence
DgN_1	GCNAARTCNTGGCCNAC
DgN_2	GCNAARTCNTGGCCNACNYT
DgN_3	YTTRTTNGTNTCRTTRTCRGG
DgN_4	TTNGTNTCRTTRTCRGCNGTNGG
$DgSeq_1$	TNCCNMGNTGGAAYTTYAC
$DgSeq_2$	RAARTCNGTRAARTTCCANC
Tsol1	CCCTGAACTTATTGATATCC
Tsol2	TGAACTTATTGATATCC
Tsol3	GAGCGGACAAACTTGAAGAT
Tsol4	TGAAGATCCAAAGTTGCTC

position system (GPS 12XL; Garmin International, Olathe, KS). The semi-variograms were estimated from the LC_{50} semi-variance data of each population and used as dependent variables in regression analysis, with the distance between sampling sites as an independent variable, as described elsewhere[21,22] and as recently emphasised.[23] The semi-variogram range is the maximum distance of interference between strains of *T. solanivora* regarding susceptibility to a given insecticide.[21,24]

2.4 Cloning of sequences encoding domain II

To clone and sequence the domain II region of the *T. solanivora* sodium channel gene, PCR reactions were initially carried out on cDNA prepared from pools of 15–20 individuals using degenerate primers designed against conserved motifs within the IIS4 and IIS6/II–III linker regions of the channel protein, following a previously described method.[25] A nested PCR approach was adopted using primers DgN1 and DgN3 in a primary PCR and primers DgN2 and DgN4 in a secondary reaction (the primer sequences are given in Table 2). The resulting fragment of the two rounds of PCR was sequenced using the forward and reverse primers $DgSeq_1$ and $DgSeq_2$. Once the tuber moth sodium channel gene sequence had been determined, specific primers were designed to perform direct PCR analysis of genomic DNA. To assess the frequency of potential mutation(s) within the five populations tested, genomic DNA was extracted from ten individual insects of each population using the phenol-chloroform-based method.[26] Gene fragments were amplified in a two-step nested PCR with specific primers Tsol1 and Tsol3 used in primary PCR followed by secondary PCR with Tsol2 and Tsol4.

PCR reactions (20 μL) consisted of 1.2 μL of template DNA, 0.75 μL of each primer (10 μM), 12 μL of GreenTaq (Fermentas) and 6 μL of sterile distilled water. The temperature cycling conditions were: 35 cycles of 95 °C for 30 s, 48–58 °C for 60 s and 72 °C for 90–120 s. Agarose gel electrophoresis (1.2%) of PCR products was carried out in 1× TBE buffer, and the Wizard SV gel and PCR Clean-up System from Promega was used to recover DNA from gel slices according to the manufacturer's recommendations. PCR products of the *para* gene fragments (470 bp) were directly sequenced (with the same primers used in PCR) employing the sequencing service of Macrogen (Seoul, South Korea). Sequences were aligned and analysed using Sequencher 5.4 software (Gene Codes Corporation, Inc., Ann Arbor, MI).

2.5 Survey of insecticides used to control *T. solanivora*

Twenty-four surveys were conducted in each county where *T. solanivora* was sampled to recognise the active ingredients used against the species, as well as to record the number of applications per cycle in the 3 years previous to the survey. The geographical coordinates were recorded for each place where the survey was performed.

2.6 Statistical analysis

The mortality rate of the tuber moth adults was corrected by the natural mortality observed in the controls (i.e. water-treated potato tubers) before analysis.[27] The concentration–mortality curves were estimated by probit analyses using the PROC PROBIT procedure (SAS Institute, 2008), and 95% confidence limits for resistance ratios at LC_{50} (RR_{50}) were estimated as reported by Robertson *et al.*[28] Resistance was considered to be significantly different ($P < 0.05$) if the 95% CI at the RR_{50} did not include the value 1. In addition, correlations between the resistance ratio, the geographical distance among the strains and the number of insecticide applications per year were also carried out (PROC CORR; SAS Institute, 2008). The assumptions of normality and homogeneity of variance were checked using the procedure UNIVARIATE (SAS Institute, 2008), and no data transformation was necessary.

3 RESULTS

3.1 Resistance to permethrin

The low χ^2 values (<5.4) and high P values (>0.05) obtained using the probit model indicated its suitability for the toxicity estimates.

Table 3. Relative toxicity of permethrin to populations of *T. solanivora*

Population	Number of insects	Degrees of freedom	Slope ± SEM[a]	LC_{50} (CI 95%)[b] (ppm)	LC_{95} (CI 95%) (ppm)	χ^2	P	RR[c] (LC_{50})
Guaitarilla	400	2	0.78 ± 0.09	1.6 (1.1–2.7)	204.1 (73.5–978.0)	0.23	0.89	1.0 (0.4–2.4)
Gualmatán	310	2	1.09 ± 0.161	6.2 (0.4–46.9)	198.1 (29.6–6252.8)	5.35	0.06	5.8 (1.4–24.6)
Iles	490	3	1.56 ± 0.12	16.2 (12.4–21.1)	182.1 (121.5–307.6)	3.97	0.26	15.0 (5.0–19.2)
Ospina nariño	300	5	1.38 ± 0.17	18.3 (1.6–31.5)	282.8 (137.8–818.9)	1.07	0.95	16.9 (4.9–25.2)
Siachoque	400	2	0.96 ± 0.08	26.3 (18.1–38.8)	1313.0 (643.9–3500.0)	4.36	0.11	24.4 (5.9–42.8)

[a] SEM: standard error of mean.
[b] LC: lethal concentration; CI: confidence interval.
[c] Resistance ratio = LC_{50} of determined strain/LC_{50} of most susceptible (Guaitarilla) population. The resistance ratios were considered to be significant if their value was greater than 1, as described elsewhere.[28]

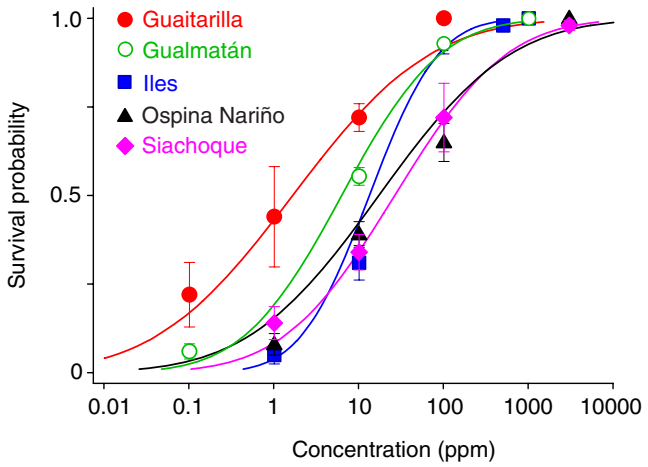

Figure 1. Toxicity of permethrin to Colombian populations of *T. solanivora*. The lines denote the lethal concentration (LC) values estimated on the basis of concentration–mortality bioassays using probit analyses. Symbols show the averaged mortality for each permethrin concentration applied in each population of *T. Solanivora*. The vertical bars represent the standard error of the average (SE).

Based on their LD_{50} values, the susceptibility to permethrin was significantly different among *T. solanivora* populations (Table 3 and Fig. 1). The resistance ratios were considered to be significant if their value was greater than 1, as described elsewhere.[28] The resistance levels to permethrin among the tuber moth populations studied varied between 5.8- and 24.4-fold (Table 3). The highest resistance level was recorded for the Siachoque population, while the Guaitarilla population was recognised as the most susceptible population.

3.2 Resistance ratio, geographic distances and number of insecticide applications

According to the surveys carried out in the geographical regions where the tuber moth populations were collected, the farmers employ a prescheduled calendar scheme of sprayings involving a considerable number of insecticide applications to control different insect pests that attack potato plants (Table 4). In the 3 year period assessed, the total number of insecticide applications varied between 62 (in Ospina Nariño) and 142 (in Guaitarilla) (Table 4). Considering that in these regions approximately four potato crops were obtained in this 3 year period, a minimum of seven insecticide applications per potato cycle were made. The pyrethroids accounted for between 16.6% (Guaitarilla) and 50.7% (Gualmatán) of the insecticide applications.

Despite this frequent rate of insecticide use, permethrin resistance was not significantly correlated with the number of applications of organophosphates ($r = 0.32$, $P = 0.44$), pyrethroids ($r = 0.26$, $P = 0.53$), carbamates ($r = -0.53$, $P = 0.17$) or other insecticides ($r = 0.13$, $P = 0.76$). The lack of correlation between permethrin and non-pyrethroid insecticides suggests a lack of cross-resistance among them, which would be due to mechanisms other than altered target-site sensibility. Furthermore, no significant semi-variogram models were obtained for the permethrin LC_{50} values and the geographical distances among the places where *T. solanivora* were collected, which indicates a lack of distance dependence at the spatial scale used and with the number of sites sampled (nugget variance 144; sill 28.9; range 3810 m; residual sum of square errors 3615; $R^2 = 0.04$; model type Gaussian). The Siachoque population was not used in this analysis because it was obtained from a laboratory population.

3.3 Sequencing of domain II of the voltage-gated sodium channel

The use of a degenerate strategy, based on primers designed against conserved sequences within the domain II region of several insect *para* sodium channel gene sequences, enabled the PCR amplification, cloning and sequencing of a 470 bp fragment of the tuber moth *para* gene (GenBank accession). The encoded amino acid sequence of this fragment is shown in Fig. 1. The sequence showed high similarity not only to insects such as the housefly *Musca domestica*, the fruit fly *Drosophila melanogaster* and the cotton aphid *Aphis gossypii* but also to lepidopteran insects such as the diamondback moth *Plutella xylostella*, the tomato leaf miner *Tuta absoluta*, the cotton leaf worm *Spodoptera litura* and the fall armyworm *Spodoptera frugiperda*.

Preliminary sequencing of RT-PCR cDNA fragments from pools of 15–20 individuals of all tuber moth populations revealed the existence of only one point mutation within this region at position 1014, based on the housefly *para* sequence numbering (GenBank accession X96668). This mutation is a substitution of the amino acid leucine by phenylalanine at position IIS6 (L1014F). In pooled samples, the sequencing indicated that the L1014F mutation was present in four out of the five populations. Only the Guaitarilla population did not exhibit this mutation. All of the four populations exhibited the L to F substitution, and the mutation appeared to be heterozygous.

The position and sequence of the two introns known to be present within the sequenced region were determined using specific primers and genomic DNA of the tuber moth *T. solanivora*. The size of the introns was 60 and 58 nucleotides respectively (Fig. 3). The sequence of both introns was highly conserved across the five populations studied, and no polymorphic bases were found.

Genomic DNA was extracted from ten individual adults of each population and used as template to amplify the IIS4–IIS6 region of the *para* gene using the specific primers designed from the cDNA sequence in order to assess the frequency of the mutation found

Table 4. Total number of insecticide applications in potato fields in a 3 year period

Strains	Organophosphates	Permethrin	Pyrethroids[a]	Carbamates	Other insecticides
Guaitarilla	18	0	18	36	36
Gualmatán	20	0	72	6	44
Iles	0	10	20	20	30
Ospina Nariño	23	6	11	14	14

[a] Also includes the number of permethrin applications.

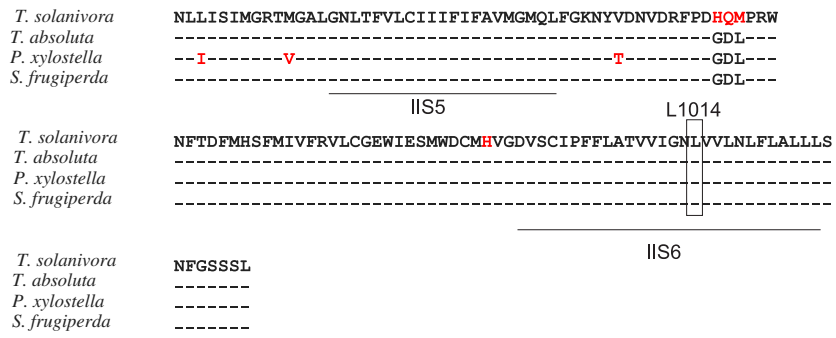

Figure 2. Amino acid alignment of the IIS4–IIS6 domain of the *T. solanivora* sodium channel with the corresponding sequence of *T. absoluta* (JQ701800), *P. xylostella* (GI2769535) and *S. frugiperda* (KC435025). Transmembrane segments (IIS5 and IIS6) are indicated by horizontal bars. The position of the L1014F is boxed.

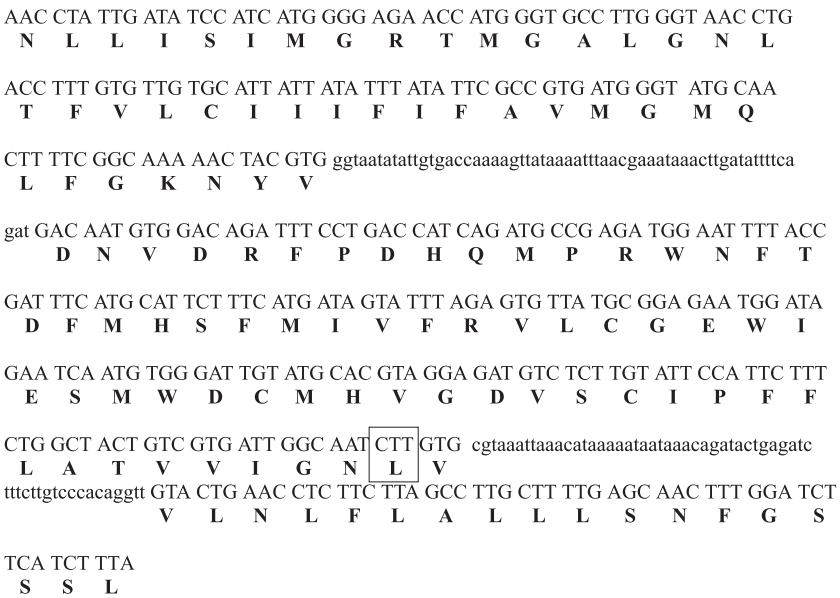

Figure 3. Sequence of the IIS4–IIS6 domain of the *T. solanivora para*-type sodium channel gene. The position of the *kdr* mutation (L1014F) is boxed. Lower-case letters indicate the intron sequences.

Table 5. Allele frequencies of the mutation L1014F genotypes among individuals from each of the five populations of *T. solanivora*

		L1014F		
Strains	Number of insects	SS	SR	RR
Guaitarilla	10	1.00	0	0
Gualmatán	10	0.80	0.20	0
Iles	10	0.20	0.75	0.05
Ospina Nariño	10	0.10	0.45	0.45
Siachoque	10	0.10	0.45	0.45

(L1014F). The results showed that the susceptible population Guaitarilla exhibited a 100% frequency of susceptible alleles (SS), while the moderately resistant population Gualmatán exhibited 20% of heterozygous resistant alleles (RS) (Table 5). The three other populations tested showed either higher frequency of the mutant heterozygous allele (Iles 75%) or high frequency of the resistant allele equally distributed between the mutant heterozygous (45%) and mutant homozygous (45%) alleles in the case of Ospina Nariño and Siachoque (Table 5).

4 DISCUSSION

Although insects and mammals have different sensitivities to pyrethroids,[19,29–32] the high toxicity of pyrethroids to non-targeted organisms (e.g. some insect predators or parasitoids or even fishes) has hindered their even wider use in agriculture.[33–35] As a potential result of the intense use of pyrethroid insecticides for the control of potato tuber moth *T. solanivora*, control failures have been frequently reported and recognised as one of the major causes of the economic losses observed in potato production in Hispanic America.[1–6] The present investigation demonstrated the presence of a single point substitution, L1014F, in the sodium channels of four permethrin-resistant Colombian populations of the potato tuber moth *T. solanivora*.

The L1014F mutation was the first target-site alteration associated with pyrethroid resistance, and it has been detected across a wide range of different insect species.[15–19] Our findings concerning the frequencies of L1014F indicated that the observed levels of pyrethroid resistance are directly related to differences in the frequencies of L1014F mutations. Furthermore, although the number of populations studied is low (i.e. only five populations), the L1014F alteration in resistant field populations seems already to be at high frequencies. Interestingly, although the Guaitarilla and Gualmatán

field populations have been exposed to other pyrethroid insecticides rather than permethrin, they were the populations that exhibited the lowest frequencies of L1014F mutation. This finding suggests that the applications of permethrin in such localities will raise the pressure in selecting resistant individuals. The development and spread of insecticide resistance depend on the selection pressure, the fitness cost associated with the resistant allele and the gene flow among populations.[36]

While the two most resistant populations (i.e. Ospina Nariño and Siachoque) exhibited the same frequencies of homozygous (RR) and heterozygous (SR) resistant genotypes, the population of Iles exhibited a higher frequency of the heterozygous (SR) resistant genotype, and the population of Gualmatán exhibited a low frequency of the resistant genotype and only in heterozygosity (SR). Such high frequencies of the L1014F mutation observed in field populations (e.g. Iles and Ospino Nariño) either are the likely consequence of a long period of selection pressure favouring this allele (although not necessarily by pyrethroid use) or derive from a higher frequency of permethrin applications targeting the control of *T. solanivora*. However, the latter seems not to be the case because populations from some sites (e.g. Guaitarilla) still do not exhibit the L1014F mutation despite having been previously exposed to other pyrethroid insecticides (Table 5). Alternatively, the L1014F frequencies in Iles and Ospino Nariño might reflect invasion by populations from other localities (e.g. Siachoque) that already have such mutations in their genomes.

The findings obtained for the Siachoque population, which has been maintained in the laboratory for many generations in a pyrethroid-free environment and showed the highest level of permethrin resistance, might result from a long period of selection pressure, reinforcing the hypothesis of a highly frequent mutation with a lack of fitness disadvantage associated with the resistant allele. There are many cases in the literature reporting no apparent disadvantages (in some cases, fitness advantages were reported) for insecticide-resistant individuals of some insect species.[37–40] Although the basis of insecticide resistance costs and its mitigation is little investigated, allelic replacement (by a less costly allele) and selection of modifier genes have been suggested as potential mechanisms to ameliorate the cost of insecticide resistance.[38,39,41,42] Lending more credence to the hypothesis that L1014F mutation is not associated with any fitness costs in Colombian populations of *T. solanivora*, we found that the other field resistant populations investigated here exhibit high frequencies of L1014F mutation and were uncorrelated with pyrethroid applications in the surveyed localities during the 3 previous years.

The wide distribution of a target-site-alteration-based resistance among geographically distant populations indicates that pyrethroids are probably losing efficacy against this pest species and may support the hypothesis of an ongoing replacement of susceptible populations by more insecticide-resistant ones with a higher capacity to invade new areas. Such findings have serious implications in the management strategies of this pest in Hispanic America, and particularly in Colombia. Recent studies have suggested gene flow of populations of *T. solanivora* from Boyacá (where the most resistant population is located) to other regions of Colombia, probably through infected potato tuber movements, emphasising the concern with the introduction of already resistant phenotypes of this invasive pest species.[6] A similar scenario has been hypothesised for another lepidopteran and closely related insect, the tomato leaf miner *Tuta absoluta*. Populations of *T. absoluta*, from various geographical regions, with low genetic variability, have been found to harbour *kdr*-like mutations, and it was suggested that its rapid expansion through South American countries and lately to the Mediterranean Basin, North Africa and Asia, may in part be mediated by the resistance of this pest to chemical insecticides.[17,43–45]

Except for a single report related to the F1515C mutation in mosquito sodium channels,[46] all *kdr* mutations reduce channel sensitivity to both type I and type II pyrethroids.[19,32] This means that, once a pest population develops target site resistance to one pyrethroid, the population is very likely to be cross-resistant to the entire class of pyrethroids. Integrated control strategies, including better monitoring of potato tuber movements across the potato-producing regions, community-based surveillance and environmental management measures, should be considered in the long term to reduce the dependence on pyrethroid insecticides to control *T. solanivora* and contribute to limiting the fast invasion of the potato tuber moths and its resistant phenotype into new geographical areas.

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REFERENCES

1 EPPO Data sheets on quarantine pests: *Tecia solanivora*. *Eur Plant Prot Org Bull* **35**:399–401 (2005).
2 Puillandre N, Dupas S, Dangles O, Zeddam J-L, Capdevielle-Dulac C, Barbin K *et al.*, Genetic bottleneck in invasive species: the potato tuber moth adds to the list. *Biol Invasions* **10**:319–333 (2008).
3 Moreno CR, London MS and Gliessman SR, Influence of a native *Solanum tuberosum* ssp. *andigenum* potato variety on management of the Guatemalan potato moth in the Venezuelan Andes. *Am J Potato Res* **93**:1–7 (2016).
4 Domínguez I, Carrero C, Ramírez W, Segovia P and Pino H, Evaluación del efecto de insecticidas sobre larvas de *Tecia solanivora*. *Agric Andina* **17**:61–73 (2009).
5 Kumar P, Ortiz EV, Garrido E, Poveda K and Jander G, Potato tuber herbivory increases resistance to aboveground lepidopteran herbivores. *Oecologia* **181**:1–11 (2016).
6 Villanueva-Mejía DF, Ramírez-Ríos V, Arango-Isaza RE and Saldamando-Benjumea CI, Microsatellite analysis reveals population structure and population expansion of *Tecia solanivora* in *Solanum tuberosum* in Colombia. *SW Entomol* **40**:37–52 (2015).
7 Dangles O, Carpio C, Barragan AR, Zeddam JL and Silvain JF, Temperature as a key driver of ecological sorting among invasive pest species in the tropical Andes. *Ecol Appl* **18**:1795–1809 (2008).
8 Carrillo D and Torrado-León E, *Tecia solanivora* Povolny (Lepidoptera: Gelechiidae), an invasive pest of potatoes *Solanum tuberosum* L. in the Northern Andes, in Potential Invasive Pests of Agricultural Crops, CABI Invasives Series Vol. 13, ed. by Peña JE. CABI, Wallingford, Oxon, UK, pp. 126–136 (2013).
9 Roblero E, Vera A and Malo E, First report of *Tecia solanivora* (Lepidoptera: Gelechiidae) attacking the potato *Solanum tuberosum* in Mexico. *Fla Entomol* **94**:1055–1055 (2011).

10 *Cooperative Agricultural Pest Survey (CAPS) Program, Solanaceous Hosts Commodity-based Survey Reference, Tecia solanivora*. [Online]. CAPS (2012). Available: http://download.ceris.purdue.edu/file/2377 [30 April 2016].
11 Tamayo FLH, *Diccionario de Especialidades Agroquímicas*. Thomson PLM, Bogotá, Colombia, 1080 pp. (2009).
12 Niño L, Revisión sobre la polilla de la papa *Tecia solanivora* en Centro y Suramérica. *Rev Latinoam Papa (Suppl.)* 4–21 (2004).
13 Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM and Lowe AJ, Adaptive evolution in invasive species. *Trends Plant Sci* **13**:288–294 (2008).
14 Facon B, Genton BJ, Shykoff J, Jarne P, Estoup A and David P, A general eco-evolutionary framework for understanding bioinvasions. *Trends Ecol Evol* **21**:130–135 (2006).
15 Miyazaki M, Ohyama K, Dunlap DY and Matsumura F, Cloning and sequencing of the *para*-type sodium channel gene from susceptible and kdr-resistant German cockroaches (*Blattella germanica*) and house fly (*Musca domestica*). *Mol Gen Genet MGG* **252**:61–68 (1996).
16 Williamson MS, Martinez-Torres D, Hick CA and Devonshire AL, Identification of mutations in the housefly para-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. *Mol Gen Genet MGG* **252**:51–60 (1996).
17 Haddi K, Berger M, Bielza P, Cifuentes D, Field LM, Gorman K *et al.*, Identification of mutations associated with pyrethroid resistance in the voltage-gated sodium channel of the tomato leaf miner (*Tuta absoluta*). *Insect Biochem Mol Biol* **42**:506–513 (2012).
18 Rinkevich FD, Du Y and Dong K, Diversity and convergence of sodium channel mutations involved in resistance to pyrethroids. *Pestic Biochem Physiol* **106**:93–100 (2013).
19 Silver KS, Du Y, Nomura Y, Oliveira EE, Salgado VL, Zhorov BS *et al.*, Voltage-gated sodium channels as insecticide targets, in *Advances in Insect Physiology*, ed. by Ephraim C. Academic Press, Burlington, MA, pp. 389–433 (2014).
20 Rincón RDF and López-Ávila A, Dimorfismo sexual en pupas de *Tecia solanivora* (Povolný) (Lepidoptera: Gelechiidae). *Rev Corpoica* **5**:41–42 (2004).
21 Silva GA, Picanço MC, Bacci L, Crespo ALB, Rosado JF and Guedes RNC, Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. *Pest Manag Sci* **67**:913–920 (2011).
22 Gontijo PC, Picanço MC, Pereira EJG, Martins JC, Chediak M and Guedes RNC, Spatial and temporal variation in the control failure likelihood of the tomato leaf miner, *Tuta absoluta*. *Ann Appl Biol* **162**:50–59 (2013).
23 Guedes R, Insecticide resistance, control failure likelihood and the first law of geography. *Pest Manag Sci* in press (2016).
24 Liebhold AM, Rossi RE and Kemp WP, Geostatistics and geographic information systems in applied insect ecology. *Annu Rev Entomol* **38**:303–327 (1993).
25 Martinez-Torres D, Devonshire AL and Williamson MS, Molecular studies of knockdown resistance to pyrethroids: cloning of domain II sodium channel gene sequences from insects. *Pestic Sci* **51**:265–270 (1997).
26 Reineke A, Karlovsky P and Zebitz C, Preparation and purification of DNA from insects for AFLP analysis. *Insect Mol Biol* **7**:95–99 (1998).
27 Abbott W, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
28 Robertson JL, Russel RM, Preisler HK and Savin NE, *Pesticide Bioassays with Arthopods*. CRC Press, Boca Raton, FL (2007).
29 Oliveira EE, Du Y, Nomura Y and Dong K, A residue in the transmembrane segment 6 of domain I in insect and mammalian sodium channels regulate differential sensitivities to pyrethroid insecticides. *NeuroToxicology* **38**:42–50 (2013).
30 Du Y, Nomura Y, Satar G, Hu Z, Nauen R, He SY *et al.*, Molecular evidence for dual pyrethroid-receptor sites on a mosquito sodium channel. *Proc Natl Acad Sci USA* **110**:11 785–11 790 (2013).
31 Vais H, Williamson MS, Hick CA, Eldursi N, Devonshire AL and Usherwood PNR, Functional analysis of a rat sodium channel carrying a mutation for insect knock-down resistance (kdr) to pyrethroids. *FEBS Lett* **413**:327–332 (1997).
32 Soderlund D, Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. *Arch Toxicol* **86**:165–181 (2012).
33 Guedes RNC, Smagghe G, Stark JD and Desneux N, Pesticidal stress in arthropod pests for optimized integraged pest management programs. *Annu Rev Entomol* **61**:43–62 (2016).
34 Desneux N, Decourtye A and Delpuech J-M, The sublethal effects of pesticides on beneficial arthropods. *Annu Rev Entomol* **52**:81–106 (2007).
35 Antwi FB and Reddy GVP, Toxicological effects of pyrethroids on non-target aquatic insects. *Environ Toxicol Pharmacol* **40**:915–923 (2015).
36 Gauthier N, Clouet C, Perrakis A, Kapantaidaki D, Peterschmitt M and Tsagkarakou A, Genetic structure of *Bemisia tabaci* Med populations from home-range countries, inferred by nuclear and cytoplasmic markers: impact on the distribution of the insecticide resistance genes. *Pest Manag Sci* **70**:1477–1491 (2014).
37 Haubruge E and Arnaud L, Fitness consequences of malathion-specific resistance in red flour beetle (Coleoptera: Tenebrionidae) and selection for resistance in the absence of malathion. *J Econ Entomol* **94**:552–557 (2001).
38 Berticat C, Boquien G, Raymond M and Chevillon C, Insecticide resistance genes induce a mating competition cost in *Culex pipiens* mosquitoes. *Genome Res* **72**:41–47 (2002).
39 Guedes RNC, Oliveira EE, Guedes NMP, Ribeiro B and Serrão JE, Cost and mitigation of insecticide resistance in the maize weevil, *Sitophilus zeamais*. *Physiol Entomol* **31**:30–38 (2006).
40 Platt N, Kwiatkowska RM, Irving H, Diabate A, Dabire R and Wondji CS, Target-site resistance mutations (kdr and RDL), but not metabolic resistance, negatively impact male mating competiveness in the malaria vector *Anopheles gambiae*. *Heredity* **115**:243–252 (2015).
41 Coustau C, Chevillon C and ffrench-Constant R, Resistance to xenobiotics and parasites: can we count the cost? *Trends Ecol Evol* **15**:378–383 (2000).
42 Raymond M, Berticat C, Weill M, Pasteur N and Chevillon C, Insecticide resistance in the mosquito *Culex pipiens:* what have we learned about adaptation? *Genetica* **112**:287–296 (2001).
43 Cifuentes D, Chynoweth R and Bielza P, Genetic study of Mediterranean and South American populations of tomato leafminer *Tuta absoluta* (Povolny, 1994) (Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers. *Pest Manag Sci* **67**:1155–1162 (2011).
44 Guedes RNC and Picanço MC, The tomato borer *Tuta absoluta* in South America: pest status, management and insecticide resistance. *Bull OEPP/EPPO Bull* **42**:21–216 (2012).
45 Guedes RNC and Siqueira HAA, The tomato borer *Tuta absoluta*: insecticide resistance and control failure. *CAB Rev* **7**:055 (2012).
46 Hu Z, Du Y, Nomura Y and Dong K, A sodium channel mutation identified in *Aedes aegypti* selectively reduces cockroach sodium channel sensitivity to type I, but not type II pyrethroids. *Insect Biochem Mol Biol* **41**:9–13 (2011).

