An EPG Study of the Probing Behavior of Adult *Bemisia tabaci* Biotype Q (Hemiptera: Aleyrodidae) Following Exposure to Cyantraniliprole

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ABSTRACT Cyantraniliprole is a novel insecticide for control of multiple chewing and sucking insect pest species including the sweetpotato whitefly *Bemisia tabaci* (Gennadius), which is one of the most important polyphagous pests in tropical, subtropical, and Mediterranean regions. This study aims to evaluate the effects of cyantraniliprole on the probing behavior of *B. tabaci* on tomato. Electrical penetration graph data indicated that on plants treated with cyantraniliprole (foliar application), adult whiteflies of the genetic variant Q2 were not able to reach the phloem and consequently did not perform the activities represented by E1 and E2 waveforms, i.e., phloem salivation (during which inoculation of geminiviruses occurs) and phloem sap ingestion (during which geminiviruses are acquired by the whiteflies), respectively. The complete failure of *B. tabaci* biotype Q adults to feed from the phloem of tomato plants treated with cyantraniliprole could be explained by rapid cessation of ingestion because of the mode of action of this insecticide. Overall, these findings indicated that cyantraniliprole might represent a useful new tool for producers to protect tomato plants from damage by *B. tabaci*.

KEY WORDS cyantraniliprole, *Bemisia tabaci*, electrical penetration graph, Q2 genetic variant, tomato yellow leaf curl virus

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is an important pest widely distributed in tropical, subtropical, and Mediterranean regions throughout the world. It is a particularly serious pest in protected environments such as greenhouses, which enable it to survive during the winter in temperate climates in North America and Europe. Numerous genetic variants and at least 36 biotypes have been assigned to the *B. tabaci* species complex (Perring 2001). The most recent concept of *B. tabaci* is that of a complex including >31 cryptic species, most of these previously reported as different biotypes (De Barro et al. 2011, Lee et al. 2013). The most widespread *B. tabaci* biotype is B (now referred to as Middle East–Asia Minor 1 or MEAM1 species), which originated in the Northeast Africa–Middle East–Arabian peninsular region (De Barro et al. 2000) and appeared around 1985 in the United States, replacing the preexisting biotype A (Costa and Brown 1991). In Southern Europe, biotype B coexists with biotype Q (now referred to as Mediterranean or MED species), which is less polyphagous and is thought to have a different origin, i.e., Mediterranean region (Guario et al. 1997). Biotype Q has become the predominant one in Spain since the year 2000 (Simón et al. 2007) and now is widely found in the Mediterranean basin, including Italy (Parrella et al. 2012), Crete (Roditakis et al. 2009), Israel (Horowitz et al. 2003), and Egypt (Farghaly 2010); as well as in East Asia including China and Japan (Zhang et al. 2005, Ueda and Brown 2006); North and Central America including Mexico; the United States; and Guatemala (Martinez-Carillo and Brown 2007, Bethke et al. 2009, Dennehy et al. 2010); and West Africa (Houndété et al. 2010). In many of these regions, biotype Q colonization has resulted in the displacement of biotype B because of the higher insecticide resistance and higher fitness of resistant Q populations (Horowitz et al. 2005, Dennehy et al. 2010, Kontsedalov et al. 2012). Recent studies have shown a considerable genetic variability among mitochondrial lineages of biotype Q. Four genetic variants have been identified, two of

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which, named Q1 (Western Mediterranean populations) and Q2 (Eastern Mediterranean populations) are highly invasive and have spread to nonnative geographic areas. The other two genetic variants, named Q3 and ASL (Africa silverleafing), consist of populations from Burkina Faso only and from Sub-Saharan Africa, respectively (Gueguen et al. 2010). Both Q1 and Q2 have been found in the United States and in Mediterranean countries like Spain and France (Chu et al. 2008, Ahmed et al. 2009, Mouton et al. 2012). Recently, in a greenhouse area of southern Italy, the frequency of Q2 whiteflies has been found to largely exceed that of Q1 whiteflies on intensive greenhouse tomato crops showing strong symptoms of tomato yellow leaf curl disease (Parrella et al. 2013).

The *B. tabaci* species complex has a wide host range of >500 plant species, for which the most polyphagous biotypes (such as A, B, and Q) probably include ≥100 host plants (Brown 2007). *B. tabaci* damages plants by extracting plant photosynthetic materials from phloem sap during ingestion, by excreting a large amount of honeydew upon which saprophytic fungi develop, and mostly by transmitting plant viruses. *B. tabaci* is a vector of >100 plant viruses, recognized as species in the genera *Begomovirus* (family, Geminiviridae), *Crinivirus* (family, Closteroviridae), *Carlavirus* (family, Betaflexiviridae), and *Ipomovirus* (family, Potyviridae) (Brown 2007). Biological performances of *B. tabaci* can be influenced by the occurrence of inherited facultative bacterial endosymbionts, including *Cardinium*, *Hamiltonella*, *Rickettsia*, *Wolbachia*, and *Arsenophonus* (Chiel et al. 2007). Specific composition of the endosymbiont community has been found frequently associated with the different mitochondrial genotypes of *B. tabaci* (Gueguen et al. 2010). Endosymbionts can affect the fitness of *B. tabaci* in different ways (Brumin et al. 2011, Himler et al. 2011), among which are increasing the susceptibility to insecticides (Kontsedalov et al. 2008, Ghanim and Kontsedalov 2009) and also possibly facilitating the transmission of geminiviruses (Gottlieb et al. 2010, Rana et al. 2012).

Within the genus *Begomovirus*, tomato yellow leaf curl virus (TYLCV) has spread beyond its origins in the Eastern Mediterranean region, and has become a major concern for tomato production in the Mediterranean and Caribbean area, in the southeastern United States and in Japan. In the United States, the estimated losses caused by whitefly-transmitted geminiviruses reach ≈20% of tomato production, but in central and South America the damage is much greater, ranging between 30 and 100% of the yield (Antignus 2007). Apparently, some of the geographic spread of TYLCV has been caused by export of virus-infected transplants or plants hosting viruliferous whiteflies, mostly belonging to biotype Q because it is very efficient in viral transmission (Brown, 2007, Matsura and Hoshino 2008, Pan et al. 2012).

TYLCV infection is poorly managed and improved strategies against the vector *B. tabaci* are needed to reduce crop losses and to prevent virus introduction into new areas. TYLCV is managed in the field primarily through the use of insecticides that reduce the whitefly populations (Antignus 2007, Polston and Lapidot 2007). Since the commercial introduction of imidacloprid, neonicotinoids have been the fastest growing class of insecticides, showing excellent contact and systemic activity; therefore, they have been used worldwide for management of *B. tabaci* in horticultural and agronomic production systems (Antignus 2007). The chitin synthesis inhibitor buprofezin and the juvenile hormone analog pyriproxifen have also been successfully used in the United States for >10 yr in rotation with neonicotinoids for control of *B. tabaci* (Toscano et al. 2001).

Another whitefly management insecticide unrelated to neonicotinoids is pymetrozine, a pyridine azomethine compound whose unique mode of action consists of interfering with feeding behavior, causing insects to die of starvation (Harrewijn and Kayser 1997). In fact, reduced spread of TYLCV was shown after pymetrozine treatments (Polston and Sherwood 2003). Recently, spiromesifen and spirotetramat, new spirocyclic phenyl-substituted derivatives of tetronic acid (Nauen and Konanz 2005), also have been used against *B. tabaci.*

Unfortunately, as a consequence of extensive exposure to insecticides, *B. tabaci* has developed resistance to many of these insecticides. The first example of *B. tabaci* resistance to neonicotinoids was detected in biotype Q in the intensive horticultural production system near Almeria, in southern Spain (Cahill et al. 1996a, Elbert and Nauen 2000), and resistance to neonicotinoids is now spread worldwide in both biotypes B and Q (Nauen and Denholm 2005, Nauen et al. 2002) due to overexpression of a cytochrome P450 monooxygenase (Karunker et al. 2009, Gorman et al. 2010, Qiong et al. 2012). This mechanism also induces cross-resistance with pymetrozine (Nauen et al. 2013). Resistance to buprofezin and pyriproxifen has been observed mainly in Israel (Cahill et al. 1996b, Horowitz and Ishaaya 1994) and the United States (Li et al. 2003, Ma et al. 2010), while no resistance to spiromesifen by biotype B has yet been reported in Florida (Mann et al. 2012).

The commercialization of insecticides with new modes of action against *B. tabaci* is advisable to ensure the availability of different effective products from which to choose and rotate for integrated resistance management programs (Castle et al. 2009). Among these new experimental insecticides, sulfoxaflor acts through the activation of nicotinic acetylcholine receptors and is also highly effective against whiteflies resistant to imidacloprid (Babcock et al. 2011, Longhust et al. 2013).

Another new insecticide active against *B. tabaci* with no cross-resistance to neonicotinoids is cyantraniliprole (DuPont Cyazypyr), an anthranilic diamide (Li et al. 2011, Caballero et al. 2013). It is a ryanodine receptor agonist, causing impairment of insect muscle function (Cordova et al. 2006), that results in rapid interruption of feeding, and consequently has the potential to reduce TYLCV transmission in the field (Castle et al. 2009).

Although field and laboratory tests may provide information about mortality caused by an insecticide application, other methods are needed to generate information on how the insecticide affects the feeding behavior of insect vectors (Cameron et al. 2013). Among many methods, the electrical penetration graph (EPG) technique is the most rigorous way to evaluate the effects of an insecticide on the feeding behavior of vectors, and consequently, on their ability to reduce transmission or spread of plant viruses (Nisbet et al. 1993; Costa et al. 2011; He et al. 2011; Butler et al. 2012; Cui et al. 2012; Jacobson and Kennedy 2013a,b).

In the present work, our EPG study aimed to determine the effects of foliar or soil treatments of cyantraniliprole on the feeding behavior of *B. tabaci* adults, using tomato as the host plant. The cyantraniliprole effects were also compared with those produced by application of other insecticides commonly used for whitefly control. The results may be helpful to evaluate the toxicity of cyantraniliprole toward *B. tabaci* and may provide valuable information about the possible effect on the reduction of TYLCV transmission.

Materials and Methods

Insects. The population of *B. tabaci* used in the EPG study was originally collected on the poinsettia, *Euphorbia pulcherrima* Willdenow ex Klotzsch, plants in 2011 from a greenhouse near Ferrara (Italy) and reared in an environmental growth chamber at 24 ± 1°C and a photoperiod of 16:8 (L:D) h on tomato plants, *Lycopersicon esculentum* L. ('Pearson'), at the Department of Life Sciences and Biotechnology, University of Ferrara (Ferrara, Italy). The genotype of *B. tabaci* was determined by molecular analysis of the mitochondrial cytochrome oxidase subunit I (*COI*) gene. DNA was extracted from single adult whiteflies as described by Parrella et al. (2012), and a 866-bp fragment of the *COI* gene was amplified using the primer pair C1-J-2195 and TL2-N-3014 (Frohlich et al. 1999). The genotype of 15 individuals was determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis of *COI* amplicons using *Apo*I endonuclease as described by Parrella et al. (2012). Further characterization of the *B. tabaci* strain used in this bioassay concerned the composition of the facultative endosymbiont community. For each genotyped whitefly, bacterial endosymbionts were screened using specific PCR primers targeting the 16S rRNA gene for *Cardinium, Hamiltonella, Rickettsia,* and *Wolbachia,* and the 23S rRNA gene for *Arsenophonus* (Chiel et al. 2007). All PCRs included a negative control (sterile water instead of DNA) to detect any DNA contamination, and a positive control to avoid false negatives.

Insecticide Applications. Foliar spray application: 1-mo-old tomato plants (≈25 cm in height) were sprayed until runoff with the insecticides to be tested. Plants were left to dry for 1 h and then returned to the greenhouse for 1 d before EPG recording. For this application, 75 mg (AI) liter^{-1} of oil-dispersion formulation of cyantraniliprole, DuPont Benevia , containing 100 g liter^{-1} DuPont Cyazypyr was used.

Soil Application. One milliliter of aqueous insecticide solution was directly injected into the wet soil of the tomato plant pot via micropipette. The 1 ml solution contained 2.5 mg of suspension concentrate formulation of cyantraniliprole (DuPont Verimark, containing 200 g liter^{-} DuPont Cyazypyr). The plants were then returned to the greenhouse for 3 d before EPG recording. Imidacloprid (Confidor 200 SL, Bayer CropScience, Milano, Italy) 150 mg (AI) liter^{-1} and pymetrozine (Plenum 50 WG, Syngenta Crop Protection, Milan, Italy) 250 mg (AI) liter^{-1} in foliar spray application were used as reference insecticides.

Experimental Setup. EPGs of adult whiteflies on tomato plants treated with cyantraniliprole or other insecticides (imidacloprid and pymetrozine) and nontreated plants (untreated control) were performed in the laboratory at 24 ± 1°C, with artificial fluorescent light (4,000 Lux) and a photoperiod of 16:8 (L:D) h. EPGs for 15 whitefly adults (72 h old) per treatment were recorded for 8 h for each treatment and control. Before each experiment, an individual whitefly was collected from the infested tomato leaves on which it was reared and then immobilized in a chilled petri dish. A small drop of water-based silver glue (Wageningen Agricultural University, Wageningen, The Netherlands) on a small piece of acetate sheet (1 by 1 cm) was placed adjacent to the whitefly in the petri dish. A stereomicroscope was mounted over the chilled petri dish so that the whiteflies could be viewed during wire attachment. A fine artist's brush was used to maneuver the whitefly next to the drop of wet silver glue. The whitefly was placed dorsal side up, and the fine condensation at the bottom of the petri dish kept the whitefly in place.

A thin (12.5 μm) gold-wire electrode (Wageningen Agricultural University, Wageningen, The Netherlands), ≈2 cm in length, was held with a pair of forceps under the stereomicroscope: the tip of thin gold-wire electrode was repeatedly dipped into the wet silver glue until a droplet of wet glue formed at the apex. The wire was then carefully maneuvered to bring the glue droplet at the apex of the wire in contact with the whitefly mesonotum. This was done relatively quickly (<10 s) to avoid drying of the droplet and loss of its sticking ability to the whitefly (Walker and Jansen 2000).

The tethered insects were individually placed on the lower surface of terminal plant leaflets (mature leaves), when plants were ≈25 cm in height. The EPG device used was a DC-type, Giga-4 model (Wageningen Agricultural University, Wageningen, The Netherlands) with an input resistance of 1 GΩ. After A–D conversion at 100 Hz (Di710 USB, Dataq, Akron, OH), the EPG signals were stored on a computer hard disk, data acquisition was mediated by PROBE 3 software (for Windows; Wageningen Agricultural University, Wageningen, The Netherlands) and signals analyzed using the same software. The EPG waveforms measured were the same as in Janssen et al. (1989), John-

son and Walker (1999), and Jiang et al. (2000). According to the typical whitefly feeding behavior, the periods of probing (stylet penetration) alternate with periods of nonprobing. During probing, three behavioral phases can be distinguished, each containing one or more waveforms: 1) pathway phase, which includes waveforms A, B, and C (intercellular penetration), and potential drop (pd), which correspond to brief intracellular punctures; 2) xylem phase, or waveform G; and 3) phloem phase, which includes waveforms E1 and E2, related to phloem salivation (during which inoculation of geminiviruses occurs) and phloem ingestion (during which geminiviruses are acquired by the whiteflies), respectively.

EPG Data Analysis. For adults, all nonsequential variables, according to the response variables provided by Backus et al. (2007), were analyzed using nonparametric analysis of variance Kruskal–Wallis test ($P < 0.05$; (software STATISTICA 6, StatSoft, Tulsa, OK). All response variables were averages calculated per insect.

Results

Whitefly Identity. On the basis of the PCR-RFLP analysis of the *COI* gene, the strain of *B. tabaci* used in this experiment was the Q2 mitochondrial type of biotype Q. All whiteflies analyzed showed identical restriction profile and the endosymbiont composition was consistent with the type of infection found in other Q2 populations (Gueguen et al. 2010). Almost all whiteflies (93%) were infected by *Rickettsia*, while 67% of individuals harbored *Wolbachia* (double infection with *Rickettsia*). *Arsenophonus*, *Cardinium*, and *Hamiltonella* were not detected (Fig. 1).

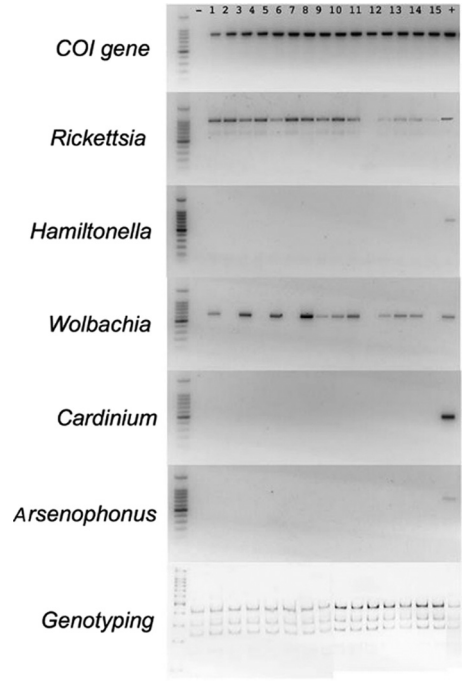

Fig. 1. PCR-RFLP profiles of the COI gene and of five endosymbionts in 15 *B. tabaci* adults.

EPG Recording. Table 1 shows that all whitefly adults tested (100%) probed their stylets within leaf tissues in both insecticide-treated and untreated tomato plants. However, in insecticide-treated plants, the mean nonprobing duration per insect was significantly longer (≈5–6 h) compared with that of untreated control plants (≈3 h), except for pymetrozine. No significant differences were detected between the insecticide treatments (Table 1).

Table 1 shows that the mean pathway waveform duration per insect was significantly higher in untreated control compared with treated samples, except for cyantraniliprole (soil application), but no significant differences were detected for the mean number of pathway events and the mean duration of each pathway event. In contrast, the number of short intracellular punctures (pd waveform), their mean duration per insect, and mean duration per event were significantly lower for all insecticide treatments compared with untreated control. However, no significant differences were found among the treatments, but only between each treatment and the untreated control.

Table 2 shows that in the untreated control, about two-thirds of *B. tabaci* adults were able to perform deep stylet penetrations and reach the phloem (adults performing the E1 waveform). In the plants treated with cyantraniliprole (foliar application), no individual was able to reach the phloem and subsequently perform the activities related to E1 and E2 waveforms. In the other treatments, cyantraniliprole (soil application), imidacloprid, and especially pymetrozine, the insects were able to reach the phloem to varying degrees but significantly less than controls. However, individuals reaching the phloem exhibited a different behavior compared with those on the untreated control plants. In more detail, only on the untreated control were the individuals (37%) able to reach the phloem more than once (with a maximum of three times), within the 8 h of recording. Moreover, for the few individuals able to reach the phloem in the tomato plants treated with insecticides, only the mean number of E1 waveform events per insect was statistically lower than that for untreated control, while the mean E1 waveform duration per insect and the mean E1 waveform duration per event were not statistically different from the untreated control (Table 2). It has been previously reported that the threshold duration of the salivation phase (E1 waveform) observed for successful inoculation of TYLCV by *B. tabaci* on tomato plants is 1.8 min (Jiang et al. 2000). In the untreated control, 37.5% of individuals were able to perform E1 waveform events longer than 1.8 min, while for imidacloprid and pymetrozine only 14.2 and 16.6% of the recorded individuals, respectively, were able to exceed this threshold. No individual was able to exceed this threshold in the plants treated with cyantraniliprole.

All individuals performing E1 waveforms were also able to perform E2 waveforms, but the mean E2 wave-

Table 1. Comparison of the nonprobing (mean ± SEM; min–max) and pathway (intercellular penetration; A–B–C waveforms) and brief intracellular punctures (pd waveform; mean ± SEM; min–max), measured by EPG (480 min of recording) of *B. tabaci* biotype Q2 on tomato plant after insecticide treatment

	Probing or stylet penetration											
		Non probing (np)			Pathway phase							
					A-B-C waveform				pd waveform			
	%[a]	Mean non-probing duration	Mean number of non-probing events	Mean duration of each non-probing event	%[a]	Mean pathway waveform duration	Mean number of pathway events	Mean duration of each pathway event	%[a]	Mean pd waveform duration (seconds)	Mean number of pd waveform events	Mean pd duration per event (seconds)
Untreated control	100	179.5 ± 25.4a (59.6–382.8)	55.7 ± 7.4ns (5.0–107.0)	5.5 ± 1.6a (0.6–23.1)	100	199.2 ± 31.1a (45.4–416.7)	56.7 ± 7.2ns (7.0–107.0)	4.2 ± 0.9ns (1.1–16.8)	81.3	124.6 ± 20.2a (18.7–259.7)	8.5 ± 1.6a (0.0–17.0)	12.28 ± 1.46a (6.2–25.6)
Cyantraniliprole (foliar application)	100	376.8 ± 22.7b (205.4–461.7)	54.9 ± 9.6ns (2.0–115.0)	54.2 ± 35.7b (2.1–52.4)	100	98.8 ± 20.4bcd (18.3–234.4)	54.7 ± 9.9ns (2.0–119.0)	5.5 ± 3.9ns (0.7–52.2)	15.4	5.0 ± 1.4b (3.5–6.5)	0.2 ± 0.1b (0.0–1.0)	5.0 ± 1.4b (3.5–6.5)
Cyantraniliprole (soil application)	100	340.0 ± 18.6b (216–449.0)	63.9 ± 17.4ns (6.0–227.0)	16.7 ± 6.4ab (1.3–74.8)	100	138.0 ± 18.5ab (31.0–263.2)	63.7 ± 17.5ns (5.0–227.0)	5.3 ± 1.9ns (0.7–25.7)	15.4	66.1 ± 42.2b (23.8–108.4)	0.6 ± 0.6b (0.0–8.0)	12.7 ± 0.8b (11.9–13.5)
Imidacloprid	100	370.9 ± 34.7b (232.5–462.8)	36.3 ± 10.6ns (9.0–83.0)	73.6 ± 55.0b (3.4–401.2)	100	88.3 ± 29.6cd (17.2–247.4)	37.0 ± 10.5ns (8.0–83.0)	3.2 ± 1.0ns (0.7–8.4)	42.9	18.1 ± 9.3b (4.1–35.8)	1.3 ± 1.0b (0.0–7.0)	7.8 ± 3.2b (4.1–14.3)
Pymetrozine	100	295.4 ± 65.7ab (83.8–452.7)	54.3 ± 8.9ns (2.0–84.0)	5.8 ± 1.5ab (1.7–11.9)	100	89.9 ± 17.5cd (27.3–143.5)	55.8 ± 8.7ns (31.0–84.0)	1.8 ± 0.4ns (0.7–3.0)	33.3	14.7 ± 3.8b (10.8–18.5)	0.8 ± 0.5b (0.0–3.0)	5.8 ± 0.3b (5.4–6.1)

Time in minutes for nonprobing, A–B–C waveforms, and in seconds for pd waveform. All variables are averages calculated per insect.
Means that share the same letter in the same column are not significantly different (ns) (Kruskal–Wallis test, $P < 0.05$).
[a] Percentage of individuals that produced the waveform type out of the total number of individual recorded per treatment.

Table 2. Comparison of xylem ingestion (G waveform), phloem salivation (E1 waveform), and phloem ingestion (E2 waveform; mean ± SEM; (min–max) measured by EPG (480 min of recording) of *B. tabaci* biotype Q2 on tomato plant after insecticide treatment

	Probing or stylet penetration											
		Xylem phase			Phloem phase							
		G waveform			E1 waveform (gemini virus inoculation)				E2 waveform (gemini virus acquisition)			
	%[a]	Mean G waveform duration	Mean no. of G waveform events	Mean G duration per event	%[a]	Mean E1 waveform duration	Mean no. of E1 waveform events	Mean E1 duration per event	%[a]	Mean E2 waveform duration	Mean no. of E2 waveform events	Mean E2 duration per event
Untreated control	25.0	35.3 ± 11.1ns (6.0–59.7)	0.6 ± 0.3ns (0.0–4.0)	13.2 ± 2.8ns (6.0–19.5)	68.8	8.0 ± 4.2ns (0.0–49.0)	1.3 ± 0.3a (0.0–3.0)	3.6 ± 1.6ns (0.6–16.3)	68.8	109.5 ± 28.0a (0.0–310.8)	0.9 ± 0.2a (0.0–3.0)	79.8 ± 16.4a (17.1–167.8)
Cyantraniliprole (foliar application)	30.8	14.3 ± 8.9ns (1.5–40.2)	0.5 ± 0.3ns (0.0–4.0)	12.2 ± 9.4ns (1.5–40.2)	0	0ns	0b	0ns	0	0b	0b	0b
Cyantraniliprole (soil application)	15.4	8.9 ± 0.1ns (8.8–8.9)	0.2 ± 0.1ns (0.0–1.0)	8.9 ± 0.1ns (8.8–8.9)	7.7	0.9 ± 0.9ns (0.9–0.9)	0.1 ± 0.1b (0.0–1.0)	0.9 ± 0.9ns (0.9–0.9)	7.7	7.1 ± 7.1b (7.1–7.1)	0.1 ± 0.1b (0.0–1.0)	7.1 ± 7.1b (7.1–7.1)
Imidacloprid	14.3	122.2 ± 122.2ns (122.0–122.0)	0.1 ± 0.1ns (0.0–1.0)	122.2 ± 122.2ns (122.0–122.0)	14.3	7.6 ± 7.6ns (7.6–7.6)	0.1 ± 0.1b (0.0–1.0)	7.6 ± 7.6ns (7.6–7.6)	14.3	16.0 ± 16.0b (16.0–16.0)	0.1 ± 0.1b (0.0–1.0)	16.0 ± 16.0b (16.0–16.0)
Pymetrozine	50.0	56.3 ± 37.7ns (18.7–94.0)	0.8 ± 0.5ns (0.0–3.0)	101.5 ± 60.0ns (22.0–219.0)	33.3	2.3 ± 1.2ns (1.1–3.5)	0.3 ± 0.2b (0.0–1.0)	2.3 ± 1.2ns (1.1–3.5)	33.3	56.3 ± 37.7b (18.7–94.0)	0.3 ± 0.2b (0.0–1.0)	56.3 ± 37.7ab (18.7–94.0)

Means that share the same letter in the same column are not significantly different (ns) (Kruskal–Wallis test, $P < 0.05$).
[a] Percentage of individuals that produced the waveform type out of the total number of individual recorded per treatment.

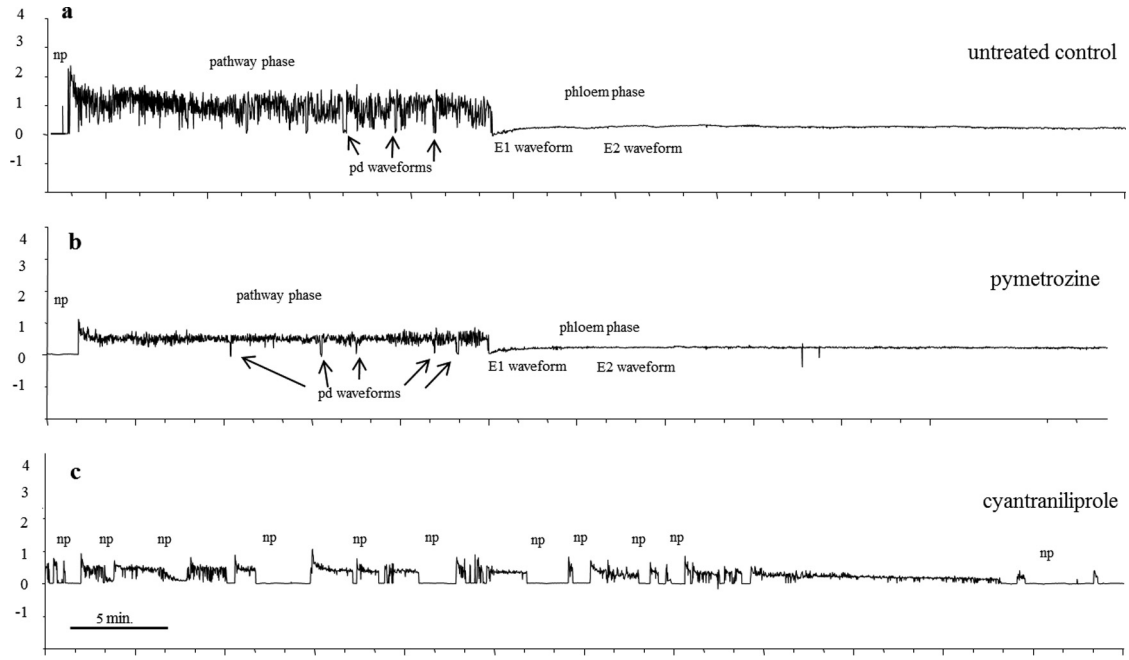

Fig. 2. Overview of a 1-h recording by EPG of adults of *B. tabaci* biotype Q2 on untreated tomato plants (a), and tomato plants treated with pymetrozine (b) and cyantraniliprole (c). The waveform amplitude in volts is reported on y-axis and time on x-axis. Scale bar indicates 5 min time interval.

form duration per insect, the mean number of E2 waveform events, and the mean E2 duration per event were statistically shorter than in the untreated control, except for pymetrozine, where the phloem ingestion (E2) was rather long (Table 2).

In Fig. 2, it is possible to observe a decreased amplitude of A–B–C waveforms for all insecticides tested, unlike what was observed in the untreated control when the same gain was used for the recordings.

Discussion

Effective management of insect vectors of plant pathogens is critical for the guest to minimize vector-borne disease in crops (Perring et al. 1999). Indeed, most insecticides reduce the number of vectors, but not all of them also hamper the feeding of insects before death, allowing pathogen transmission by insect vectors. Jiang et al. (2000) clarified why whitefly-transmitted viruses are so difficult to control with chemical insecticides alone—because only one viruliferous adult is able to efficiently transmit TYLCV with only one phloem contact lasting ≈2 min. Therefore, suitable pest management strategies should integrate an efficient control of whitefly-transmitted viruses by interfering both with virus acquisition and inoculation cycles. Besides lethal toxicity, some insecticides exhibit other interesting properties that affect feeding behavior or interfere with virus transmission. The reduction in efficiency of transmission of TYLCV by *B. tabaci* primarily depends on how much the insecticide is active in preventing any phloem penetration and, above all, in hindering the repetition of this behavior for long periods before insect death (Jiang et al. 2000).

The new diamide insecticide cyantraniliprole (DuPont Cyazypyr), a ryanodine receptor agonist, both controls *B. tabaci* population and interferes with pest feeding behavior (Cordova et al. 2006, Cameron et al. 2013). Recent field trials on different crops confirm the direct action of this insecticide on *B. tabaci* populations in Italy (Wiles et al. 2012) and India (Mandal 2012). With the use of a fluorescent technique, Cameron et al. (2013) recently demonstrated that cyantraniliprole produced a significant reduction of *B. tabaci* nymphs feeding when compared with nymphs fed on plants treated with other insecticides such as imidacloprid or spirotetramat. In addition, cyantraniliprole is not affected by commonly occurring resistance mechanisms that are known to confer resistance to other insecticide chemistries. In laboratory tests, both biotypes B and Q showed susceptibility to cyantraniliprole on *B. tabaci* populations from Arizona and Florida that are resistant to imidacloprid and pyriproxyfen (Li et al. 2011, Caballero et al. 2013). The same results were obtained in the European strain of *B. tabaci* biotype Q (Bielza et al. 2011). Besides the direct toxic action on *B. tabaci*, cyantraniliprole showed field reduction of TYLCV transmission in the United States (Castle et al. 2009, Schuster et al. 2010). The same result was obtained for tomato spotted wilt virus transmitted by thrips on pepper (Jacobson and Kennedy 2011, 2013a).

In this EPG study, all *B. tabaci* adults biotype Q2 exposed to tomato plants with cyantraniliprole foliar application failed to reach the phloem sieve elements

and perform their salivation (E1 waveform) and ingestion (E2 waveform) activities. The failure of adults to feed from the phloem in comparison with untreated control is because of the fast action of cyantraniliprole: most of its effects are via ingestion, but the insecticide could also cause intoxication via contact (as shown in several insect groups including nonsucking insects such as fruit flies). Another relevant observation during stylet penetration in the intercellular pathway phase is the low tendency to perform brief intracellular punctures (pd waveform) by whiteflies in the treated plants. Indeed, in plants treated with different insecticides, all recordings showed a general decrease in amplitude of pathway waveforms in comparison with untreated control (Fig. 2a–c). Such results had previously been observed in a study on aphids with pymetrozine, and were attributed to negative effects of the insecticide on the aphid's salivary pump (Harrewijn and Kayser 1997).

In our study, the final consequence of cyantraniliprole intoxication was that *B. tabaci* adults were unable to feed from their target tissue. If stylets are prevented from reaching the phloem, the inoculation related to phloem salivation and the acquisition associated with phloem ingestion of a phloem-restricted virus such as TYLCV does not occur. However, further field trials are required to test this hypothesis.

Indeed, geminivirus inoculation occurs when the insect salivates into a phloem sieve element (E1 waveform) by its stylets, during the first period of sieve element penetration, while geminivirus acquisition occurs only upon phloem sap ingestion (E2 waveform). For *B. tabaci*, a strong correlation exists between the efficiency of inoculation of TYLCV and the two measures of probing: mean duration per insect of salivation within sieve elements (E1 waveform duration) and the number of phloem penetrations by the vector (number of E1 waveform events per insect). As previously reported, for a successful inoculation of TYLCV by *B. tabaci* on tomato plants the salivation phase (E1 waveform) should continue for at least 1.8 min (Jiang et al. 2000).

The EPG technique was used by Johnson et al. (2002) to study the mechanism of transmission of a semipersistent virus, lettuce chlorosis virus, family Closteroviridae, by *B. tabaci.* In this case, it was shown that inoculation also occurs during phloem phase.

It is also possible that brief intracellular punctures of mesophyll tissues by *B. tabaci* (pd waveform) are involved in the transmission of nonpersistent viruses in the genus *Ipomovirus* or *Carlavirus* (family Potyviridae), such as the cowpea mild mottle virus, because nonpersistent viruses are not restricted to the phloem and are mainly detected in the epidermis and mesophyll (Muniyappa and Reddy 1983). However, experimental evidence for this assumption is lacking and further studies are required (Fereres and Moreno 2009).

Overall, the data presented indicate that cyantraniliprole may represent a useful new tool for producers to protect tomato plants from *B. tabaci* feeding, and possibly to reduce infection by TYLCV and other viruses transmitted in both persistent and nonpersistent manners by this species complex. Based on the above results, cyantraniliprole is expected to be effective within an integrated approach to virus management. Its unique mechanism of action and the absence of cross-resistance with neonicotinoids (Bielza et al. 2011, Li et al. 2011, Caballero et al. 2013) make it a useful tool for plant protection in integrated resistance management and integrated pest management programs.

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