RESEARCH

Impact of biopesticides on the probing and feeding behavior of *Aphis gossypii*

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Abstract

Background Pesticides-induced mortality has traditionally been the toxicological endpoint on which a chemical is selected for pest management strategies. However, the exposure to a pesticide might also cause behavioral alterations that can dramatically affect the dynamics of pest-plant interaction. Understanding these non-lethal effects is crucial for developing comprehensive and sustainable pest control measures.

Methods Here, using the Electrical Penetration Graph (EPG) technique, we evaluated whether biopesticides routinely used in organic or conventional pest management might alter the probing and feeding behavior of the cotton aphid *Aphis gossypii* on treated plants. The post exposure persistence of feeding alterations when moving the insect onto clean plants was also assessed. The tested biopesticides were chosen for being aphicides or because used in those crops hosts of *A. gossypii*. Generalised linear mixed models were fitted to analyse the effects of biopesticides on the probing and feeding behavior of aphids, using untreated control data as a model baseline.

Results Residuals of *Bacillus thuringiensis*, *Beauveria bassiana*, and sulfur significantly affected the dynamics of the stylets intercellular route compared to the control (namely, shorter pathways, fewer and shorter brief intracellular stylets probes, higher and longer derailed stylets events). Mineral oil, orange essential oil and pyrethrin delayed or impaired the onset of phloem sap ingestion. On the other hand, copper and potassium salts promoted feeding. Pyrethrin was the only biopesticides inducing persistent behavioral alterations, with insects displaying a higher frequency of occurrence of xylem ingestion events when moved to untreated plants after exposure.

Conclusions Overall, this study demonstrates that biopesticides modulate the aphid-plant interactions by impairing or facilitating the exploitation of the host plant, also affecting the patterns conducive to plant pathogens transmission. This study also highlights the importance of considering in toxicological studies the impact of all the compounds the pest could be exposed to within the agroecosystem, including those not designed for that specific pest.

Keywords Electrical Penetration Graph technique (EPG), Sublethal effects, Botanicals, Entomopathogens, Organic farming

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Background

Synthetic pesticides have long been the major tool for controlling arthropod pests, diseases, and weeds in agricultural cropping systems worldwide (Guedes et al. 2016). However, the strong reliance on these chemicals has led to an increase in pest resistance (Mahas et al. 2022), secondary pest outbreaks and resurgence, besides posing a serious threat to the environment, human health, and the provision of ecosystem services (Desneux

et al. 2007; Rani et al. 2021). Therefore, sustainable alternatives to synthetic pesticides for pest management are urgently needed.

In this context, the interest in biopesticides is growing significantly in parallel with the global endorsement of Integrated Pest Management (IPM) as the paradigm for crop protection (Fira et al. 2018). Biopesticides are defined as substances derived from naturally occurring materials and organisms such as plants, animals, bacteria and minerals (Copping and Menn 2000). They are reported to have low environmental impacts, rapid degradation, low toxicity for humans and reduced acute toxicity on beneficial insects (Monsreal-Ceballos et al. 2018). Nevertheless, the efficacy of biopesticides, and of pesticides in general, is often evaluated mainly in terms of acute toxicity, thus overlooking sublethal (physiological and behavioral) effects on the target pest (Guedes et al. 2016), including the possible alteration of probing behaviors underlying host acceptance and, in case of pest vectors of plant disease, pathogen transmission (Mokrane et al. 2020). Indeed, pesticides can interfere with behaviors underlying host plant location and acceptance by the exposed insects in different ways, repelling the insect or masking the cues leading to host plant recognition. This reduces the olfactory capacity of insects and prevents or promotes probing and feeding (Desneux et al. 2007).

Any toxicant causing sublethal effects could therefore interfere with or disrupt insect-plant interaction, as well as promoting feeding, potentially affecting the overall pest population dynamics (Passos et al. 2022). The interference of biopesticides with pests' physiology and behavior can open new scenarios for their use in rational and sustainable pest management strategies as effective alternatives to synthetic pesticides. Additionally, toxicological studies are generally designed to assess the efficacy of insecticidal compounds on target species, while other target insects could be exposed to other types of agrochemicals, including fungicides, which are not developed to display a toxic action against insect pests (Ziaei et al. 2017). Nevertheless, even these molecules could affect the insects in subtle ways, eluding the current understanding. This is crucial information for reaching the final goal of transitioning toward sustainable integrated pest control packages.

Therefore, the aim of the present study was to evaluate whether some of the biopesticides commonly used in both organic and conventional agriculture, including substances applied to control aphids, insecticides targeting pests other than aphids, and fungicides, might alter aphids' probing and feeding behavior and aphid-plant interaction. The occurrence of alterations in aphid behavioral patterns first on treated plants (exposure phase), and then on untreated plants after the access to treated ones (post-exposure phase or recovery bioassay) was investigated. We used as study model the polyphagous pest *Aphis gossypii* Glover (Hemiptera: Aphidoidea) that among the general sup-sucking damage is considered an important vector of various plant diseases, such as the Citrus Tristeza Virus (Campolo et al. 2014).

Methods

Plant and aphid rearing

Zucchini plants (*Cucurbita pepo* L., variety Zucchino bianco di Sicilia) were grown in a greenhouse facility, in plastic pots filled with a mixture of peat and perlite (1:1) (24 °C, RH) and irrigated daily. Ten-day old zucchini plants (first true leaf stage) were used for the Electrical Penetration Graph (EPG) recording.

Aphis gossypii (colony established at CIHEAM Bari in 2019) was reared on 2-week-old zucchini seedlings in plastic and mesh rearing cages (30 × 25 × 30) in a climatic chamber at a 24 ± 2 °C temperature, 60% RH and photoperiod of 16:8 (Light: Dark). The plants were replaced every 2 weeks. Five-day-old apterous adults were used for the EPG recording.

Tested biopesticides

The focus of this study was on the biopesticides' impact on aphids probing and feeding behavior, particularly *Aphis gossypii*, including products not specifically designed/applied against aphids, but to which aphids are indeed exposed to, i.e., fungicides, or entomopathogens not supposed to target aphids, etc.… Hence, in the present study, the biopesticides commonly used in organic and conventional agriculture in citrus orchards to control aphids, other target pests and/or fungi were selected to assess whether these products, which might not target aphids, can cause significant alterations in probing behaviors underlying host plant selection, acceptance, and transmission of aphid-borne plant viruses.

The biopesticides were tested at their maximum label rate and were: (i) Azadirachtin (1.5 ml/L); (ii) *Beauveria bassiana* (1 ml/L); (iii) *Bacillus thuringiensis* (1 g/L); (iv) Copper (2.5 g/L); (v) Orange essential oil (OEO) (6 ml/L); (vi) Mineral oil (20 ml/L); (vii) Potassium salt of fatty acids (4 ml/L); (viii) Pyrethrin (1.5 ml/L); (ix) Sulfur (2.5 g/L); and tap water was used as untreated control. Details on the biopesticides used (commercial name, active ingredient, dose used, mode of action, target pest, and their sublethal effects on *A. gossypii* observed in the present study) are reported in additional file 1 (Table S1). These compounds were chosen among those approved for use in EU organic agriculture under EU Regulation (EC) No 889/2008.

Zucchini plants were sprayed by a hand-handle sprayer until runoff and left drying under controlled laboratory

conditions (24 °C, RH) for 24 h before the EPG recording (see details below).

Probing and feeding behavior of aphids on treated zucchini plants and recovery bioassay

The effect of the biopesticides on the probing (all the patterns from stylet insertion into the host plant to withdrawal) and feeding (phloem activities, i.e., salivation in and passive ingestion from sieve elements) behavior of *A. gossypii* was evaluated using the EPG technique, exposing the insects to plants previously sprayed.

Aphis gossypii were collected with a paintbrush from zucchini plants. Afterward, the insects were immobilized with a vacuum device (Welch—WOB-L 2534) (Wolflabs, Pocklington, UK), then attached to a thin gold wire (18 μm diameter, 3 cm length), using a water-based silver glue (EPG-Systems, Wageningen, The Netherlands). The gold wire was glued, with acetone-based silver glue (Ted Pella, INC), to a copper wire (2 cm length) welded to a brass nail. After tethering, aphids were starved for one hour. The aphids were placed on the abaxial side of the plant leaf, and their probing and feeding behavior was recorded for 8 h. At the end of the recording, individuals' survival was checked and still living aphids were starved for one hour. In the meantime, the electrode and wiring quality was checked. Then, the aphids were transferred to untreated zucchini plants for another 8 h EPG recording (recovery bioassay). Twenty to twenty-three replicates per treatment were performed, with single combinations of aphids/plants, i.e., one plant per tested aphid. The position of each treated plant was switched at each replicate to avoid any positional bias. A recording, i.e., a replicate, was considered valid, thus included in the analysis, when the aphid was active (crawling, probing, and/or touching the plant with the labium) for at least the first hour.

Aphid probing and feeding behavior was monitored using a Giga-8dd device (EPG Systems, Wageningen, The Netherlands). Each replicate corresponded to a single combination of aphid-plant. Six to eight channels (i.e., 6 to 8 plants, one plant per treatment) were used per EPG round. Recordings were performed inside a Faraday cage, in an acclimatized room (24 ± 2 °C, 60% RH) with the lights on during the whole period of the recording. EPG signals were recorded and analyzed using Stylet + software for Windows. The EPG waveforms considered were: np (non-probing behavior), C (stylet pathway, i.e. stylets intercellular penetration searching for phloem vessels), pd (potential drop, i.e. brief intracellular probes performed during the intercellular pathway to evaluate the chemical components of the host plant) (Jiménez et al. 2020b), E1e (extracellular salivation, i.e. salivation events occurring out of sieve elements), E1 (phloem salivation), E2 (phloem ingestion), G (xylem feeding), waveform F

(derailed stylets, i.e. indicating difficulties in the intercellular route toward sieve elements), phloem-pd (potential drops in phloem, i.e. brief stylets penetration within phloem vessels and/or companion cells possibly aimed at sense the chemical constituents of phloem sap) (Jiménez et al. 2020b) and short-E1 (phloem salivation events lasting few seconds and not followed by phloem ingestion) (Jiménez et al. 2020a).

Differences in probing and feeding behavior compared to the water control (either on treated plant, or on the untreated plant post-exposure) were evaluated by calculating EPG non-sequential and sequential variables as described by Backus et al. (2007). Namely, per each waveform, the following non-sequential variables were calculated and compared: (i) number of waveform events per insect (NWEI) (namely, the sum of the number of events of a particular waveform during the 8 h recording); (ii) waveform duration per insect (WDI) (namely, the sum of duration of each event of a particular waveform during the 8 h recording); (iii) waveform duration per event per insect WDEI (namely, the average duration of single waveform events); (iv) proportion of insects performing a specific waveform (PPW); (v) total probing time; (vi) number of probes. The sequential variables considered were: (i) time to first probe from start of EPG; (ii) time from the beginning of the first probe to first pd; (iii) time from start of EPG to first E1; (iv) time from first probe to first E1; (v) time from start of EPG to first E2; (vi) time from start of EPG to first sustained E2 (longer than 10 min); (vii) time from first probe to first E1; (viii) time from first probe to first E2; (ix) time from first probe to first sustained E2; (x) time from start of EPG to first phloem-pd; (xi) time from the beginning of first probe to first phloem-pd; (xii) and time from first phloem-pd to first E2. All behavioral data were processed using the MS Excel Workbook for automatic EPG data calculation, developed by Sarria et al. (2009).

Statistical analyses

Statistical analyses were carried out using R, version 4.1.0 (R Core Team, 2021). Mortality following biopesticide exposure and feeding behavioral differences between the biopesticides and the control (i.e., sequential, and non-sequential EPG variables) were analysed by a Generalised Linear Mixed Model using Template Model Builder (GLMM.TMB), using the treatment as fixed factor, and untreated control data as a model baseline. When necessary, the data were transformed with ln $(x + 1)$ or $\sqrt{x}$ to reduce heteroscedasticity and improve normal distribution. Models were run using the "glmmTMB" package, while residual distribution was checked using the "DHARMa" package. Summary statistics of significant EPG variables are reported in additional file S2.

Additional file S3 reports the significant PPW (proportion of individuals that performed a specific waveform). Figure 1 illustrates the progression of spittlebugs' probing activity over time, examined at 30-min intervals for all EPG-tested treatments.

Results

Probing and feeding behavior of *Aphis gossypii* on sprayed plants

Aphid mortality due to exposure to biopesticides was not significantly different between the control and the tested biopesticides. Number of dead individuals per treatment is reported in additional fileS4. Replicates in which aphids did not engage in any probing activity but remained alive throughout the eight hours of the EPG recordings were excluded from the statistical analysis (Additional file S5) (Fig. 1).

Non-sequential EPG variables

The analysis of non-sequential EPG variables showed that the total time spent by aphids probing (total probing time) was significantly shorter on plants treated with mineral oil (370.54 ± 18.25 min) compared to the control (437.69 ± 13.56 min) ($z = -2.2$, $p = 0.030$). Moreover, the number of probes performed was higher on mineral oil-treated plants (29.5 ± 3.36) compared to control plants (14 ± 2.58) ($z = 2.39$, $p = 0.017$).

Furthermore, the number of non-probing events (np NWEI) was significantly higher on plants treated with mineral oil (30 ± 3.39) compared to the control (14 ± 2.62) ($z = 2.45$, $p = 0.014$). Moreover, the total duration of non-probing (np WDI) was marginally longer than the control (42.31 ± 13.56 min) on mineral oil-treated plants (109.46 ± 18.25 min) ($z = 1.91$, $p = 0.056$).

The total duration (WDI) of the stylets' pathway (C waveform) was shorter than the control (150.28 ± 12.19 min) on plants treated with *B. thuringiensis* (126.56 ± 22.5 min; $z = -2.064$; $p = 0.039$) and potassium salts (114.43 ± 21.55 min; $z = -2.139$; $p = 0.032$). Additionally, the average duration of a single stylets pathway event (C WDEI) was shorter than the control (8.82 ± 0.88 min) for aphids exposed to residues of *B. thuringiensis* (5.75 ± 0.93 min; $z = -2.726$; $p = 0.006$), *B. bassiana* (6.18 ± 0.43 min; $z = -2.345$; $p = 0.019$), pyrethrin (6.44 ± 0.63 min; $z = -2.711$; $p = 0.006$) and sulfur (5.97 ± 0.62 min; $z = -2.979$; $p = 0.003$). The number of pathway events (C NWEI) was greater on mineral oil-treated plants (32.5 ± 3.56) compared to control plants (18 ± 2.75) ($z = 2.259$, $p = 0.023$) (Figs. 2, 3).

The total duration and the number of pd waveforms (pd WDI; pd NWEI) were significantly reduced on plants treated with *B. thuringiensis* (pd WDI: 3.45 ± 1.28 min; $z = -2.109$; $p = 0.034$; pd NWEI: 53 ± 19.06; $z = -2.072$;

$p = 0.038$) compared to control plants (pd WDI: 8.43 ± 0.87 min; pd NWEI: 115 ± 13.04). No significant differences were observed in the number (NWEI) and durations (WDI and WDEI) of phloem-pds.

E1e and short E1 waveforms were not observed in the EPG recordings. Moreover, none of the biopesticides tested induced significant alterations of the dynamics of phloem salivation (E1 waveform).

The duration of either the total (E2 WDI) or the single phloem ingestion bouts (E2 WDEI) were significantly longer than the control (E2 WDI: 158.18 ± 25.79 min; E2 WDEI: 127.14 ± 20.94 min) on plants treated with potassium salts (E2 WDI: 313.12 ± 34.52 min; $z = 2.251$, $p = 0.024$; E2 WDEI: 313.12 ± 35.57 min; $z = 3.083$; $p = 0.002$).

On the other hand, the proportion of individuals that produced E2 waveforms (E2 PPW) was significantly lower on plants treated with mineral oil (50%; $z = -2.438$; $p = 0.015$), OEO (58%; $z = -2.039$; $p = 0.041$) and pyrethrin (56%, $z = -2.146$, $p = 0.032$) compared to the control (87%).

The number of F events (F NWEI) was significantly higher on plants treated with *B. thuringiensis* and OEO (3 ± 1.11, $z = 2.113$, $p = 0.035$; 2 ± 0.58, $z = 2.349$, $p = 0.019$, respectively) compared to the control (1 ± 0.2). Additionally, the total duration of F waveform (F WDI) was significantly longer on plants treated with *B. thuringiensis* (119.88 ± 46.14 min, $z = 3.814$, $p = 0.0001$), *B. bassiana* (66.17 ± 21.64 min, $z = 2.901$, $p = 0.003$), OEO (68.54 ± 38.57 min, $z = 2.868$, $p = 0.004$), and sulfur (55.01 ± 37.69 min, $z = 3.254$, $p = 0.001$) compared to the control (22.29 ± 9.27 min). The average duration of single F event (F WDEI) was significantly longer on plants treated with *B. thuringiensis* (36.28 ± 25.68, $z = 2.22$, $p = 0.03$) compared to the control (13.55 ± 9.72).

Sequential EPG variables

Aphids exposed to mineral oil and OEO residues required a significantly longer time (either calculated from the start of the EPG recording or from the first probe) to perform the first phloem ingestion (E2) and initiate a sustained ingestion (sustained E2) (Mineral oil: Time to 1st E2: 470.31 ± 35.39 min, $z = 2.01$, $p = 0.045$; Time from 1st probe to 1st E2: 464.48 ± 37.02 min, $z = 2.24$, $p = 0.025$; Time to 1st sustained E2: 470.31 ± 35.39 min, $z = 2.4$, $p = 0.016$; Time from 1st probe to 1st sustained E2: 464.48 ± 37.02 min, $z = 2.17$, $p = 0.030$; OEO: Time to 1st E2: 427.43 ± 35.5 min, $z = 1.923$, $p = 0.049$; Time from 1st probe to 1st E2: 425.66 ± 34.86 min, $z = 2.21$, $p = 0.027$; Time to 1st sustained E2: 427.43 ± 35.5 min, $z = 2.11$, $p = 0.035$; Time from 1st probe to 1st sustained E2: 425.43 ± 34.86 min, $z = 2.13$, $p = 0.033$) compared to
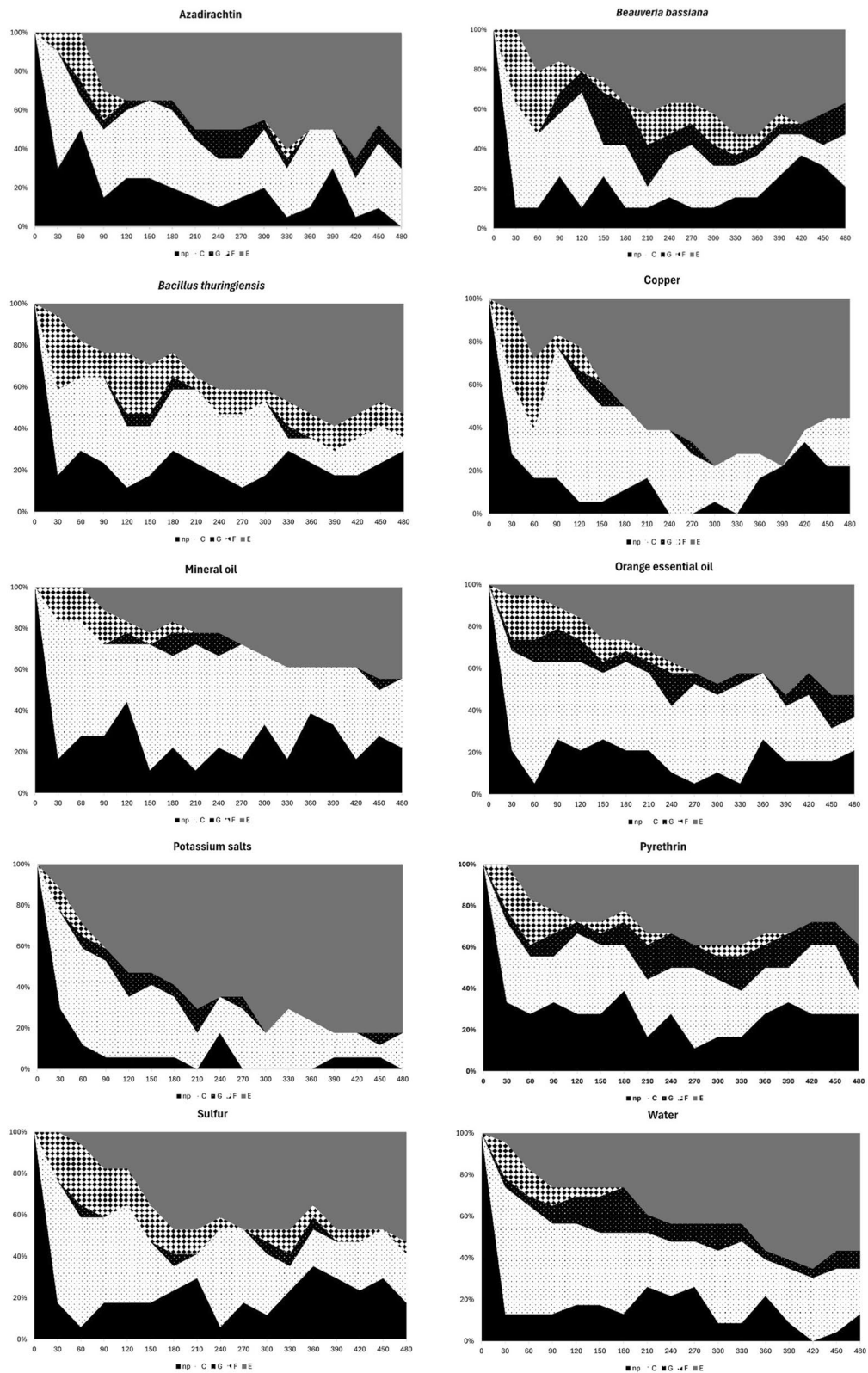
Fig. 1 The progression of spittlebugs' probing activity over time, examined at 30-min intervals for all EPG-tested treatments
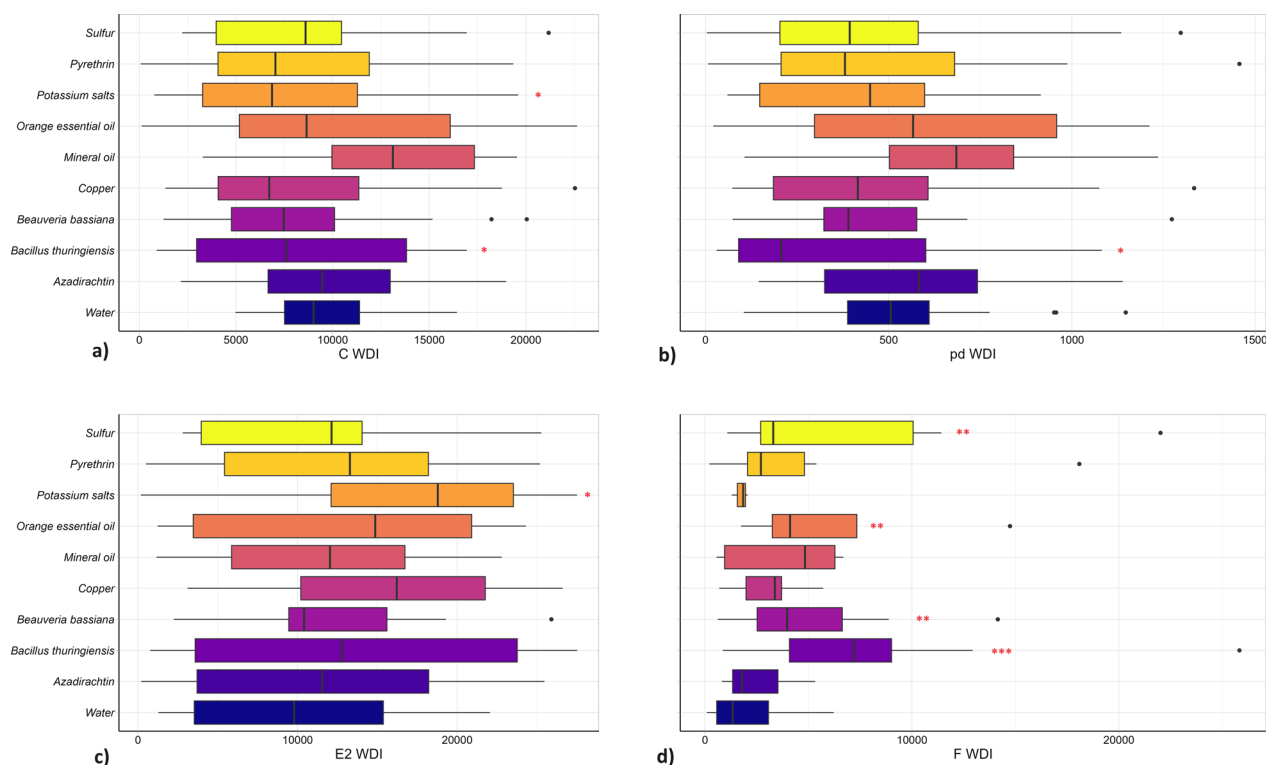
Fig. 2 Waveforms duration per insect (WDI)

the control (Time to 1st E2: 227.83 ± 30.44 min; Time from 1st probe to 1st E2: 223.45 ± 30.28 min; Time to 1st sustained E2: 236.38 ± 30.46 min; Time from 1st probe to 1st sustained E2: 231.41 ± 30.31 min).

By contrast, the time needed for the first phloem pd (either calculated from the beginning of the EPG recording or from the first probe) was significantly shorter for aphids given access to copper and potassium salts-treated plants (Time to 1st ppd: 153.33 ± 31.13 min, z = − 2.22, p = 0.026; Time from 1st probe to 1st ppd: 144.58 ± 30.19 min, z = − 2.23, p = 0.026; Time to 1st ppd: 188.99 ± 44.62 min, z = − 2.31, p = 0.020; Time from 1st probe to 1st ppd: 181.37 ± 44.15 min, z = − 2.51, p = 0.012, respectively) compared to the control (Time to 1st ppd: 338.07 ± 35.63 min; Time from 1st probe to 1st ppd: 336.99 ± 35.67 min) (Fig. 4).

Aphis gossypii recovery bioassays

Probing and feeding behavior of aphids on untreated plants after being exposed to sprayed plants was significantly different from the control only for pyrethrin. Aphids that were previously exposed to pyrethrin-treated plants showed a significant increase in the proportion of

individuals performing xylem feeding events (G PPW) (63%; z = 2.018; p = 0.044) compared to the control (29%).

Discussion

The control of phytophagous insects strongly relies on the use of synthetic pesticides. Besides the development of resistance by pests and the hazardous effect on the environment, human health, and ecosystem services, these chemicals are strictly prohibited in organic agriculture (Isman 2006). Biopesticides represent a putatively sustainable tool (and the only chemical tool admitted in organic agriculture) for pest management, particularly considering their relative safety for non-target organisms. Nevertheless, the efficacy of these biopesticides is often evaluated only in terms of acute toxicity, overlooking their impact on the physiology and behavior on the insects surviving the exposure (Ricupero et al. 2022). Indeed, pesticides that do not cause mortality might alter behaviors such as settling on the host plant, probing and feeding, and oviposition.

In the present study, the EPG technique was used to characterize the effects of nine biopesticides, all of natural origin thus authorized for use in organic agriculture, on the probing and feeding behavior of *A. gossypii*. It is important to mention that EPG-results might not completely explain the mode of action of the
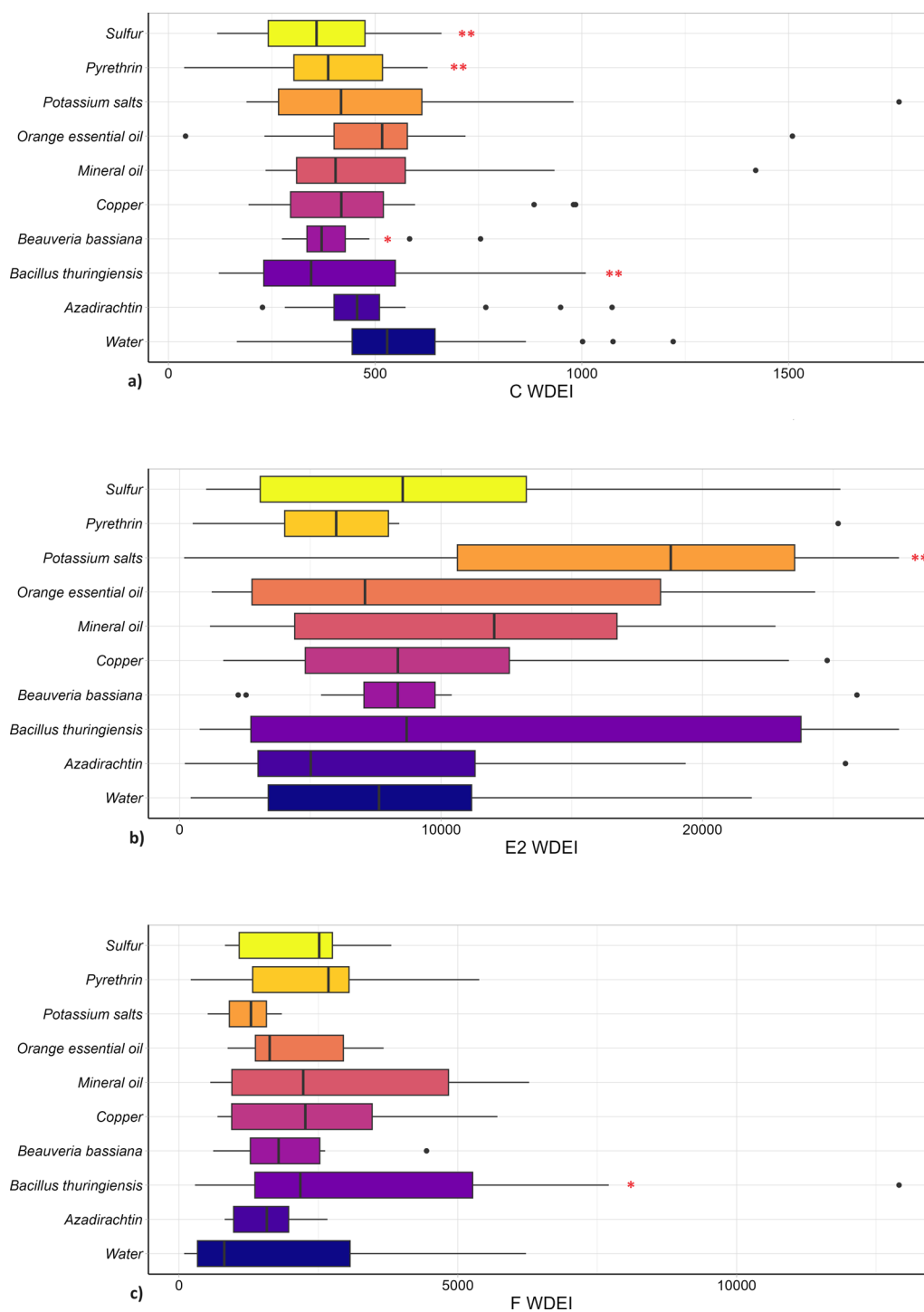
Fig. 3 Waveforms duration per event per insect (WDEI)

biopesticides tested, given tethering might affect (even if to a minor extent) probing and feeding activities (Tjallingii 1986; Nisbet et al. 1993), and prevent possible aphids' escape in response to repellent compounds. Therefore, further dedicated studies should dig into the

possible biopesticides impact on behaviors other than probing and feeding. However, the EPG represents the most rigorous method of real-time characterization of probing and feeding of piercing-sucking insects. The data presented here represent a reliable indication of
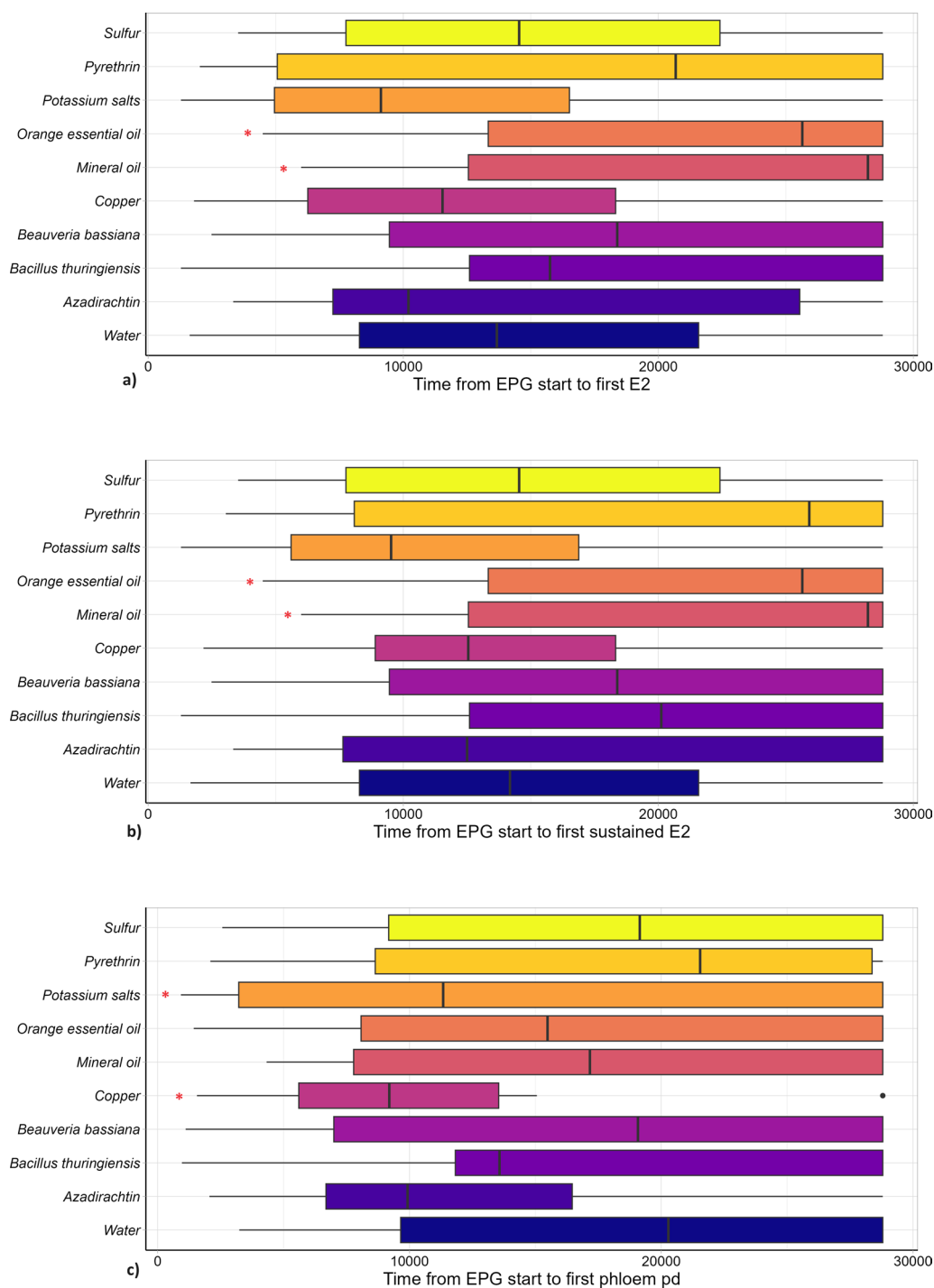
Fig. 4 Sequential variables

how the exposure to biopesticides applied to the recommended field dose might alter aphids' interaction with the host plant.

Overall, most of the biopesticides tested, either insecticides specifically targeting aphids, or insecticides applied against other pests and fungicides, altered behaviors that are pivotal for aphids' recognition and exploitation of the host plant. The exposure to some of the residuals, as those of pyrethrin, may even cause persistent behavioral alterations. On the other hand,

residuals of compounds as potassium salt and copper, rather than disturbing probing and feeding activities, acted as feeding promoters.

The orange essential oil (OEO) residuals delayed or impaired the access to sieve elements and the onset of phloem sap ingestion. Moreover, individuals exposed to OEO residues performed more numerous and longer derailed stylets pattern events. The latter is an electrical pattern theoretically associated with the production of gelling saliva while the stylets do not proceed in their regular intercellular pathway toward the sieve elements (Tjallingii 2006). These effects could possibly be related to essential oil components reported as antifeedant as terpenoids and phenols, whose perception by the insect may have affected the regular intercellular stylets route, delaying the access to phloem vessels (Sayed et al. 2022). The absence of persistent toxicological outcomes upon the exposure to OEO (i.e., absence of significant behavioral alteration on the untreated zucchini plants the aphids were moved to post-exposure) could have been associated with the relatively short time of OEO residuals exposure and successive recovery bioassay (8 h exposure, followed by 1 h starvation and 8 h of recovery bioassay) adopted in the present study. Further studies testing longer exposure and post-exposure intervals might provide relevant insight into how OEO, and biopesticides in general, might impact the plant-pest interaction in the long term.

Aphids given access to plants treated with *B. thuringiensis* exhibited shorter pathways, and shorter and fewer pds (brief intracellular stylets probes) compared to the control. In addition, more numerous and longer derailed stylets pattern events were recorded on *B. thuringiensis* treated plants compared to the control. Rashid et al. (2017) reported that the treatment with *Bacillus velezensis* strain YC7010 administered by root drench significantly reduced the settling, feeding, and reproduction of the green peach aphid *Myzus persicae* on *Arabidopsis thaliana* leaves. These effects were associated with *Bacillus*-induced plant systemic resistance, with the upregulation of the senescence-promoting gene Phytoalexin Deficient 4 (*PAD4*), and the downregulation of Botrytis-induced kinase 1 (*BIK1*). Bacterial-induced systemic resistance and aphid infestation led to higher hydrogen peroxide H_2O_2 production, cell death, and callose deposition. Indeed, hydrogen peroxide accumulation induces cell death and in turn limits the flow of photo-assimilates to the feeding sites, which could deter aphids and limit their feeding (Hao et al. 2008; Rashid et al. 2017). However, compared to previous studies, here significant behavioral alterations just 24 h after *B. thuringiensis* application to the plant by foliar spray were observed (in the work of Rashid and colleagues the aphids were given

access to the treated plants 5 days after *Bacillus* application by root drench). We thus hypothesize that the biopesticide interference with the regular intracellular stylets route toward the phloem vessels, and with the host recognition process (the intracellular probes (pds) mainly serve to evaluate the chemical constituents of the plant and its palatability for the aphid) observed here could have been associated with the combined effects of *Bacillus*-produced lipopeptides (Kim et al. 2004) and induced systemic resistance.

Mineral oil has been reported to cause a physical impairment of stylets penetration into host plant tissues caused by the surfactant layer covering the treated plant (Powell et al. 1998). In contrast, consistently with Norris et al. (2023), here we observed that rather than delaying/impairing stylets insertion, the treatment with mineral oil triggered a higher probing frequency, with aphids on treated plants performing more numerous and shorter probes. This in turn has led to a delayed access to phloem vessels, and a reduction of the proportion of individuals engaging with phloem sap ingestion during the EPG recording time. Generally, a long time required to perform the first probe (stylets insertion) is mainly caused by physical (e.g., wax or toughness of the plant leaf surface) and chemical (e.g., repellent volatiles) stimuli present on the plant leaf surface, thus "externally", repelling/impairing the insect. On the contrary, a more "hectic" probing activity can be associated with the perception of "internal" deterring stimuli that insects perceive once the stylets are within the host plants. Tan et al. (2005) reported that mineral oil moves within intercellular spaces and into various cells of citrus leaves, including phloem and xylem. The oil droplets accumulating within plant tissues upon mineral oil application could therefore have influenced aphids probing, leading to an increased number of stylets insertion, delaying or impairing the access to phloem vessels and the onset of passive ingestion (Tan et al. 2005).

On plants treated with the entomopathogenic fungus *B. bassiana*, aphids exhibited shorter average duration of pathways and longer duration of the derailed stylets pattern events (F waveform). Entomopathogenic fungi, besides acting directly against the target pest by infecting it, and producing fungal metabolites with insecticidal properties, can also endophytically colonize the host plant, triggering the activation of plant defenses impairing insect settling on the host (González-Mas et al. 2019; Homayoonzadeh et al. 2022). Homayoonzadeh et al. (2022) reported that the inoculation of cucumber plants with *B. bassiana* increased the levels of plant secondary metabolites, which in turn altered aphids' physiology. The same authors found that high levels of phenolic and hydrogen peroxide contents in plants treated with *B.*

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bassiana disrupted the enzymatic activity in aphids, and had toxic effects on their midguts, damaging the insect digestive system. However, the effects on the insects of the high phenolic and H_2O_2 contents reported by Homayoonzadeh et al. (2022), were marked 28 days after the treatment. Expanding on previous findings, similarly to *B. thuringiensis*, our data suggest that treatments with *B. bassiana* (and the putative endophytic colonization) significantly affect the aphid-plant interaction as soon as 24 h after the application.

Toxicological studies generally focus on lethal and sublethal effects of chemicals specifically applied to target the species of interest. Therefore, in pest management programs, the possible effects of chemicals as fungicides on insect pests is generally overlooked. However, within the agroecosystem, a pest is exposed to a whole array of chemicals, including those applied against species belonging to different *taxa* or even *phyla*, that can affect the non-target pest in ways that escape our comprehension. Our data indeed suggest fungicides as copper and sulfur may significantly alter the aphid-plant interaction. Aphids given access to plants treated with sulfur displayed shorter pathway events and longer duration of stylets derailment. On the other hand, aphids exposed to copper-treated plants required a shorter time to perform the first phloem pd (the first stylets insertion in sieve elements and/or companion cells) compared to control. Therefore, while sulfur may disturb the pest altering the probing activity, copper can even act as feeding promoter, facilitating the access to phloem. Overall, the fungicide-induced behavioral alterations observed could have been associated with a direct effect of the chemical on the insect, or with a chemical-induced change of plant traits affecting the insect-plant interaction. However, the current lack of knowledge on how fungicides interact/interfere with the insect-plant interaction only permits to muster a guess regarding the causes underlying our findings. For example, sulphur-fertilization was reported to increase the content of glucosinolates in cabbage plants (Santos et al. 2018); the presence within plant tissues of compounds as glucosinolates affecting insect-plant interaction (Hopkins et al. 2009) is consistent with the alterations in the probing activities observed. On the other hand, copper was reported to activate the immune system in exposed insects, with the maintenance of unnecessarily high immune functions being compensated by increased resource intake (Polkki 2016). We could therefore speculate that copper exposure would lead to "hungry" insects searching for food, an explanation that would be consistent with the rapid access to the sieve elements observed in aphids offered copper-treated plants

compared to control. Besides plant-mediated effects on insects, pesticides themselves can act directly on insects sensory organs, stimulating or inhibiting probing and feeding from a treated plant (Corso and Gazzoni 1998). Independently on the underlying causes of the observed fungicides-mediated behavioral alterations, our finding highlights the importance of considering the lethal and sublethal effects on a pest of all the compounds to which the pest could be exposed under field conditions when designing rational and sustainable pest management strategies.

Similarly to copper, residuals of potassium salt likely acted as feeding promoter rather than disturbing the probing and feeding activity. *Aphis gossypii* given access to zucchini plants treated with potassium salts exhibited indeed shorter pathways, and longer total and average durations of phloem ingestion events than the control. Additionally, the insects required a shorter time to perform the first phloem pd (thus to access sieve elements) on treated plants compared to the control. The more rapid access to the sieve elements could be explained by the interaction of the salts with the plant cuticular membranes, which in turn become more permeable. For example, Elshatshat et al. (2007) found that potassium carbonate (K_2CO_3) interacts with the polymer matrix of the cuticular membrane, leading to an increase in water content and permeability of leaf cuticles. For this reason, aphid stylets could have breached plant cuticles and thus easily reached the phloem tissues sooner than in the control.

Overall, *A. gossypii* showed alterations in probing and feeding behavior, that may translate in an impairment or a facilitation in the exploitation of the host plant, on plants treated with *B. thuringiensis, B. bassiana*, OEO, mineral oil, potassium salts, sulfur, and copper compared to the control. However, these effects were non-persistent, i.e., during the recovery bioassay insects exposed to the biopesticides behaved similarly to those given access to control plants. Namely, these biopesticides (or possibly the biopesticide-induced plant defenses) caused significant changes in aphid interaction with the host plant but were not directly toxic against the insect that indeed behaved "normally" once moved to an untreated plant. However, further studies applying longer exposure and time after exposure intervals, or testing other routes of exposure (e.g., topical application), might unveil further crucial information regarding how biopesticides interfere with the pest-plant interaction.

In contrast to the other compounds tested, pyrethrin residuals, besides interfering with probing and feeding on treated plants, induced persistent behavioral alterations

in exposed aphids. Specifically, aphids on pyrethrin-treated plants displayed shorter pathway events, and fewer individuals engaging with phloem ingestion (E2 waveform) compared to the control. Additionally, during the recovery bioassay, aphids exposed to pyrethrin engaged with xylem sap ingestion more frequently than the control ones. According to previous studies, xylem feeding in aphids is more frequent after experiencing a starvation period (Powell and Hardie 2002; Jhou et al. 2021). Therefore, aphids that did not ingest phloem sap during the EPG round on pyrethrin-treated plants, being starved and dehydrated, engaged with xylem ingestion as soon as moved to untreated plants for the recovery bioassay. Nonetheless, no significantly higher proportion of individuals performing xylem ingestion was observed after exposure to other biopesticides that like pyrethrin reduced the occurrence of phloem ingestion, namely mineral oil and OEO. Therefore, we hypothesize that the alteration of the probing and feeding dynamics upon exposure to pyrethrin could have been potentially due to the neurotoxicity of the compound, exerted by modulating activities of voltage-gated sodium channels in the insects' nervous systems (Kojima et al. 2022). Further investigations should address the persistence of these pyrethrin-induced behavioral effects and their implication for the biology of the pests and the pest-plant interaction in the long term.

Conclusions

Overall, our findings highlight that even if less effective than old-generation pesticides (Ricupero et al. 2020) for rapidly suppressing pest populations (low or null acute toxicity), biopesticides can interfere with insect's probing and feeding behaviors underlying the host plant location, acceptance, and exploitation (and conducive to the acquisition and inoculation of vector-borne plant pathogens), and crucial for pest population dynamics. The behavioral alterations can either be associated with the biopesticide itself, or, as for *B. thuringiensis* and *B. bassiana*, might possibly result from the activation of host plant defenses. However, some of the chemical residuals an aphid can be exposed to, as those of copper and potassium salt, rather than negatively impact the pest-plant interaction, may act as feeding promoter. Furthermore, our data highlight the importance of extending toxicological studies on the lethal and sublethal effects of pesticides to the whole array of compounds used for crop protection that the pest can be exposed to, besides those specifically devised to target that specific insect species.

Finally, the results gathered on entomopathogens such as *B. bassiana* and *B. thuringiensis* open new scenarios for their use as elicitors of plant defense response, leading to a reduction in the host plant palatability for the pest, or promoting the synthesis of metabolites with insecticidal activity.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43170-024-00269-4.

Additional file 1: Table S1: Details on the biopesticides used for the exposure of *Aphis gossypii* and their main effects in the presented study.

Additional file 2: Table S2: Summary statistics of the significant EPG variables of aphids exposed to treated plants (exposure phase). Values are expressed in seconds.

Additional file 3: Table S31: The proportion of individuals that produced E2 waveform after being exposed to treated plants (exposure phase). Table S3.2: The proportion of individuals that produced G waveform after being exposed to untreated plants (recovery bioassays).

Additional file 4: Table S4: Number of dead individuals per treatment after exposure to treated plants (8 h).

Additional file 5: Table S51: Number of unactive individuals per treatment after the exposure to treated plants (8 h) (exposure phase). Table S5.2: Number of unactive individuals per treatment after the exposure to untreated plants (8 h) (recovery bioessays).

Author contributions
DC and ZH conceived and designed the study; ZH and GC collected the data; ZH, CZ, AbB analyzed the EPG data; ZH and GT performed the statistical analysis; ZH and DC wrote the first draft of the paper; AB substantively revised the paper. KD, VV and DC provided fundings. All authors read and approved the final manuscript.

Availability of data and materials
Further data are available from the corresponding author (daniele.cornara@uniba.it) upon request.

Declarations

Ethic approval and consent to participate
Not applicable.

Consent for publication
All authors consented to the publication of this manuscript.

Competing interests
The authors declare that they have no competing interests.

References
Backus EA, Cline AR, Ellerseick MR, Serrano MS. *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton: new methods and parameters for analysis of nonsequential electrical penetration graph data. Ann Entomol Soc Am. 2007;100(2):296–310. https://doi.org/10.1603/0013-8746(2007)100[296:lhhmfo]2.0.co;2.
Campolo O, Chiera E, Malacrinò A, Laudani F, Fontana A, Albanese GR, et al. Acquisition and transmission of selected CTV isolates by *Aphis gossypii*. J

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Asia Pac Entomol. 2014;17(3):493–8. https://doi.org/10.1016/j.aspen.2014.04.008.

Copping LG, Menn JJ. Biopesticides: a review of their action, applications and efficacy. Pest Manag Sci. 2000;56(8):651–76. https://doi.org/10.1002/1526-4998(200008)56:8%3c651::aid-ps201%3e3.0.co;2-u.

Corso IC, Gazzoni DL. Sodium chloride: an insecticide enhancer for controlling pentatomids on soybeans. Pesq Agrop Brasileira. 1998;33:1563–72.

Desneux N, Decourtye A, Delpuech J-M. The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol. 2007;52(1):81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440.

Elshatshat S, Schreiber L, Schönherr J. Some cesium and potassium salts increase the water permeability of astomatous isolated plant cuticles. J Plant Nutr Soil Sci. 2007;170(1):59–64. https://doi.org/10.1002/jpln.200625042.

Fira D, Dimkić I, Berić T, Lozo J, Stanković S. Biological control of plant pathogens by *Bacillus* species. J Biotechnol. 2018;285:44–55. https://doi.org/10.1016/j.jbiotec.2018.07.044.

González-Mas N, Cuenca-Medina M, Gutiérrez-Sánchez F, Quesada-Moraga E. Bottom-up effects of endophytic *Beauveria bassiana* on multitrophic interactions between the cotton aphid, *Aphis gossypii*, and its natural enemies in melon. J Pest Sci. 2019;92(3):1271–81. https://doi.org/10.1007/s10340-019-01098-5.

Guedes RNC, Smagghe G, Stark JD, Desneux N. Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu Rev Entomol. 2016;61(1):43–62. https://doi.org/10.1146/annurev-ento-010715-023646.

Hao P, Liu C, Wang Y, Chen R, Tang M, Du B, et al. Herbivore-induced callose deposition on the sieve plates of rice: an important mechanism for host resistance. Plant Physiol. 2008;146(4):1810–20. https://doi.org/10.1104/pp.107.111484.

Homayoonzadeh M, Esmaeily M, Talebi K, Allahyari H, Reitz S, Michaud JP. Inoculation of cucumber plants with Beauveria bassiana enhances resistance to *Aphis gossypii* (Hemiptera: Aphididae) and increases aphid susceptibility to pirimicarb. Eur J Entomol. 2022;119:1–11. https://doi.org/10.14411/eje.2022.001.

Hopkins RJ, van Dam NM, van Loon JJ. Role of glucosinolates in insect-plant relationships and multitrophic interactions. Ann Rev Entomol. 2009;54:57–83.

Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol. 2006;51(1):45–66. https://doi.org/10.1146/annurev.ento.51.110104.151146.

Jhou Y-S, Poovendhan S, Huang L-H, Tsai C-W. Host acceptance and plant resistance: a comparative behavioral Study of *Myzus persicae* and *Acyrthosiphon pisum*. InSects. 2021;12(11):975. https://doi.org/10.3390/insects12110975.

Jiménez J, Arias-Martín M, Moreno A, Garzo E, Fereres A. Barley yellow dwarf virus can be inoculated during brief intracellular punctures in phloem cells before the sieve element continuous salivation phase. Phytopathol. 2020a;110(1):85–93. https://doi.org/10.1094/PHYTO-07-19-0260-FI.

Jiménez J, Garzo E, Alba-Tercedor J, Moreno A, Fereres A, Walker GP. The phloem-pd: a distinctive brief sieve element stylet puncture prior to sieve element phase of aphid feeding behavior. Arthropod Plant Interact. 2020b;14(1):67–78. https://doi.org/10.1007/s11829-019-09708-w.

Kim PI, Bai H, Bai D, Chae H, Chung S, Kim Y, et al. Purification and characterization of a lipopeptide produced by Bacillus thuringiensis CMB26. J Appl Microbiol. 2004;97(5):942–9. https://doi.org/10.1111/j.1365-2672.2004.02356.x.

Kojima T, Yamato S, Kawamura S. Natural and synthetic pyrethrins act as feeding deterrents against the black blowfly, *Phormia regina* (meigen). Insects. 2022;13(8):678. https://doi.org/10.3390/insects13080678.

Mahas JW, Steury TD, Huseth AS, Jacobson AL. Imidacloprid resistant *Aphis gossypii* populations are more common in cotton dominated landscapes. Pest Manag Sci. 2022;79(3):1040–7. https://doi.org/10.1002/ps.7274.

Mokrane S, Cavallo G, Tortorici F, Romero E, Fereres A, Djelouah K, et al. Behavioral effects induced by organic insecticides can be exploited for a sustainable control of the Orange Spiny Whitefly *Aleurocanthus spiniferus*. Sci Rep. 2020;10(15746):1–12. https://doi.org/10.1038/s41598-020-72972-x.

Monsreal-Ceballos RJ, Ruiz-Sánchez E, Ballina-Gómez HS, Reyes-Ramírez A, González-Moreno A. Effects of botanical insecticides on hymenopteran parasitoids: a meta-analysis approach. Neotrop Entomol. 2018;47(5):681–8. https://doi.org/10.1007/s13744-017-0580-5.

Nisbet AJ, Woodford JAT, Strang RHC, Connolly JD. Systemic antifeedant effects of azadirachtin on the peach-potato aphid *Myzus persicae*. Entomol Exp Appl. 1993;68(1):87–98. https://doi.org/10.1111/j.1570-7458.1993.tb01692.x.

Norris RH, Silva-Torres CS, Lujan M, Wilson-Rankin EE, Mauck KE. Footprints of predatory lady beetles stimulate increased dispersal of aphid prey, but do not alter feeding behavior or spread of a non-persistently transmitted plant virus. Food Webs. 2023;37: e00325. https://doi.org/10.1016/j.fooweb.2023.e00325.

Passos LC, Ricupero M, Gugliuzzo A, Soares MA, Desneux N, Carvalho GA, et al. Does the dose make the poison? Neurotoxic insecticides impair predator orientation and reproduction even at low concentrations. Pest Manag Sci. 2022;78(4):1698–706. https://doi.org/10.1002/ps.6789.

Pölkki M. The effects of copper exposure on life-history traits in insects. Turun Yliopiston Julkaisuja Annales Universitatis Turkuensis Sarja-Biologica Geographica Geologica. 2016;2(328):1–44.

Powell G, Hardie J. Xylem ingestion by winged aphids. Entomol Exp Appl. 2002;104(1):103–8. https://doi.org/10.1046/j.1570-7458.2002.00996.x.

Powell G, Hardie J, Pickett JA. The effects of antifeedant compounds and mineral oil on stylet penetration and transmission of potato virus Y by *Myzus persicae* (Sulz.) (Hom., Aphididae). J Appl Entomol. 1998;122:331–3. https://doi.org/10.1111/j.1439-0418.1998.tb01507.x.

Rani L, Thapa K, Kanojia N, Sharma N, Singh S, Grewal AS, et al. An extensive review on the consequences of chemical pesticides on human health and environment. J Clean Prod. 2021;283: 124657. https://doi.org/10.1016/j.jclepro.2020.124657.

Rashid MHO, Khan A, Hossain MT, Chung YR. Induction of systemic resistance against aphids by endophytic *Bacillus velezensis* YC7010 via expressing PHYTOALEXIN DEFICIENT4 in Arabidopsis. Front Plant Sci. 2017;8:211. https://doi.org/10.3389/fpls.2017.00211.

Ricupero M, Desneux N, Zappalà L, Biondi A. Target and non-target impact of systemic insecticides on a polyphagous aphid pest and its parasitoid. Chemosphere. 2020;247: 125728. https://doi.org/10.1016/j.chemosphere.2019.125728.

Ricupero M, Biondi A, Cincotta F, Condurso C, Palmeri V, Verzera A, Zappalà L, Campolo O. Bioactivity and physico-chemistry of garlic essential oil nanoemulsion in tomato. Entomol Gen. 2022;42(6):921–30. https://doi.org/10.1127/entomologia/2022/1553.

Santos NA, Teixeira NC, Valim JOS, Almeida EFA, Oliveira MGA, Campos WG. Sulfur fertilization increases defense metabolites and nitrogen but decreases plant resistance against a host-specific insect. Bull Entomol Res. 2018;108(4):479–86.

Sarria E, Cid M, Garzo E, Fereres A. Excel Workbook for automatic parameter calculation of EPG data. Comput Electron Agric. 2009;67(1–2):35–42. https://doi.org/10.1016/j.compag.2009.02.006.

Sayed S, Soliman MM, Al-Otaibi S, Hassan MM, Elarrnaouty S-A, Abozeid SM, et al. Toxicity, deterrent and repellent activities of four essential oils on *Aphis punicae* (Hemiptera: Aphididae). Plants. 2022;11(3):463. https://doi.org/10.3390/plants11030463.

Tan BL, Sarafis V, Beattie GAC, White R, Darley EM, Spooner-Hart R. Localization and movement of mineral oil in plants by fluorescence and confocal microscopy. J Exp Bot. 2005;56(420):2755–63. https://doi.org/10.1093/jxb/eri269.

Tjallingii WF. Wire effects on aphids during electrical recording of stylet penetration. Entomol Exp Appl. 1986;40(1):89–98. https://doi.org/10.1111/j.1570-7458.1986.tb02159.x.

Tjallingii WF. Salivary secretions by aphids interacting with proteins of phloem wound responses. J Exp Bot. 2006;57(4):739–45. https://doi.org/10.1093/jxb/erj088.

Ziaei Madbouni MA, Samih MA, Qureshi JA, Biondi A, Namvar P. Compatibility of insecticides and fungicides with the zoophytophagous mirid predator *Nesidiocoris tenuis*. PLoS ONE. 2017;12(11): e0187439. https://doi.org/10.1371/journal.pone.0187439.

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