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Review: Prospects for management of whitefly using plant semiochemicals, compared with related pests

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Abstract

Whitefly (Hemiptera: Sternorrhyncha: Aleyrodidae) pests are economically important in agriculture, including the tobacco whitefly, *Bemisia tabaci,* and the greenhouse whitefly, *Trialeurodes vaporariorum*. Whiteflies are mainly controlled by synthetic insecticides but resistance to these

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insecticides is rapidly evolving. A semiochemical-based management strategy could provide an alternative to the use of insecticides, by exploiting natural volatile signalling processes to manipulate insect behaviour. Whitefly behaviour is affected by differences in plant odour blends. Selected compounds have been suggested as putative semiochemicals, but in only a few studies, potential volatiles were eventually characterised by electrophysiology or olfactometry. The application of antennal preparation methods from the closely related families, the aphids (Hemiptera: Aphididae) and psyllids (Hemiptera: Psyllidae), may help to facilitate whitefly electroantennography. Behavioural bioassays are essential to identify the repellent or attractant effect of each semiochemical. The relevance of the semiochemicals in whitefly management needs to be evaluated in the respective cultivation system. Although the value of semiochemicals has not been demonstrated in the field against whiteflies, there is an emerging range of possible field applications and some promising prospects. Overall, the olfactory system of whiteflies needs to be elucidated in more detail.

Keywords: whitefly, semiochemicals, volatile organic compounds, repellents, olfaction, pest control

1 INTRODUCTION

Whiteflies (Hemiptera: Sternorrhyncha: Aleyrodidae), including the tobacco whitefly, *Bemisia tabaci,* and the greenhouse whitefly, *Trialeurodes vaporariorum*, are common agricultural pests that damage a wide range of economically important crop plants, such as tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*) and watermelon (*Citrullus lanatus*), especially by acting as vectors of devastating plant viruses.[1] Management of whitefly populations relies predominantly on the deployment of broad-spectrum synthetic insecticides, but because of rapid evolution of insecticide resistance,[2] new interventions are urgently needed. Semiochemical-based approaches are considered as environmentally benign alternatives to the use of insecticides, because semiochemicals act only as signals and are not toxic at the levels deployed. Semiochemicals also have an essential evolutionary role and, although their use in pest management could cause selection for resistance, other related semiochemicals would need to evolve in order to fulfil the crucial signalling role targeted originally. The newly evolved chemicals could be readily identified and used rationally to replace the semiochemicals to which resistance had earlier evolved.[3] Semiochemicals are perceived by the olfactory organs of the insect that are mostly located on the antennae. Olfaction was not thought to play a significant role in whitefly host plant selection until the beginning of the 21st century, with research focussing mainly prior to that on whitefly vision. However, an increasing number of studies shows that whitefly behaviour is affected by plant-emitted volatile organic compounds (VOCs). In behavioural bioassays, whitefly preference varies between potential host plants and even between host plant varieties,[4-6] cultivars,[4] and accessions.[7] Furthermore, whiteflies can discriminate between different qualitative conditions of host plants, for example differences in nitrogen fertilization,[8] leaf position,[9] aphid colonisation and virus infection.[10-12] Moreover, the ultrastructure of certain antennal sensilla of *B. tabaci*, *T. vaporariorum* and *Aleyrodes proletella* indicates olfactory function.[13-15] Odorant binding proteins and chemosensory proteins have been detected in *B. tabaci* by transcript analysis,[16] and certain volatile compounds have been shown to bind to chemosensory proteins of *B. tabaci*.[17] The whitefly olfactory system is so highly developed that it can even differentiate between stereoisomers of VOCs.[18]

In this review, we focus on plant-produced, volatile semiochemicals and their potential for application in whitefly management. The aim is to identify gaps in whitefly olfaction research and to encourage work on this topic. The literature focuses exclusively on the economically important whitefly species and biotypes of *B. tabaci* and *T. vaporariorum,* but they will be compared with other hemipterous pests in the suborder of the Sternorrhyncha, the aphids (Hemiptera: Aphididae) and psyllids (Hemiptera: Psyllidae). All adults of these families are phloem-feeders on host plants and are vectors of plant pathogens.

2 IDENTIFICATION AND EVALUATION OF PLANT-EMITTED WHITEFLY SEMIOCHEMICALS

2.1 Isolation of putative semiochemicals

Bemisia tabaci and *T. vaporariorum* are extreme generalists and thus must be able to detect the different volatiles specific to many host plants.[19,20] An expedient approach to identify whitefly semiochemicals is to compare the volatile collections from different physiological conditions of the host plant evoking different behavioural responses from the whiteflies. For example, VOCs released from less attractive plants might be repellent compounds. Furthermore, it is important to include compounds that differ in their proportions because the ratio of the compounds can be crucial.[21,22] A principal component analysis of the volatile collections can be helpful to identify putative semiochemicals within the mixture.[4,23] There are various techniques available for collecting plant volatiles, such as solvent extraction, steam distillation or air entrainment.[5] The latter is preferred because headspace collections represent the actual released quantities of the naturally occurring compounds. In addition, this non-destructive sampling method does not risk extracting compounds formed from damaged plant tissue. For instance, green leaf volatiles are only detected in headspace collections from tomato plants after mechanical wounding.[7] The technical set up of the sampling method, e.g. the choice of adsorbent material, needs to be investigated to achieve the best possible outcome.[24]

2.2 Whitefly electrophysiology towards semiochemicals

Usually an extract of collected plant volatiles includes a complex and diverse range of compounds, but only a subset of them is likely to have a semiochemical role.[22] High resolution gas chromatography (GC) coupled with electroantennography (GC-EAG) and a detector (e.g. flame ionization detector) is a powerful tool for the identification of semiochemicals within a blend of compounds (Fig. 1). Here, the ability of a compound to be perceived at the olfactory level is indicated by a measured voltage deflection caused by olfactory receptor neurones localized in sensilla on the antennae.[25] The bioactive compounds are then further identified by GC-coupled mass spectrometry and/or nuclear magnetic resonance spectroscopy after purification by preparative GC. However, no GC-EAG study with whiteflies has so far been published. Apparently, EAG is not a favoured technique for whiteflies. Among all studies on whitefly olfaction, only two include EAG measurements.[7,18] In these studies, excised antenna of *B. tabaci* adults were mounted on a custom-made holder and volatile terpenoids emitted from less-preferred accessions of the wild tomato plants *Solanum pennellii, S. habrochaites,* and *S. peruvianum* were puffed individually over the antenna. The excised whitefly antennae did not remain viable for the duration of a GC-EAG experiment using a collected plant volatile extract.[18] Thus, the method is not yet adapted sufficiently for whiteflies to identify active compounds by GC-EAG. Whitefly electrophysiology might learn from the longer ongoing research on aphid olfaction where GC-EAG and EAG are widely used for detecting semiochemicals.[26] The longevity of the EAG preparation might be increased by a different antennal preparation technique, for example a whole insect preparation. An EAG study on the black bean aphid *Aphis fabae* lasted longer with a whole insect preparation compared to the excised antennae.[27] Here, the aphid is immobilized with a copper wire restraint. A different and successful approach comprising fixing insects within pipette tips was used for psyllids, where whole insect preparations were used for GC-EAG and EAG.[28] The whole insect preparation reduces the risk of drying antennae with the resistance increasing to a level too high for measurements. Another advantage of the longer usable life of the antennal preparation is the option for an extended recovery time between the single stimulations. The antennal responsiveness of *A. fabae* was higher with a longer recovery time by using the whole insect preparation technique.[27]

A different technique for investigating the olfactory response of insects towards semiochemicals utilises single sensillum recordings. Here, the electrical activity of one sensillum is measured instead of all sensilla on the antennal flagellum.[29] This method, also in combination with GC, has been used for decades in aphid olfaction research and, more recently, successfully applied in psyllid olfaction research.[23] This technique can help to receive antennal responses when there is a low number of sensilla, shown in the carrot psyllid *Trioza apicalis*.[30] No absolute number of antennal sensilla was presented in *B. tabaci* biotypes but the study indicates a similar sparse sensillar setup compared to *T. apicalis*.[13,30,31]

Overall, the research on whitefly olfaction would benefit from more electrophysiological studies. GC-EAG analysis of a volatile collection can greatly facilitate the search for semiochemicals. EAG-active VOCs are detected by insect antennae and are most likely affecting whitefly behaviour. The EAG method can also be used for dose-response tests comparing the antennal responses between different doses of the same semiochemical. This investigation can help to identify the most efficient dose in whitefly management.

2.3 Evaluation of whitefly behaviour towards semiochemicals

EAG studies do not reveal the behavioural activity of an identified plant-emitted semiochemical, i.e. whether it is a repellent or an attractant. For this, insect behaviour towards olfactory cues needs to be evaluated, for example in an olfactometer assay. Olfactometers are sealed devices with a system of channels with or without directed airstreams providing different odour sources. The insect is released into the system where it can move freely and decide between the channels permeated with the test odour and with the solvent or air only. A solvent is often needed to apply semiochemicals on the release device (usually filter paper) and it serves as a control. The evaluation of the insect behaviour can be based on the choice of channel or on the time spend in the respective channel. It is expected that the insect will respond positively to channels containing an attractant stimulus compared with the solvent/air and positively with the solvent/air compared with a repellent stimulus. There is a wide variation in the procedure for measuring whitefly responses towards semiochemicals (e.g. the number of released whiteflies at a time, dimensions of the olfactometer or the evaluation method), thus making it difficult to compare different studies. However, dual-choice olfactometers (Fig. 2), where the insect chooses between two channels (one containing the stimulus and the other only the solvent or air), have been used in all tests with whiteflies responding to individual VOCs (Table 1). The tubular olfactometer is a linear device where the whiteflies are released into the middle of the tube and they can move directly to either side (Fig. 2A). The behavioural bioassay is conducted without airflow. The tested airborne semiochemicals have been considered as repellents using the avoidance index as evaluation method (Table 1). This index is the number of whiteflies counted in the zone of the repellent subtracted from the number of whiteflies counted in the zone of the control divided by the total number of counted whiteflies. The bioassays in the T-shaped olfactometer are conducted without airstream (Fig. 2B). The whiteflies are released at the base of the device and need to move via the junction into one of both branches. Their choice is evaluated by the number of individuals counted in the decision chambers. The Y-shaped olfactometer bioassay is performed with a directed, charcoal-filtered and humidified airstream (Fig. 2C). Here, the whiteflies are also released at the base of the olfactometer. They need to walk into the junction and make a choice. The preference of the whiteflies is evaluated by counting the number of individuals which entered the respective channel in a defined way (e.g. moving at least one third into the respective channel). Repellency olfactometer tests predominate due to the greater interest in preventing the settlement of viruliferous whiteflies on crop plants (Table 1). Two types of olfactory-mediated repellents have been defined depending on their effect on insect behaviour.[35] So-called true repellents cause insects actively to move away from the odour source, whilst odour-masking repellents either reduce or disrupt the attractiveness of the host plant. The Y-shaped olfactometer might be difficult for investigation of true repellents because the whitefly will not go into the choice region.[35] This might be resolved by including the response rate of all tested whiteflies (responders vs. non-responders) or excluding responses below a set minimum in the evaluation.[36,37] However, none of the studies has addressed the behaviour of the whitefly towards a true repellent by evaluating an oriented movement away from the odour source. Overall, the evaluation of whitefly repellents or attractants is rated based on the proportion of responding whiteflies in relation to the used solvent or clean air. The degree of the semiochemical effect on whitefly behaviour is also dose-dependent.[5,6] The terms repellency and attractancy should be used with caution and always in context.

The four-arm olfactometer (Fig. 3) is a different device in insect olfactometry and a standard bioassay in aphid studies used for either repellents and attractants.[38,39] In this experimental setup, the insect can move freely in an arena divided into four areas with respective airstreams. Two four-arm olfactometer tests have been so far published with *B. tabaci*. These studies investigate the effect of odour masking of non-host plants or aphid-induced plant volatiles instead of selected semiochemicals.[40,10] The four-arm olfactometer might receive little consideration for whitefly bioassays because the design of this device needs insects actively exploring all arms. Whiteflies are known to be flyers also for short distances. For example, they fly up the host plant when being disturbed. However, they can walk actively in a four-arm and Y-shaped olfactometer (Schlaeger S, 2016, pers. obv.).

In contrast to the evaluation of a true repellent, experimental setups for host odour-masking repellents should include the emitted volatiles of the host plant. Beyond that, the crop plant has visual cues that could also interfere with the effect of the semiochemical on whitefly behaviour. For instance, the visual attractiveness of the crop plant might override the effect of a host odour masking repellent. In general, all potential semiochemicals must be tested in the respective cultivation system for its relevance in whitefly control. Under field conditions, whitefly semiochemicals are exposed to all kinds of odorants from the environment. The effect of the whitefly semiochemical could be diminished in this mixture of VOCs.

An indirect but important approach to control whiteflies is to identify plant-mediated semiochemicals that attract natural enemies. For example, (*Z*)-3-hexen-1ol, (*E*)-4,8-dimethyl-1,3,7-

nonatriene (DMNT) and 3-octanone are emitted in higher quantities from *T. vaporariorum*-infested bean plants, *Phaseolus vulgaris*, than from uninfested plants.[41] The synthetic versions of these VOCs, when presented individually or in a blend, increased the attractancy of the whitefly parasitoid, *Encarsia formosa,* in wind tunnel bioassays. *Arabidopsis thaliana* plants emit myrcene when attacked by *B. tabaci*.[42] In a Y-shaped olfactometer, applying synthetic myrcene to the odour of uninfested *A. thaliana* plants attracted more *E. formosa* parasitoids compared to the odour of plants that were not treated with the semiochemical.[42]

3 POSSIBLE APPLICATIONS OF SEMIOCHEMICALS TO WHITEFLY MANAGEMENT

Keeping whitefly infestation on crop plants below an economic threshold level is a part of an integrated pest management strategy. The settlement and feeding of one individual on a crop plant is enough for virus transmission as the vectored virus diseases are systemic, affecting the whole plant. Thus, the economic threshold is very low. Consequently, the odour-masking effect of a repellent appears not to be sufficient, because members of the whitefly population may still land on the crop plant. For control of whitefly virus vectors, a true repellent that prevents almost complete whitefly colonisation is the preferable choice.

Semiochemicals are an important component of push-pull technology which combines repellents and attractants in the same cropping system. The pest is deterred from the crop plant (push) and lured to a more attractive source (pull) at the same time.[43] This strategy is especially suited for the control of greenhouse pests, such as *T. vaporariorum*, because of the confined area.[43]

Semiochemicals can be integrated into the cropping system by cultural practices, e.g. intercropping with semiochemical-emitting plants. Adult *B. tabaci* infestation of tomato plants was reduced by intercropping with either coriander (*Coriandrum sativum*) or Greek basil (*Ocimum minimum*) plants, or a citronella grass (*Cymbopogon* spec.) mulch.[44] Where economically viable, another possibility for field application of active semiochemicals is deployment of synthesized VOCs via sprays or slow-release dispensers.[43] There have been no field studies reported with selected whitefly semiochemicals. However, *B. tabaci* settlement on tomato plants was reduced in a greenhouse experiment using bottles with a 1 % mixture (*R*)-limonene, citral and, as a slow release agent and antioxidant, olive oil (in a ratio of 63:7:30), as a 'push' treatment and yellow sticky traps as the pull.[45] The application of the sesquiterpene hydrocarbon and aphid alarm pheromone (*E*)-β-farnesene or methyl salicylate in a paraffin oil-formulation released from a rubber septum in a wheat (*Triticum aestivum*) field centered in a wheat-pea (*T. aestivum-Pisum sativum*) strip intercropping system reduced aphid infestation and increased the number of parasitized aphids.[46] The potential of using a push-pull strategy for management of the Asian citrus psyllid *Diaphorina citri*, which is the vector of *Candidatus Liberibacter asiaticus*, the causative agent for citrus greening disease, has been reviewed.[47] It has been stated that more knowledge about psyllid-host interactions needs to be generated before more applied studies can be performed. However, potential psyllid semiochemicals have been identified, such as the homoterpenes DMNT and (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT). A synthetic mixture of DMNT and TMTT reduced the attractiveness of the hosts orange jasmine*, Murraya paniculata,* and sweet orange Pera D6, *Citrus sinensis*, in a four-arm-olfactometer bioassay.[48] In y-shaped and four-arm olfactometer assays, dimethyl disulphide, identified from the non-host guava, *Psidium guajava*, reduced the attractiveness of volatiles from *C. sinensis*.[49] Furthermore, dimethyl disulphide released from polyethylene vials reduced the infestation of *D. citri* in an orchard of Valencia oranges, *C. sinensis*, for up to four weeks.

A different approach to the deployment of semiochemicals is to modify the emitted VOCs of the crop plant by genetic engineering, such that the plant either is not attractive for pest insects (odour masking) or becomes repellent.[50] This mode of direct pest management can be supplemented with attraction of beneficial natural enemies for conservation biological control.[51] The most prominent example of this strategy to date for pest management is the engineering of elite wheat to release (*E*)-β-farnesene, which was confirmed in laboratory bioassays.[52] However, the repellent effect on aphids could not be confirmed in field studies. The missing effect in the field might be due to bad weather conditions during the trial. Another explanation might be the difference in the release of (*E*)-β-farnesene from the plant (emitted continuously) in comparison with release from the aphid (sudden burst release). Plant glandular trichomes are sources of semiochemicals, thus making them targets for genetic engineering in pest resistance.[53] A promising attempt for the modification of the semiochemical biosynthesis in trichomes for whitefly management was shown in the cultivated tomato.[54] The wild tomato *S. habrochaites* accession PI127826 is naturally less attractive towards *B. tabaci* and releases distinct quantities of the sesquiterpene hydrocarbon 7-epizingiberene.[7,18] The application of 7-epizingiberene to the cultivated tomato reduced the settlement of *B. tabaci* adults in a free-choice bioassay.[18] The introduction of the biosynthetic pathway of 7-epizingiberene into the glandular trichomes of the cultivated tomato with trichome specific promotors led to the production of this whitefly semiochemical.[54] The repellent property against *B. tabaci* was not investigated with the transgenic lines in this study.

Semiochemicals can be expensive to synthesise and chemically unstable, which is unfavourable for field application. A possible approach to overcome these challenges is the rational design of analogues of the semiochemicals by chemoenzymatic synthesis. In this method, the acceptance of unnatural substrate by the specifically responsible biosynthesis enzyme leads to analogues of the natural product which might have superior properties. This synthetic biology approach has recently been successfully demonstrated for the aphid sesquiterpene semiochemical (*S*)-germacrene D and has led to the rational discovery of novel semiochemicals.[55] This approach is also now being tested with the whitefly semiochemical 7-epizingiberene and its biosynthesis enzyme, epizingiberene synthase. The biosynthetic pathways to the production of the new analogues have the potential of being engineered into crop plants and therefore the library of semiochemical tools for whitefly management widened. The availability of a wide range of tools provides an opportunity to mitigate the evolution of whitefly resistance to semiochemicals.[3]

4 CONCLUSION

Plant-produced VOCs can alter whitefly behaviour, but few studies have investigated the effects of the actual putative semiochemicals through electrophysiology and behavioural work with whiteflies. More information about the olfactory system of whiteflies is needed, for example which sensilla are responsible for olfaction. This knowledge is necessary to identify semiochemicals for subsequent use in whitefly management.

For a better general understanding, it might be useful to broaden the research on whitefly olfaction to other species with economic importance in addition to *B. tabaci*. Only few studies deal with *T. vaporariorum* or *A. proletella*. *A. proletella* is of particular interest, because it is a specialist in comparison with the generalists *B. tabaci* and *T. vaporariorum*, feeding primarily on cruciferous plant species. In addition, semiochemical interventions against *Trialeurodes* species could be more advantageous because of the higher value of glasshouse products in comparison to arable production.

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Table 1 Overview of olfactometer tests evaluating the behavioural response of *Bemisia tabaci* towards plant-produced, individual volatile organic compounds

Type of olfactometer	Compound	Stated effect	Evaluation method	Reference
T-shaped	(*R*)-Limonene	Attractancy	Preference	5
T-shaped	(*E*)-Caryophyllene	Attractancy	Preference	5
Tubular	(*R*)-Limonene	Repellency	Avoidance index	6
Tubular	Myrcene	Repellency	Avoidance index	6
Tubular	(*E*)-Ocimene	Repellency	Avoidance index	6
Tubular	(*R*)-Limonene	Repellency	Avoidance index	45
Tubular	Limonene†	Repellency	Avoidance index	45
Tubular	Citronellal	Repellency	Avoidance index	45
Tubular	Citral	Repellency	Avoidance index	45
Tubular	α-Pinene†	Repellency	Avoidance index	45
Tubular	Geranyl nitrile	Repellency	Avoidance index	45
Y-tube	2-Ethyl-1-hexanol†	Attractancy	Preference	32

Y-tube	o-Xylene	Repellency	Preference	32
Y-tube	Phenol	Attractancy	Preference	32
Y-tube	α-Pinene†	Repellency	Preference	32
Y-tube	Salicylic acid	Repellency	Preference	33
Y-tube	Limonene†	Repellency	Preference	33
Y-tube	1,8-Cineole	Repellency	Residence time	34
Y-tube	Linalool†	Attractancy	Residence time	34
Y-tube	(*E*)-2-Hexenal	Attractancy	Response/attraction rate	36
Y-tube	3-Hexen-1-ol†	Attractancy	Response/attraction rate	36
Y-tube	Limonene†	Repellency	Response/attraction rate	36
Y-tube	(*R*)-Limonene	Repellency	Response	37
Y-tube	Geranyl nitrile	Repellency	Response	37

†Isomeric composition not given.

Figure 1 Overview of the technical set up of a gas chromatography – flame ionization detection coupled with electroantennography.

Figure 2 Designs of different dual-choice olfactometers. The directions for the whitefly's choices and the locations of the stimuli/control are illustrated.

Figure 3 Design of a four-arm olfactometer. The 4 areas of the arena for the whitefly's choices and the locations of the stimuli/control are illustrated.

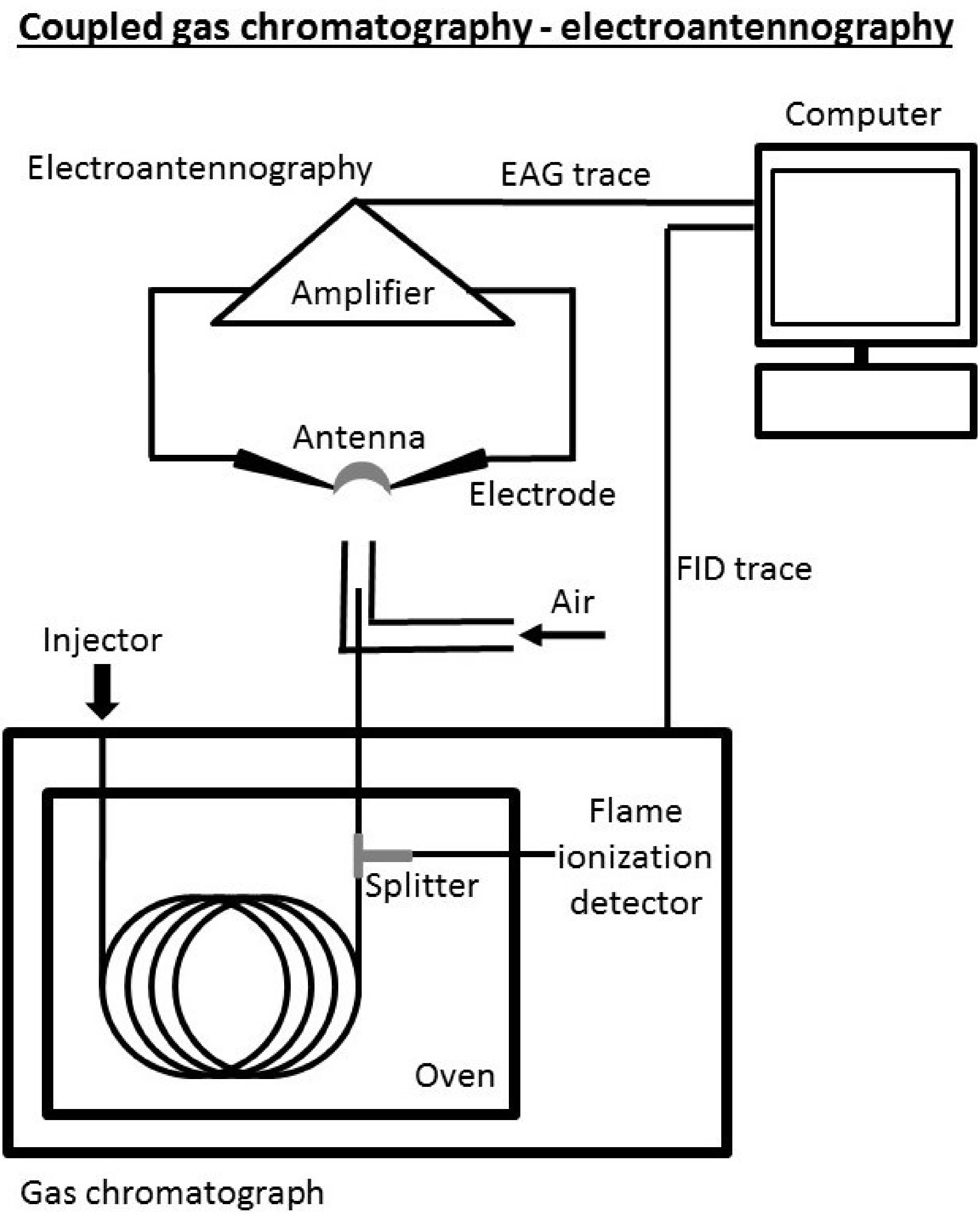
Coupled gas chromatography - electroantennography
Computer
Electroantennography
EAG trace
Amplifier
Antenna
Electrode
FID trace
Air
Injector
Flame
ionization
detector
Splitter
Oven
Gas chromatograph

Figure 1.jpg

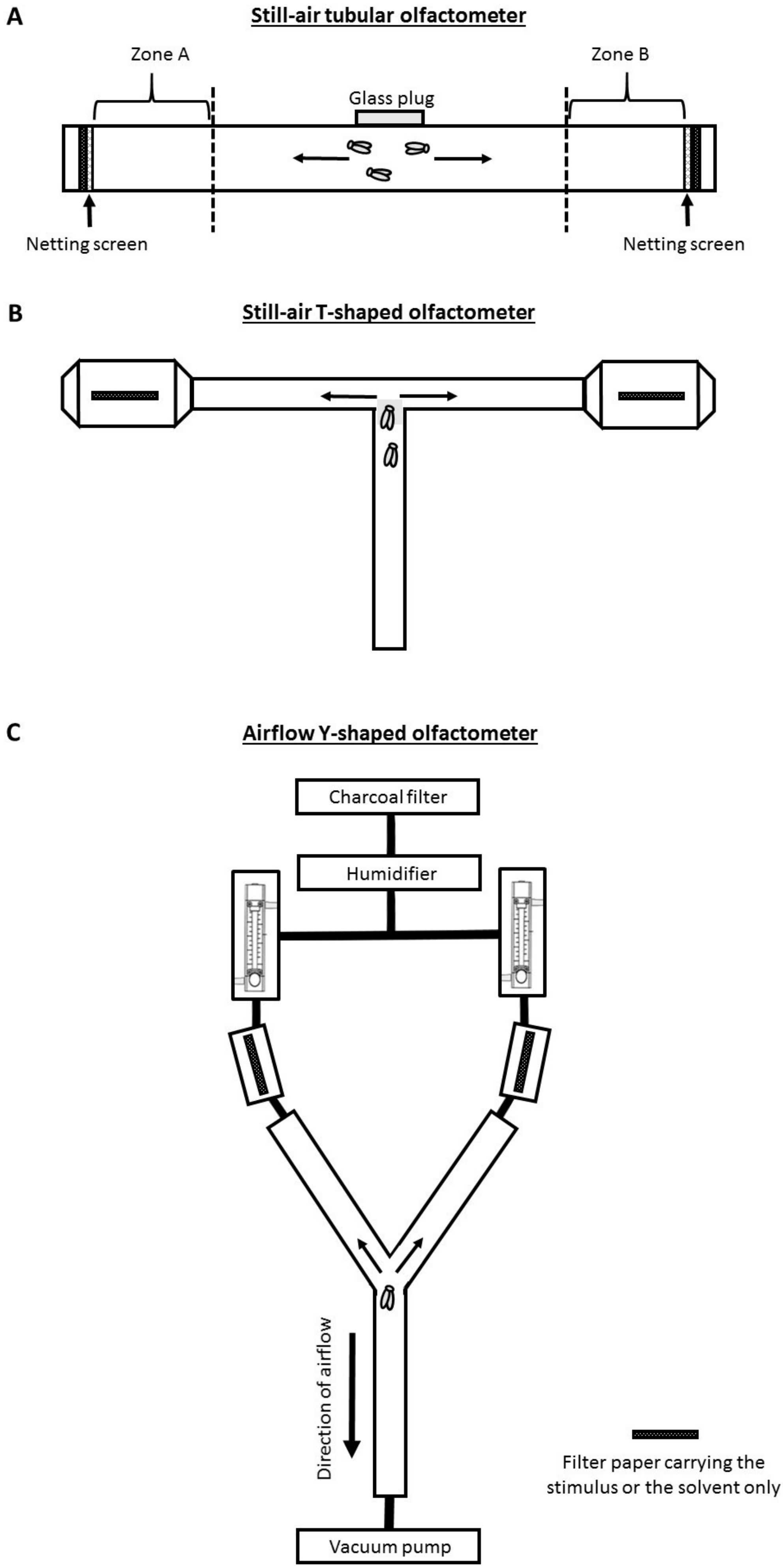
A
Still-air tubular olfactometer
Zone A
Zone B
Glass plug
Netting screen
Netting screen
B
Still-air T-shaped olfactometer
C
Airflow Y-shaped olfactometer
Charcoal filter
Humidifier
Direction of airflow
Filter paper carrying the
stimulus or the solvent only
Vacuum pump

Figure 2.jpg

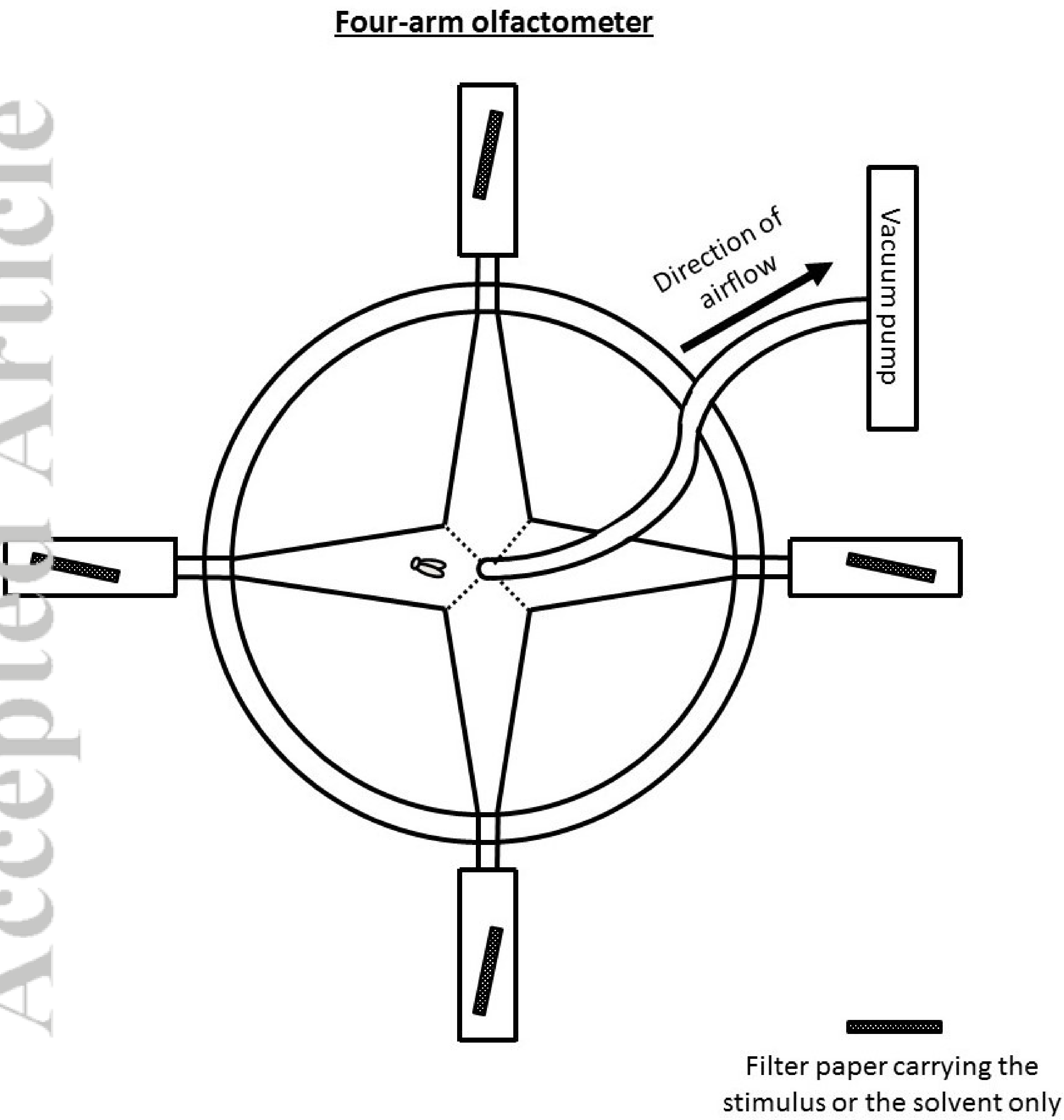
Four-arm olfactometer
Direction of airflow
Vacuum pump
Filter paper carrying the stimulus or the solvent only

Figure 3.jpg