Morphological and chemical plant properties mediate host plant selection of whiteflies (Hemiptera: Aleyrodidae)

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1 General introduction

1.1 Whiteflies

With more than 1,550 species, whiteflies constitute a large group of hemipterans that differ in their biology and distribution, thus highlighting their adaptability to a wide range of ecological conditions and host plant species (BÄHRMANN 2002; OUVRARD AND MARTIN 2019). Consequently, important crop plants are also found among their host plant range. Today, whiteflies are among the most important agricultural pests causing severe direct and indirect damage to numerous crops and ornamentals in fields and greenhouses worldwide (BYRNE ET AL. 1990; BRØDSGAARD AND ALBAJES 2002; WRAIGHT ET AL. 2017). Considering only the tropics and subtropics, the estimated economic losses amount to hundreds of millions of dollars per year; moreover, an increase of the whitefly outbreaks in wide areas beyond the tropics is known for decades (DUFFUS 1987; BINK-MOENEN AND MOUND 1990; CAPINERA 2008).

Whitefly life cycle and biology

Overall, the life cycle of whiteflies comprises six developmental stages (Figure 1). Whiteflies feed, mate and oviposit almost exclusively on the leaf undersides of their host plants (BÄHRMANN 2002). Whitefly eggs are pear-shaped or ovoid and carry an egg pedicel, by which they are anchored into the leaf tissue and stomata together with a glue-like substance produced by the female whitefly during oviposition (PAULSON AND BEARDSLEY 1985; BYRNE AND BELLOWS 1991; BUCKNER ET AL. 2002; VOIGT ET AL. 2019). After a few days, eggs become increasingly darker until hatching of the first instar larva (BÄHRMANN 2002).

All nymphal stages are characterized by an oval body shape, but only the first larval instar possesses well-developed legs as well as antennae (BYRNE AND BELLOWS 1991; BÄHRMANN 2002; WALKER ET AL. 2010). As the first instar is the only larval stage capable of moving over short distances, they are also called crawlers (BYRNE AND BELLOWS 1991; SUMMERS ET AL. 1996). Immediately after hatching crawlers search suitable feeding sites on phloem within reach, which must fulfil all nutritional needs for further development (WEBER 1931; WALKER ET AL. 2010). As a result, they usually settle very close to their hatching site on the leaf underside and start feeding (VAN LENTEREN AND NOLDUS 1990; PRICE AND TABORSKY 1992).

Henceforth, length and width of the following larval instars increase with every moult and the body extremities show a regressive development leading to a sessile life form (BÄHRMANN 2002; GELMAN ET AL. 2002). Right before each moult, the larvae need to pull out their stylets from leaf tissues to completely shed their cuticles (LEI ET AL. 1996).

The preimaginal development of whiteflies is a special case of hemimetabolism, named allometaboly, with a strong deferral of the formation of adult morphological features, which is made up in the fourth nymphal instar. This fourth nymphal instar is characterized by a process of metamorphosis and forms a kind of a pupal stage, which is also referred to as puparium (BYRNE AND BELLOWS 1991; BÄHRMANN 2002). It includes several changes on the morphological and physiological level: The early fourth larval instar is flattened and translucent, it then soon develops an expanded and opaque-white appearance with species-specific patterns of dorsal and lateral spine-like extensions (BYRNE AND BELLOWS 1991; BÄHRMANN 2002). At some point, feeding as well as the production of honeydew are stopped (LEI ET AL. 1996; COSTA ET AL. 1999). The first externally visible feature of metamorphosis is the occurrence of two red spots, which later develop into compartment eyes, giving rise to the term red-eyed nymph (GELMAN ET AL. 2002; WALKER ET AL. 2010). It is hypothesized that the puparium is the result of the suppression of a nymphal stage and, therefore, misses one moult (WEBER 1934). After completing metamorphosis, adults hatch and the empty puparial case is left on the leaves (BÄHRMANN 2002).

In adult whiteflies, males are smaller than females, and both sexes carry four membranous wings (BYRNE AND BELLOWS 1991). Moreover, adults are completely covered in extracuticular waxes which are produced by themselves and spread on their entire bodies except for the eyes (BYRNE AND HADLEY 1988; NELSON ET AL. 2000). Due to this wax coverage, whiteflies resemble tiny moths, which already led to taxonomic mismatches in the past (MARTIN ET AL. 2000). Most whiteflies show parthenogenesis and reproduce by arrhenotoky (BYRNE AND BELLOWS 1991). Under summer conditions, the first mating and oviposition take place only a few hours after adult emergence either on the same leaves or after migration to different plants or plant parts (BÄHRMANN 2002).

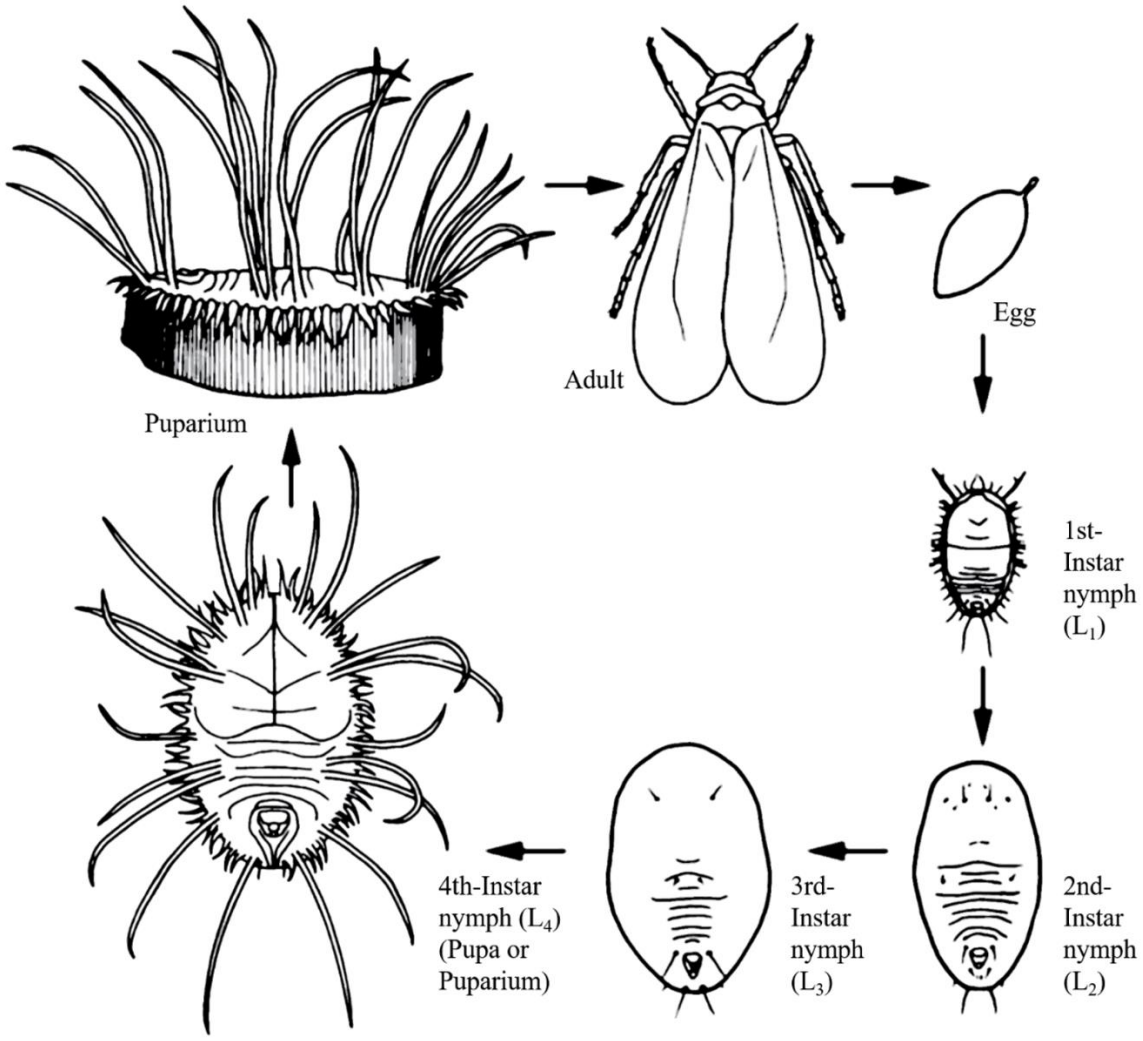

Figure 1: The life cycle of the greenhouse whitefly *Trialeurodes vaporariorum* (Westw.) (modified after GULLAN AND MARTIN 2009; original whitefly images after GILL 1990)

The cabbage whitefly

In temperate latitudes the cabbage whitefly *Aleyrodes proletella* (L.) is distributed around the world (MOUND AND HALSEY 1978; BÄHRMANN 2002). In earlier times, *A. proletella* was not considered an agricultural threat, whereas the insect is seen as a serious pest today (CARDEN 1972; DALE ET AL. 1976; NEBREDA ET AL. 2005). The cabbage whitefly is considered polyphagous but infests only few plant families compared to some of its family members. While its main host plants are *Brassica* species, *A. proletella* is also known to infest plants of the Compositae and Papaveraceae family (HILL 1987; BÄHRMANN 2002). In middle Europe, females can be observed laying eggs on their hosts throughout the year (BÄHRMANN 2002). In areas with temperatures below zero in the winter season, however, egg deposition of the overwintering female *A. proletella* starts in the spring with temperatures above 8–10 °C (IHEAGWAM 1978; BÄHRMANN 2002). If female cabbage

whiteflies are not disturbed, egg deposition is practiced in circular patterns, as the female rotates around the stylet insertion point while still feeding on its host (EL-HELALY ET AL. 1972; BÄHRMANN 2002). Furthermore, *A. proletella* is a potential polyvoltine species with the ability to develop more than two but usually less than four generations per year, which is mainly dependent on ambient temperatures (BÄHRMANN 2002). The adult cabbage whitefly measures approximately 1.4 mm in body length (DESHPANDE 1933). Their outer appearance is slightly darker coloured in comparison to other whitefly species, especially the abdominal part, and the wings carry dark spots (BÄHRMANN 2002) (Figure 2).

The silverleaf whitefly

The silverleaf whitefly *Bemisia tabaci* (Genn.) has a worldwide distribution and inhabits every continent except Antarctica (EPPO 2019; OUVRARD AND MARTIN 2019). It is a pest of the tropics and subtropics but can be found in greenhouses of temperate environments as well (EPPO 2019). Demonstrating its high degree of polyphagia, *B. tabaci* infests more than 500 plant species in 63 plant families, including important vegetables and ornamentals (BAUFELD AND UNGER 1994; BÄHRMANN 2002). The damage potential of the silverleaf whitefly is further enhanced by its ability to transmit numerous viral diseases, particularly gemini viruses, such as the economically important Tomato leaf curl virus, African cassava mosaic virus or Pepper leaf curl virus (COHEN 1990). Another defining feature of the silverleaf whitefly is its genetic plasticity, which is expressed by the presence of several biotypes of this species complex (BURBAN ET AL. 1992; JIMENEZ ET AL. 1994). These biotypes differ not only on the molecular level but also in their preferences towards their host plants (BÄHRMANN 2002). Due to this genetic plasticity and its huge damage potential, *B. tabaci* is a successful invasive species that has led to several historical outbreaks in the past such as the 1920s in India, the 1930s and 1940s in Israel, as well as the 1970s in Brazil (NARANJO ET AL. 2010). During their life span, female silverleaf whiteflies can lay over 300 eggs, which are often deposited in an arc shape (GANGWAR AND GANGWAR 2018). Moreover, *B. tabaci* can form 11–14 generations per year in the field of suitable areas (GERLING AND MAYER 1996). The adult *B. tabaci* is about 0.85–0.91 mm long with a white to yellowish body colour and white wings, which are typically resting in a roof-shaped manner (BÄHRMANN 2002) (Figure 2).

The greenhouse whitefly

The greenhouse whitefly *Trialeurodes vaporariorum* (Westw.) is present in Africa, America, Asia, Europe, and Oceania (BÄHRMANN 2002; EPPO 2019). It is predominantly found in the fields of the tropics and subtropics but has become an important key pest on vegetable crops in greenhouses

all around the world (LANGE AND BRONSON 1981; VAN LENTEREN AND WOETS 1988). As a distinct generalist species, the greenhouse whitefly shows an extremely high degree in polyphagia with a host plant range consisting of approximately 859 crop and ornamental species of a total of 121 plant families (CABI 2019). Together with other species of the *Trialeurodes* and *Bemisia* genera, *T. vaporariorum* is a vector of viral diseases (JONES 2003). Females can lay more than 500 eggs during their lives on suitable hosts, which are deposited in circles on hair-less leaves and without a pattern on pubescent leaves (WEBER 1931; CABI 2019). Depending on local conditions, the greenhouse whitefly can form between 7 and 11 generations per year (GAMARRA ET AL. 2016). Adults measure between 1 to 1.1 mm and have a pale-yellow body with white wings, which are held flat (BYRNE AND BELLOWS 1991; CABI 2019). In comparison to *B. tabaci*, *T. vaporariorum* has a more triangular shape and can additionally be well distinguished according to the outer appearance of the puparium, which carries several waxy setae (BÄHRMANN 2002) (Figure 2).

Figure 2: Adults of *A. proletella* (1), *B. tabaci* (2), and *T. vaporariorum* (3)

Crop damage and whitefly management strategies

Plant damage and crop yield loss from whitefly infestations are mainly caused by three different reasons resulting in various consequences. As whiteflies feed on the phloem of their host plants to ingest assimilates and amino acids, reduced plant growth with decreased number and size of leaves is a typical damage symptom (GANGE AND BROWN 1989; BYRNE AND MILLER 1990; BYRNE AND BELLOWS 1991; BÄHRMANN 2002). Furthermore, the honeydew secreted in high quantities by whitefly adults and nymphs reflects light and promotes sooty mould fungi. Light reflectance by honeydew and coverage by sooty moulds both reduce direct radiation by increased diffuse light (honeydew) or light absorbance (sooty mould), leading to a decreased photosynthesis rate of the plant (RABBINGE AND BASTIAANS 1989; BYRNE AND MILLER 1990; HONG AND RUMEI 1993; MIBEY

1997). In addition, gas exchange can also be affected causing early plant ageing, which can be accelerated by stomatal occlusion by whitefly wax excretions and honeydew (KLINGAUF AND ŞENGONCA 1982). As a result, agricultural plant products have lower marketability due to substantial reductions in quality, which can even lead to complete yield loss (BYRNE AND BELLOWS 1991; SAUCKE ET AL. 2011). Thirdly, some whitefly species act as plant virus vectors with 90 % of the transmitted viruses belonging to the genus Begomovirus of the family Geminiviridae, which causes important diseases of numerous dicotyledonous crops worldwide (JONES 2003; NAVAS-CASTILLO ET AL. 2011). An important representative of this genus is, for instance, the Tomato yellow leaf curl virus (CZOSNEK ET AL. 2017).

The most common way to control whiteflies is the use of insecticides in the field and by parasitoids in greenhouses. Nevertheless, several circumstances may lead to control failure and fast pest resurgence. Chemical measures become more and more limited, as resistances against multiple classes of insecticides have been reported and are widespread in global whitefly populations today (NAUEN AND DENHOLM 2005; BASS ET AL. 2015; DÂNGELO ET AL. 2018). Furthermore, contact insecticides may constitute a problem, as whiteflies mainly sit on the lower leaf sides, which are difficult to reach directly by spray applications (BÄHRMANN 2002). Another limiting factor in the field is the passive distribution of whiteflies by wind, which causes repeatedly introductions of the pest from outside or neighbouring fields despite a successful control in the first place (VAN LENTEREN AND NOLDUS 1990; BYRNE ET AL. 1996; BÄHRMANN 2002). While biological control alone is generally less effective in the field, it is widely performed in greenhouses using predominantly parasitic wasps of the genera *Encarsia* and *Eretmocerus* (VAN LENTEREN AND WOETS 1988; VAN LENTEREN 2000; LIU ET AL. 2015). Other biological control agents used are the mirid *Macrolophus caliginosus* (Wagner), predacious mites, particularly *Amblyseius swirskii* (Athias-Henriot), as well as entomopathogenic fungi (OSBORNE AND LANDA 1992; ALOMAR ET AL. 2006; KNAPP ET AL. 2018). Nevertheless, successful augmentation and parasitization by antagonists in greenhouses are accompanied by several obstacles. On the one hand, physical factors such as greenhouse temperature, crop spacing, and fertilization regime can affect pest control; on the other hand, plant factors, for instance plant species and variety, plant trichomes, morphological leaf characteristics, and changing canopy influences performance of whitefly antagonists (HODDLE ET AL. 1998). While yellow sticky traps are mainly used for whitefly monitoring, they have been discussed as a control method in greenhouses as well (STEINER ET AL. 1999). In contrast, yellow sticky traps proved to be ineffective in whitefly suppression in the field (LU ET AL. 2012). However, cultural control methods such as trap crops, intercropping, and mulching were recently reported to represent a useful supportive component in whitefly management strategies (PERRING ET AL. 2018).

1.2 Host plant selection by whiteflies

The selection of hosts that offer suitable sites for feeding and oviposition is a central evolutionary element in herbivorous insect-plant associations. This allows insects to ensure the best prerequisites for themselves and their offspring's performance (SINGER 1986; THOMPSON 1988; VAN LENTEREN AND NOLDUS 1990). As potential host plants grow together with non-hosts in complex vegetation, host recognition is crucial for species survival (SCHOONHOVEN ET AL. 2005). Hence, insects have developed individual strategies to identify a suitable host plant. According to the general conception, insects follow an individual sequence of behavioural elements in a fixed order (SCHOONHOVEN ET AL. 2005). At any stage of these reaction chains, plants or plant parts that are evaluated as potential sites for feeding and oviposition so far can be rejected when positive stimuli are absent or negative stimuli dominate at a certain behavioural step, and insects return to the first behavioural step, searching, again (SCHOONHOVEN ET AL. 2005). Decisions over acceptance or rejection are driven by the sensory information based on plant cues, but the physiological status and previous experiences may affect the host selection process of an herbivorous insect as well (BROWNE 1993; SCHOONHOVEN ET AL. 2005). Host plant selection is particularly important in female whiteflies, as feeding and oviposition occur on the same leaves and are even done simultaneously. Moreover, larval stages are predominantly sessile, so host selection occurs only in adult whiteflies with profound effects on the offspring's fitness (VAN LENTEREN AND NOLDUS 1990; BÄHRMANN 2002). According to this "mother-knows-best-principle", host preference in whiteflies is often associated with host suitability and whitefly performance (LEVINS AND MACARTHUR 1969; JAENIKE 1978; THOMPSON 1988; VAN LENTEREN AND NOLDUS 1990; BLUA ET AL. 1995; MAYHEW 1997; GRIPENBERG ET AL. 2010; TARAVATI ET AL. 2018). As potential hosts can be recognized by insects from a distance as well as after landing, the process of host plant selection in whiteflies can be divided into (1) searching and (2) contact-testing (VAN LENTEREN AND NOLDUS 1990; SCHOONHOVEN ET AL. 2005). While the first phase describes the selection of a host plant before landing, the second part comprises the selection of a host plant after landing including the stylet penetration process.

Host plant selection before landing

Colour is an important first cue to which whiteflies react from a distance. For the selection of landing sites, specific wavelengths in the ranges of 400–600 nm play an important role, while object size and shape do not have an influence (DOWELL 1979; COOMBE 1982). Consequently, whiteflies orientate towards the blue sky and tend to land on green plants, as wavelengths around 400 nm correspond to the blue sky and wavelengths between 500 and 600 nm coincide with the transmission spectrum of green leaves (MACDOWALL 1972; VAISHAMPAYAN ET AL. 1975; COOMBE 1982). Additionally, short-wavelength UV radiation provokes migratory behaviour with enhanced

locomotory functions and longer wavelengths trigger vegetative behaviour such as feeding and reproduction (MOUND 1962; AFFELDT ET AL. 1983; VAN LENTEREN AND NOLDUS 1990). First studies on whitefly olfaction could only find limited evidence of an odour-assisted host plant localization (MOUND 1962; VAISHAMPAYAN ET AL. 1975; DOWELL 1979). However, *A. proletella* reacts to odours of crushed cabbage leaves and recent studies could indeed show that *B. tabaci* and *T. vaporariorum* use olfactory cues for selection of landing sites among different plant varieties, cultivars and accessions (BUTLER 1938; BLEEKER ET AL. 2009; DARSHANEE ET AL. 2017; SADEH ET AL. 2017; TU AND QIN 2017). Moreover, whiteflies can distinguish different qualities of potential host plants concerning their nitrogen supply, leaf position, and health status (TAN AND LIU 2014; TSUEDA ET AL. 2014; FERERES ET AL. 2016; ISLAM ET AL. 2017; SCHLAEGER ET AL. 2018).

Host plant selection after landing and stylet penetration

After choosing a landing site, whiteflies are influenced by visual stimuli to select a suitable feeding site as a starting point for stylet penetration. While whiteflies usually land on the upper and more intensively illuminated leaf side, they walk to the shaded side regardless of whether it is the adaxial or abaxial leaf side (COOMBE 1982; VAN LENTEREN AND NOLDUS 1990). Moreover, young leaves are more preferred than old leaves, which might be due to probing deterrents on leaf cuticles, differences in the penetrability of the leaf cuticles or strategic advantages for subsequent generations (OHNESORGE ET AL. 1981; WALKER 1987; WALKER 1988; WALKER AND ZAREH 1990; BÄHRMANN 2002). Leaf characteristics such as epicuticular surface waxes or leaf hairs can be perceived by several contact sense organs in whiteflies, which are located on antennae, stylets, tarsi as well as ovipositors (BERLINGER 1986; WALKER AND GORDH 1989). While only little is known about the influence of epicuticular surface waxes on whiteflies, the effect of leaf hairs is ambivalent. On one hand, leaf hairs can represent a physical barrier for oviposition and larval development; on the other hand, leaf hairs may contribute to a favourable microclimate (BÄHRMANN 2002).

In the beginning of the stylet penetration process, whiteflies penetrate the leaf tissue by inserting their stylets between epidermal cells or use stomata as an entry (POLLARD 1955; WALKER 1985; VAN LENTEREN AND NOLDUS 1990). Thenceforth, parenchyma penetration is performed mainly intercellular, whereby penetration between adjacent cell walls and intercellular air spaces in the leaf tissue are common (POLLARD 1955; COHEN ET AL. 1998). As intracellular punctures are rare in whiteflies, only limited damage of leaf tissues occurs through penetration (POLLARD 1955; JANSSEN ET AL. 1989; WALKER ET AL. 2010). The objective is the penetration of a phloem sieve element and the continuous feeding on phloem sap, as this represents the main food source in whiteflies (LEI ET AL. 2001; BÄHRMANN 2002; WALKER ET AL. 2010). Feeding on xylem sap might occur as well, although it contains fewer nutrients compared to the phloem sap (POMPON ET AL. 2011). Instead,

xylem feeding was associated with supporting water balance and regulating osmotic potential in phloem sap feeders (SPILLER ET AL. 1990; POMPON ET AL. 2011). Overall, leaf penetration and localization of phloem sieve elements are time-consuming, and several probing attempts are necessary before the phloem is successfully reached (LEI ET AL. 1997). In conclusion, the stylet penetration process is based on various internal chemical and physical properties of the different leaf tissue types of a leaf (VAN LENTEREN AND NOLDUS 1990). Besides properties of the leaf epidermis, various factors of the mesophyll tissue, vascular bundles, and even intracellular information affect host choice in whiteflies (LEI ET AL. 1998; LEI ET AL. 1999; LEI ET AL. 2001).

1.3 Host plant resistance against whiteflies

Host plant resistance is defined as any reduction in the growth of an insect population, as influenced by heritable host plant characteristics, compared to a susceptible variety or genotype (PAINTER 1951; DE PONTI ET AL. 1990). Integrated pest control systems, which also utilize plant resistance, are therefore a powerful and effective form of pest control that is also considered to be very economical and environmentally friendly (RUSSELL 1978; DE PONTI ET AL. 1990; PALANISWAMY 1996; BROEKGAARDEN ET AL. 2011; VAN DOORN AND VOS 2013). According to PAINTER (1951) and KOGAN AND ORTMAN (1978) host plant resistance can be grouped into three categories including (i) antixenosis (non-preference), (ii) antibiosis (non-performance), as well as (iii) tolerance. Antixenosis defines a group of plant properties that deter insects from the use of the plant as a source for food, oviposition and/or shelter (PAINTER 1951; KOGAN AND ORTMAN 1978; SCHOONHOVEN ET AL. 2005). Therefore, repellent, deterrent or antifeedant effects interfere with mating, oviposition, and feeding in insects (PAINTER 1951; SCHOONHOVEN ET AL. 2005). Furthermore, interference of insect behaviour can be already caused by the absence of a stimulus required for host recognition within the host selection process (PANDA AND KHUSH 1995). Antibiosis, on the other hand, refers to plant characteristics that have adverse effects on an insect's physiology including its growth, development, reproduction, and survival (PANDA AND KHUSH 1995; SCHOONHOVEN ET AL. 2005). Biophysical and biochemical plant defences, as well as nutritional factors, are involved in antibiosis disrupting the normal metabolic processes of an insect (PANDA AND KHUSH 1995). Consequently, antixenosis is related to behavioural aspects, while antibiosis occurs from direct lethal effects (DE PONTI ET AL. 1990). In contrast to antixenosis and antibiosis, tolerance describes the ability of a plant to withstand pest infestation or to compensate loss or injury (STONER 1996). As a result, insect pests do not experience a selection pressure from tolerance, as the rate of pest population increase is not affected (PANDA AND KHUSH 1995; SCHOONHOVEN ET AL. 2005). Therefore, tolerance is no longer considered as a subcategory of plant resistance today but rather seen as a plant defence mechanism (SCHOONHOVEN ET AL. 2005).

Plant resistance to herbivores is observed frequently; however, the identification of plant characteristics contributing to plant resistance is difficult (STONER 1992). To detect resistance in a large assortment of plant varieties and genotypes, two basic test methods can be used to evaluate insect responses to host plants: (i) dual- or multi-choice tests for detection of antixenosis, and (ii) no-choice tests for detection of antibiosis (DAVIS 1985; SMITH ET AL. 1994). While antixenosis is evaluated by counting adults and/or eggs as a measure for preference on two or more choice alternatives, antibiosis is assessed by comparing life-history data of whitefly oviposition rates, adult and nymph survival as well as development times on specific hosts without a choice alternative (BAS ET AL. 1992; MUIGAI ET AL. 2002; MUIGAI ET AL. 2003; BALDIN AND BENEDUZZI 2010). Screening techniques in the greenhouse and laboratory include tests with whole plants or plant leaves in combination with leaf clip cages, whereas other approaches successfully established *in vitro* bioassays with detached leaves or leaf discs, which are less cost intensive and space consuming (ROMANOW ET AL. 1991; ERB ET AL. 1994; SHARMA ET AL. 2005; FIRDAUS ET AL. 2012; GUO ET AL. 2013).

Even though host plant resistance has been observed repeatedly in the past, the origin and mechanisms of the resistance were less studied and, therefore, often remained unidentified. Most studies on host plant resistance to whiteflies have focused on cotton, tomato, sweet pepper, eggplant, cucumber, soybean and cassava (DE PONTI ET AL. 1990; LAMBERT ET AL. 1995; BELLOTTI ET AL. 1999). The mechanisms causing plant resistance against whiteflies can be of physical, chemical, or morphological nature and determine the respective testing and breeding methods, which are required to introduce resistant traits into other desired plants (DE PONTI ET AL. 1990). Among the plant characteristics known to date that contribute to whitefly resistance are the presence, morphology and appearance of leaf trichomes, epicuticular lipid composition, tissue hardness and cuticle thickness, leaf structure, leaf shape and plant canopy closure, plant height, plant pH, secondary metabolites, as well as plant chemicals that may occur in all plant tissues and structures (BERLINGER ET AL. 1983; WALKER 1985; BERLINGER 1986; SIPPELL ET AL. 1987; WALKER 1987; BUTLER ET AL. 1988; WALKER 1988; DE PONTI ET AL. 1990; CHANNARAYAPPA ET AL. 1992; SNYDER ET AL. 1998; TOSCANO ET AL. 2002; LAMBERT ET AL. 1995; SÁNCHEZ-PEÑA ET AL. 2006; FIRDAUS ET AL. 2011; HASANUZZAMAN ET AL. 2016; HASANUZZAMAN ET AL. 2018; VOIGT ET AL. 2019). Nevertheless, the combinations and potential interactions between these plant characteristics and the final resistance mechanism are still not fully understood, yet.

1.4 Objectives

Despite intensive studies on whiteflies with focus on its biology, behaviour, ecology, damage, and control, many aspects of this species-rich complex are still unknown to date. As current control measures do not provide adequate success, the worldwide whitefly problematic is constantly growing (DUFFUS 1987; BINK-MOENEN AND MOUND 1990; NAUEN AND DENHOLM 2005; CAPINERA 2008; BASS ET AL. 2015; DÂNGELO ET AL. 2018). However, a deep understanding of the host plant selection process of whiteflies is essential for the development of alternative control strategies (VAN LENTEREN AND NOLDUS 1990). While the mediating factors of the whitefly host choice behaviour before landing are well known, host plant selection after landing as well as the stylet penetration process still raise numerous questions.

Based on the results of bioassays with several host plant species and cultivars on host preferences of three whitefly species, this work should shed light on potential antixenotic traits affecting whitefly host selection. Electrical penetration graph (EPG) analysis should elucidate potential sources of host plant resistance affecting the stylet penetration process of whiteflies. Subsequently, the role of epicuticular leaf waxes of cruciferous plants in the host plant selection process of *A. proletella* should be investigated. Furthermore, the contribution and association of single amino acids present in the phloem sap of several vegetable crops on the host choice behaviour of *T. vaporariorum* should be evaluated. Due to the heterogeneity of the issue and the methodology, the different topics are covered by the following separate chapters set up as manuscripts to be submitted for publication.

2 Host plant species and cultivar preferences of three whitefly species

Abstract: Studies on host plant adaption and host plant selection in whiteflies require bioassays on host plant preference as a reference. In dual choice tests, host preferences of *Aleyrodes proletella* (L.), *Bemisia tabaci* (Genn.), and *Trialeurodes vaporariorum* (Westw.) were compared using various host plants. To assess and rank host attractiveness for each whitefly species, preference indices were determined for each host-whitefly combination. For *A. proletella*, host preference was found in decreasing order by oilseed rape > kale > savoy cabbage > blue turnip cabbage > cauliflower > white turnip cabbage > white cabbage. For *B. tabaci* and *T. vaporariorum* host preference was found to be superior for eggplant followed in decreasing order by tobacco > tomato > cucumber > bean > sweet pepper. This study provides insight into whitefly-host adaption of three whitefly species and may be used as a reference for further studies. As significant differences within host rankings not necessarily presupposed a significant outcome in dual choice tests of this study, it is recommended to assess host preferences individually following the research question and study design.

Keywords: behaviour, host plant resistance, plant cultivars

2.1 Introduction

Characterization and quantification of specific behaviours associated with host plant choice are essential studies to describe host plant adaptation and chemical ecology of herbivorous insects (KNOLHOFF AND HECKEL 2014). Choice tests are commonly used to quantify the effects of a wide range of environmental factors, as well as hereditary and anthropogenic influences on insect behaviour (LOCKWOOD 1998; RAFFA ET AL. 2002). Bioassays on host plant preference are particularly used in crop cultivar screenings for resistant varieties, which are an effective control measure against insect pests in integrated pest control systems (RUSSELL 1978; DE PONTI ET AL. 1990; PALANISWAMY 1996; RAFFA ET AL. 2002; SCHOONHOVEN ET AL. 2005; BROEKGAARDEN ET AL. 2011; VAN DOORN AND VOS 2013). In whiteflies, host plant selection is of paramount importance as females feed and oviposit simultaneously on the same host plant leaves (BÄHRMANN 2002). Additionally, larval stages are predominantly immobile, so that host selection is limited to adult whiteflies with profound effects on the offspring's preimaginal development and physical fitness of adults. As a result, host preference in whiteflies is strongly associated with host suitability and the subsequent pest performance (LEVINS AND MACARTHUR 1969; JAENIKE 1978; THOMPSON 1988; VAN LENTEREN AND NOLDUS 1990; BLUA ET AL. 1995; MAYHEW 1997; GRIPENBERG ET AL. 2010; TARAVATI ET AL. 2018). Consequently, disruption of the host plant selection process caused by host

recognition failure in adult whiteflies would lead to lower infestation levels, and may even affect pest performance, best represented by lifetable parameters (PAINTER 1951; SCHOONHOVEN ET AL. 2005). The standard approach of preference testing is the simultaneous offer of choice alternatives to assess an insect's relative response (RAFFA ET AL. 2002). Such screening techniques can include tests with whole plants or plant leaves in greenhouse and laboratory, whereas other approaches successfully established *in vitro* bioassays with detached leaves or leaf discs (ERB ET AL. 1994; SHARMA ET AL. 2005; FIRDAUS ET AL. 2012; GUO ET AL. 2013). The objective of the present study was to assess host plant preferences of several host plant species and cultivars offered for adult *Aleyrodes proletella* (L.), *Bemisia tabaci* (Genn.) and *Trialeurodes vaporariorum* (Westw.). Therefore, dual choice bioassays under greenhouse conditions using leaf clip cages were performed, thus extending the knowledge of the food spectrum of these economically important whitefly species.

2.2 Materials and methods

Insects and plants

Adults of *Aleyrodes proletella* (L.), *Bemisia tabaci* (Genn.), and *Trialeurodes vaporariorum* (Westw.) were obtained from the institute's stock rearings (Department of Applied Entomology, Institute of Phytomedicine, University of Hohenheim) reared in the greenhouse on broccoli and poinsettia (25/23 ± 2 °C each, L18/D6 photoperiod, 50 ± 5 % RH).

Seven *Brassica* cultivars and six vegetables were grown in pots (LC 14, Pöppelmann GmbH & Co. KG, Lohne, Germany): blue and white turnip cabbage (BTC, WTC) (*Brassica oleracea* L. convar. *acephala* var. *gongylodes*, cv. "Delikateß Blauer", cv. "Delikateß Weißer"), cauliflower (CA) (*Brassica oleracea* L. convar. *botrytis* var. *botrytis*, cv. "Erfurter Zwerg"), kale (KA) (*Brassica oleracea* L. convar. *acephala* var. *sabellica*, cv. "Grüner Krauser"), savoy cabbage (SC) (*Brassica oleracea* L. convar. *capitata* var. *sabauda*, cv. "Vertus"), white cabbage (WC) (*Brassica oleracea* L. convar. *capitata* var. *alba*, cv. "Brunswijker"), oilseed rape (OR) (*Brassica napus* L. subsp. *napus*, cv. "Attila"), bean (BE) (*Phaseolus vulgaris* L., cv. "Rakker"), cucumber (CU) (*Cucumis sativus* L., cv. "Delikateß"), eggplant (EG) (*Solanum melongena* L., cv. "Falcon"), sweet pepper (SP) (*Capsicum annuum* L., cv. "California Wonder"), tobacco (TB) (*Nicotiana tabacum* L., cv. "Orient Xanthi"), and tomato (TO) (*Solanum lycopersicum* L., cv. "Resi"). Experimental plants were grown under greenhouse conditions (22/18 ± 2 °C each, L18/D6 photoperiod, 50 ± 5 % RH), irrigated daily, and fertilized weekly with 30 ml 0.5 % Wuxal® Super (8 % N, 8 % P, 6 % K, Aglukon GmbH, Düsseldorf, Germany). The soil mixture was composed of 50 % potting soil (Floradur®, Floragard Vertriebs-GmbH, Oldenburg, Germany), 30 % compost soil (institute's production), and 20 % sand. The plants were used in experiments when they reached BBCH stage 17–18.

Host attractivity screening

Attractiveness levels of host plants were assessed in a series of dual choice cage experiments with different combinations of whiteflies and host plant species in the greenhouse (Table 1). Overall, single tests were carried out as a completely randomized block design which was repeated eight times at different time intervals. For each choice test, whiteflies were offered a young expanded leaf of each test plant in opposite position. At least 20 randomly selected adult whiteflies were taken from the stock rearing by a suction tube and placed into one clip cage made of clear plastic Petri dishes (8.5 cm diam., 1.5 cm height) fitted with foam seal on edges to prevent any mechanical damage to the leaves and with an organdy-covered window in the lid for ventilation. The leaf area covered by the cage was kept as small as possible to minimize potential negative effects on photosynthetic traits of leaves (CRAFTS-BRANDNER AND CHU 1999). Cages were mounted on leaves using aluminium hair clips, which were retained by thin split bamboo sticks to not bend or even damage the plant leaves. After two days, the number of whitefly individuals was counted on each leaf.

Table 1: Whitefly-host plant combinations tested in host attractivity screening experiments

Whitefly species	Host plants
Aleyrodes proletella	blue turnip cabbage (BTC) *vs.* white turnip cabbage (WTC) *vs.* cauliflower (CA) *vs.* kale (KA) *vs.* oilseed rape (OR) *vs.* savoy cabbage (SC) *vs.* white cabbage (WC)
Bemisia tabaci	Bean (BE) *vs.* cucumber (CU) *vs.* eggplant (EG) *vs.* sweet pepper (SP) *vs.* tobacco (TB) *vs.* tomato (TO)
Trialeurodes vaporariorum	Bean (BE) *vs.* cucumber (CU) *vs.* eggplant (EG) *vs.* sweet pepper (SP) *vs.* tobacco (TB) *vs.* tomato (TO)

Statistics

All obtained data were analysed using JMP® 14.1.0 (SAS Institute Inc., Cary, NC, USA). The respective statistical procedures and statistical core data are provided in the legends of the tables. For determination of host plant attractiveness of two host plant choices, preference indices (PI) were calculated using the following equation based on KOGAN AND GOEDEN (1970):

$$Preference\ index\ (PI) = \frac{n_A}{n_A + n_B}$$

where n_A is the number of whitefly adults on host plant choice A and n_B is the number of whitefly adults on host plant choice B. In this scale, PI = 1 and PI = 0 represent an absolute preference for one of the two choice alternatives, whereas PI = 0.5 implies no preference between both choice alternatives.

2.3 Results

Significant differences in host attractiveness was found for each whitefly species in dual choice tests of various host plant combinations (Figure 3, 4, 5). To determine the overall attractiveness of each host plant within one bioassay, the mean preference index was formed from all dual tests of the respective host (Table 2). As a result, each host could be ranked according to its mean preference index within one bioassay. For *A. proletella*, host attractiveness was ranked as OR > KA > SC > BTC > CA > WTC > WC. While oilseed rape, kale and savoy cabbage were preferred hosts with preference indices higher than 0.5, white turnip cabbage and white cabbage were less attractive within this scale. Host preferences of *B. tabaci* and *T. vaporariorum* could both be ranked as EG > TB > TO > CU > BE > SP. Eggplant, tobacco and tomato were classified as attractive, whereas cucumber bean and sweet pepper were overall less preferred when compared to an alternative.

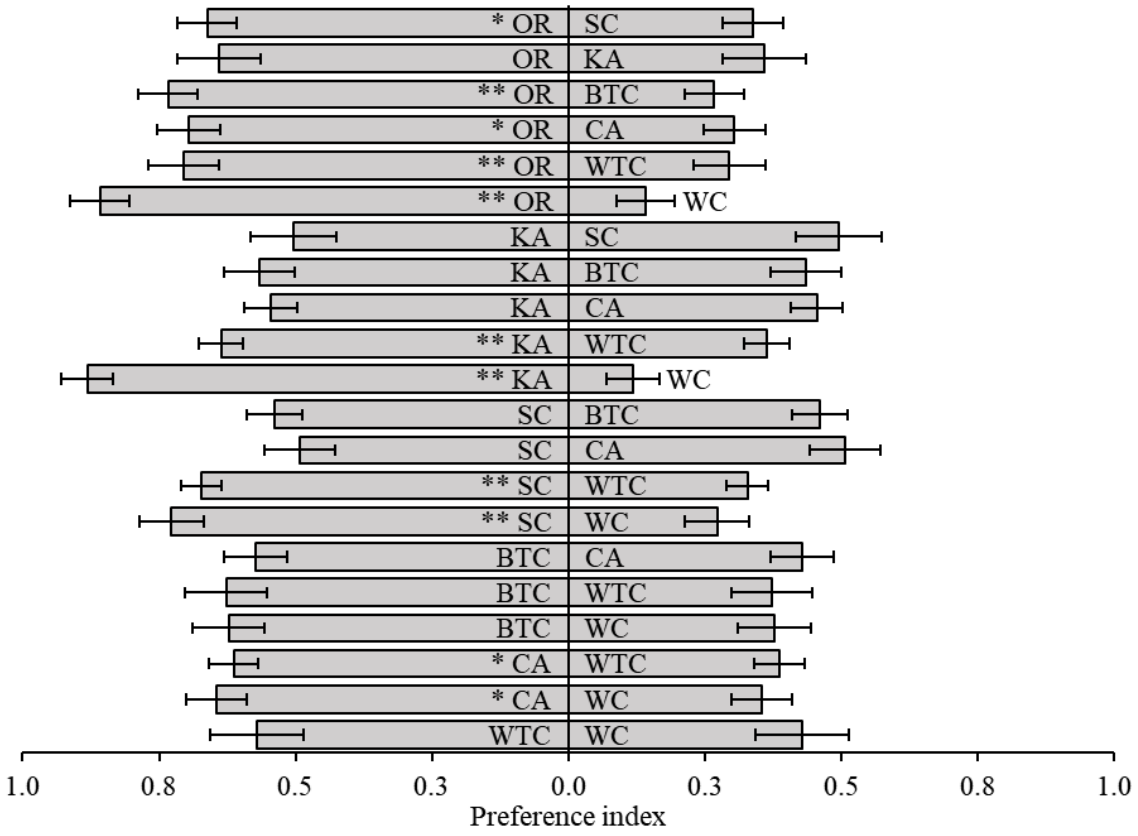

Figure 3: Preference indices (mean ± s.e.m.) of *A. proletella* adults in dual choice tests
(OR = oilseed rape, SC = savoy cabbage, KA = kale, BTC = blue turnip cabbage, CA = cauliflower, WTC = white turnip cabbage, WC = white cabbage)
Wilcoxon signed-rank test for each whitefly species ($H_0 = 0.5$ two-sided, $\alpha = 0.05$, $n = 8$ for each combination, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$)

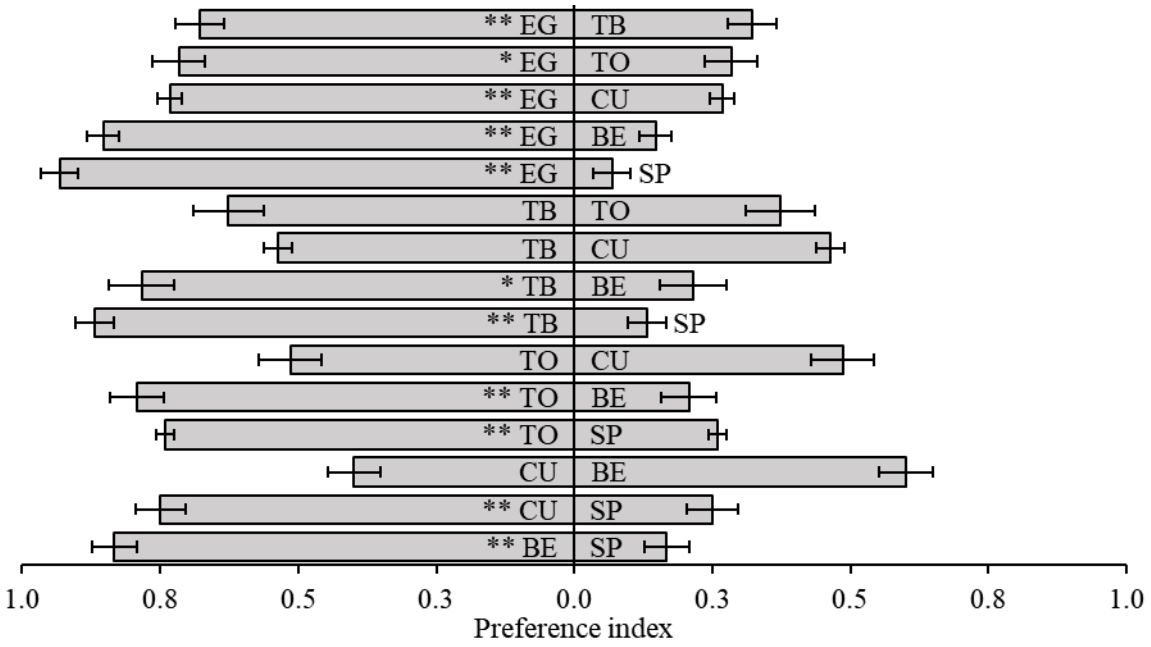

Figure 4: Preference indices (mean ± s.e.m.) of *B. tabaci* adults in dual choice tests (EG = eggplant, TB = tobacco, TO = tomato, CU = cucumber, BE = bean, SP = sweet pepper) Wilcoxon signed-rank test for each whitefly species (H_0 = 0.5 two-sided, α = 0.05, n = 8 for each combination, **P* < 0.05, ***P* < 0.01, ****P* < 0.001)

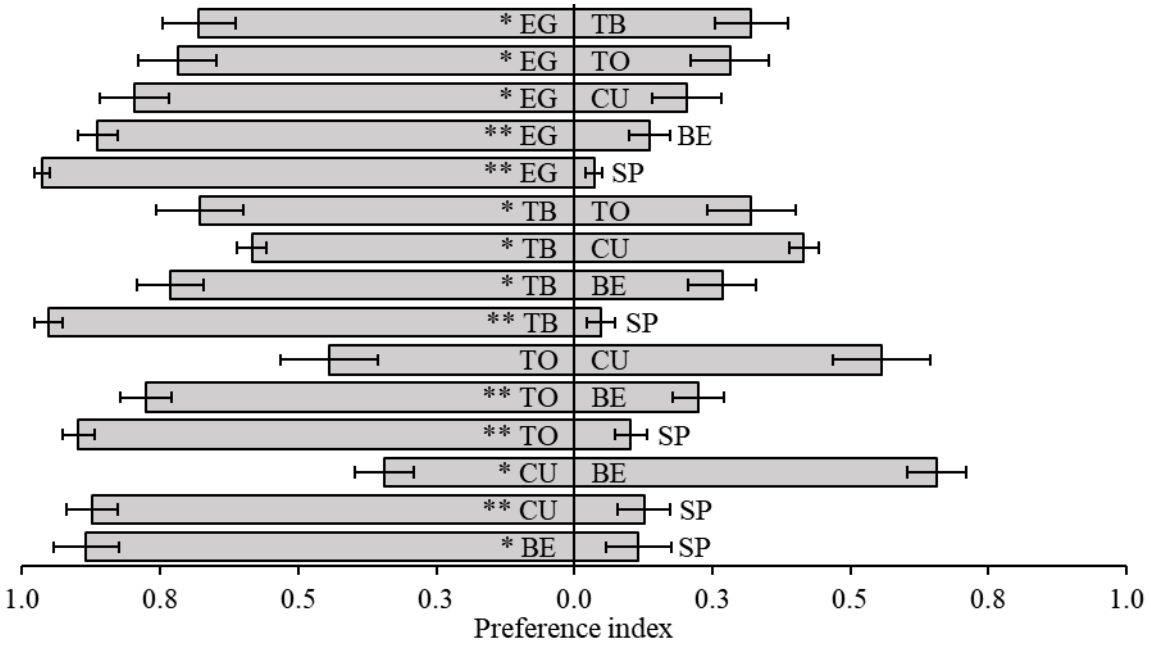

Figure 5: Preference indices (mean ± s.e.m.) of *T. vaporariorum* adults in dual choice tests (EG = eggplant, TB = tobacco, TO = tomato, CU = cucumber, BE = bean, SP = sweet pepper) Wilcoxon signed-rank test for each whitefly species (H_0 = 0.5 two-sided, α = 0.05, n = 8 for each combination, **P* < 0.05, ***P* < 0.01, ****P* < 0.001)

Table 2: Mean preference indices of whiteflies on different host plants

Host plant	*A. proletella* mean ± s.e.m.	Host plant	*B. tabaci* mean ± s.e.m.	*T. vaporariorum* mean ± s.e.m.
OR	0.71 ± 0.03 a	EG	0.71 ± 0.02 a	0.80 ± 0.03 a
	n = 57		n = 40	n = 40
KA	0.58 ± 0.03 b	TB	0.59 ± 0.03 b	0.65 ± 0.04 b
	n = 55		n = 40	n = 40
SC	0.54 ± 0.03 bc	TO	0.54 ± 0.04 bc	0.54 ± 0.05 bc
	n = 56		n = 40	n = 40
BTC	0.50 ± 0.03 bc	CU	0.47 ± 0.03 cd	0.48 ± 0.04 c
	n = 57		n = 40	n = 40
CA	0.50 ± 0.03 c	BE	0.38 ± 0.03 d	0.43 ± 0.05 c
	n = 62		n = 40	n = 40
WTC	0.39 ± 0.03 d	SP	0.21 ± 0.03 e	0.09 ± 0.02 d
	n = 60		n = 40	n = 40
WC	0.30 ± 0.03 e			
	n = 56			
X^2	99.5942	X^2	96	106.0986
d.f.	6	d.f.	5	5
P	< 0.0001	*P*	< 0.0001	< 0.0001

Kruskal-Wallis one-way test followed by Wilcoxon Each Pair at $\alpha = 0.05$
Means with s.e.m. in a column followed by the same index letter are not statistically different
OR = oilseed rape, SC = savoy cabbage, KA = kale, BTC = blue turnip cabbage,
CA = cauliflower, WTC = white turnip cabbage, WC = white cabbage, EG = eggplant,
TB = tobacco, TO = tomato, CU = cucumber, BE = bean, SP = sweet pepper

2.4 Discussion

Host plant choice in herbivorous insects is determined by an array of factors affecting the immediate outcome of an encounter between insect and plant (SINGER 1986). Thus, both preferential and non-preferential plant factors are responsible for the overall attractiveness of a host plant towards the herbivore. As it was highlighted in this study, polyphagous whitefly species do not colonize their available host plants evenly. Instead, whiteflies prefer some hosts over others, with plant attractiveness determined by plant species, variety and genotype (VAN LENTEREN AND NOLDUS 1990; CALVITTI AND REMOTTI 1998; BÄHRMANN 2002; BALDIN AND BENEDUZZI 2010; DA COSTA ZACHÉ ET AL. 2013; HUTAPEA ET AL. 2019). Determination of host plant attractiveness to phloem feeders is difficult, as host preferences vary according to numerous factors such as the number of choice alternatives, the presence of potential competitors and antagonists, the experimental setup and duration, as well as previous experiences of the insect (HEARD 2000; RAFFA ET AL. 2002; STOCKTON ET AL. 2016; STAM ET AL. 2017). Additionally, host plant preference can be affected by abiotic factors and cultural conditions such as light, temperature, humidity, nutrient and water supply, as

well as the overall health status of experimental plants (ZEBITZ 1988; ZEBITZ 1990; BÖHNKE AND ZEBITZ 1990; ZEBITZ ET AL. 1990; PRÜTER AND ZEBITZ 1991; ZEBITZ AND KEHLENBECK 1991; BALE ET AL. 2002; STALEY ET AL. 2010; PATHANIA ET AL. 2020). In the past, most studies on whitefly preference focused on comparisons of several plant species or genotypes in multi-choice tests with more than two host choice alternatives (COSTA ET AL. 1991a; BLUA ET AL. 1995; MEAGHER ET AL. 1997; BALDIN AND BENEDUZZI 2010; RAKHA ET AL. 2017; DOMINGOS ET AL. 2018). Indeed, multi-choice tests represent a powerful tool used for evaluation of insect behaviour and were found to be more sensitive than no-choice tests (RAFFA ET AL. 2002). Furthermore, testing several choice alternatives at once is less cost intensive and time-consuming. However, in multi-choice preference experiments few host plants with increased attractiveness might mask preference differences of less attractive hosts. Therefore, insect preference is seen as a relative concept, and it is concluded that host preference needs to be assessed for each set of plants individually that are available to the insect (SCHOONHOVEN ET AL. 2005). In dual choice tests of this study, significant differences in host attractiveness were determined between various host plants in the whitefly species tested. Moreover, the calculation of mean preference indices for each host plant and whitefly species was well suited for evaluation of attractiveness ranks and interpretation of the overall host attractiveness. *Aleyrodes proletella* preferred oilseed rape most, followed in decreasing order by kale, savoy cabbage, blue turnip cabbage, cauliflower, white turnip cabbage and white cabbage. For *B. tabaci* and *T. vaporariorum*, host preference was found in decreasing order by eggplant, tobacco, tomato, cucumber, bean and sweet pepper. Nevertheless, it should be noted that statistically differences in the overall attractiveness between hosts not necessarily presuppose a significant outcome in dual choice tests. The mechanisms causing plant resistance against whiteflies need to be known as they determine the testing and breeding methods, which are required to introduce resistant traits into the desired plants (DE PONTI ET AL. 1990). Among the plant characteristics possibly mediating host plant selection in whiteflies are numerous factors. Whitefly densities and intra-varietal preferences are determined by plant architecture, canopy closure, leaf shape as well as leaf morphological features such as leaf hairs and trichomes density (BERLINGER 1986; SIPPELL ET AL. 1987; BUTLER ET AL. 1988; DE PONTI ET AL. 1990; CHANNARAYAPPA ET AL. 1992; SNYDER ET AL. 1998; TOSCANO ET AL. 2002; SÁNCHEZ-PEÑA ET AL. 2006; HASANUZZAMAN ET AL. 2016). Whiteflies are strongly dependent on environmental factors such as temperature and relative air humidity, which in turn are influenced by morphological characteristics of plants and leaves (BERLINGER 1986). A reduced canopy and certain leaf shapes provide a better air movement with lower relative air humidity as well as higher temperatures, resulting in a less favourable environment for whiteflies (SIPPELL ET AL. 1987; BERLINGER 1986). While the presence of leaf hairs and trichome density can alter the microclimate on leaves as well, heavily pubescent leaves often restrict whitefly movement as leaf hairs can also provide a physical barrier (BERLINGER 1986; BÄHRMANN 2002). Nevertheless, *B. tabaci* was found to exert a strong preference for egg deposition at the base of leaf hairs, which

explains the attractiveness of pubescent leaves towards this whitefly species (OMRAN AND EL KHIDIR 1978). Moreover, glandular leaf hairs exude chemical compounds, which can contribute to whitefly resistance due to adverse effects on whitefly biology or due to its sticky texture (TINGEY AND GIBSON 1978; WILLIAMS ET AL. 1980; KISHA 1981; BERLINGER 1986). Cuticle properties such as the structure of the leaf epidermis and epicuticular lipids can directly affect infestation levels and oviposition rates of whiteflies (WALKER 1985; WALKER 1987; WALKER 1988; LAMBERT ET AL. 1995; FIRDAUS ET AL. 2011; KHAN ET AL. 2011; VOIGT ET AL. 2019). The penetration force needed during stylet penetration of whitefly feeding as well as during implanting the egg pedicel into the leaf epidermis during whitefly oviposition might be directly correlated with the thickness and flexibility of the leaf epidermis (VOIGT ET AL. 2019). The epicuticular wax layer of leaves may also provide a hindrance to whiteflies (KHAN ET AL. 2011). Besides, specific components of epicuticular lipids were previously discussed to correlate with population densities of whiteflies due to deterrent effects (LAMBERT ET AL. 1995). Finally, internal leaf chemistry as determined by amino acids and sugars in the phloem, phloem pH, as well as secondary metabolites and plant chemicals such as phenolics, alkaloids, saponins, terpenes, lipids and carbohydrates occurring in all plant tissues and structures are important factors mediating host plant selection in whiteflies (BERLINGER ET AL. 1983; BERLINGER 1986; CHANNARAYAPPA ET AL. 1992; HASANUZZAMAN ET AL. 2016; HASANUZZAMAN ET AL. 2018). The nutritional value of a host plant strongly affects the host selection and performance of whiteflies. Therefore, a high concentration of nitrogen, glucose, amino acids, and lower moisture content was shown to be highly preferred and *vice versa* (HASANUZZAMAN ET AL. 2018). In addition, it has been suggested that the pH of cotton leaves serves as a cue in finding suitable host plants for *B. tabaci* since it increases with the age of the plant, can be altered by environmental changes, and thus could reflect the nutritional value of the plant itself (BERLINGER 1986). On the other side, host preference of whiteflies depends on the presence of secondary plant chemicals that are part of an anti-herbivore defence mechanism of plants. For example, high amounts of phenolic compounds were shown to impair whitefly performance (HASANUZZAMAN ET AL. 2018). However, digestive enzymes and protective enzymes of whiteflies might play a substantial role in their settling and oviposition ability resulting in sometimes high population densities on host plants with rather high contents of toxic secondary compounds (BERLINGER 1986; LIN ET AL. 2018). Nevertheless, still little is known about the host choice behaviour of whiteflies and further research is needed to elucidate the mediating factors in host selection process. As previously discussed, plant traits that mediate host plant selection in whiteflies can occur in every plant tissue layer. Accordingly, a useful next step would be to localize the potential sources of host plant resistance more precisely. Experiments using the electrical penetration graph (EPG) method are particularly suited to this task, as they allow detection of potential sources of host plant resistance at the level of individual plant tissue layers (JANSSEN ET AL. 1989; LEI ET AL. 1998; LEI ET AL. 1999; LEI ET AL. 2001). Subsequently, further experiments can be derived from the results of the EPG analysis.

3 Potential sources of host plant resistance against three whitefly species elucidated by electrical penetration graph analysis

Abstract: Recording probing behaviour of phloem feeders by electrical penetration graphs (EPG) is a common approach to elucidate host plant quality and/or host plant antixenosis. In EPG analyses, which compare probing and feeding activities of *Aleyrodes proletella* (L.), *Bemisia tabaci* (Genn.) and *Trialeurodes vaporariorum* (Westw.) on two host plants each, potential sources of host plant resistance could be identified. It was found that whiteflies decide on host plant acceptance by multiple plant factors located in different plant tissues. On more attractive hosts all whiteflies had significantly prolonged probes, and pathway phases implying the presence of a plant factor that determines host choice before the actual phloem-feeding. Additional host plant variants with mechanically removed leaf surface wax furthermore proved that epicuticular leaf waxes play a key role in the host selection process of *A. proletella*. The removal of epicuticular leaf waxes led to early interrupted probes and absent phloem phases. It is therefore concluded that constituents of leaf surface waxes act as feeding stimulants for *A. proletella* promoting stylet penetration and phloem accession. Additionally, phloem-feeding of *B. tabaci* and *T. vaporariorum* was significantly shorter on less preferred hosts indicating that phloem sap quality mediates host choice in these whitefly species. Overall, this study identifies a new source of resistance against *A. proletella* and sheds light onto the underlying mechanisms of host plant selection in whiteflies.

Keywords: electrical penetration graph, stylet penetration, host plant selection, antixenosis

3.1 Introduction

Whiteflies are serious pests of vegetables, ornamentals and agricultural crops of increasing economic importance worldwide (MOUND AND HALSEY 1978; DUFFUS 1987; BINK-MOENEN AND MOUND 1990; CAPINERA 2008). As piercing-sucking phloem feeders, whiteflies pose a serious risk for plant production and global food security. Plant injury and yield reduction is caused by (i) direct damage due to feeding activity (LLOYD 1922; BYRNE AND BELLOWS 1991), (ii) secondary damage resulting from the secretion of honeydew often followed by infestations with sooty mould fungi (BYRNE AND MILLER 1990; BÄHRMANN 2002; SAUCKE ET AL. 2011), and (iii) damage by transmission of plant viruses (JONES 2003; LEGG 2010). In the field, whiteflies are mainly controlled by insecticides, but their efficacy has been severely limited because insecticide resistance evolved in almost all insecticide classes (HOROWITZ ET AL. 1998; NAUEN AND DENHOLM 2005; BASS ET AL. 2015; DÂNGELO ET AL. 2018). In contrast, biological control is successfully applied in greenhouses; however, its implementation and success are often dependent on several constraints such as physical

factors and plant factors, for instance, greenhouse climate and leaf morphology (HODDLE ET AL. 1998). Thus, alternative methods are required that can replace chemical measures or support biocontrol (GERLING 1992; BÄHRMANN 2002).

In the complex strategy of integrated pest management, plant resistance to whiteflies contributes in several ways due to their economic and ecological advantages (RUSSEL ET AL. 1978; DE PONTI ET AL. 1990; PALANISWAMY 1996; BROEKGAARDEN ET AL. 2011; VAN DOORN AND VOS 2013). Knowledge of the mediating factors of host plant selection by whiteflies could help develop efficient resistance breeding programs with pronounced antixenosis to reduce plant damage and virus transmission. Once whiteflies are attracted and land on a potential host plant, the further selection process is characterized by contact-testing and intensive probing of plant tissues. At the beginning of each probing process, whiteflies get into contact with morphological, physical and chemical leaf characteristics on the leaf surface such as leaf hairs and epicuticular surface waxes (BERLINGER 1986). For this reason, whiteflies examine the outer leaf surface with their labium, which carries several contact chemoreceptors (WALKER AND GORDH 1989). To continue probing by intercellular stylet penetration of the successive leaf tissue layers, whiteflies must penetrate the epidermis. The epidermal thickness, in turn, varies according to plant species, leaf age as well as prevailing abiotic environmental conditions and may, therefore, interfere with reaching the mesophyll tissues (WALKER 1985; WALKER 1987; WALKER 1988; LEI ET AL. 2001; VOIGT ET AL. 2019). During mesophyll penetration, stimulants or deterrent factors in the intercellular fluids may be detected by additional chemosensilla of the alimentary canal of whitefly stylets (CAMPBELL ET AL. 1986; HUNTER ET AL. 1996; LEI ET AL. 1998). Intracellular compounds of the mesophyll, however, seem to play only a minor role, as whiteflies use only a few intracellular punctures during the probing process (JANSSEN ET AL. 1989; LEI ET AL. 1996; LEI ET AL. 2001). Subsequently, whiteflies may then access the phloem tissue. Before feeding, they first inject watery saliva to avoid sealing mechanisms and to prevent turgor loss (TJALLINGII 2006; WALKER ET AL. 2010). Evaluation of the nutritional quality of the phloem sap and the possible presence of phloem-mobile secondary compounds then ultimately determines over sustainable phloem-feeding and host acceptance. Besides that, xylem sap consumption might occur as well, which is assumed to support water balance and to regulate osmotic potential in phloem sap feeders (SPILLER ET AL. 1990; POMPON ET AL. 2011). Although the probing process of whiteflies is well understood, the determining factors mediating the selection of a host plant or a feeding site often remain unclear. Therefore, subsequent studies are required to identify the origin and location of such potential resistance-giving factors.

The electrical penetration graph (EPG) method is particularly suitable to study whitefly feeding behaviour since it represents a powerful tool to record penetration behaviour and food uptake in piercing-sucking insects. The basic principle is an electrical circuit, which was introduced by MCLEAN AND KINSEY (1964), further developed by TJALLINGII (1978) and recently reviewed by

BACKUS ET AL. (2019). Overall, the goal of this study was to assess host preferences of three whitefly species on two host plants each and to record probing and feeding behaviour for each whitefly-host combination using the EPG method. While potential sources of host plant resistance were investigated, the host selection process of whiteflies is discussed in the context of host plant range and insect-plant adaptation.

3.2 Materials and methods

Insects and plants

Adults of *Aleyrodes proletella* (L.), *Bemisia tabaci* (Genn.), and *Trialeurodes vaporariorum* (Westw.) were obtained from the institute's stock cultures (Department of Applied Entomology, Institute of Phytomedicine, University of Hohenheim) reared on broccoli, poinsettia, and tobacco respectively in the greenhouse (25/23 ± 2 °C each, L18/D6 photoperiod, 50 ± 5 % RH).

Savoy cabbage (SC) (*Brassica oleracea* L. convar. *capitata* var. *sabauda*, cv. "Vertus"), white cabbage (WC) (*Brassica oleracea* L. convar. *capitata* var. *alba*, cv. "Brunswijker"), cucumber (CU) (*Cucumis sativus* L., cv. "Delikateß") and sweet pepper (SP) (*Capsicum annuum* L., cv. "California Wonder") were sown in pots (LC 14, Pöppelmann GmbH & Co. KG, Lohne, Germany). Experimental plants were grown under greenhouse conditions (22/18 ± 2 °C each, L18/D6 photoperiod, 50 ± 5 % RH)), irrigated daily, and fertilized weekly with 30 ml 0.5 % Wuxal® Super (8 % N, 8 % P, 6 % K, Aglukon GmbH, Düsseldorf, Germany). The soil mixture was composed of 50 % potting soil (Floradur®, Floragard Vertriebs-GmbH, Oldenburg, Germany), 30 % compost soil (institute's production), and 20 % sand. The plants were used in experiments when they reached BBCH stage 17–18.

Electrical penetration graph studies

Probing and feeding behaviour of each whitefly species was studied on each two host plants with known host attractiveness (see Chapter 2) using the electrical penetration graph (EPG) technique (Table 3). Furthermore, additional cabbage variants with waxy (+) and dewaxed (–) leaf undersides were subjected to EPG experiments to examine the effect of epicuticular waxes of cabbage on host plant acquisition by *A. proletella*. Instead of using nonpolar solvents, which might have had a detrimental effect on the leaf tissues, wax has been removed gently using cotton wool. As wax removal was not possible on sweet pepper and cucumber leaves without harming subjacent leaf tissues, the influence of leaf surface waxes was only studied in whitefly-host combinations with *A. proletella*.

Table 3: Whitefly-host plant combinations tested in EPG experiments

Whitefly species	Host plants
Aleyrodes proletella	Savoy cabbage (SC+, SC−), white cabbage (WC+, WC−)
Bemisia tabaci	Cucumber (CU), sweet pepper (SP)
Trialeurodes vaporariorum	Cucumber (CU), sweet pepper (SP)

+ denotes the presence and − denotes the absence of epicuticular waxes on abaxial leaf surfaces

Adult female whiteflies randomly taken from a synchronized colony (max. 24 h old) were anaesthetized with CO_2 and integrated into a DC-Giga-4 EPG system (manufactured by Wageningen University, Netherlands) (Figure 6). Four whiteflies were recorded simultaneously by attaching them separately onto EPG electrodes using a gold wire (12 µm diam., 2 cm length) and water-based silver conductive glue on their dorsa (both: EPG Systems, Wageningen, Netherlands). Before being glued, the wax layer covering the whitefly's dorsum was removed using a fine brush and water. A copper rod (2 mm diam., 10 cm length) was inserted into pots of test plants as second electrodes, which closed the electrical circuit. The experimental set was placed in a Faraday cage situated in a room completely insulated from possibly disturbing electromagnetic fields of the environment. Probing activities were monitored in a Faraday cage for 8 h under artificial light (SON-T Agro, Philips, 2,000 K, 16,000 lm) at room temperature (20 ± 2 °C). While whiteflies were only used once, plants were used twice. Eight replicates per whitefly-host combination served to calculate EPG parameters associated with non-phloem feeding, whereas at least 16 EPG recordings were used for the analysis of phloem-feeding. Signals were recorded and analysed using NextView/NT software (plug-in card: PCI-Base 50/300, A/D-conversion-module: MAD12; both BMC-Schetter, Germany) on a standard PC.

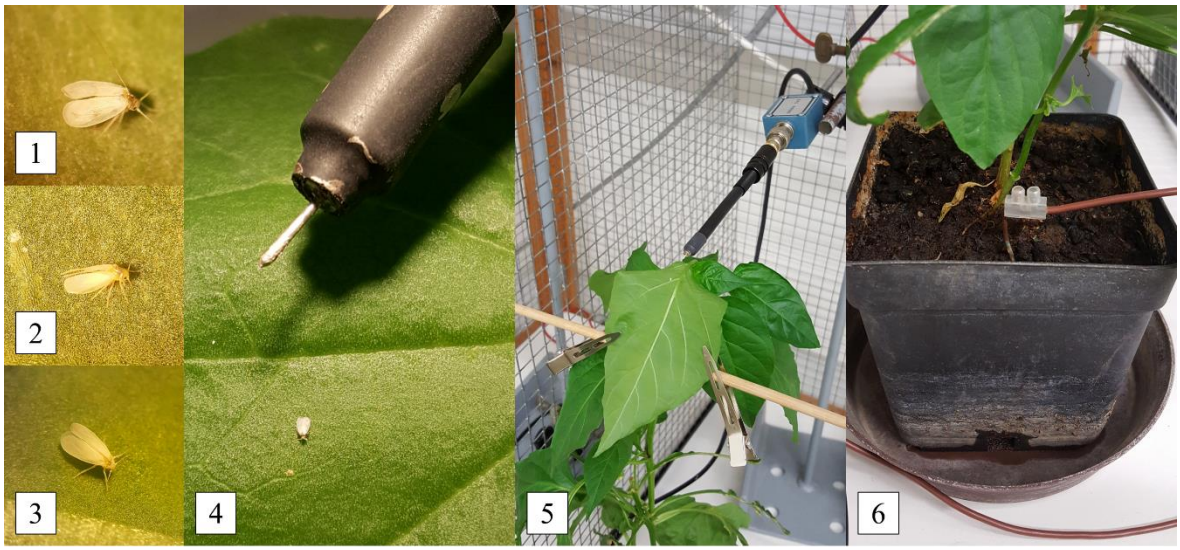

Figure 6: Experimental setup of the EPG system
Whiteflies attached to gold wires: *A. proletella* (1), *B. tabaci* (2), *T. vaporariorum* (3); whitefly wired to EPG electrode (4); EPG electrode mounted to EPG system (5); plant electrode in a plant pot (6)

For analysis of EPG experiments, the position of the whitefly stylets and the associated probing activities were interpreted according to EPG waveforms previously defined by TJALLINGII (1978) and LEI ET AL. (1996). Overall, six waveforms occurring during EPG recording could be identified, including waveform C (stylet pathway phase), np (non-probing period), F (penetration difficulties), G (ingestion of xylem), E(pd)1 (phloem salivation) and E(pd)2 (phloem ingestion). Potential drops occurred only rarely and were therefore excluded from data analysis. Thirteen sequential and non-sequential EPG parameters associated with insect preference towards specific host plant tissue layers were calculated from these data according to SARRIA ET AL. (2009) (Table 4).

Table 4: EPG parameters associated with insect preference towards specific host plant tissue layers measured in the study

EPG parameter	Related tissue
Total probing time	All tissues
Total number of probes	All tissues
Mean probe duration	All tissues
Mean duration of C	All tissues
Mean duration of np	All tissues
Mean duration of G	Xylem
Mean duration of F	Epidermis and mesophyll
Total duration of E(pd)	Phloem
Number of E(pd)	Phloem
Mean duration of E(pd)	Phloem
Mean duration of E(pd)1	Phloem
Mean duration of E(pd)2	Phloem

Statistics

All obtained data were analysed using JMP® 14.1.0 (SAS Institute Inc., Cary, NC, USA). Before statistical analysis, the residuals were tested for normal distribution by Shapiro-Wilk test. All continuous data were found normally distributed. When reasonable, an outlier analysis by Mahalanobis-procedure was performed. All data were subjected to an analysis of variance, procedure "Generalized Linear Models", before ensuing further statistical analyses. The respective statistical procedures and statistical core data are provided in the legends of the tables.

3.3 Results

Non-phloem and phloem probing activities differed with whitefly species, host plant, and leaf surface (Tables 5, 6 and 7). The time *A. proletella* and B. *tabaci* spent probing during one recording session (total probing time) differed between host plants but showed no differences in the number of probes (Table 5). In contrast, the number of probes during one recording session (total number of probes) by *T. vaporariorum* differed significantly between host plants within an almost similar total probing time on cucumber and sweet pepper leaves. Calculation of the average probing time for each recording session (mean probe duration = total probing time divided by the total number of probes) was therefore used to compare the general probing activity for all whitefly species. The mean probe duration of *A. proletella* was superior on savoy cabbage with epicuticular waxes, whereas less time was spent on white cabbage and cabbage cultivars without epicuticular waxes. *Bemisia tabaci* and *T. vaporariorum*, on the other hand, probed on average longer on cucumber.

Comparisons of general probing activities as well as of specific probing behaviours reflected by typical waveform patterns allowed detailed insights into the stylet penetration process of whiteflies (Figure 7). The stylet pathway activity (mean duration of waveform C) of *A. proletella* was longest on savoy cabbage with epicuticular waxes, whereas wax removal led to an earlier interruption of waveform C for both cabbage cultivars (Table 6). In contrast, *A. proletell*a had significantly shorter periods of non-probing activity (mean duration of waveform np) on savoy cabbage with epicuticular waxes than on white cabbage and cabbage variants without epicuticular waxes. A similar pattern could be observed for *B. tabaci*, as the stylet pathway activity was longer and non-probing periods were shorter on cucumber in comparison to sweet pepper. The mean duration of waveforms C and np of *T. vaporariorum*, however, were both significantly longer on cucumber. Xylem feeding (mean duration of waveform G) was longer on sweet pepper for both, *B. tabaci* and *T. vaporariorum*, while xylem feeding activity of *A. proletella* did not differ between host plants. Moreover, *A. proletella* suffered most from problems during penetration (mean duration of waveform F) on savoy cabbage without epicuticular leaf waxes and suffered least on white cabbage with epicuticular waxes. For *T. vaporariorum*, mean duration of waveform F was significantly longer on cucumber, whereas for *B. tabaci* no significant differences could be found between the host plants.

While phloem-associated EPG parameters of *A. proletella* were similar for both host plants, *B. tabaci* and *T. vaporariorum* showed significant differences in their phloem activity. This could be seen in the phloem-specific waveforms per recording session (Table 7). The total duration of the phloem phase (total duration = sum of all E(pd) per session) and the average discrete E(pd) of *B. tabaci* and *T. vaporariorum* were both significantly longer on cucumber, whereas the number of phloem events (number of E(pd)) did not differ between the host plant. As the phloem activity can further be distinguished in phloem salivation (mean duration of waveform E(pd)1) and phloem-feeding (mean duration of waveform E(pd)2), significant differences could be observed only for the phloem-feeding periods of *B. tabaci* and *T. vaporariorum*.

Comparing the probing behaviour of *B. tabaci* and *T. vaporariorum*, *B. tabaci* distinguished between hosts by different probing durations, whereas for *T. vaporariorum* different numbers of feeding attempts and a more intensive probing let the adults discriminate the host plants. However, the later phloem-feeding activity did not differ between both whitefly species.

Table 5: General probing parameters (total number of probes, total probing time, and mean duration of probes) of whiteflies on different host plants

			Probing parameter			
			Total number of probes[1]		Total probing time[2] (min)	Mean probe duration[2] (min)
Whitefly species	Host plant		mean ± s.e.m.		mean ± s.e.m.	mean ± s.e.m.
A. proletella	SC+		32.50 ± 5.08 a		407.46 ± 9.27 a	12.54 ± 2.13 a
			n = 8		n = 8	n = 252
	SC−		51.63 ± 6.71 a		323.69 ± 23.14 b	6.27 ± 1.19 bd
			n = 8		n = 8	n = 405
	WC+		44.88 ± 3.90 a		278.18 ± 24.28 bc	6.20 ± 0.92 c
			n = 8		n = 8	n = 351
	WC–		42.88 ± 3.90 a		227.60 ± 36.97 c	5.31 ± 1.00 d
			n = 8		n = 8	n = 336
		X^2	5.4098	F	9.0454	10.0227*
		d.f.	3	d.f.	3, 28	3, 672*
		P	0.1441	*P*	0.0002	< 0.0001*
B. tabaci	CU		39.00 ± 4.92 a		360.22 ± 19.82 a	4.66 ± 0.47 b
			n = 8		n = 8	n = 302
	SP		36.29 ± 4.12 a		201.46 ± 37.87 b	3.09 ± 0.21 c
			n = 7		n = 8	n = 323
T. vaporariorum	CU		14.50 ± 2.39 b		253.61 ± 37.30 ab	9.30 ± 1.51 a
			n = 8		n = 8	n = 107
	SP		36.14 ± 2.02 a		242.01 ± 21.97 b	3.77 ± 0.30 bc
			n = 7		n = 8	n = 300
		X^2	16.1124	F	4.9574	8.5418*
		d.f.	3	d.f.	3, 28	3, 356*
		P	0.0008	*P*	0.0069	< 0.0001*

[1] Kruskal-Wallis one-way test followed by Wilcoxon Each Pair-test, $\alpha = 0.05$
[2] one-way ANOVA followed by Tukey–Kramer HSD-test, $\alpha = 0.05$
Means with s.e.m. in a column followed by the same index letter are not statistically different
* F, d.f., and *P* values were corrected by Welch-ANOVA test because of variance inhomogeneity after Levene's test
+ denotes presence and – denotes absence of epicuticular surface waxes on cabbage leaves

Table 6: Mean duration of non-phloem activity of whiteflies probing on different host plants

			EPG parameter separated by waveform and specific probing event			
Whitefly species	Host plant		Mean duration of C (min) mean ± s.e.m.	Mean duration of np (min) mean ± s.e.m.	Mean duration of G (min) mean ± s.e.m.	Mean duration of F (min) mean ± s.e.m.
A. proletella	SC+		4.17 ± 0.25 a	1.73 ± 0.11 c	46.29 ± 15.06 a	5.39 ± 0.89 bc
			n = 280	n = 253	n = 10	n = 56
	SC–		1.71 ± 0.10 c	2.48 ± 0.15 bc	25.89 ± 3.54 a	12.63 ± 3.99 a
			n = 444	n = 412	n = 24	n = 27
	WC+		2.81 ± 0.15 b	3.25 ± 0.23 b	48.03 ± 10.64 a	2.55 ± 0.27 c
			n = 420	n = 352	n = 5	n = 95
	WC–		1.58 ± 0.10 c	4.31 ± 0.33 a	62.51 ± 19.66 a	8.04 ± 1.51 ab
			n = 368	n = 342	n = 10	n = 67
		F	56.7510*	27.3223*	2.4381*	8.5993*
		d.f.	3, 744*	3, 724*	3, 12*	3, 73*
		P	< 0.0001*	< 0.0001*	0.1146*	< 0.0001*
B. tabaci	CU		2.93 ± 0.11 b	2.11 ± 0.17 c	32.47 ± 4.30 b	0.77 ± 0.15 b
			n = 428	n = 306	n = 11	n = 26
	SP		2.17 ± 0.08 c	4.44 ± 0.41 b	53.70 ± 2.21 a	0.67 ± 0.13 b
			n = 396	n = 332	n = 4	n = 29
T. vaporariorum	CU		6.07 ± 0.56 a	9.82 ± 1.49 a	25.11 ± 4.90 b	11.26 ± 3.37 a
			n = 135	n = 117	n = 8	n = 10
	SP		3.11 ± 0.19 b	4.78 ± 0.27 b	37.09 ± 1.06 ab	2.16 ± 0.67 b
			n = 319	n = 304	n = 7	n = 12
		F	26.4798*	34.7034*	5.6485	4.5975*
		d.f.	3, 440*	3, 380*	3, 26	3, 22*
		P	< 0.0001*	< 0.0001*	0.0041	0.0116*

One-way ANOVA followed by Tukey-Kramer HSD-test, $\alpha = 0.05$
Means with s.e.m. in a column followed by the same index letter are not statistically different
* F, d.f., and *P* values were corrected by Welch-ANOVA test because of variance inhomogeneity after Levene's test
+ denotes presence and – denotes absence of epicuticular surface waxes on cabbage leaves

Table 7: Number, total duration, and mean duration of phloem activity of whiteflies feeding on different host plants

			EPG parameter				
			Number of E(pd)[1]	Total duration of E(pd)[2] (min)	Mean duration of E(pd)[2] (min)	Mean duration of E(pd)1[2] (min)	Mean duration of E(pd)2[2] (min)
Whitefly species	Host plant		mean ± s.e.m.	mean ± s.e.m.	mean ± s.e.m.	mean ± s.e.m.	mean ± s.e.m.
A. proletella	SC+		1.25 ± 0.25 a	127.57 ± 43.08 a	102.05 ± 37.99 a	1.08 ± 0.08 a	100.98 ± 29.21 a
			n = 8	n = 8	n = 10	n = 10	n = 10
	WC+		1.60 ± 0.40 a	42.36 ± 18.30 a	26.48 ± 11.00 a	0.87 ± 0.14 a	25.61 ± 10.97 a
			n = 5	n = 5	n = 8	n = 8	n = 8
		X^2	0.9848	F 1.8203*	1.9109*	1.4008	1.9067*
		d.f.	1	d.f. 3, 9*	1, 10*	1, 16	1, 10*
		P	0.3120	*P* 0.1012*	0.0837*	0.1804	0.0844*
B. tabaci	CU		1.10 ± 0.10 a	121.07 ± 30,17 a	110.06 ± 29.13 a	0.36 ± 0.08 a	109.70 ± 29.17 a
			n = 10	n = 10	n = 11	n = 11	n = 11
	SP		1.63 ± 0.32 a	24.75 ± 8.84 b	15.23 ± 4.13 b	0.81 ± 0.38 a	14.43 ± 3.82 b
			n = 8	n = 8	n = 13	n = 13	n = 13
T. vaporariorum	CU		1.29 ± 0.18 a	79.68 ± 23.93 ab	86.28 ± 30.77 a	0.31 ± 0.04 a	85.97 ± 30.76 a
			n = 7	n = 7	n = 10	n = 10	n = 14
	SP		2.20 ± 0.44 a	27.54 ± 10.72 b	13.58 ± 12.16 b	0.61 ± 0.10 a	12.98 ± 3.37 b
			n = 10	n = 10	n = 22	n = 22	n = 22
		X^2	6.4449	F 5.1278	5.0528*	2.6934*	5.0823*
		d.f.	3	d.f. 3, 31	3, 20*	3, 25*	3, 20*
		P	0.0919	*P* 0.0054	0.0090*	0.0672*	0.0087*

[1] Kruskal-Wallis one-way test followed by Wilcoxon Each Pair-test, α = 0.05
[2] one-way ANOVA followed by Tukey-Kramer HSD test for *B. tabaci* and *T. vaporariorum*, α = 0.05
Means with s.e.m. in a column followed by the same index letter are not statistically different
* denotes F, d.f., and *P* values were corrected by Welch-ANOVA test because of variance inhomogeneity after Levene's test
+ denotes presence and – denotes absence of epicuticular surface waxes on cabbage leaves

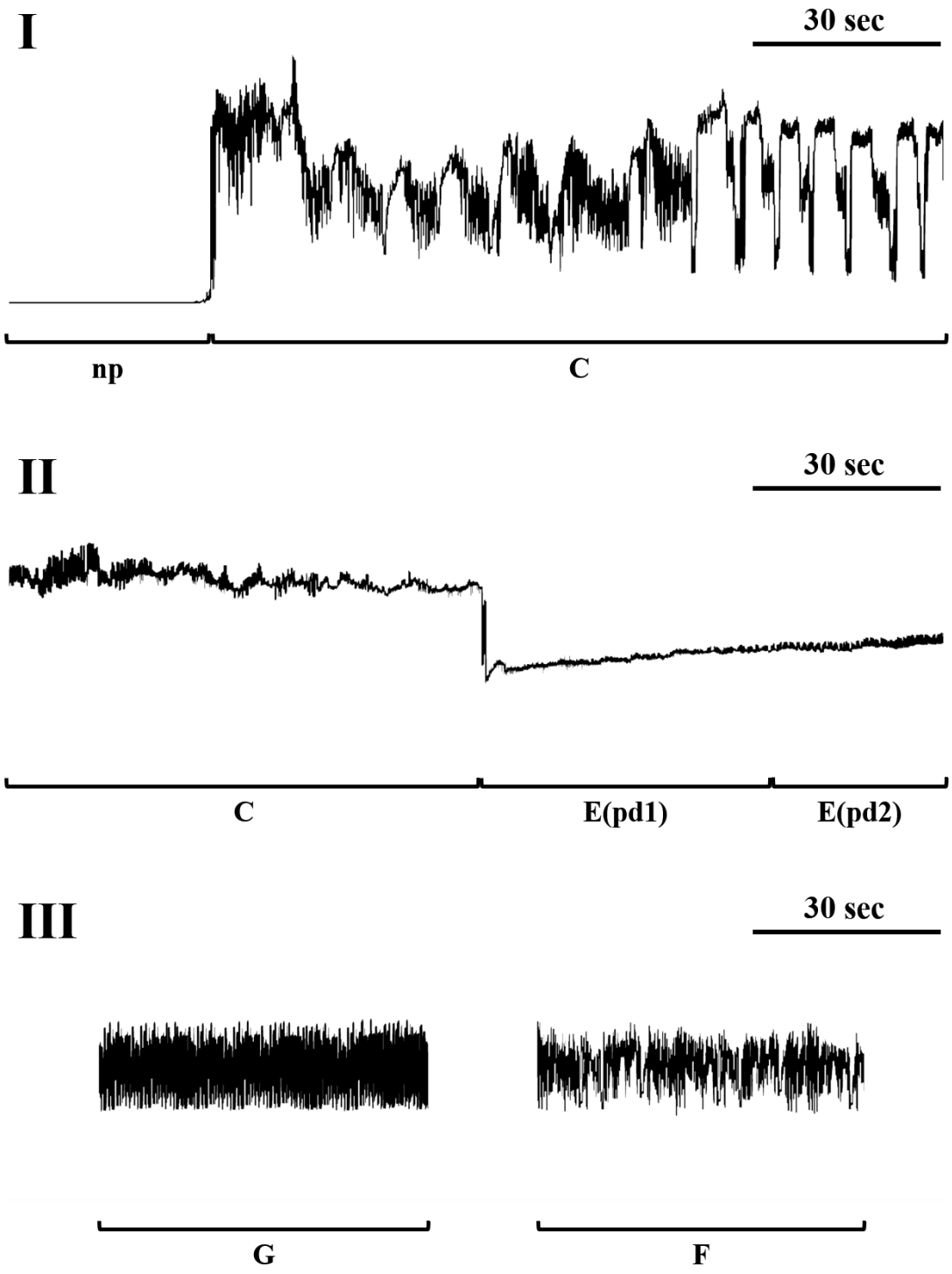

Figure 7: Typical waveform patterns recorded during the study representing specific probing behaviours of the whitefly stylet penetration process

I: non-probing activity (waveform np), beginning of a probe and stylet pathway phase with typical sawtooth shaped waveforms (waveform C); II: stylet pathway phase (waveform C), phloem phase with salivation into the phloem (waveform E(pd1)) followed by phloem feeding (waveform E(pd2)); III xylem phase (waveform G) and stylet penetration difficulties (waveform F); vertical axis represents voltage level, bar represents time scale

3.4 Discussion

Identification of determining factors mediating host plant selection in whiteflies

While host plant selection of whiteflies already starts before landing by receiving visual or olfactory information of the potential host, host plant acceptance or host rejection mainly takes place after landing during initial contact with the host (VAN LENTEREN AND NOLDUS 1990; XU ET AL. 1994; DARSHANEE ET AL. 2017). Probing behaviour on potential feeding sites consequently includes the evaluation of a variety of different plant factors (JANSSEN ET AL. 1989; LEI ET AL. 1998; LEI ET AL. 1999; LEI ET AL. 2001). General probing parameters reflect the overall preference of a host plant best, as whiteflies probe less but longer on attractive hosts and more often but for shorter on less preferred host plants (MONTLLOR AND TJALLINGII 1989; SAUGE ET AL. 1998; BOOIJ ET AL. 2013). Therefore, EPG results of this study confirm the observed host plant preferences, as *A. proletella* probed on average more intensively on the more preferred host savoy cabbage, whereas probing activities of *B. tabaci* and *T. vaporariorum* were more persistent on the more preferred host cucumber. However, probing activities were characterized either by a longer total probing time or by a smaller number of probes on preferred hosts, while the opposite was the case on unattractive host plants with a shorter total probing time or by a higher number of probes. As a result, the calculation of the mean probe duration for each whitefly-host combination was well suited for interpretation in this case. Overall, the general probing parameters elucidate that prolonged probe durations with later probe interruptions correlate with the observed host preferences and that whiteflies need a considerable amount of time to accept a plant as a host.

In contrast to the general probing parameters, the attractiveness and acceptance of single plant tissues by whiteflies is not necessarily reflected by the overall attractiveness of a potential host (LEI ET AL. 1998; JIANG AND WALKER 2007). Therefore, comparisons of single EPG parameters associated with specific probing behaviours help to identify and localize the mediating factors of host plant selection. All whitefly species tested were found to have increased pathway phase periods (waveform C) on preferred hosts, which might be related to their characteristic probing activities with only a few intracellular punctures and different sub-patterns of the C waveform (JANSSEN ET AL. 1989; GIVOVICH AND NIEMEYER 1995; LEI ET AL. 1998). Non-probing activities (waveform np) of *A. proletella* and *B. tabaci* were on average shorter on preferred hosts, leading to the assumption that an intensive examination of the parenchyma tissue not only contributes to host acceptance but might, therefore, even promote phloem accession. Consequently, factors mediating host plant selection in whiteflies would be already present during the pathway phase, as postulated by VAN LENTEREN AND NOLDUS (1990) before. Although the non-probing activity of *T. vaporariorum* was higher on the more preferred host plant cucumber, this might be attributed to the distinctly lower total number of probes as well as to the increased occurrence of the F pattern. The F pattern waveform

is used as an indicator of probing difficulties during the pathway phase, which are perceived during EPG recordings with aphids and whiteflies (CAILLAUD ET AL. 1995). However, different scenarios may be related to the incidence of the F pattern. While it may be more present on preferred hosts with more persistent pathway phases, missing probing stimuli or the presence of deterrents may provoke an increased occurrence of the F waveform.

A sustained phloem sap uptake shows the acceptance of a potential host plant with a higher acceptance rank and *vice versa* (LEI ET AL. 1998; LEI ET AL. 2001). Nevertheless, plant factors within all leaf tissues can affect the phloem uptake continuity by whiteflies and therefore influence host choice behaviour. Furthermore, xylem phases are negatively associated with phloem consumption, as unsuitable host plants are characterized by few and short phases of phloem ingestion and long phases of xylem ingestions (LEI ET AL. 2001). As *A. proletella* did not show any significant differences, neither regarding their phloem-feeding continuity nor regarding their xylem phase activity, it is suggested that the preference behaviour of *A. proletella* is mainly based on non-phloem factors in this study. However, once *B. tabaci* and *T. vaporariorum* reached the phloem phase (waveform E), both fed longer and more continuously on cucumber. Additionally, xylem consumption is negatively associated with phloem consumption for *B. tabaci*. As a result, this study presents evidence that phloem factors such as sugars, amino acids, and possibly also proteins, vitamins, secondary compounds, and phloem pH contribute to host acceptance of *B. tabaci* and *T. vaporariorum.* In order to feed continuously from a potential host plant, phloem feeders including whiteflies must deal with chemical and mechanical plant defence mechanisms first. While chemical defence includes the presence of deterrent compounds in the phloem, mechanical defence strategies occur by blockage of the whitefly stylet or the phloem sieve element during attempted feeding (WILL ET AL. 2013). Although the effect of mechanical defence mechanisms by callose or protein blockage could not be completely excluded in our study, we suppose that chemical factors are responsible for differences in phloem acceptance between host plants. This assumption is supported by the fact that salivation prior to phloem feeding is related to inhibition of plant defence mechanisms in piercing-sucking herbivores and no differences of feeding attempts as well as phloem salivation periods could be measured for all whitefly-host comparisons in this study (TJALLINGII 2006; WALLING 2008). Consequently, only chemical phloem factors are assumed to be the cause of the observed differences in phloem feeding continuity. However, it remains unclear whether feeding deterrents are present in the phloem sap and to what extent phloem acceptance in whiteflies is determined by the phloem quality only.

Role of epicuticular factors affecting host selection strategy in A. proletella

Various cues on the plant surface such as the wax structure and chemical composition of epicuticular waxes are known to affect host plant selection and feeding behaviour of aphids (POWELL ET AL. 1999; POWELL ET AL. 2006; WÓJCICKA 2015). So far only limited indications concerning epicuticular waxes influencing the feeding behaviour of whiteflies exist without a direct proof (LAMBERT ET AL. 1995; KHAN ET AL. 2011). In this study, strong evidence for epicuticular leaf waxes having a key function in the host selection process of *A. proletella* was found, as wax removal had drastic impacts on the whole probing process of *A. proletella*. In summary, whitefly individuals feeding on the cabbage leaves without epicuticular wax showed an earlier probe interruption, a less intensive examination of epidermal and mesophyll tissues, increased phases without probing activities, and more stylet penetration difficulties during probing activity. It is further important to mention that no phloem events could be detected on dewaxed cabbage cultivars during 8 h of EPG recording. Within such a time frame, it seems extremely unrealistic that whiteflies consistently probe on one potential feeding site without rejection. Nonetheless, it should be noted that *A. proletella* individuals could be observed to settle down and oviposit on dewaxed cabbage cultivars for multiple days under greenhouse conditions, which would normally not happen without continuous feeding on the host plant. Also, stress caused by insect wiring can never be fully excluded, which generally leads to less phloem events to be detected during EPG experiments (LEI ET AL. 1997). As a result, it is concluded that the number of phloem events performed by *A. proletella* on leaves without epicuticular waxes was reduced to some extent but would normally not be completely impeded. This could be explained by constituents of the leaf surface waxes of cabbage cultivars acting as feeding stimulants for *A. proletella*, which promote stylet penetration and, therefore, host acceptance. However, further research is required to evaluate this hypothesis. The primary wax components of cabbage plants are alkanes, alcohols, fatty acids, ketones, and aldehydes, with their proportions influenced by the cultivation method of the plants (SUTTER 1984; JUN ET AL. 2015). Triterpenoids such as lupeol as well as α- and β-amyrins are present in cabbage plants in smaller quantities but have been recognized to influence insect-plant interactions including whiteflies (EIGENBRODE ET AL. 1991; LAMBERT ET AL. 1995; EIGENBRODE AND PILLAI 1998; MARTELANC ET AL. 2007). Moreover, it should be stated that the host selection process of *B. tabaci* and *T. vaporariorum* might be affected by epicuticular stimulants as well. Even though it was not possible to test this in our experiments due to a missing methodological realization of removing leaf surface waxes from cucumber and sweet pepper without harming subjacent leaf tissues. Furthermore, the composition of epicuticular waxes of cucumber and sweet pepper was studied intensively of fruits, but not from leaf surfaces of plants (LOWNDS ET AL. 1993; SMITH ET AL. 2006; WANG ET AL. 2015).

4 Epicuticular leaf waxes acting as phagostimulants towards *Aleyrodes proletella* (L.)

Abstract: Since epicuticular waxes are located at the interface between plants and their aerial environment, they are often involved in the primary steps of host plant selection by herbivorous insects. In this study, the role of leaf epicuticular waxes of several *Brassica* cultivars in the host selection process of the cabbage whitefly *Aleyrodes proletella* (L.) was investigated. Dual choice experiments were carried out on waxy and dewaxed plant leaves as well as on Parafilm® treated with leaf wax extracts. Life-history traits on waxy and dewaxed leaves were monitored. The feeding behaviour was recorded on Parafilm® treated with and without leaf wax extracts. Furthermore, scanning electron microscopy (SEM) imaging was used to visualize epicuticular leaf wax crystals on the plant surface. It could be shown that epicuticular waxes served as an arrestant stimuli triggering whitefly settlement. On the other side, life-history traits were impaired on leaves without epicuticular wax. Moreover, whitefly feeding was enhanced on Parafilm® treated with leaf wax extracts of preferred hosts. As the shapes of individual leaf wax crystals varied on natural leaf surfaces, it was assumed that leaf waxes of host plant cultivars differ in their chemical composition. Therefore, it was hypothesized that *A. proletella* evaluates host plant quality especially by properties of leaf epicuticular waxes. Consequently, it could be proved that epicuticular waxes promote feeding of *A. proletella* and act as phagostimulants. Overall, these findings offer breeding potential for the development of whitefly resistant crop cultivars.

Keywords: host plant selection, host plant preference, life-history traits, electrical penetration graph, epicuticular wax, arrestant, phagostimulant

4.1 Introduction

The aerial surface of leaf epidermal cells in most plants is impregnated with a lipid polymer layer covered by a waxy deposit, both constituting the plant cuticle (DOMÍNGUEZ ET AL. 2011). The lipid polymer layer forms a translucent film containing cutin, whereas epicuticular wax is deposited on top (JEFFREE 2006). According to KERSTIENS (1996) and RIEDERER (2006), the plant cuticle is responsible for numerous functions of crucial importance for plant life, including its primary role of transpiration control, control over uptake and loss of solved polar substances, control over the exchange of gases and vapours, transport of lipophilic substances, repel of water and particles, attenuation of UV and photosynthetic active radiation, protection from mechanical influences, and is an important component for plant development. However, the high diversity in structure and chemical composition of epicuticular waxes suggest a range of ecological functions of the plant

cuticle, as waxes vary among plant species, genotypes and plant parts (EGLINTON AND HAMILTON 1967; EIGENBRODE AND ESPELIE 1995; BARTHLOTT ET AL. 1998; DHANYALAKSHMI ET AL. 2019). Epicuticular waxes are located at the interface between a plant and its aerial environment. Therefore, they often represent the first physical contact between insect and plant, mediating interactions between both (KERSTIENS 1996; RIEDERER 2006). Until today, numerous relationships between insects and plants are known to be mediated by epicuticular waxes (EIGENBRODE AND ESPELIE 1995). The chemical composition, the physical structure and optical properties of plant surface waxes provide information about the suitability of the host plant and might even influence the ability of an insect to move (EIGENBRODE AND ESPELIE 1995; JENKS AND ASHWORTH 1999; EIGENBRODE 2004; MÜLLER AND RIEDERER 2005). The absence or a reduced quantity of epicuticular waxes was observed to correlate with increased susceptibility of the host plant or led to a reduced infestation density of aphids, *Plutella xylostella* (L.), *Eurydema* spp., *Phyllotreta* spp., *Artogeia rapae* (L.), *Mamestra brassicae* (L.) and *Tetranychus ludeni* (Zacher) (WAY AND MURDIE 1965; TSUMUKI ET AL. 1989; STONER 1990; EIGENBRODE ET AL. 1991; BODNARYK 1992b; EIGENBRODE AND PILLAI 1998; BOHINC ET AL. 2014; CASTRO ET AL. 2019). In other cases, epicuticular waxes affect oviposition rates of *Delia radicum* (L.) and several lepidopteran species such as *Plutella xylostella* (L.), *Artogeia rapae* (L.), *Eupoecilia ambiguella* (Hübner) and *Lobesia botrana* (Denis and Schiffermüller) (PROKOPY ET AL. 1983; UEMATSU AND SAKANOSHITA 1989; STONER 1990; SILVA ET AL. 2017; RID ET AL. 2018). While the movement of fall armyworm larvae *Spodoptera frugiperda* (J.E. Smith) was triggered on corn leaves with leaf surface waxes, Coleoptera and larva of the lacewing *Chrysoperla carnea* (Steph.) were shown to have impaired mobility on plant surfaces with epicuticular wax crystals (STORK 1980; BODNARYK 1992a; BODNARYK 1992b; EIGENBRODE ET AL. 1996; GORB AND GORB 2002; GORB AND GORB 2006; GORB ET AL. 2008; GORB ET AL. 2017; VOIGT ET AL. 2018). The epicuticular wax layer can take a wide variety of forms and appear as films, layers and crusts, granules, platelets, rods, threads, tubes, and crystalloid transitional forms (BARTHLOTT ET AL. 1998). The individual chemical composition of epicuticular lipids determines the appearance and determines visual properties of the epicuticular wax (PROKOPY ET AL. 1983; HOLMES AND KEILLER 2002; OLASCOAGA ET AL. 2014). As a result, the heavy wax blooms from *Brassica* cultivars increase reflectivity in other wavelengths and let the plants appear whiter (PROKOPY ET AL. 1983). While optical properties are already perceived by insects from a distance, chemical composition and fine structure are recognized during physical contact only. Therefore, plant epicuticular waxes affect herbivorous insects at different behavioural steps within their host selection process. Consequently, plant surface waxes provide much potential for breeding insect-resistant cultivars (EIGENBRODE AND ESPELIE 1995). Especially *Brassica* species may have a great breeding potential due to their heavy epicuticular wax bloom. Indeed, epicuticular waxes of cruciferous plants have been reported to affect host plant acquisition of aphids, flea beatles, Lepidoptera, stink bugs and thrips (STONER 1990; EIGENBRODE ET AL. 1991; EIGENBRODE ET AL. 2000; TRDAN ET AL. 2004; ŽNIDARČIČ ET AL. 2008;

BOHINC ET AL. 2014). The goal of the present study was to investigate the role of leaf epicuticular waxes of various *Brassica* cultivars in the host selection process of *Aleyrodes proletella* (L.). For this purpose, whitefly settlement was compared on waxy and dewaxed leaves as well as on Parafilm® that was treated with leaf wax extracts to clarify whether *A. proletella* can identify qualitative differences between leaf wax of different host cultivars. Additionally, scanning electron microscopy (SEM) imaging was used to characterize epicuticular wax crystal types and to visualize leaf surfaces. Determination of life-history traits and electrical recording of the feeding activity of *A. proletella* identified the underlying mechanism in host selection provided by epicuticular leaf waxes of cruciferous plants.

4.2 Materials and methods

Insects and plants

Aleyrodes proletella (L.) adults obtained from the institute's stock rearings (Department of Applied Entomology, Institute of Phytomedicine, University of Hohenheim) have been maintained on broccoli under controlled conditions in the greenhouse (25/23 ± 2 °C, L18/D6 photoperiod, 50 ± 5 % RH).

Seven *Brassica* cultivars were used in the study with known attractiveness (see Chapter 2): blue and white turnip cabbage (BTC, WTC) (*Brassica oleracea* L. convar. *acephala* var. *gongylodes*, cv. "Delikateß Blauer", cv. "Delikateß Weißer"), cauliflower (CA) (*Brassica oleracea* L. convar. *botrytis* var. *botrytis*, cv. "Erfurter Zwerg"), kale (KA) (*Brassica oleracea* L. convar. *acephala* var. *sabellica*, cv. "Grüner Krauser"), savoy cabbage (SC) (*Brassica oleracea* L. convar. *capitata* var. *sabauda*, cv. "Vertus"), white cabbage (WC) (*Brassica oleracea* L. convar. *capitata* var. *alba*, cv. "Brunswijker"), and oilseed rape (OR) (*Brassica napus* L. subsp. *Napus*, cv. "Atilla"). Experimental plants were grown under greenhouse conditions (22/18 ± 2 °C each, L18/D6 photoperiod, 50 ± 5 % RH), irrigated daily, and fertilized weekly with 30 ml 0.5 % Wuxal® Super (8 % N, 8 % P, 6 % K, Aglukon GmbH, Düsseldorf, Germany). The soil mixture was composed of 50 % potting soil (Floradur®, Floragard Vertriebs-GmbH, Oldenburg, Germany), 30 % compost soil (institute's production), and 20 % sand. The plants were used in experiments when they reached BBCH stage 17–18.

Host plant preference with and without leaf epicuticular waxes

Attractiveness of host plants with waxy (+) and dewaxed (−) leaf undersides was assessed in a series of dual choice cage experiments. Overall, eight repetitions were carried out for each single test in the greenhouse. Therefore, epicuticular wax has been removed gently using cotton wool as nonpolar solvents might have had a detrimental effect on the leaf tissues. For each choice test, whiteflies were offered the youngest expanded leaf of each test plant in opposite position. At least 20 randomly selected adult whiteflies were taken from the stock rearing by a suction tube and placed into one clip cage made of a clear plastic Petri dish (8.5 cm diam., 1.5 cm height) fitted with foam seal on edges to prevent any mechanical damage to the leaves and with an organdy-covered window in the lid for ventilation. The leaf area covered by the cage was kept as small as possible to minimize potential negative effects on photosynthetic traits of leaves (CRAFTS-BRANDNER AND CHU 1999). Cages were mounted on leaves using aluminium hair clips, which were retained by thin split bamboo sticks to not bend or even damage the plant leaves. After two days, the number of whitefly individuals was counted on each leaf.

Wax extraction and application onto Parafilm®

Epicuticular leaf wax of a total 50 leaves of each *Brassica* cultivar was collected by gently stroking the leaf surface using pre-washed cotton wool to mechanically remove the leaf wax. Subsequently, cotton wool with wax was washed three successive times in glass beakers containing pure chloroform. Wax extracts were then combined, stored and dried in a desiccator at room temperature to constant weight. Wax yields were then determined gravimetrically on an analytical balance (MC1 Research RC 210P, Sartorius AG, Göttingen, Germany). To utilize similar amounts of wax in the experiment as in nature, leaf area measurements using the software package ImageJ® served to calculate the naturally occurring amount of epicuticular waxes per cm^2 leaf surface. Finally, epicuticular waxes were resolved again in chloroform, and comparable wax volumes were transferred onto pieces of Parafilm® with a surface area of 16 cm^2. Parafilm® treated with chloroform only served as control variant. Treated Parafilm® was stored two days in a desiccator to assure complete evaporation of chloroform residues.

Epicuticular wax preference

Attractiveness levels of epicuticular leaf wax extracts were assessed in a series of dual choice experiments. For each single test, eight repetitions were carried out in the laboratory. Parafilm® with or without epicuticular waxes was stretched to cover glass Petri dishes (3.5 cm diam., 1 cm height) filled with a 20 % sucrose solution. The so prepared Petri dishes were placed upside down onto self-

built plastic covers with holes that allowed whiteflies to access and choose between two Parafilm® variants. The plastic covers fitted onto glass containers (15 cm diam., 10 cm height), which contained at least 20 randomly selected adult whiteflies for one day to choose feed. Subsequently, the number of *A. proletella* individuals on each variant was counted. As light was found to affect whitefly settlement, direct light sources were excluded from the experimental setup during the whole test period and glass containers were covered with an opaque foil. Nevertheless, diffuse light could still pass through the glass Petri dishes, thus making both choice alternatives easily accessible to the whiteflies.

SEM imaging

Samples subjected to SEM-imaging were prepared from fresh leaves of oilseed rape, blue turnip cabbage and white cabbage. After each leaf was cut into small pieces with a clean razor blade, leaf samples were fixed with their adaxial side onto aluminium stubs using silver glue. Between cuttings razor blades were washed first with pure methanol and then with distilled water. Subsequently, aluminium stubs were stored in a desiccator until the leaf samples were completely dry. For SEM analyses, samples were coated with gold-palladium (80:20) using a sputter coater (SCD 040, Balzers Union AG, Balzers, Liechtenstein) and investigated with an SEM DSM 940 (Carl Zeiss AG, Oberkochen, Germany) at 5 kV. The micrographs were digitized with Orion (software version 6.38, Orion Microscopy, Eli, Belgium).

Life-history traits

Mortality, duration of preimaginal development, longevity and fecundity of *A. proletella* were assessed on oilseed rape, blue turnip cabbage and white cabbage in a climate chamber to ensure controlled conditions (24 ± 1 °C, L18/D6 photoperiod, 50 ± 5 % RH). At least 20 randomly selected adult whiteflies were collected from the colony and confined together in one clip cage on the abaxial side of a waxy leaf, whereas another 20 whiteflies were confined in a second clip cage on the abaxial side of a dewaxed leaf of the same plant. Again, wax removal was attained by using cotton wool. After 24 h of oviposition, clip cages and adult whiteflies were removed to record the number of eggs deposited on each leaf and cultivar. Every day and until all living immatures had completed development, developmental times and mortality were recorded. Emerging adults were immediately sexed visually using a magnifying glass, and single pairs were confined separately in clip cages on the same plant. For each female, the total number of laid eggs and the adult longevity was counted. As males usually died earlier than females, males from the same treatment were replaced if necessary. Overall, eight replicates of each variant were used.

Feeding behaviour

Whitefly feeding activities were studied using the electrical penetration graph (EPG) technique (Tjallingii 1978). Adult female whiteflies (max. one day old) were anaesthetized with CO_2 and integrated into the electrical circuit of a DC-Giga-4 EPG system (manufactured by Wageningen University, Netherlands). Four whiteflies were recorded simultaneously by attaching them onto EPG electrodes using a gold wire (12 µm diam., 2 cm length) and water-based silver glue on their dorsa (both: EPG Systems, Wageningen, Netherlands). Before being glued, the wax layer covering the whitefly's dorsum was removed using a fine brush and water. Instead of plants, Petri dishes were used that contained a 20 % sucrose solution and were covered with Parafilm® pieces with or without epicuticular leaf waxes. For each Petri dish, a copper rod (2 mm diam., 10 cm length) was inserted into the sucrose solution as the second electrode. Probing activities were monitored in a Faraday cage for 6 h during the day at room temperature (20 ± 2 °C). Both Petri dishes and whiteflies were only used once. Whitefly feeding behaviour was recorded on eight Petri dishes per variant. Signals were recorded and analysed using NextView/NT software (plug-in card: PCI-Base 50/300, A/D-conversion-module: MAD12, both BMC-Schetter, Germany) on a standard PC. The position of the whitefly stylets, as well as their feeding activities, were interpreted according to EPG waveforms previously defined by TJALLINGII (1978) and LEI ET AL. (1996) on plants. Waveforms occurring during EPG recording associated with non-probing activity, probing-activity and whitefly feeding could be identified.

Statistics

All obtained data were analysed using JMP® 14.1.0 (SAS Institute Inc., Cary, NC, USA). Before statistical analysis, the residuals were tested for normal distribution by Shapiro-Wilk test. All continuous data were found normally distributed. All data were subjected to an analysis of variance, procedure "Generalized Linear Models", before ensuing statistical analyses. The respective statistical procedures and statistical core data are provided in the legends of the tables.

For determination of host plant attractiveness of two host plant choices, preference indices (PI) were calculated using the following formula based on KOGAN AND GOEDEN (1970):

$$Preference\ index\ (PI) = \frac{n_A}{n_A + n_B}$$

where n_A is the number of whitefly adults on host plant choice A and n_B is the number of whitefly adults on host plant choice B. In this scale, PI = 1 and PI = 0 represent an absolute preference for one of the two choice alternatives, whereas PI = 0.5 implies no preference between both choice alternatives.

4.3 Results

Host choice behaviour of *A. proletella* depended not only on the presence or absence of epicuticular waxes but also on leaf wax quality (Figure 8, 9, 10). In dual choice experiments with *Brassica* leaves, epicuticular waxes significantly attracted more whiteflies compared to leaves without wax (Figure 8). While significant differences were found for each variety, a mean preference index of 0.86 ± 0.02 (n = 56) was determined for *Brassica* leaves with leaf waxes regardless of the cultivar. In dual choice tests with extracted leaf wax, which was then applied onto Parafilm® and offered for selection together with a clean Parafilm® control, epicuticular waxes again considerably contributed to host attractiveness (Figure 9). Except for wax extracts from white turnip cabbage, significant differences could be measured for each wax variant. Accordingly, for Parafilm® that had been treated with leaf wax, an average preference index of 0.71 ± 0.02 (n = 56) could be determined to demonstrate the promoting effect of *Brassica* leaf waxes on whitefly settlement. When two leaf wax extracts of two different host cultivars were offered, whiteflies preferred leaf wax extracts to varying degrees (Figure 10). For example, it was found that epicuticular leaf wax of oilseed rape was significantly more attractive than leaf wax of blue turnip cabbage and white cabbage, whereas leaf wax of blue turnip cabbage was more preferred over waxes of white cabbage.

SEM-images of leaf undersides of oilseed rape, blue turnip cabbage and white cabbage before and after treatment with cotton wool displayed considerable optical differences (Figure 11). Images of leaf undersides after treatment with cotton wool proved a successful removal of the epicuticular wax with only few residues. Furthermore, epicuticular wax crystals of all three cabbage varieties differed in shape. Wax crystals on abaxial leaf sides of oilseed rape were characterized by many irregular plates and few rod-like projections, whereas waxes of blue turnip cabbage displayed many thread- and rod-like structures, as well as few irregular plates. In contrast, epicuticular waxes of white cabbage appeared as polygonal rodlets.

Life-history traits of *A. proletella* differed between host cultivar and between leaf surfaces with and without epicuticular waxes (Table 8). While total preimaginal mortality was significantly higher on dewaxed leaves compared to waxy leaf surfaces of oilseed rape, mortality did not differ between cultivars and leaf surfaces of other cultivars. The time needed from egg deposition until adult emergence was fastest on oilseed rape, slowest on white cabbage, and preimaginal developmental times were significantly reduced on leaves without surface waxes on all host cultivars. Additionally, female adult whiteflies survived longest on oilseed rape; however, longevity did not statistically differ between blue turnip cabbage and white cabbage. Moreover, female *A. proletella* survived significantly longer on leaves with surface waxes of oilseed rape and blue turnip cabbage. Most eggs were laid by female whiteflies on oilseed rape, whereas fecundity was lowest on white cabbage. On leaves without surface waxes fecundity was significantly reduced.

Feeding behaviour of whiteflies on different Parafilm® surfaces depended on the treatment (Table 9). Although the number of feeding attempts did not differ between Parafilm® surface qualities, statistical differences could be detected for the total and the mean feeding duration. While the total feeding time was highest on surfaces treated with epicuticular waxes of oilseed rape, food consumption of *A. proletella* was significantly reduced on Parafilm® surfaces with leaf wax of white cabbage. The mean feeding duration was significantly prolonged on surfaces with epicuticular waxes of oilseed rape and blue turnip cabbage, whereas feeding was interrupted earlier on white cabbage. However, the mean feeding times of *A. proletella* on the Parafilm® control did not differ from surfaces treated with leaf waxes in this case.

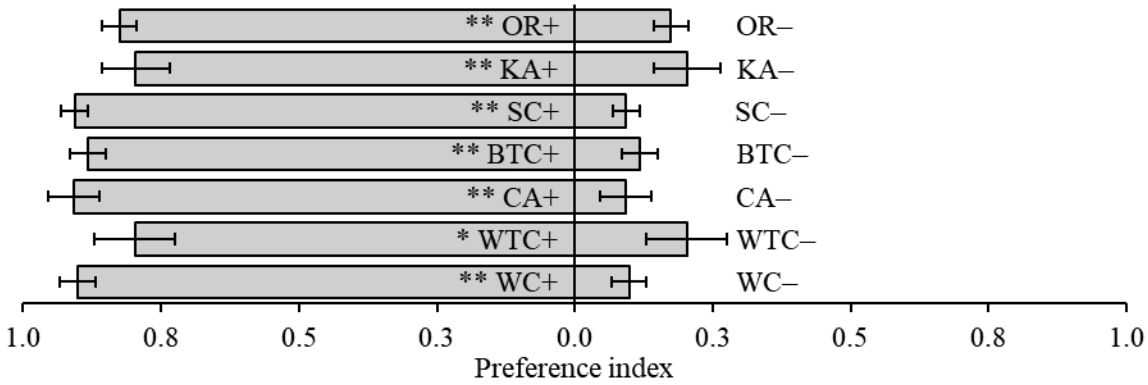

Figure 8: Host plant preference indices (mean ± s.e.m.) of *A. proletella* adults in dual choice tests on different cabbage cultivars

(OR = oilseed rape, SC = savoy cabbage, KA = kale, BTC = blue turnip cabbage, CA = cauliflower, WTC = white turnip cabbage, WC = white cabbage, + denotes presence and – denotes absence of epicuticular surface waxes on cabbage leaves)

Wilcoxon signed-rank test ($H_0 = 0.5$ two-sided, $\alpha = 0.05$, $n = 8$ for each combination, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$)

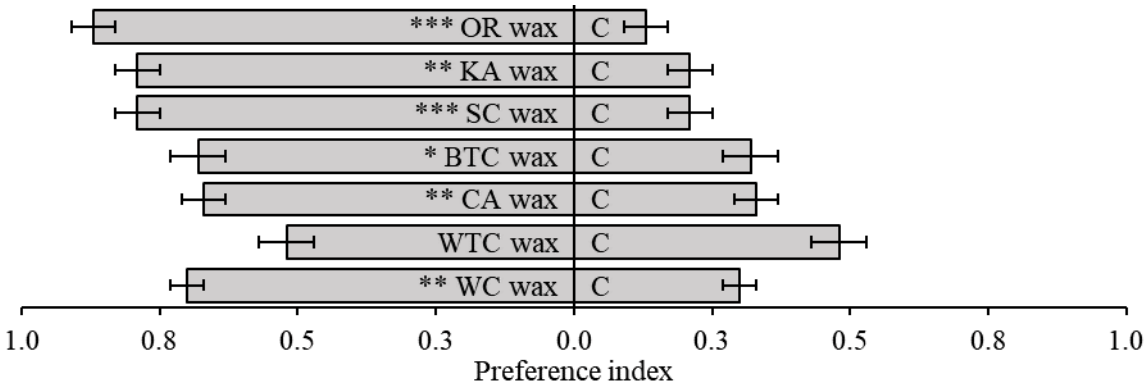

Figure 9: Leaf wax preference indices (mean ± s.e.m.) of *A. proletella* adults in dual choice tests on Parafilm® treated with epicuticular leaf waxes of various cabbage cultivars

(OR = oilseed rape, SC = savoy cabbage, KA = kale, BTC = blue turnip cabbage, CA = cauliflower, WTC = white turnip cabbage, WC = white cabbage, C = Parafilm® control)

Wilcoxon signed-rank test ($H_0 = 0.5$ two-sided, $\alpha = 0.05$, $n = 8$ for each combination, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$)

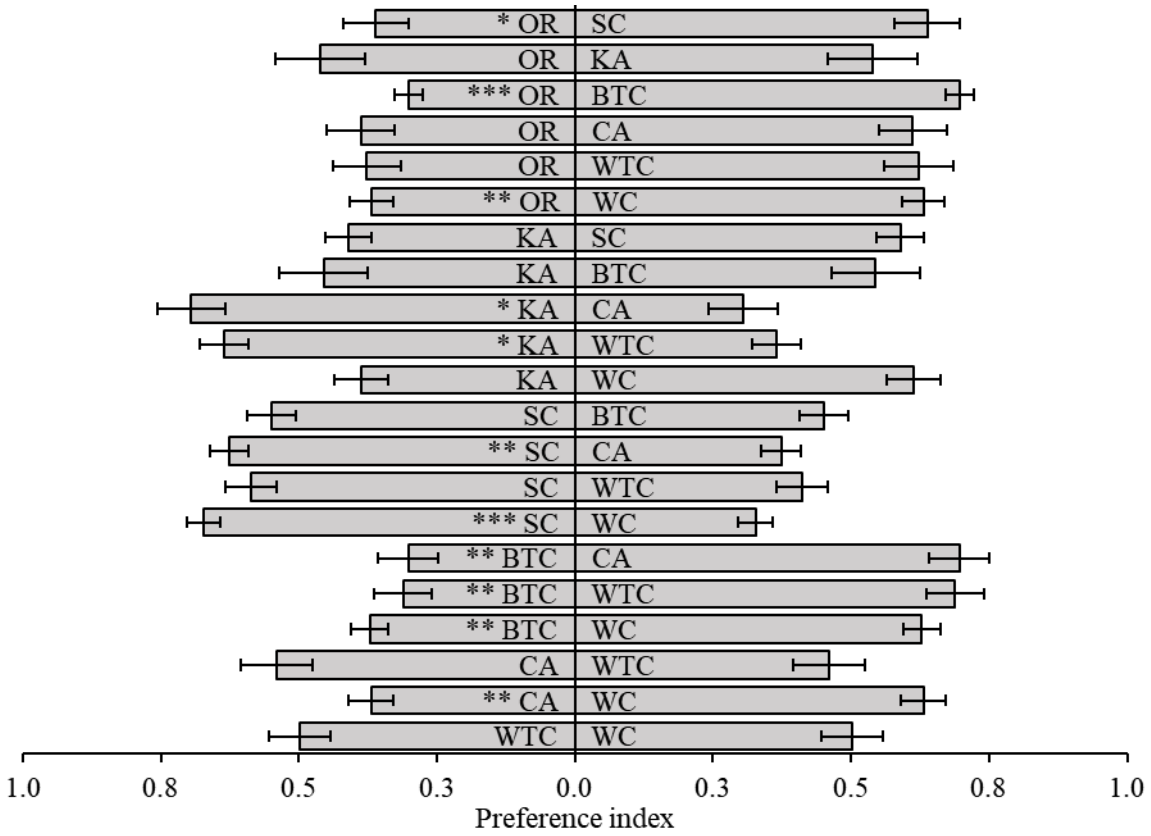

Figure 10: Leaf wax preference indices (mean ± s.e.m.) of *A. proletella* adults on Parafilm® treated with epicuticular leaf waxes of various cabbage cultivars

(OR = oilseed rape, SC = savoy cabbage, KA = kale, BTC = blue turnip cabbage, CA = cauliflower, WTC = white turnip cabbage, WC = white cabbage)

Wilcoxon signed-rank test ($H_0 = 0.5$ two-sided, $\alpha = 0.05$, n = 10 for each combination, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$)

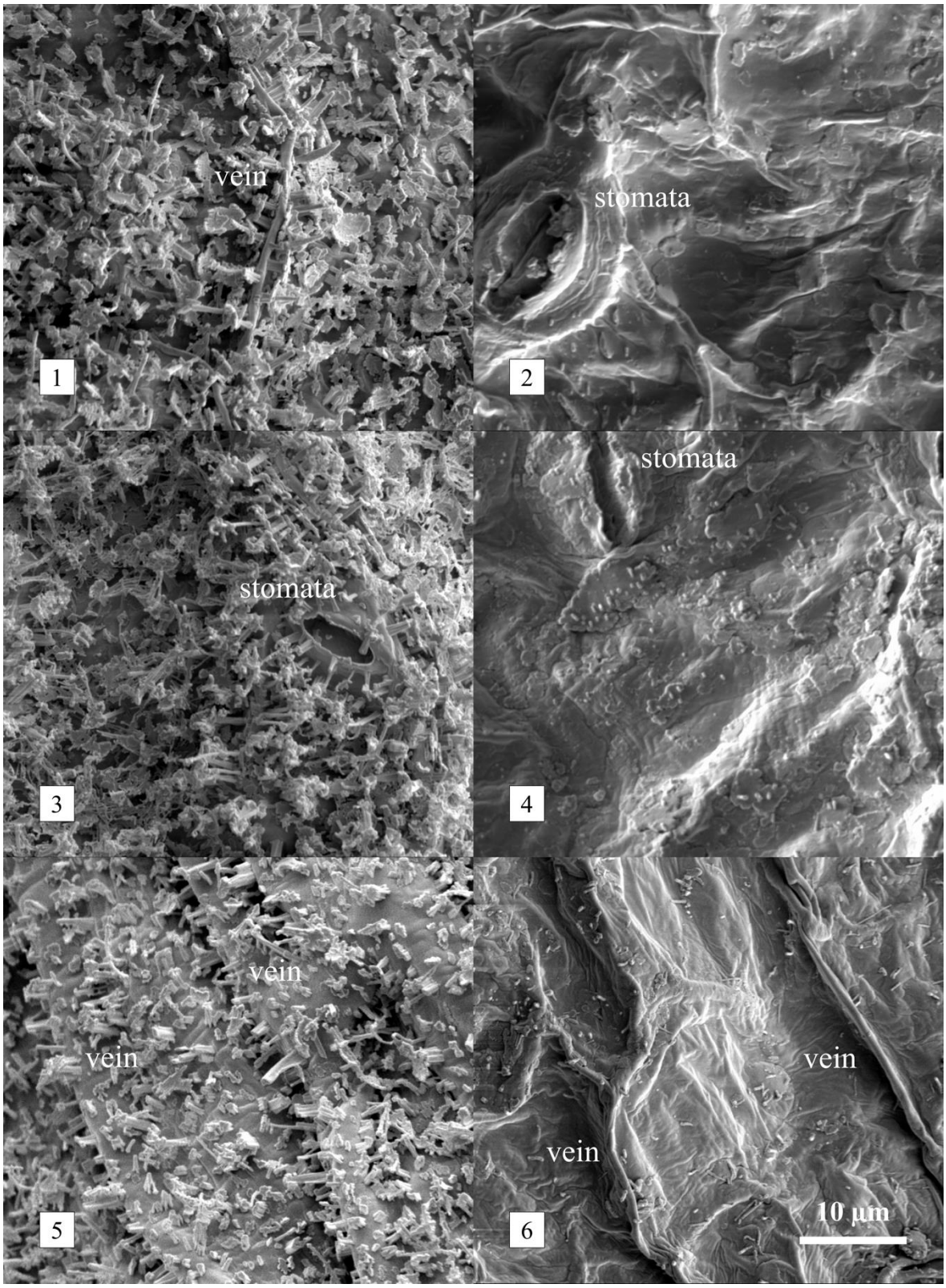

Figure 11: SEM images of abaxial leaf sides of various *Brassica* cultivars with waxy (+) and dewaxed (–) leaf surfaces

(1): oilseed rape +, (2): oilseed rape –, (3): blue turnip cabbage +, (4): blue turnip cabbage –, (5): white cabbage +, (6): white cabbage –; pictures were taken at 5 000 V, focal length 5 mm, 2 000x magnification

Table 8: Life-history traits of *A. proletella* on different cabbage cultivars with and without epicuticular waxes

Host plant		Total preimaginal mortality[1] (%) mean ± s.e.m.		Duration of preimaginal development[2] (days) mean ± s.e.m.	Female longevity[2] (days) mean ± s.e.m.	Total fecundity[2] (eggs/female) mean ± s.e.m.
OR+		12.37 ± 3.57 b		24.99 ± 0.08 f	57.50 ± 2.31 a	540.60 ± 25.21 a
		n = 10		n = 699	n = 10	n = 10
OR–		53.17 ± 7.19 a		26.04 ± 0.10 e	40.00 ± 1.78 b	254.60 ± 22.84 b
		n = 10		n = 553	n = 10	n = 10
BTW+		35.18 ± 6.05 ab		27.01 ± 0.15 d	31.10 ± 1.32 c	191.30 ± 15.45 b
		n = 10		n = 539	n = 10	n = 10
BTW–		43.54 ± 9.50 a		29.59 ± 0.26 c	24.60 ± 1.47 d	94.20 ± 9.48 cd
		n = 10		n = 305	n = 10	n = 10
WC+		26.61 ± 3.80 ab		30.93 ± 0.27 b	25.10 ± 1.93 cd	97.60 ± 10.74 c
		n = 10		n = 160	n = 10	n = 10
WC–		27.97 ± 7.92 ab		34.10 ± 0.50 a	21.10 ± 1.51 d	66.20 ± 9.44 d
		n = 10		n = 126	n = 10	n = 10
	F	4.4535	X^2	780.8296	42.2832	49.0737
	d.f.	5, 54	d.f.	5	5	5
	P	0.0016	*P*	< 0.0001	< 0.0001	< 0.0001

[1] one-way ANOVA followed by Tukey-Kramer HSD test at $\alpha = 0.05$, [2] Kruskal-Wallis one-way test followed by Wilcoxon Each Pair at $\alpha = 0.05$
Means with s.e.m. in a column followed by the same index letter are not statistically different
OR = oilseed rape, BTC = blue turnip cabbage, WC = white cabbage
+ denotes presence and – denotes absence of epicuticular surface waxes on cabbage leaves

Table 9: Number of feeding attempts, total feeding duration, and mean feeding duration of *A. proletella* on Parafilm® surfaces with epicuticular waxes of different host plants

Surface		Number of feeding attempts[1] mean ± s.e.m.		Total feeding duration[2] (min) mean ± s.e.m.	Mean feeding duration[2] (min) mean ± s.e.m.
OR+		22.88 ± 5.06 a		101.65 ± 13.18 a	4.46 ± 0.50 a
		n = 8		n = 8	n = 183
BTW+		13.50 ± 2.58 a		76.80 ± 16.20 ab	5.69 ± 0.83 a
		n = 8		n = 8	n = 108
WC+		17.50 ± 2.91 a		39.06 ± 9.26 b	2.23 ± 0.31 b
		n = 8		n = 8	n = 140
C		12.13 ± 2.58 a		37.66 ± 9.61 b	3.11 ± 0.53 ab
		n = 8		n = 8	n = 97
	X^2	4.2236	F	6.2869	9.3211*
	d.f.	3	d.f.	3, 28	3, 260*
	P	0.2383	*P*	0.0021	< 0.0001*

[1] Kruskal-Wallis one-way test followed by Wilcoxon Each Pair at $\alpha = 0.05$
[2] one-way ANOVA followed by Tukey-Kramer HSD test at $\alpha = 0.05$
Means with s.e.m. in a column followed by the same index letter are not statistically different
* F, d.f., and *P* values were corrected by Welch-ANOVA test because of variance inhomogeneity after Levene's test
OR = oilseed rape, BTC = blue turnip cabbage, WC = white cabbage, C = Parafilm® control
+ denotes presence of epicuticular surface waxes on cabbage leaves

4.4 Discussion

Cuticular waxes in plants are a diverse composition of aliphatic components belonging to nine major classes, which are *n*-alkanes, wax esters, aldehydes, ketones, secondary alcohols, β-diketones, fatty alcohols, and triterpenoids (EIGENBRODE AND ESPELIE 1995). Furthermore, each class comprises a homologous series of isomers, with usually one compound dominating the total composition of the plant cuticular wax (MÜLLER AND RIEDERER 2005). This study demonstrates that epicuticular leaf waxes of cruciferous plants are of great importance for the recognition of a potential host plant by *A. proletella*. While the removal of leaf epicuticular waxes led to considerable reductions in host plant attractiveness of various host cultivars, leaf surface waxes applied onto Parafilm® served as arrestant stimuli. Although the true food, phloem sap, should have the same quality when offered from waxy and dewaxed leaves of the same cultivar, the epicuticular waxes must be taken as a key factor in the host selection process by *A. proletella*. Moreover, epicuticular leaf waxes revealed qualitative differences, as leaf wax attractiveness could be ranked as follows: oilseed rape wax > blue turnip cabbage wax > white cabbage wax. Consequently, it is hypothesized that *A. proletella* evaluates host plant suitability especially by physical contact with epicuticular leaf

waxes. Sensing of epicuticular waxes might occur by chemo- and mechanosensory sensillae located at the apex of the labium in whiteflies (WALKER AND GORDH 1989). This assumption could be additionally supported by visual characteristics of leaf surfaces. Due to the varying morphology of epicuticular wax crystals visualized by SEM, it is furthermore assumed that chemical compositions differ between leaf waxes of host cultivars used in the study. However, the interpretation of intact surface crystals is difficult, as the density of wax crystals on *Brassica* leaves is relatively high and is dependent from various environmental conditions such as light intensity, humidity, or temperature (BAKER 1974; JEFFREE 2006; KOCH ET AL. 2006). In general, *Brassica* waxes are considered to consist of (i) a continuous film, (ii) flat crystals and (iii) upright columns, with flat crystals and upright columns being the predominant forms varying strongly in their shape (GÓMEZ-CAMPO ET AL. 1999; JEFFREE 2006). While alkanes, ketones, and secondary alcohols were previously ascribed to form column or plate-shaped wax crystals in *Brassica* plants, ketones are attributable to form dendritic structures as well (MEUSEL ET AL. 1999; MEUSEL ET AL. 2000; JEFFREE 2006). In several studies, wax amounts and chemical profiles of epicuticular surface lipids of different cultivated plants affected host plant selection in herbivorous insects. Accordingly, high levels of *n*-alkanes, fatty alcohols and triterpenoids were associated with insect resistance towards Lepidoptera, aphids, as well as the azalea lace bug *Stephanitis pyrioides* (Scott) (JOHNSON AND SEVERSON 1984; BERGMAN ET AL. 1991; ROBERTSON ET AL. 1991; YANG ET AL. 1993b; YANG ET AL. 1993c; BALSDON ET AL. 1995; EIGENBRODE AND ESPELIE 1995). However, *n*-alkanes, fatty acids and triterpenoids were also correlated with insect susceptibility of host plants to Lepidoptera and thrips (YANG ET AL. 1993a; UDAYAGIRI AND MASON 1997; LI AND ISHIKAWA 2006; KARMAKAR ET AL. 2016; RID ET AL. 2018). The epicuticular wax blend of leaf surfaces may also contain a range of other polar components including amino acids, nonprotein amino acids, sugars, sucrose and glucose esters, sesquiterpenes, diterpenes, phenolics, phenolic glycosides, glucosinolates and other plant metabolites acting as major cues in the host selection process of herbivorous insects (EIGENBRODE AND ESPELIE 1995; KERSTIENS 1996; MÜLLER AND RIEDERER 2005). Both primary and secondary metabolites can be associated with leaf waxes, as they can be deposited by leakage or diffusion to plant surfaces, whereas some components are exuded by glandular trichomes (DERRIDJ ET AL. 1996; MALUF ET AL. 2001; MÜLLER AND RIEDERER 2005). However, this is not assumed in the case of glucosinolates, as it is controversially discussed whether glucosinolates may or may not be present on the leaf surface. By mechanical removal of the epicuticular wax layer of *Brassica* leaves using gum arabic, glucosinolates could not be detected at the leaf surface. In contrast, leaf surface wax extraction by rinses with organic solvents revealed a correlation between glucosinolate concentrations and stomatal conditions (GRIFFITHS ET AL. 2001; MÜLLER AND RIEDERER 2005; REIFENRATH ET AL. 2005; STÄDLER AND REIFENRATH 2009). As a result, it was postulated that polar glucosinolates are washed from the inner leaf tissue to the outside through open stomata during solvent extraction but are not naturally present on the leaf surface (REIFENRATH ET AL. 2005; STÄDLER AND REIFENRATH 2009). In addition to the

reduction in host plant attractiveness, the absence of epicuticular waxes also had a significant effect on life-history traits and feeding behaviour of *A. proletella*. Life-history traits generally vary greatly between whitefly species, host plants species and health, as well as environmental impacts (COSTA ET AL. 1991b; SHISHEHBOR AND BRENNAN 1996; BLACKMER AND BYRNE 1999; BÄHRMANN 2002; ALONSO ET AL. 2009; ASKOUL ET AL. 2019). In this study, it was additionally found that the absence of epicuticular waxes led to increased mortality, delayed development times, decreased adult longevity, and lower fecundity in *A. proletella*. According to COLE AND RIGGALL (1992) the water stress susceptibility could be increased in *Brassica* plants due to the removal of epicuticular leaf wax, leading to elevated concentrations of deterrent compounds on the leaf surfaces. However, the dewaxed leaf areas made up only a few cm^2 of the whole plant surface, suggesting water stress was not present. It is more likely that the absence of the host-finding stimulus provided by the leaf waxes led to a change in the feeding behaviour of the whiteflies. Although no statistical differences between the phloem uptake per unit of time (mean feeding durations) on Parafilm® with and without leaf wax extracts could be measured, the total phloem uptake (total feeding time) within 6 h was significantly higher on surfaces treated with leaf wax extracts of oilseed rape as well as blue turnip cabbage. In summary, cruciferous leaf waxes might function as feeding stimulants towards *A. proletella*. Therefore, the absence of epicuticular leaf waxes could lead to a decreased food intake, which may result in a poor supply with important nutrients such as essential amino acids. In the long-term consequence, the life cycle of *A. proletella* was prolonged and life-history traits were impaired. Similar conclusions could be drawn for aphids, orthopterans, lepidopterans and coleopterans. Feeding of *Aphis fabae* (Scopoli), *Chaitophorus leucomelas* (Koch), *Microtylopteryx hebardi* (Rehn), *Locusta migratoria* (L.), *Anthonomus grandis* (Boheman), *Bombyx mori* (L.) and *Plutella xylostella* (L.) was promoted by the presence of epicuticular waxes of susceptible host plants and feeding was delayed or reduced when leaf surface waxes were not present (BERNAYS ET AL. 1976; MORI 1982; MCKIBBEN ET AL. 1985; EIGENBRODE ET AL. 1991; BRAKER AND CHAZDON 1993; POWELL ET AL. 1999; ALFARO-TAPIA ET AL. 2007). In addition, *Choristoneura fumiferana* (Clemens) showed varying feeding preferences for various epicuticular wax fractions of its host plant indicating that wax components may act as feeding stimulants (MALONEY ET AL. 1988). Overall, these findings offer the breeding potential for the development of resistant crop cultivars. Further investigations are needed to determine the chemical composition of leaf epicuticular waxes on *Brassica* leaves and to identify which wax components are mediating host choice in *A. proletella*. Once the mediating wax components are known, breeding of *Brassica* cultivars with certain leaf wax characteristics could result in *A. proletella* no longer being able to recognize its host plants.

5 Phloem amino acid composition affect host plant preferences of *Trialeurodes vaporariorum* (Westw.)

Abstract: Amino acids ingested with the phloem sap of plants strongly affect whitefly performance. The goal of this study was to investigate the influence of phloem amino acids on the host choice behaviour of whiteflies. Therefore, the phloem amino acid profiles of six vegetable crops varying in their host plant attractiveness towards the greenhouse whitefly *Trialeurodes vaporariorum* (Westw.) were analysed by liquid chromatography-mass spectrometry (LC-MS). In a second step, multiple regressions of the amino acid compositions and the host plant preferences of *T. vaporariorum* were performed. To verify the contribution and association of single amino acids on host choice, feeding preferences were assessed in dual choice experiments using sucrose media with and without single added amino acids. Glutamic acid, threonine, phenylalanine and serine were the most relevant amino acids to explain host plant attractiveness. Furthermore, essential, aromatic, and hydroxylated amino acid groups affected most host plant selection of *T. vaporariorum*. On the other hand, dual choice experiments proved that lysine, asparagine, threonine, valine, glutamine, leucine, tryptophan, glutamic acid, tyrosine, aspartic acid, cysteine, and alanine exert gustatory stimuli determining feeding preferences. Overall, the effects of single amino acids in natural hosts only partially agreed with the effects of individual amino acids *in vitro*. While the presence and concentration of other plant compounds in the phloem sap of host plants might have additionally affected the influence of single amino acids, other plant factors not associated with the phloem sap might have determined host plant selection of *T. vaporariorum* as well.

Keywords: host plant susceptibility, phloem sap, amino acids, multiple regressions

5.1 Introduction

Nitrogen is a vital nutrient for herbivorous insects, and it takes a central role in the growth of all organisms (MATTSON 1980). Together with a wide range of other compounds such as sugars, proteins, sugar alcohols, and hormones, nitrogen is present in the form of free amino acids in the phloem sap of plants (ANSTEAD ET AL. 2013). Furthermore, phloem sap is usually characterized by the absence or at least low concentrations of secondary compounds compared to other plant parts (DOUGLAS 2006). Consequently, herbivorous insects utilize the phloem sap as their nutrient source and nitrogen supply. However, phloem sap represents an unbalanced source of nutrition. On the one hand, phloem sap consists mainly of carbohydrates and only low concentration of nitrogen; on the other hand, the ratio between essential and non-essential amino acids is in favour of the non-essential amino acids (DOUGLAS 1993; DIXON 1998; SANDSTROM AND MORAN 1999; DOUGLAS 2006).

Nevertheless, some of the most significant pests in agriculture and horticulture, such as whiteflies almost exclusively use phloem sap as their food source (BLACKMER AND BYRNE 1999). Whiteflies penetrate the phloem sieve elements of a plant by their piercing-sucking mouthparts, and as a result of the high hydrostatic pressure, phloem sap exudes out into their stylets and is consumed (WALKER ET AL. 2010). They successfully exploit this niche because: (i) whiteflies have endosymbionts providing their hosts with certain essential amino acids, which may occur in too low concentrations or even are lacking in the phloem sap (HOUK AND GRIFFITHS 1980; CAMPBELL 1989; THAO AND BAUMANN 2004; SKIDMORE AND HANSEN 2017), (ii) whiteflies excrete excess dietary sugars, which are otherwise lethal to them due to the ability of sugars to lower the osmotic pressure (BYRNE AND BELLOWS 1991; DOUGLAS 2006; WALKER ET AL. 2010), and (iii) whiteflies are assumed to compensate lower levels of amino acids by increasing their feeding rates as proved for aphids (PROSSER ET AL. 1992).

Insect performance is strongly affected by the different nutritional value of individual amino acids ingested and, therefore, amino acid compositions affect host plant suitability (AUCLAIR 1963; ROCK AND KING 1967; DADD AND KRIEGER 1968; BRODBECK AND STRONG 1987; WILKINSON AND DOUGLAS 2003; CHIOZZA ET AL. 2010; DHILLON AND KUMAR 2017). To study the influence of phloem sap nitrogen in host plant suitability of herbivorous insects amongst different host plant species or cultivars, measurements of free amino acid compositions are often utilized (ROCK AND KING 1967; WEIBULL 1988; COLE 1997; CHIOZZA ET AL. 2010; DHILLON AND KUMAR 2017). Amino acid compositions are often statistically correlated with life-history parameters as a measure for host suitability, however, most of these studies focused exclusively on aphids as model organisms (DOUGLAS 1993; WILKINSON AND DOUGLAS 2003; DOUGLAS 2006). The goal of this study was the profiling and relative quantification of 20 free amino acids in the phloem sap of six vegetable species with varying levels of host plant attractiveness *vis-à-vis* the greenhouse whitefly *Trialeurodes vaporariorum* (Westw.). Multiple regressions of the relative amino acid proportions in the phloem of host plants, with the preference of *T. vaporariorum* towards these hosts, should identify the most relevant amino acids and amino acid groups explaining host plant selection. In addition, dual choice experiments using sucrose solutions with and without added single amino acids should verify the contribution and association of individual amino acids in host selection of the whitefly.

5.2 Materials and methods

Insects and plants

Trialeurodes vaporariorum (Westw.) adults were obtained from the institute's stock cultures (Department of Applied Entomology, Institute of Phytomedicine, University of Hohenheim) reared on poinsettia under controlled conditions in the greenhouse (25/23 ± 2 °C each, L18/D6 photoperiod, 50 ± 5 % RH).

Seven vegetable plant species were used in the study with known attractiveness (see Chapter 2): bean (BE) (*Phaseolus vulgaris* L., cv. "Mombacher Speck"), cucumber (CU) (*Cucumis sativus* L., cv. "Delikateß"), eggplant (EG) (*Solanum melongena* L., cv. "Falcon"), sweet pepper (SP) (*Capsicum annuum* L., cv. "California Wonder"), tobacco (TB) (*Nicotiana tabacum* L., cv. "Orient Xanthi"), and tomato (TO) (*Solanum lycopersicum* L., cv. "Resi"). Experimental plants were grown under greenhouse conditions (22/18 ± 2 °C each, L18/D6 photoperiod, 50 ± 5 % RH), irrigated daily, and fertilized weekly with 30 ml 0.5 % Wuxal® Super (8 % N, 8 % P, 6 % K, Aglukon GmbH, Düsseldorf, Germany). The soil mixture was composed of 50 % potting soil (Floradur®, Floragard Vertriebs-GmbH, Oldenburg, Germany), 30 % compost soil (institute's production), and 20 % sand. The plants were used in experiments when they reached BBCH stage 17–18.

Phloem sap sampling

The leaf exudation technique was used for phloem sap collection according to KING AND ZEEVAART (1974) as well as URQUHART AND JOY (1981). For this purpose, the upper fully expanded leaves of eight plants of each vegetable plant species were cut at their petioles under distilled water with a razor blade, directly transferred into individual test tubes containing a solution of 20 mM ethylenediaminetetraacetic acid (EDTA, adjusted to pH 7.0 with NaOH) and incubated in a dark chamber kept at 100 % RH. After 6 h, plants were removed from the test tubes and separate phloem sap fractions from each leaf were frozen at -20 °C until chemical analyses.

Amino acid analysis

In order to determine the composition of amino acids, phloem exudates were analysed by controlled liquid chromatography-mass spectrometry (LC-MS) (MS-detector: LTQ Velos Dual Pressure Linear Ion Trap, Thermo Scientific Inc., Waltham, MA, USA). The phloem exudates were lyophilised (Christ® ALPHA 1-4, vacuum level: 0.375 mbar, temperature: -30 °C, Martin Christ GmbH, Osterode am Harz, Germany), dissolved in 0.5 ml water and transferred into microtubes to centrifuge for 5 min at 12,000 rpm. The supernatant was diluted with 25 % methanol and samples

were incubated at 55 °C. After 10 min samples were then diluted with 25 % acetonitrile. Pre-column derivatization took place in an UHP Accela autosampler (Thermo Scientific Inc., Waltham, MA, USA) using the AccQ-Fluor Reagent Kit (WAT052880, Waters Corporation, Milford, MA, USA) and AccQ-Fluor reagent (15 µl AccQ-Fluor reagent, 75 µl borate buffer, 10 µl phloem sample).

LC-MS analysis was performed at 33 °C using a UHPLC Accela 1250 pump (Thermo Scientific Inc., Waltham, MA, USA) and an AccQ-TagTM column (3.9 x 150 mm, 4 µ, WAT052885, Waters Corporation, Milford, MA, USA). Elution buffers were ammonium formate buffer (10 mM, pH 6.3) + 2 % methanol, and acetonitrile. The flow rate was constant at 0.5 ml min^{-1} and the injection volume was 3 µL. Peak identification of amino acids was confirmed by standard addition and quantified by an eternal standard with 20 amino acids each at a concentration of 250 mmol ml^{-1}. With this method all proteinogenic amino acids can be analysed in their free forms.

Feeding preference for single amino acids

Gustatory properties of single amino acids were assessed in a series of dual choice experiments. Overall, eight repetitions were carried out for each single test in the laboratory. For each choice test, whiteflies were offered two Petri dishes covered by Parafilm® in the opposite position. While both Petri dishes contained a 20 % sucrose solution, only one additionally contained 3 mg/ml of a single amino acid. At least 20 randomly selected adult whiteflies were taken from the stock rearing by a suction tube and placed into glass containers (10 cm diam., 8 cm height). Containers were then closed with lids that were fitted with organdy windows for ventilation and with holes that allowed the whiteflies access to the Petri dishes placed upside-down on the glass containers. After seven days, the number of whitefly individuals was counted once on each Petri dish variant, as it was found that whiteflies made their final choice within this time.

Statistics

All obtained data were analysed using JMP® 14.1.0 (SAS Institute Inc., Cary, NC, USA). Before data were subjected to one-way analysis of variance (ANOVA), the residuals were tested for normality by Shapiro-Wilk test as well as for homogeneity of variance following the Levene-test. When data did not meet the assumptions of homogeneity of variance, Welch-ANOVA was used for interpretation. The respective statistical procedures and statistical core data are provided in the legends of the tables.

For determination of amino acid preference in the dual choice tests, preference indices (PI) were calculated using the following formula based on KOGAN AND GOEDEN (1970):

$$Preference\ index\ (PI) = \frac{n_A}{n_A + n_B}$$

where n_A is the number of whitefly adults on host plant choice A and n_B is the number of whitefly adults on host plant choice B. In this scale, PI = 1 and PI = 0 represent an absolute preference for one of the two choice alternatives, whereas PI = 0.5 implies no preference between both choice alternatives.

To analyse the general patterns of trait covariation, relationships between relative amino acid proportions in the phloem sap of host plants and preference indices of *T. vaporariorum* towards these hosts were analysed using principal components analysis. Correlations between relative amino acid compositions of the phloem sap and the host plant preference of *T. vaporariorum* towards these hosts was performed using stepwise multiple regression analyses that bidirectionally select for minimum BIC. As a measure for the host plant preference of *T. vaporariorum*, the mean preference indices (PI) were used (see Chapter 2). Additionally, amino acids were grouped according to their physiological and chemical properties (Table 10). Therefore, correlations were performed for relative proportions of individual amino acids as well as for amino acid groups separately.

Table 10: Classification of amino acids groups according to physiological and chemical properties

Group	Amino acids
Essential	Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val
Aromatic	Phe, Trp, Tyr
Heterocyclic	Pro
Aliphatic	Ala, Gly, Ile, Leu, Pro, Val
Amidated	Asn, Gln
Hydroxylated	Ser, Thr,
Basic	Arg, His, Lys
Acidic	Asp, Glu

5.3 Results

The following amino acids were detected: alanine (Ala), arginine (Arg), asparagine (Asn), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val), whereas aspartic acid (Asp), cysteine (Cys), lysine (Lys), and methionine (Met)

levels were below the detection limit. Relative proportions of free amino acids in the phloem sap differed significantly within each host plant species (Table 11). Glutamine was the predominant amino acid in the phloem of cucumber, eggplant, sweet pepper, tobacco, and tomato. In contrast, asparagine and glutamic acid were present in the highest proportions in the phloem sap of bean. The relative amino acid levels also varied significantly between host plant species. However, proportions of histidine and tryptophan did not differ depending on the host plant.

When the relative proportions of free amino acids in the phloem sap of plants were grouped according to their physiological and chemical properties, relative amino acid proportions varied significantly within host plant species (Table 12). Amidated amino acids were the predominant group in the phloem sap of all host plant species. Additionally, phloem sap of bean was dominated by acidic amino acids as well. Furthermore, relative proportions of each amino acid group differed significantly between host plant species.

The patterns of covariation among the percentage composition of individual amino acids in the phloem sap of host plants and the mean preference indices of *T. vaporariorum* towards these hosts show that two axes explained 52.9 % of the variance (Figure 12). Considering the number of variables accounted for in the model, this represents an acceptable value that is open to interpretation. On the other hand, patterns of covariation among the relative proportions of amino acid groups in the phloem sap of host plants as well as the mean preference indices of *T. vaporariorum* show that two axes explained 59.8 % of the variance (Figure 13). This represents a more robust value that explains variance even better than the first analysis of trait covariation by including amino acid classification. While the first axis was associated with the amino acid proportions in the phloem of host plants, the second axis was associated with the preference indices of *T. vaporariorum* in both analyses. Within the two axis dimensions, the different host plant species were often aggregated.

Stepwise multiple regressions between the relative proportions of individual amino acids in the phloem sap of host plants and the preference indices of *T. vaporariorum* revealed that glutamine, phenylalanine, serine, and threonine were the most relevant amino acids to explain host plant preference of *T. vaporariorum* (Table 13). According to this model, glutamine and threonine decreased host plant attractiveness, whereas phenylalanine and serine had a positive effect. Furthermore, stepwise multiple regressions between relative proportions of amino acid groups in the phloem sap of host plants and the preference indices of *T. vaporariorum* determined that essential, aromatic, as well as hydroxylated amino acids, explained the host plant preference the most (Table 14). While aromatic and hydroxylated amino acids were positively affecting host plant preference, essential amino acids had negative effects.

In dual choice preference experiments, feeding preferences of adult *T. vaporariorum* differed significantly between single amino acids (Figure 14). While lysine had the highest preference index (0.70 ± 0.06), alanine was at the least preferred amino acid (0.03 ± 0.02). Within this spectrum, proline, methionine, glycine, serine, phenylalanine, histidine, and isoleucine were more preferred, whereas asparagine, threonine, valine, glutamine, leucine, tryptophan, glutamic acid, tyrosine, aspartic acid, cysteine, as well as alanine were less preferred. In contrast, feeding preference of *T. vaporariorum* towards arginine did not statistically differ from that of other amino acids. However, measured preferences for one of the two choice alternatives within each single test were only significant for lysine, asparagine, threonine, valine, glutamine, leucine, tryptophan, glutamic acid, tyrosine, aspartic acid, cysteine, and alanine.

Table 11: Relative contents of free amino acids in phloem exudates of different host plants

		Amino acid			
		% Ala	% Arg	% Asn	% Gln
Host plant		mean ± s.e.m.	mean ± s.e.m.	mean ± s.e.m.	mean ± s.e.m.
BE		6.52 ± 1.10 a ^{BCDE}	1.77 ± 0.24 a ^{EFG}	19.69 ± 1.49 a ^{A}	10.68 ± 0.79 d ^{B}
CU		5.43 ± 0.42 ab ^{BCD}	1.60 ± 0.50 ab ^{CDE}	2.29 ± 0.47 c ^{CDE}	66.34 ± 2.96 a ^{A}
EG		1.89 ± 0.18 c ^{EFG}	1.32 ± 0.36 abc ^{FG}	7.61 ± 2.18 b ^{BC}	35.07 ± 2.72 c ^{A}
SP		1.48 ± 0.57 c ^{C}	0.06 ± 0.06 c ^{C}	3.24 ± 0.54 bc ^{C}	53.27 ± 5.64 ab ^{A}
TB		1.46 ± 0.31 c ^{E}	0.34 ± 0.34 bc ^{E}	5.92 ± 0.92 bc ^{CDE}	48.37 ± 3.47 bc ^{A}
TO		3.54 ± 0.43 bc ^{D}	0.11 ± 0.11 c ^{D}	5.11 ± 0.54 bc ^{CD}	45.89 ± 2.73 bc ^{A}
	F	16.1072*	11.3566*	23.7954*	105.5034*
	d.f.	5, 18*	5, 18*	5, 19*	5, 17*
	P	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
		% Glu	% Gly	% His	% Ile
		mean ± s.e.m.	mean ± s.e.m.	mean ± s.e.m.	mean ± s.e.m.
BE		24.47 ± 2.15 a ^{A}	1.46 ± 0.16 ab ^{EFG}	0.42 ± 0.18 a ^{FG}	1.81 ± 0.41 b ^{EFG}
CU		7.94 ± 1.64 b ^{B}	2.32 ± 0.49 a ^{CDE}	2.09 ± 0.71 a ^{CDE}	0.74 ± 0.37 b ^{DE}
EG		9.64 ± 1.01 b ^{B}	1.04 ± 0.19 bc ^{FG}	0.29 ± 0.11 a ^{G}	5.92 ± 0.79 a ^{BCDEF}
SP		14.48 ± 2.79 b ^{B}	0.22 ± 0.14 c ^{C}	0.16 ± 0.11 a ^{C}	0.14 ± 0.09 b ^{C}
TB		9.29 ± 1.55 b ^{CD}	0.05 ± 0.05 c ^{E}	0.05 ± 0.05 a ^{E}	0.17 ± 0.17 b ^{E}
TO		14.88 ± 2.37 b ^{B}	0.12 ± 0.12 c ^{D}	0.09 ± 0.09 a ^{D}	0.13 ± 0.13 b ^{D}
	F	9.2902	18.4430*	2.5498*	12.4411*
	d.f.	5, 42	5, 18*	5, 18*	5, 18*
	P	< 0.0001	< 0.0001*	0.0633*	< 0.0001*

Table 11: Continued

Host plant		Amino acid			
		% Leu mean ± s.e.m.	% Phe mean ± s.e.m.	% Pro mean ± s.e.m.	% Ser mean ± s.e.m.
BE		3.38 ± 0.77 b ^{DEFG}	1.80 ± 0.88 b ^{EFG}	7.58 ± 2.47 bc ^{BCD}	9.47 ± 1.06 a ^{BC}
CU		0.67 ± 0.44 c ^{E}	0.04 ± 0.04 b ^{E}	1.56 ± 0.21 c ^{CDE}	5.69 ± 0.98 ab ^{BC}
EG		6.65 ± 0.93 a ^{BCDE}	10.09 ± 0.67 a ^{B}	2.79 ± 0.57 c ^{CDEFG}	7.62 ± 0.38 ab ^{BC}
SP		0.33 ± 0.22 c ^{C}	0.51 ± 0.35 b ^{C}	15.87 ± 2.58 ab ^{B}	3.52 ± 1.75 b ^{C}
TB		0.61 ± 0.61 c ^{E}	0.13 ± 0.13 b ^{E}	19.38 ± 3.54 a ^{B}	10.23 ± 0.90 a ^{C}
TO		0.12 ± 0.12 c ^{D}	0.42 ± 0.42 b ^{D}	12.74 ± 0.70 ab ^{B}	10.58 ± 1.75 a ^{BC}
	F	11.4207*	39.4768*	50.2937*	4.0268*
	d.f.	5, 18*	5, 16*	5, 17*	5, 18*
	P	0.0001*	< 0.0001*	< 0.0001*	0.0122*
		% Thr mean ± s.e.m.	% Trp mean ± s.e.m.	% Tyr mean ± s.e.m.	% Val mean ± s.e.m.
BE		4.49 ± 0.19 a ^{CDEFG}	0.04 ± 0.03 a ^{G}	0.73 ± 0.14 b ^{FG}	5.68 ± 0.90 ab ^{BCDEF}
CU		1.88 ± 0.49 ab ^{CDE}	0.05 ± 0.05 a ^{E}	0.89 ± 0.27 b ^{DE}	0.49 ± 0.49 c ^{E}
EG		0.14 ± 0.11 b ^{G}	0.44 ± 0.14 a ^{G}	2.48 ± 0.37 a ^{DEFG}	7.00 ± 0.67 a ^{BCD}
SP		4.72 ± 1.70 a ^{C}	0.06 ± 0.05 a ^{C}	0.25 ± 0.16 b ^{C}	1.70 ± 1.12 bc ^{C}
TB		2.81 ± 0.67 ab ^{DE}	0.05 ± 0.05 a ^{E}	0.36 ± 0.23 b ^{E}	0.77 ± 0.77 c ^{E}
TO		4.53 ± 1.01 a ^{D}	0.12 ± 0.12 a ^{D}	0.18 ± 0.18 b ^{D}	1.44 ± 1.44 c ^{D}
	F	71.9166*	1.3833*	7.0214*	8.4873
	d.f.	5, 17*	5, 18*	5, 19*	5, 42
	P	< 0.0001*	0.2747*	0.0007*	< 0.0001

One-way ANOVA followed by Tukey-Kramer HSD test at α = 0.05; for comparisons within amino acids F, d.f., and *P* values are given in the columns, for comparisons within host plants: BE = bean, F = 70.9346*, *P* = < 0.0001*, d.f. = 15, 40*; CU = cucumber, F = 46.3037*, *P* = < 0.0001*, d.f. = 15, 41*; EG = eggplant, F = 57.1051*, *P* = < 0.0001*, d.f. = 15, 41*; SP = sweet pepper, F = 11.4761*, *P* = < 0.0001*, d.f. = 15, 41*; TB = tobacco, F = 25.4387*, *P* = < 0.0001*, d.f. = 15, 41*; TO = tomato, F = 45.7755*, *P* = < 0.0001*, d.f. = 15, 42*, n = 8 for each variant

Means with s.e.m. in columns (minuscule letters) and lines (capital letters) followed by the same index letter are not statistically different

* F, d.f., and *P* values were corrected by Welch-ANOVA test because of variance inhomogeneity after Levene's test

Table 12: Free amino acid contents grouped according to their physiological and chemical properties in phloem exudates of different host plants

		Amino acid group			
Host plant		% essential mean ± s.e.m.	% aromatic mean ± s.e.m.	% heterocyclic mean ± s.e.m.	% aliphatic mean ± s.e.m.
BE		19.39 ± 2.46 b ^{BC}	2.57 ± 0.92 b ^{E}	7.58 ± 2.47 bc ^{DE}	20.77 ± 2.85 a ^{BC}
CU		7.54 ± 1.71 c ^{BCD}	0.98 ± 0.30 b ^{E}	1.56 ± 0.21 c ^{DE}	10.71 ± 0.81 b ^{B}
EG		31.86 ± 3.11 a ^{B}	13.02 ± 0.89 a ^{CD}	2.79 ± 0.57 c ^{E}	18.29 ± 1.90 ab ^{C}
SP		7.68 ± 2.61 c ^{BC}	0.81 ± 0.45 b ^{C}	15.87 ± 2.58 ab ^{B}	18.04 ± 2.58 ab ^{B}
TB		4.94 ± 1.49 c ^{DE}	0.54 ± 0.26 b ^{E}	19.38 ± 3.54 a ^{BC}	21.67 ± 3.27 a ^{B}
TO		6.96 ± 2.50 c ^{CD}	0.71 ± 0.71 b ^{D}	12.74 ± 0.70 ab ^{BC}	16.65 ± 0.80 ab ^{B}
	F	19.6609	32.3896*	50.2937*	2.9863
	d.f.	5, 42	5, 18*	5, 17*	5, 45
	P	< 0.0001	< 0.0001*	< 0.0001*	0.0215
		% amidated mean ± s.e.m.	% hydroxylated mean ± s.e.m.	% basic mean ± s.e.m.	% acidic mean ± s.e.m.
BE		30.37 ± 2.00 c ^{A}	13.96 ± 1.16 ab ^{CD}	2.18 ± 2.0.22 ab ^{E}	24.47 ± 2.15 a ^{AB}
CU		68.63 ± 2.60 a ^{A}	7.57 ± 0.96 b ^{BCD}	3.69 ± 0.89 a ^{CDE}	7.94 ± 1.64 b ^{BC}
EG		42.68 ± 3.98 bc ^{A}	7.76 ± 0.41 b ^{DE}	1.61 ± 0.40 bc ^{E}	9.64 ± 1.01 b ^{CDE}
SP		56.51 ± 5.53 ab ^{A}	8.24 ± 2.70 ab ^{BC}	0.22 ± 0.19 c ^{C}	14.48 ± 2.79 b ^{B}
TB		54.29 ± 3.59 ab ^{A}	13.04 ± 1.00 ab ^{BCD}	0.40 ± 0.34 bc ^{E}	9.29 ± 1.55 b ^{CDE}
TO		51.00 ± 43.84 b ^{A}	15.11 ± 2.61 a ^{BC}	0.20 ± 0.20 c ^{D}	14.88 ± 2.37 b ^{BC}
	F	26.1278*	8.9703*	13.7773*	9.2902
	d.f.	5, 19*	5, 18*	5, 19*	5, 42
	P	< 0.0001*	0.0002*	< 0.0001*	< 0.0001

One-way ANOVA followed by Tukey-Kramer HSD test at $\alpha = 0.05$; for comparisons within amino acid groups F, d.f., and *P* values are given in the columns; for comparisons within host plants: BE = bean, F = 57.3424*, *P* = < 0.0001*, d.f. = 7, 21*; CU = cucumber, F = 102.8988*, *P* = < 0.0001*, d.f. = 7, 22*; EG = eggplant, F = 53.5138*, *P* = < 0.0001*, d.f. = 7, 23*; SP = sweet pepper, F = 27.5766*, *P* = < 0.0001*, d.f. = 7, 21*; TB = tobacco, F = 56.8026*, *P* = < 0.0001*, d.f. = 7, 22*; TO = tomato, F = 117.8637*, *P* = < 0.0001*, d.f. = 7, 22*, n = 8 for each variant
Means with s.e.m. in columns (minuscule letters) and lines (capital letters) followed by the same index letter are not statistically different
* F, d.f., and *P* values were corrected by Welch-ANOVA test because of variance inhomogeneity after Levene's test

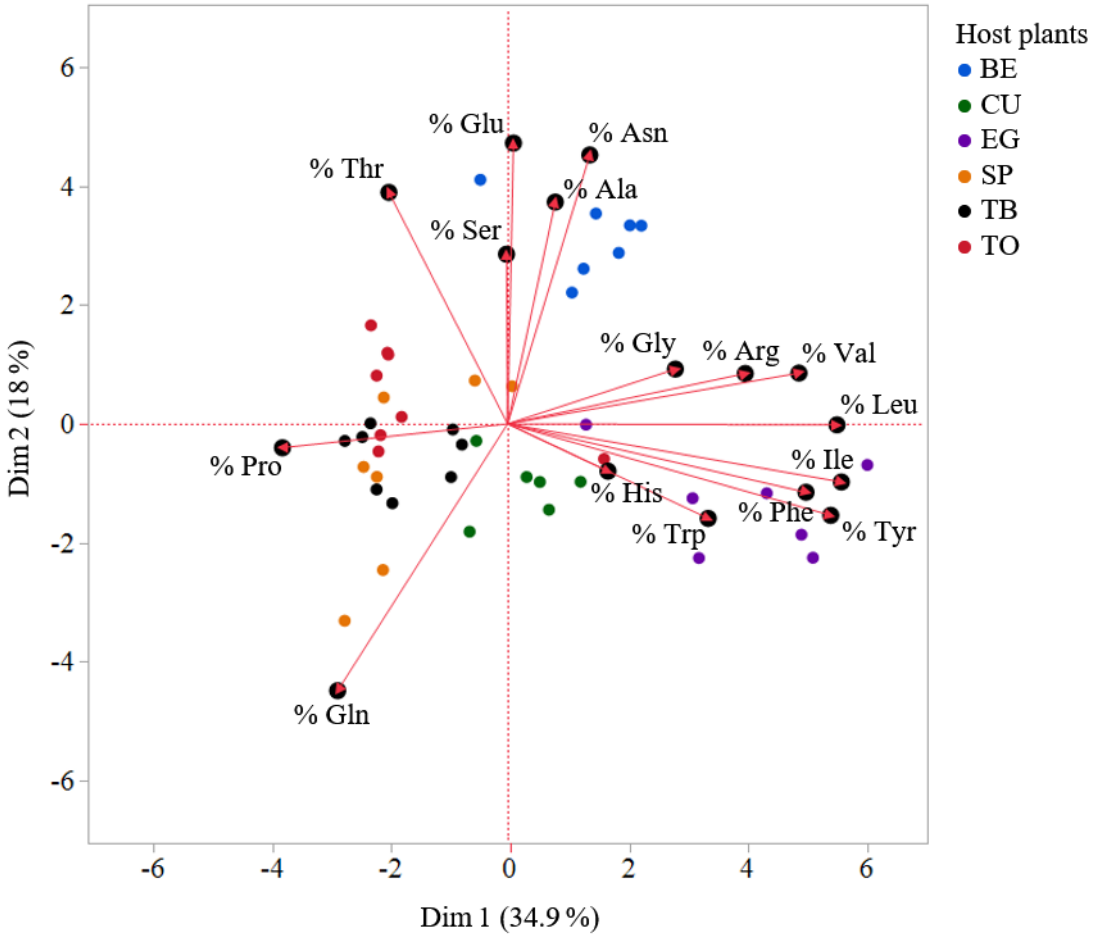

Figure 12: Biplot of relationships between relative proportions of amino acids in the phloem sap of host plants with varying attractiveness towards *T. vaporariorum*
(EG = eggplant, TB = tobacco, TO = tomato, CU = cucumber, BE = bean, SP = sweet pepper)
Principal components analysis of general patterns of trait covariation

Table 13: Results of stepwise multiple regressions correlating relative proportions of single amino acids in the phloem sap of different host plant species with the mean preference index of these host plants towards *T. vaporariorum*

Dependent variable	Estimator	t	d.f.	F	*P*
% Glu	-0.006136291	-2.14	1, 43	4.5670	0.0383
% Phe	0.0233520225	3.88	1, 43	15.0640	0.0004
% Ser	0.0239485327	4.69	1, 43	22.0010	< 0.0001
% Thr	-0.023027387	-2.72	1, 43	7.4040	0.0094

Stepwise multiple regression model, $r^2 = 0.61$, F = 16.5773, *P* = < 0.0001, d.f. = 4, 43
PI = 0.4163962814 + (-0.006136291Glu) + (0.0233520225Phe) + (0.0239485327Ser) + (-0.023027387Thr)
AICc = -40.357, RMSE = 0.1451

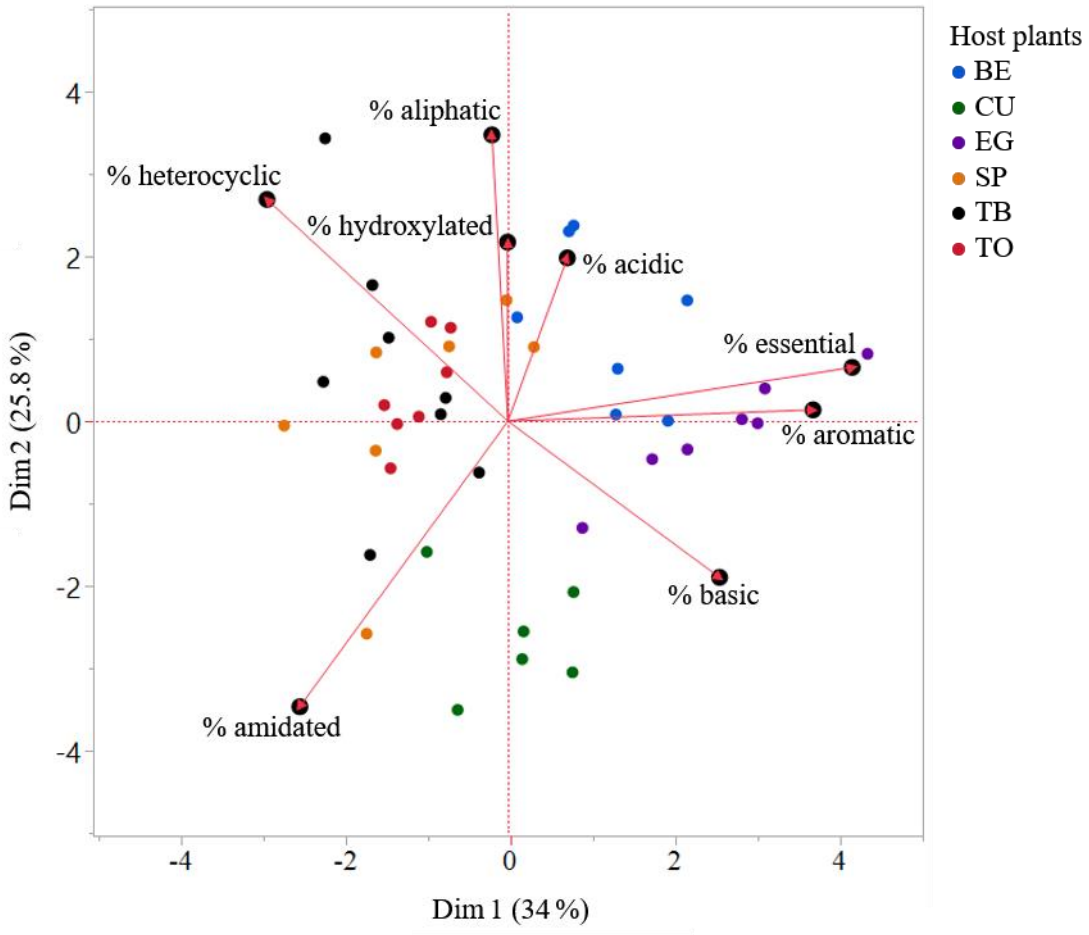

Figure 13: Biplot of relationships between relative proportions of amino acid groups in the phloem sap of host plants with varying attractiveness towards *T. vaporariorum*
(EG = eggplant, TB = tobacco, TO = tomato, CU = cucumber, BE = bean, SP = sweet pepper)
Principal components analysis of general patterns of trait covariation

Table 14: Results of stepwise multiple regressions correlating relative proportions of single amino acid groups in the phloem sap of different host plant species with the mean preference index of these host plants towards *T. vaporariorum*

Dependent variable	Estimator	t	d.f.	F	*P*
a = % Essential	-0.011058134	-2.20	1, 44	4.5670	0.0334
b = % Aromatic	0.0502404368	4.27	1, 44	15.0640	0.0001
c = % Hydroxylated	0.012423429	2.53	1, 44	22.0010	0.0149

Stepwise multiple regression model, $r^2 = 0.44$, F = 11.7333, *P* = < 0.0001, d.f. = 3, 44
PI = 0.3195285654 + (-0.011058134a) + 0.0502404368b + 0.012423429c
AICc = -26.4079, RMSE = 0.1704

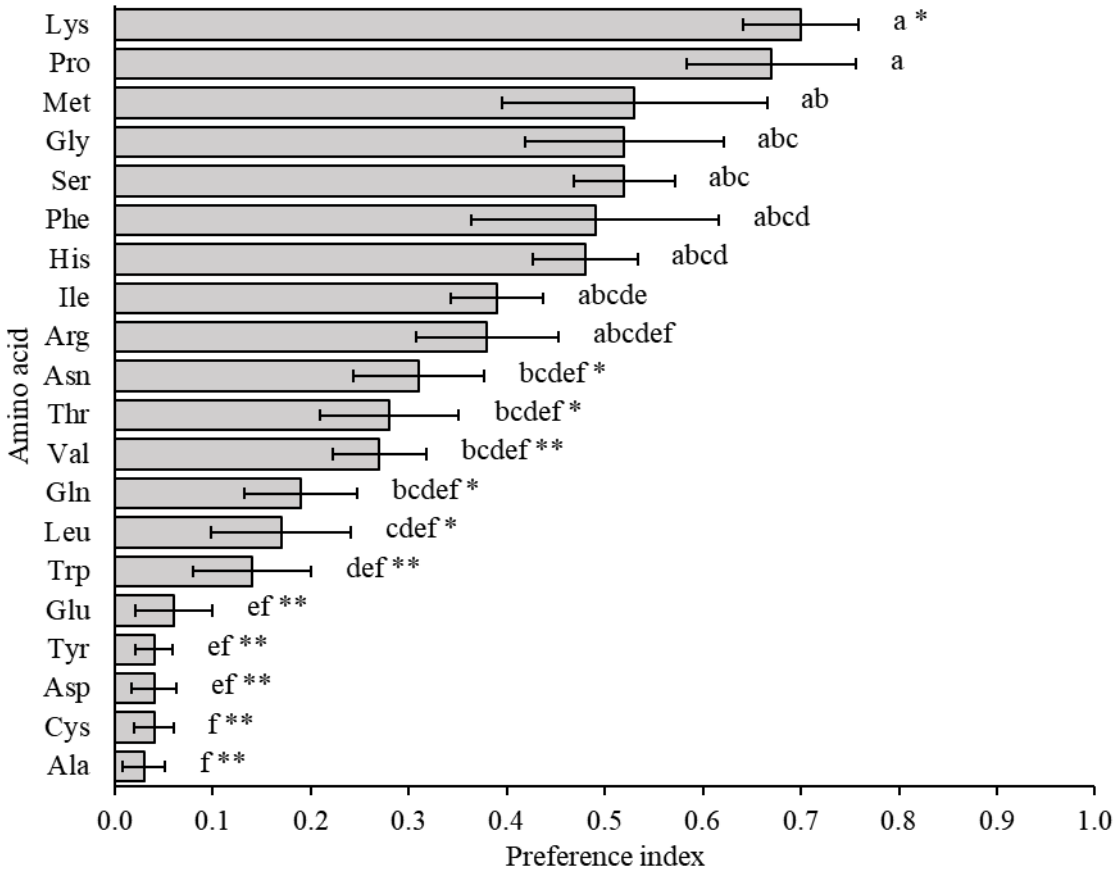

Figure 14: Preference indices (mean ± s.e.m.) of *T. vaporariorum* adults choosing between sucrose solutions with added single amino acids against a control sucrose solution

One-way ANOVA followed by Tukey-Kramer HSD test at $\alpha = 0.05$, means with s.e.m. in a column followed by the same index letter are not statistically different ($n = 8$ for each amino acid; $F = 17.4465$*; d.f.* = 19, 50; *P** = < 0.0001; F, d.f., and *P* values were corrected by Welch-ANOVA test because of variance inhomogeneity after Levene's test)

Wilcoxon signed-rank test for each amino acid ($H_0 = 0.5$ two-sided, $\alpha = 0.05$, $n = 8$ for each combination, *P < 0.05, **P < 0.01, ***P < 0.001)

5.4 Discussion

Overall, amino acid composition could explain the observed host preferences to a large extend and, therefore, proved to be an important factor in the host selection of *T. vaporariorum*. According to the principal component analysis of the general patterns of trait covariation, scatter plots indicate clustering of individual host plant variables, suggesting the presence of effects that determine the host plant attractiveness of *T. vaporariorum*.

Especially glutamine but also asparagine and glutamic acid dominated the amino acid spectra of the phloem sap samples collected from various vegetable crops in this study, which has already been demonstrated for *Avena sativa* (L.), *Cucumis melo* (L.), *Hordeum vulgare* (L.), *Lycopersicon esculentum* (L.) and *Solanum tuberosum* (L.) (WEIBULL ET AL. 1990; MITCHELL ET AL. 1992; VALLE ET AL. 1998; KARLEY ET AL. 2002; DINANT ET AL. 2010). Since these amino acids are involved in central functions in the plant metabolism, they are constantly synthesized in high concentrations (NOVAK 2008). Within the plant, aspartic acid and glutamic acid are converted by the uptake of a second amino group into glutamine and asparagine (HELDT ET AL. 2011). The resulting amidated amino acids act as storage and transport molecules of nitrogen and can be translocated by both the xylem as well as the phloem of the plant (MOHR AND SCHOPFER 1992; NOVAK 2008). Although amidated amino acids had the highest proportion among all amino acid groups in the phloem sap of vegetable crops in this study, they did not explain host plant preferences of *T. vaporariorum* neither by principal component analysis of general patterns of trait covariation nor by stepwise multiple regressions. However, the multiple regressions of individual amino acids revealed that high levels of glutamic acid in the phloem sap of plants negatively affected host plant attractiveness. Glutamic acid was also found to reduce whitefly performance, as survival and oviposition of *B. tabaci*, feeding on artificial diets containing high concentrations of glutamic acid, were reduced (THOMPSON 2006; THOMPSON 2011). Furthermore, glutamic acid concentrations in the phloem sap of resistant barley cultivars were higher compared to susceptible varieties towards the bird cherry-oat aphid *Rhopalosiphum padi* (L.) (WEIBULL 1988).

According to the principal component analysis of general patterns of trait covariation and the multiple regressions of single amino acids, host plant preference of *T. vaporariorum* was clearly determined by arginine, histidine, isoleucine, leucine, phenylalanine, threonine, tryptophan, and valine proportions in the phloem sap of host plants. These are essential amino acids in insect nutrition (DADD 1973; BRODBECK AND STRONG 1987). While phenylalanine had a positive effect, threonine was found to negatively affect host plant preference in multiple regressions of single amino acids. On sucrose solution with added phenylalanine, *B. tabaci* had a better survival (THOMPSON 2006; THOMPSON 2011). However, no negative effects of threonine associated with whitefly performance could be determined. Additionally, essential amino acids were found to negatively affect the host preference of *T. vaporariorum* in multiple regressions. The importance of essential and non-essential amino acids for insect feeding and growth has been reported by several researchers for decades (DADD AND KRIEGER 1968; VAN EMDEN AND BASHFORD 1971; BRODBECK AND STRONG 1987; DOUGLAS 1993; DOUGLAS 2003; DOUGLAS 2006). On diets containing predominantly non-essential amino acids, *B. tabaci* and several aphid species were found to perform well (DOUGLAS 2003; THOMPSON 2011). While it was shown for the aphid-bacteria symbiosis that only a few amino acids are essential, for aphids deprived of their symbionts, the ten essential amino acids are indeed required

for aphid growth (MITTLER 1971). When the black bean aphid *Aphis fabae* (Scop.) fed on artificial diets with deleted amino acids histidine, methionine, threonine, and valine, aphid performance was depressed (WILKINSON AND DOUGLAS 2003). Deletion of one of the other essential amino acids had no negative effect, and interclonal variation in the dietary requirements of aphids occurred. Endosymbionts are thus able to compensate for nutritional imbalances by synthesizing essential amino acids and making them available to their host (BAUMANN ET AL. 2006). However, the prevalence and diversity of endosymbionts differ in whiteflies depending on the whitefly species and its host association (MARUBAYASHI ET AL. 2014; GÓMEZ-DÍAZ ET AL. 2019). Besides the primary endosymbiont *Candidatus* Portiera aleyrodidarum, which is present in all whitefly species, whiteflies also harbour a variable number of secondary endosymbionts of several genera including *Arsenophonus*, *Cardinium*, *Fritchea*, *Hamiltonella*, *Hemipteriphilus*, *Rickettsia* and *Wolbachia* (ANDREASON ET AL. 2020). Furthermore, in endosymbioses with multiple partners, the synthesis of essential amino acids is often divided between the different endosymbionts, meaning that each endosymbiont mediates the biosynthesis of a subset of essential amino acids or a subset of the reactions in the biosynthetic pathway of a single essential amino acid (DOUGLAS 2016). Consequently, the effect of individual essential amino acids on host plant selection of whiteflies might strongly depend on their association with endosymbionts.

Aromatic amino acids in the phloem sap of plants strongly correlated with the host preference of adult *T. vaporariorum* according to the principal component analysis of general patterns of trait covariation. In the stepwise multiple regressions, aromatic amino acids were shown to have a positive effect on host plant attractiveness. Phenylalanine, tryptophan and tyrosine have already been related to the nutritional requirements of insects associated with cuticle synthesis (DENNEL 1958a; DENNELL 1958b; BRUNET 1980; BERNAYS AND WOODHEAD 1984). Damage by larvae of the western flower thrips *Frankliniella occidentalis* (Perg.) towards four vegetable crops was also shown to positively correlate with high aromatic amino acid concentrations in plant leaves (MOLLEMA AND COLE 1996). In this study, aromatic amino acids could be preferred by adult *T. vaporariorum* to meet nutritional requirements necessary for whitefly development. However, the requirements of aromatic amino acids due to cuticle formation should be obsolete in adult whiteflies. Accordingly, diets with high contents of phenylalanine were preferred by nymphs of the large-headed grasshopper *Phoetaliotes nebrascensis* (Thomas) but not by adults (BEHMER AND JOERN 1993). Nevertheless, high levels of aromatic amino acids in the phloem sap of a potential host plant might still represent a positive cue in the host selection of whiteflies for the provision of the best nutritional prerequisites for the offspring development.

In addition, serine was positively correlated with host plant preference of *T. vaporariorum* according to multiple regression analysis of single amino acids found in the phloem of host plants. Serine is formed as an intermediate product of the photorespiration in plants and represents a

considerable proportion of the amino acids supplied by the mesophyll cells (HELDT ET AL. 2011; ROS ET AL. 2014). Therefore, serine is usually present in relatively high proportions in the phloem sap of plants, which could be confirmed for the vegetable crops of this study. Serine also positively affected survival and oviposition of *B. tabaci* feeding on sucrose solutions with added amino acids (THOMPSON 2006; THOMPSON 2011). Additionally, the importance of serine in the growth of the green peach aphid *Myzus persicae* (Sulzer) was reported by DADD AND KRIEGER (1968). Serine and threonine together represent the group of hydroxylated amino acids that positively affected host plant preference of *T. vaporariorum* in stepwise multiple regressions. Hydroxylated amino acids were found to consistently evoke strong arrestant responses in larvae of the southwestern corn borer *Diatraea grandiosella* (Dyar) (HEDIN ET AL. 1993).

Results of the feeding preference experiments proved that *T. vaporariorum* could differentiate between individual amino acids by their gustatory properties. Along with other nutrient compounds, some amino acids serve as phagostimulants for different insect species including orthopterans, hemipterans, coleopterans and lepidopterans (MITTLER 1967; SRIVASTAVA ET AL. 1983; BERNAYS AND CHAPMAN 1994). In this study, lysine was the only amino acid enhancing the acceptance of sucrose media. In contrast, alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, leucine, threonine, tryptophan, tyrosine and valine deterred adult *T. vaporariorum*. Asparagine, aspartic acid, cysteine, glutamic acid and tyrosine deterred the pea aphid *Acyrthosiphon pisum* (Harris) feeding on artificial diets (SRIVASTAVA ET AL. 1983). Glutamic acid also decreased the acceptability of sucrose towards *M. persicae* (MITTLER 1967). Overall, the effects of single amino acids only partially agreed between the results of the stepwise multiple regressions and the feeding preference experiments. This could be explained as phloem sap contains numerous compounds, including sugars and proteins, which additionally determine its gustatory properties (KEHR 2006; ANSTEAD ET AL. 2013). Nonprotein amino acids such as γ-aminobutyric acid (GABA) are present in the phloem as well and may act as feeding stimulants or deterrents in aphids (Srivastava et al. 1983; Montllor 1991; DIXON 1998). Although studies indicate similar effects of GABA in whiteflies, relative proportions of GABA in the phloem sap were not determined in this study (BLACKMER AND BYRNE 1999). On the other hand, consideration should be given to the concentration of amino acids, as different concentrations of amino acids have varying effects on whitefly performance (THOMPSON 2006; THOMPSON 2011). A typical example is the ratio of carbohydrates to amino acids, which was discussed to be a factor influencing the feeding behaviour of whiteflies (BLACKMER AND BYRNE 1999). Therefore, the resultant effects of individual amino acids in natural hosts might be, as opposed to those *in vitro*, rather associated with the presence and the concentration of other plant compounds. Moreover, the results indicate that a dominant presence of amino acids with strong gustatory effects might influence phloem sap uptake, thus contributing to host plant resistance towards *T. vaporariorum*.

6 General discussion

6.1 Host plant adaption affects host plant selection strategies in whiteflies

As whiteflies are a group of insects with a distinct inherent ecological potency, numerous crops and ornamental plants are part of their host plant range (DOWELL AND STEINBERG 1979; BYRNE AND BELLOWS 1991; BÄHRMANN 2002). Two whitefly species, *T. vaporariorum* and *B. tabaci*, are also characterized by an extreme degree of polyphagy and have caused severe damage to important crops by significant pest outbreaks in the past (MOHAN ET AL. 1988; LOURENÇÃO ET AL. 2008; NARANJO ET AL. 2010; NASRUDDIN AND MOUND 2016). In contrast, *A. proletella* has mainly adapted to cruciferous crops as their main host plants (BÄHRMANN 2002). Both, climate change and global trade, contribute to the spread of whiteflies and the phytopathogenic viruses they transmit, aggravating the global crop health status (WARD AND MASTERS 2007; CANTO ET AL. 2009). Furthermore, rising ambient temperatures and carbon dioxide concentrations in the atmosphere may lead to an alteration of life cycles, reproductive patterns, as well as trophic interactions between plants, whiteflies and their antagonists (BEZEMER AND JONES 1998; CURNUTTE ET AL. 2014; AREGBESOLA ET AL. 2019).

To understand the host plant range of herbivorous species, the degree of specialization—which is usually classified in monophagous, oligophagous and polyphagous according to the number of plant families that are infested—is of considerable importance (SCHOONHOVEN ET AL. 2005). Host plant specialization in insects represents the rule. With only less than 10 % of all herbivorous insect species feeding within a host plant range consisting of three plant families by maximum, specialists (monophagous and oligophagous species) and generalists (polyphagous species) are both awarded several advantages (BERNAYS AND GRAHAM 1988; BERNAYS AND CHAPMAN 1994; SCHOONHOVEN ET AL. 2005; OVČARENKO ET AL. 2016). It has been suggested that each host feeding strategy involves specific adaptions and that specialisation in particular is a "trade-off" (DETHIER 1954). For example, specialization allows an herbivorous pest insect to adapt in extreme to one plant species, while it is not or less adapted to others (BERNAYS AND GRAHAM 1988). Although the high percentage of specialist herbivores indicates that specialists have been more successful in the past, climate and habitat change are predicted to endanger especially specialist insect species with narrow diet widths (BERNAYS AND GRAHAM 1988; WARREN ET AL. 2001; KOTIAHO ET AL. 2005; MATTILA ET AL. 2011).

Generalists are characterized by a large food spectrum and have a higher probability to become pests, as they can adapt to different habitat types and cropping systems. Therefore, polyphagous herbivores which can choose from various plant species are more likely to find a suitable host. In contrast, oligo- or even monophagous herbivores are restricted to a few plant species (WARD AND

MASTERS 2007). Often, generalists are also euryoecious and can easily adapt to global change (BYRNE AND BELLOWS 1991; KENNEDY AND STORER 2000; VÁZQUEZ 2006; CLAVEL ET AL. 2011). Considering climate and habitat changes, generalists are more likely to persist by expanding their geographic range and crowding out specialists (MCKINNEY AND LOCKWOOD 1999; ANDREW AND HUGHES 2004; WARD AND MASTERS 2007).

As generalist species have greater availability of resources and can use food mixtures from different plant species, they can benefit from the option to improve their food quality by increasing their nutrient balance or by avoidance of allelochemicals (BERNAYS ET AL. 1994; BERNAYS AND MINKENBERG 1997). However, generalists must have the flexibility to react to changing environmental influences such as increasing temperatures to ensure resource acquisition (BERNAYS AND WCISLO 1994). Since generalists are affected by the chemistry and morphology of their host plants, they reveal different performance on different host species (VIA 1990). In addition, generalist insects may be less capable in dealing with reduced nitrogen conditions and increased concentrations of phenolic compounds, as it is predicted to occur under CO_2 enrichment (BEZEMER AND JONES 1998; WARD AND MASTERS 2007). Specialists, on the other hand, can cope better with variations in nutrient balance within their host plant and make faster decisions in the host plant selection process (WARD AND MASTERS 2007; OVČARENKO ET AL. 2016). As a result, specialists are more effective in host finding, recognition and discrimination (BERNAYS AND WCISLO 1994; BERNAYS 2001). Moreover, it was often hypothesized that specialists can tolerate or even resist host plant defence by specific circumvention mechanisms (ALI AND AGRAWAL 2012). The general assumption is, therefore, that the strong evolutionary adaptation of a specialist allows better exploitation of its host, whereas a generalist can find access to a broader host spectrum to take advantage of diversity.

Another aspect affecting the host selection strategy in herbivorous insects is their previous host experience. Preconditioning by long-term experience on one host plant species can lead to changes in host preference as well as pest performance, as was previously shown for the extreme generalist species *B. tabaci* and *T. vaporariorum* (PAPAJ AND PROKOPY 1989; BYRNE AND BELLOWS 1991; BERNAYS AND MINKENBERG 1997; LEI ET AL. 1998; LEE ET AL. 2010; HU ET AL. 2011; OVČARENKO ET AL. 2016). While conducting experiments on host selection and performance in this study, different host plants were used in the experiments than the ones used for maintenance breeding. In this way, preconditioning effects could be excluded.

Herbivorous insects generally differ greatly in their adaption to host plants amongst insect orders, families and species, which is expressed, *inter alia*, in a species-specific process of host plant selection (BERNAYS AND CHAPMAN 1994; SCHOONHOVEN ET AL. 2005). Ultimately, this applies to whiteflies as well, but only limited research was carried out to compare the host selection behaviour of several whitefly species to understand the underlying mechanisms in whitefly-host adaptions. To

this date, studies mainly concentrated on comparisons between different biotypes of *B. tabaci* (JIANG ET AL. 1999; JIAO ET AL. 2014; MILENOVIC ET AL. 2019). In this study, the host selection strategy of whiteflies was found to differ between species and host plant preference was based on multiple plant characteristics. It is assumed that these differences in strategy are the result of an evolutionary adaptation between whiteflies and their host ranges, which have formed individually according to the ecological niche they have occupied. The more specialized species, *A. proletella*, has mainly adapted to cruciferous plants and makes efficient decisions within the host selection process by evaluating epicuticular leaf waxes on the leaf surface of potential host plants. In contrast, the extreme generalists *B. tabaci* and *T. vaporariorum* can use a broad host plant spectrum. A key factor mediating decisions on host choice preferences in these species is the nutritive quality of the phloem sap. Therefore, the generalist approach is more laborious and time-consuming since numerous probing attempts are necessary before the phloem is successfully reached (LEI ET AL. 1998). The "neural hypothesis of diet width" suggests that decision making is more efficient when based on simple or exaggerated cues instead of making choices among numerous complex sensory inputs (BERNAYS 1998). Therefore, a specialist can recognize its hosts by more specific plant factors, whereas a generalist species would need to be capable of processing much more information to recognize its host plant species in a similar way (BERNAYS AND FUNK 1999; BERNAYS 2001). Additionally, long decision times may involve an ecological risk, which is significantly reduced in specialists (BERNAYS 1998). Since the nervous system of insects is simple, generalists need to focus on more general characteristics—such as nutritional cues—to be able to select between potential hosts. According to this "neural-constraints hypothesis", the observed differences in the host selection behaviour between three whitefly species in the study can be explained (DETHIER 1954; KENNEDY AND BOOTH 1954; BERNAYS AND FUNK 1999; BERNAYS 2001).

Another general approach, the "mother-knows-best-principle" also known as the "preference-performance hypothesis", proposes that female herbivorous insects prefer to oviposit on host plants that provide the best prerequisites for their offspring (LEVINS AND MACARTHUR 1969; JAENIKE 1978; THOMPSON 1988; MAYHEW 1997). In whiteflies, feeding and oviposition are performed by females on the same leaves and are even done simultaneously (VAN LENTEREN AND NOLDUS 1990; BÄHRMANN 2002). Therefore, host plant selection is particularly important, and the "mother-knows-best-principle" can be applied to whiteflies as well. According to a study of GRIPENBERG ET AL. (2010), host choice was affected by diet width and, therefore, the preference for "high-quality hosts" was stronger in oligophagous insects than in polyphagous insects. In other studies, distinct host plant preferences were found for whiteflies and demonstrated that host attractiveness is often linked with host suitability (VERSCHOOR-VAN DER POEL AND VAN LENTEREN 1978; VAN LENTEREN AND NOLDUS 1990; ASKOUL ET AL. 2019). As a result, host preference of *A. proletella* might correlate stronger with its performance compared to *B. tabaci* and *T. vaporariorum*.

6.2 Epicuticular leaf waxes affecting host plant preference in whiteflies

The external surface of leaf epidermal cells is coated by the multi-layered plant cuticle, which fulfils numerous functions crucial for plant life (KERSTIENS 1996; DOMÍNGUEZ ET AL. 2011). In chemical ecology, it is important to examine the interface between plants and their environment to gain a better understanding of the co-evolution between plants and herbivorous insects (MÜLLER AND RIEDERER 2005). At the aerial surface of the plant cuticle are the epicuticular surface waxes, which vary significantly in composition between plant species, genotype and plant parts (EGLINTON AND HAMILTON 1967; EIGENBRODE AND ESPELIE 1995; BARTHLOTT ET AL. 1998; DHANYALAKSHMI ET AL. 2019). Additionally, the composition of epicuticular waxes differs between leaf age and leaf side, and is influenced by environmental conditions as well as agricultural chemicals (BAKER 1974; HOLLOWAY ET AL. 1977; EIGENBRODE AND SHELTON 1992; BERNAYS AND CHAPMAN 1994; KANNO AND HARRIS 2000a; KANNO AND HARRIS 2000b; MÜLLER AND HILKER 2001; KOCH ET AL. 2006). As epicuticular surface waxes often represent the first physical contact between an herbivorous insect and its host plant, numerous interactions between plants and insects are known to be mediated by epicuticular waxes (EIGENBRODE AND ESPELIE 1995; KERSTIENS 1996).

Certain properties of epicuticular surface waxes can affect the behaviour of herbivorous insects positively or negatively. In many cases, the absence or a reduced quantity of epicuticular waxes led either to an increased susceptibility of the host plant or to a reduced infestation density of aphids, *Eurydema* spp., *Phyllotreta* spp., *Artogeia rapae* (L.), *Mamestra brassicae* (L.) and *Tetranychus ludeni* (Zacher) (WAY AND MURDIE 1965; TSUMUKI ET AL. 1989; STONER 1990; BODNARYK 1992b; BOHINC ET AL. 2014; CASTRO ET AL. 2019). In addition, epicuticular waxes may affect oviposition. While most insect herbivores lay more eggs on plants without epicuticular waxes, others show decreased oviposition rates (PROKOPY ET AL. 1983; UEMATSU AND SAKANOSHITA 1989; STONER 1990; SILVA ET AL. 2017; RID ET AL. 2018).

As has already been shown in scanning electron microscopy, the epicuticular wax layer often consists of epicuticular wax crystals exhibiting great micromorphological diversity such as films, layers and crusts, granules, platelets, plates, rodlets, threads, tubules and transitional crystalloid forms (BARTHLOTT ET AL. 1998). The varying chemical composition of epicuticular lipids affects the appearance and, consequently, determine the visual properties of these wax crystals (PROKOPY ET AL. 1983; HOLMES AND KEILLER 2002; OLASCOAGA ET AL. 2014). In addition to the yellow and green portions of the reflected light spectrum, heavy wax blooms such as those from *Brassica* cultivars increase reflectivity in other wavelengths, and let the plants appear whiter (PROKOPY ET AL. 1983). A changed epicuticular microstructure or variations in epicuticular wax chemical composition may, therefore, lead to a changed spectral reflectance and can affect insect behaviour on different behavioural steps of host plant acquisition (EIGENBRODE AND ESPELIE 1995).

Once an herbivorous insect has direct contact with a potential host, epicuticular surface waxes might determine host susceptibility as they interfere with insect attachment (EIGENBRODE AND ESPELIE 1995). Coleoptera and larva of the lacewing *Chrysoperla carnea* (Steph.) showed mobility on plant surfaces with epicuticular wax crystals due to reduction of the contact area, epicuticular wax dissolving, and/or fluid absorption caused by these epicuticular waxes (STORK 1980; BODNARYK 1992a; BODNARYK 1992b; EIGENBRODE ET AL. 1996; GORB AND GORB 2002; GORB AND GORB 2006; GORB ET AL. 2008; GORB AND GORB 2017; GORB ET AL. 2017; VOIGT ET AL. 2018).

Besides the physical structure of epicuticular surface waxes, the chemical composition mediates host selection of herbivorous insects as well (EIGENBRODE AND ESPELIE 1995). Plant cuticular waxes are often characteristic blends of aliphatic components including *n*-alkanes, wax esters, aldehydes, ketones, secondary alcohols, β-diketones, fatty alcohols, and triterpenoids (BERNAYS AND CHAPMAN 1994; YEATS AND ROSE 2013). Usually, each compound class consisting of a homologous series of isomers is dominated by a main component (MÜLLER AND RIEDERER 2005). In experiments with neonate diamondback moth *Plutella xylostella* (L.) individuals, cabbage leaves with a reduced amount of epicuticular waxes were less attractive than leaves with high amounts of leaf waxes (EIGENBRODE ET AL. 1991; EIGENBRODE AND PILLAI 1998). The same non-preference behaviour occurred using only leaf wax extracts on glass surfaces. This led to the assumption that epicuticular wax composition was responsible for the observed resistance, as epicuticular lipids were assumed to have a different crystalline microstructure after evaporation of the organic solvent used for extraction. However, recent studies could prove that epicuticular waxes can recrystallise from chloroform extracts on artificial surfaces under *in vitro* conditions with a similar micromorphology as waxes on the intact leaves (MEUSEL ET AL. 1999; MEUSEL ET AL. 2000; KOCH AND ENSIKAT 2008; GANEVA ET AL. 2015). In other studies, high levels of *n*-alkanes, fatty alcohols and triterpenoids were correlated with insect resistance in several cultivated plants (EIGENBRODE AND ESPELIE 1995). Accordingly, movement of fall armyworm larvae *Spodoptera frugiperda* (J.E. Smith) was triggered on corn leaves with leaf surface waxes containing high proportions of *n*-alkanes (YANG ET AL. 1993b; YANG ET AL. 1993c). The fatty alcohol docosanol (C_{22}) in surface waxes of tobacco leaves was associated with resistance against the tobacco budworm *Heliothis virescens* (Fab.), whereas triacontanol (C_{30}) in epicuticular waxes of alfalfa could be correlated with resistance against the spotted alfalfa aphid *Therioaphis maculata* (Buck.) (JOHNSON AND SEVERSON 1984; BERGMAN ET AL. 1991). High levels of α- and β-amyrin triterpenols were attributed to confer resistance of azalea against the azalea lace bug *Stephanitis pyrioides* (Scott) as well as of raspberry against the raspberry aphid *Amphorophora idaei* (Börner) (ROBERTSON ET AL. 1991; BALSDON ET AL. 1995). However, *n*-alkanes, fatty acids and triterpenols were also found to be present in higher levels in epicuticular waxes of susceptible hosts to the several Lepidoptera species and thrips (YANG ET AL. 1993a; UDAYAGIRI AND MASON 1997; LI AND ISHIKAWA 2006; KARMAKAR ET AL. 2016; RID ET AL. 2018).

The effects of epicuticular surface lipids on insect behaviour are quite diverse. Some components of surface waxes developed as stimulants or deterrents during co-evolution and are, therefore, used by insects as cues for host plant recognition (BENNETT AND WALLSGROVE 1994; EIGENBRODE AND ESPELIE 1995; KERSTIENS 1996). Insect feeding was stimulated or deterred by *n*-alkanes, fatty alcohols, fatty acids, and triterpenoids of Orthoptera, Lepidoptera, Coleoptera, as well as the green peach aphid *Myzus persicae* (Sulzer) (BERNAYS ET AL. 1976; MORI 1982; MCKIBBEN ET AL. 1985; MALONEY ET AL. 1988; BRAKER AND CHAZDON 1993; EIGENBRODE AND ESPELIE 1995). Furthermore, *n*-alkanes, fatty acids, and the triterpenoid oleanolic acid were found to stimulate oviposition in Lepidoptera (UDAYAGIRI AND MASON 1997; LI AND ISHIKAWA 2006; RID ET AL. 2018). Moreover, epicuticular lipid compositions may have toxic effects on herbivorous insects, as growth of Lepidoptera was better on diets lacking epicuticular waxes and mortality of the grain aphid *Sitobion avenae* (Fab.) was higher on diets with epicuticular surface waxes (YANG ET AL. 1991; YANG ET AL. 1992; WÓJCICKA 2016). Additionally, life parameters of Lepidoptera as well as the greenbug aphid *Schizaphis graminum* (Rond.) were found to be negatively affected by triterpenoids (SHANKARANARAYANA ET AL. 1980; VARANDA ET AL. 1992). Nevertheless, evidence on toxic effects is inconclusive, as postingestive activities were not clearly distinguished from actual deterrence (EIGENBRODE AND ESPELIE 1995).

Aside from the various components of epicuticular surface waxes, a range of other polar components including amino acids, nonprotein amino acids, sugars, sucrose and glucose esters, sesquiterpenes, diterpenes, phenolics, phenolic glycosides and glucosinolates located at the plant surface can be involved in host recognition or herbivore deterrence (EIGENBRODE AND ESPELIE 1995; KERSTIENS 1996). Some of these components are exuded by glandular trichomes or are internal components that reach the leaf surface by diffusion across the cuticle (DERRIDJ ET AL. 1996; MALUF ET AL. 2001; MÜLLER AND RIEDERER 2005). On one side, epicuticular surface lipids may influence the perception of polar components by herbivorous insects and, on the other side, epicuticular waxes and polar components may together compose a chemical signature used by herbivores for host plant recognition (EIGENBRODE AND ESPELIE 1995). Therefore, *n*-propyl disulfide was more effective as an oviposition stimulant towards the onion fly *Delia antiqua* (Meigen) on model plants in combination with paraffin than without (HARRIS ET AL. 1987). In another example, sinigrin in combination with paraffin or an *n*-alkane mixture increased oviposition by *P. xylostella* even more, than sinigrin alone (SPENCER 1996).

Additionally, indirect effects of epicuticular surface waxes can affect herbivory performance, as the accessibility of certain components beneath or within surface lipids may vary depending on the amount of wax (EIGENBRODE AND ESPELIE 1995). For example, plants with reduced epicuticular surface waxes are more susceptible to water stress, which leads to increased concentrations of deterrent compounds that in turn reduce feeding by the cabbage aphid *Brevicoryne brassicae* (L.) (COLE AND RIGGALL 1992).

In this study, epicuticular leaf waxes proved to play a central role in the host selection process of *A. proletella* by several experiments. Epicuticular leaf waxes of cruciferous plants acted as arrestant stimulant during searching phase and feeding stimulant during contact-testing phase that promote stylet penetration and phloem accession. As feeding and oviposition are simultaneously performed in whiteflies (VAN LENTEREN AND NOLDUS 1990; BÄHRMANN 2002), it is assumed that epicuticular waxes must also function as oviposition stimulant. Furthermore, KHAN ET AL. (2011) showed that viruliferous whiteflies could successfully transfer the Cotton leaf curl virus to *Gossypium hirsutum* (L.) as well as to a wax mutant, while resistant *G. arboreum* (L.) with 50% more wax compared to test plants could not be infected. It was concluded that the epicuticular leaf wax of cotton may act as a physical barrier towards whiteflies and provide hindrance in the transfer of the virus. Although it was not tested, epicuticular leaf waxes must have affected whitefly infestation and feeding patterns as well. Moreover, the chemical composition of epicuticular leaf waxes seems to determine qualitative differences between surface lipids of different host plants which result in varying leaf wax attractiveness towards *A. proletella*. Consequently, it is hypothesized that *A. proletella* pre-evaluates host plant quality only by physical contact with epicuticular leaf waxes alone by sensing of epicuticular waxes with their chemo- and mechanosensory sensillae located at the apex of the whitefly labium (WALKER AND GORDH 1989). LAMBERT ET AL. (1995) analysed epicuticular lipid compositions of several soybean genotypes and found that low levels of the triterpenoid lupeol tended to have higher populations of *Bemisia argentifolii* (Genn.) and *Trialeurodes abutilonea* (Hald.). The different shapes of epicuticular wax crystals visualized by SEM in this study imply that chemical compositions differ between the leaf surface waxes of host cultivars used in the study. Besides various components of epicuticular surface waxes, a range of other primary and secondary plant metabolites present at the leaf surface could be responsible for mediating host selection behaviour of *A. proletella*.

Especially glucosinolates are a prominent group of secondary plant compounds in Brassicaceae and are known to stimulate feeding and oviposition in cabbage pests (STÄDLER 1992; BENNETT AND WALLSGROVE 1994; HOPKINS ET AL. 1997; MARAZZI ET AL. 2004). As already mentioned, SPENCER (1996) found that sinigrin increased oviposition by the diamondback moth *P. xylostella* even more in combination with paraffin or an *n*-alkane mixture than sinigrin alone. Nevertheless, it is controversially discussed whether glucosinolates may or may not be present on the leaf surface due

to unfavourable physicochemical properties (GRIFFITHS ET AL. 2001; MÜLLER AND RIEDERER 2005; REIFENRATH ET AL. 2005). Several investigators used chemical solvents to prepare leaf wax extracts from Brassicaceae in which glucosinolates could be detected (HOPKINS ET AL. 1997; GRIFFITHS ET AL. 2001). However, methods involving leaf surface washings with solvents may lead to extraction of compounds from epidermal or mesophyll as well, whereas, on the contrary, no traces of glucosinolates could be found when mechanical wax removal was applied (REIFENRATH ET AL. 2005; STÄDLER AND REIFENRATH 2009). Nevertheless, physical properties of epicuticular wax crystals might also affect whiteflies not only by their structure during contact with the plant but also by their visual characteristics on a distance during the host plant searching phase.

6.3 Phloem amino acids affecting host plant preference in whiteflies

The principal function of the phloem sieve elements in plants is the transport of nutrients and organic metabolites over long distances from the regions of acquisition or production to the physiological sinks, *i.e.* the sites of usage or storage (SJOLUND 1997). Therefore, phloem sap is loaded with compounds like sugars, hormones, amino acids, proteins, sugar alcohols, and other organic compounds in varying proportions to fulfil the physiological needs of dependent cells in the different plant tissues (ANSTEAD ET AL. 2013). Phloem sap contains no or very low concentrations of secondary compounds and is generally considered free from toxins and deterrents (DOUGLAS 2006). Consequently, the phloem sieve elements of a plant are targeted by herbivorous insects that utilize the phloem sap as their nutrient source. Hemipterans, especially members of the Sternorrhyncha such as whiteflies and aphids, even exclusively rely on phloem sap as their food source (DOLLING 1991). For ingestion, whiteflies penetrate the sieve elements with their stylets, and as a result of the high hydrostatic pressure, phloem sap exudes out into their stylets to be consumed (WALKER ET AL. 2010).

A plant that is successfully used as a host by an herbivorous insect, should provide a holistic diet to support insect growth, reproduction and development (BECK 1972; DHILLON AND KUMAR 2017). The utilized carbon and nitrogen sources present in the phloem sap are sugars and free amino acids, respectively. As nitrogen takes a central role in the metabolic processes, cellular structure and genetic coding, the nitrogen content of plants is vitally important to herbivorous insects (MATTSON 1980). Therefore, amino acids are of special importance within the ecological context (SCRIBER 1984; DOUGLAS 2003). However, nitrogen is a limiting factor and phloem sap composition is unbalanced (MATTSON 1980; DOUGLAS 2006). Phloem feeders must face two nutritional barriers: the nitrogen barrier and the sugar barrier. The nitrogen barrier results from the ratio of essential and nonessential amino acids, which is 1:4–1:20 favouring nonessential amino acids, whereas the sugar barrier is due to the low nitrogen concentration in the phloem sap in contrast to the high ratio of

sugars (DIXON 1998; SANDSTROM AND MORAN 1999; DOUGLAS 2003; DOUGLAS 2006; WALKER ET AL. 2010). Nevertheless, whiteflies have overcome these obstacles by several adaptions to their ecological niche. First, all whiteflies harbour obligatory nutritional symbionts, primarily "*Candidatus* Portiera aleyrodidarum", which are endosymbiotic and transmitted vertically from mother to offspring (SZKLARZEWICZ AND MOSKAL 2001; THAO AND BAUMANN 2004; SKIDMORE AND HANSEN 2017). These microorganisms synthesize and provide their hosts with certain essential amino acids allowing them to survive on poor diets (HOUK AND GRIFFITHS 1980; CAMPBELL 1989; DOUGLAS AND PROSSER 1992; THAO AND BAUMANN 2004; SKIDMORE AND HANSEN 2017). The high concentration of the sugar in the phloem sap often exceeds 1 M sugar and thus leads to an osmotic pressure that is 2–5 times higher than the osmotic pressure of the insect body (DOUGLAS 2006). As a result, whiteflies excrete excess dietary sugars through a filter chamber, which would be otherwise lethal to them (BYRNE AND BELLOWS 1991; DOUGLAS 2006; WALKER ET AL. 2010). The filter chamber is a modified part of their gut that directs excess water and/or sugar directly to the hindgut, where it is quickly excreted (WALKER ET AL. 2010). Additionally, aphids can compensate for lower levels of amino acids by increasing their feeding rates, which may also be a feature in whiteflies (PROSSER ET AL. 1992).

Another nitrogen limiting factor and difficulty posed to phloem feeders is that phloem sap composition varies depending on the seasonal and diurnal cycle, the developmental stage and the nutritional status of the plant, as well as abiotic factors (GEIGER AND SERVAITES 1994; PEUKE ET AL. 1994; BOGGIO ET AL. 2000; CORBESIER ET AL. 2001; KARLEY ET AL. 2002; DOUGLAS 2006). Apart from low average quality, potential host plants can also represent a challenge for insect herbivores due to their variable nutrient content (WETZEL ET AL. 2016). Whiteflies likely respond to long-term changes in phloem composition by altered behaviours such as varying the feeding rate according to nutrient content and the resulting osmotic pressure as well as withdrawing their stylets to find a different sieve element. Furthermore, post-ingestive responses including changes of the gut sucrase activity and transporter functions in sugar and amino acid assimilation are plausible but have not been considered yet (DOUGLAS 2006).

Dietary nitrogen concentration is a major determinant of population increase in phloem feeding insects (SCRIBER 1984; FEBVAY ET AL. 1988; DOUGLAS 2003). Insect performance is strongly influenced by the different effects caused by individual amino acids ingested and, therefore, amino acid compositions affect host plant suitability (AUCLAIR 1963; ROCK AND KING 1967; DADD AND KRIEGER 1968; BRODBECK AND STRONG 1987; WILKINSON AND DOUGLAS 2003; CHIOZZA ET AL. 2010; DHILLON AND KUMAR 2017). In general, the ten amino acids considered essential are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, as they are not synthesized by insects (DADD 1973). Nutrients are considered essential when their deletion from the diet would prevent further insect growth, development and/or reproduction

(THOMPSON AND SIMPSON 2009). Consequently, essential amino acids must either be taken up through the diet or provided by symbionts. Beyond the need for protein synthesis, essential amino acids fulfil additional physiological functions. For instance, arginine is a precursor for the muscle phosphagen phosphoarginine (THOMPSON AND SIMPSON 2009). Although tyrosine and cysteine are not essential, they have special significance, as they are synthesized from the essential amino acids phenylalanine and methionine respectively (DADD 1973; SANDSTROM AND MORAN 1999). Phenylalanine and tyrosine are both important to produce phenolic and quinone metabolites, which are key components for the cross-linking of proteins during sclerotization (BEHMER 2008; THOMPSON AND SIMPSON 2009; ANDERSEN 2010). Other amino acids are generally considered non-essential. Nevertheless, non-essential amino acids are still required to a certain extent for normal growth and development (THOMPSON AND SIMPSON 2009). The reason for this is that the synthesis of non-essential amino acids as well as the subsequent elimination of remnant compounds are metabolically expensive (BEHMER 2008).

Furthermore, insects can taste amino acids, which was shown for orthopterans, hemipterans, coleopterans and lepidopterans (MITTLER 1967; SRIVASTAVA ET AL. 1983; BERNAYS AND CHAPMAN 1994). In choice tests with aphids feeding on sucrose media, alanine, asparagine, isoleucine, leucine, methionine, phenylalanine, tryptophan, and y-amino butyric acid (GABA) were found to have phagostimulatory effects. On the other hand, arginine, asparagine, aspartic acid, cysteine, cystine, histidine, glutamic acid, glycine, proline, serine, and tyrosine were found to be deterrent (MITTLER 1967; SRIVASTAVA ET AL. 1983). Thus, phagostimulatory and antifeedant effects derived from amino acids and other dietary components determine the attractiveness and susceptibility of a host plant. This could also be highlighted in this study with *T. vaporariorum*. On the one hand, this study could correlate the amino acid composition in the phloem sap with host susceptibility of several host plants towards *T. vaporariorum*. On the other hand, it was proven that gustatory properties of single amino acids determine the acceptance of sucrose media. However, the actual concentration of amino acids in the phloem sap of host plants remains unknown due to the phloem sampling methodology and gustatory properties of single amino acids were measured for one concentration level only. The amino acid concentration is highly relevant for the attractiveness of the host plant, as exceptionally high concentrations of single amino acids were found to be unusable and even result in toxicity (BRODBECK ET AL. 1990). Moreover, the gustatory properties of the phloem are not only defined by amino acids, as phloem sap contains numerous compounds, including sugars and proteins (KEHR 2006; ANSTEAD ET AL. 2013). Consequently, the resultant effects of individual amino acids *in vitro* were different to those observed in natural hosts in this study.

6.4 Outlook

This thesis may provide the basis for new approaches used in breeding whitefly resistant crops. Based on the finding of this thesis, whitefly resistant cultivars with a dual mode of resistance that combine an epidermal/mesophyll factor as well as a phloem factor would be particularly effective in integrated pest management strategies to control whiteflies. In this context, crop varieties with altered leaf epicuticular wax compositions could contribute to host plant resistance. Targeted breeding of such crop cultivars with modified epicuticular surface waxes is becoming increasingly realistic, since there has been significant progress in the last 25 years in the identification and characterization of the genes involved in epicuticular wax synthesis (AARTS ET AL. 1995; LEMIEUX 1996; SUH ET AL. 2005; LEE AND SUH 2013; FICH ET AL. 2016). Another conceivable approach could be the application of artificial wax components on the leaf surfaces of host plants to deter whiteflies. Furthermore, the dominant presence of phloem compounds exerting strong inhibitory effects on whitefly feeding, such as amino acids, could additionally determine host susceptibility towards whiteflies. However, further research is necessary, and the following questions derived from this thesis should be answered in future:

(i) which compounds of the epicuticular surface wax blend of cruciferous leaves are responsible for the observed antixenotic effects on *A. proletella*;
(ii) are leaf epicuticular waxes also mediating host plant selection in other whitefly species;
(iii) which amino acids determine the feeding preference of whiteflies in the phloem sap of host plants;
(iv) which compounds of the phloem sap additionally affect host plant choice in whiteflies and to what extend;
(v) what are further mediating factors of host plant resistance in whiteflies.

Summary

Whiteflies are a species-rich insect family that is characterized by high adaptability to a variety of ecological conditions. Consequently, they are among the most important pests causing severe damage to numerous cultivated and ornamental plants worldwide. With their piercing-sucking mouthparts, whiteflies penetrate the leaf tissue of their hosts and feed on the phloem sap of the plants. The present dissertation comprises four studies and contributes to the knowledge of the host plant selection process by whiteflies.

In the first study, host preferences were determined in dual choice tests for *Aleyrodes proletella* (L.), *Bemisia tabaci* (Genn.), and *Trialeurodes vaporariorum* (Westw.) on several host plants. Subsequent calculation of preference indices was used to obtain host rankings serving as references in the following chapters. Host attractiveness towards *A. proletella* could be ranked in decreasing order by oilseed rape, kale, savoy cabbage, blue turnip cabbage, cauliflower, white turnip cabbage and white cabbage. Host preferences to both *B. tabaci* and *T. vaporariorum* were found in decreasing order by eggplant, tobacco, tomato, cucumber, bean and sweet pepper. On the one hand, this study extends the knowledge on the food spectrum of these economically important pests; on the other hand, the results highlight the host adaptation of whiteflies.

The second study elucidated potential sources of host plant resistance against *A. proletella*, *B. tabaci*, and *T. vaporariorum* by recording their probing and feeding behaviour on two host plants each using the electrical penetration graph (EPG) method. All whitefly species used prolonged probes and pathway phases on more attractive hosts. Additionally, probes of *A. proletella* were interrupted earlier and lacked phloem phases on host plant leaves with mechanically removed surface wax. The phloem phases of *B. tabaci* and *T. vaporariorum* were shorter on less preferred host plants. It is concluded that whiteflies decide upon host plant acceptance by evaluation of multiple plant factors located in epidermal and/or mesophyll tissues of leaves as well as in the phloem sap of plants. Moreover, epicuticular leaf waxes are a key factor in the host selection process of *A. proletella*. It is hypothesized that constituents of the leaf surface wax act as stimulants promoting leaf penetration and phloem accession. The findings of this study shed light on the whitefly-host adaptation.

The goal of the third study was to identify the role of epicuticular leaf waxes of several *Brassica* cultivars in the host selection process of *A. proletella*. For this purpose, dual choice tests were carried out on both waxy and dewaxed plant leaves as well as on Parafilm® treated with different leaf wax extracts. Also, life-history traits were monitored on waxy and dewaxed leaves, and the feeding activity of *A. proletella* was recorded on Parafilm® with and without leaf wax extracts. Scanning electron microscopy (SEM) imaging was used to visualize epicuticular leaf waxes on the plant surface. While waxy leaves were preferred, leaf wax extracts triggered whitefly settlement. In

contrast, life-history parameters were impaired on dewaxed leaves. On Parafilm® treated with leaf wax extracts of preferred hosts, feeding was furthermore enhanced. As the wax crystal morphology varied on natural leaf surfaces, it is suggested that epicuticular leaf waxes of several host plant cultivars differ in their chemical composition. Consequently, *A. proletella* evaluates the suitability of host plants, especially by characteristics of the epicuticular leaf waxes. Finally, it was proved that leaf surface waxes of host plants promote feeding and act as phagostimulants. Although the wax compounds mediating host plant selection remain unknown, these findings offer breeding potential for resistant crop cultivars.

In the fourth study, the influence of free phloem amino acids on the host plant selection of *T. vaporariorum* was investigated. Via liquid chromatography-mass spectrometry (LC-MS), the amino acid profiles in the phloem sap of six vegetable crops varying in their host plant attractiveness were analysed. Subsequently, stepwise multiple regressions of the relative amino acid compositions and the pre-determined host plant preferences were performed. To verify the contribution of single amino acids on host choice, dual choice tests on sucrose media with and without added single amino acids were carried out. According to multiple regressions, glutamic acid, threonine, phenylalanine and serine were the most relevant amino acids to explain host plant attractiveness. Furthermore, essential, aromatic, and hydroxylated amino acid groups affected host plant selection most. On the other hand, dual choice tests proved that lysine, asparagine, threonine, valine, glutamine, leucine, tryptophan, glutamic acid, tyrosine, aspartic acid, cysteine, and alanine exerted gustatory stimuli determining feeding preferences on sucrose media. However, the effects of individual amino acids in the phloem sap only partially agreed with the effects measured *in vitro*. Besides non-phloem plant factors mediating host choice behaviour of *T. vaporariorum* on natural hosts, the presence and concentration of other phloem compounds might have additionally influenced host attractiveness. Nevertheless, single amino acids play an active role in phagostimulation, whereas some amino acids exert strong inhibitory effects. This indicates that the dominant presence of such amino acids might reduce phloem sap uptake, thus contributing to host plant resistance towards *T. vaporariorum*.

Overall, this research compared the host selection process of three whitefly species to identify their underlying mechanisms. It is hypothesized that the observed host selection strategies are the result of evolutionary adaptations between whiteflies and their host plants. Depending on the occupied ecological niche, species-specific host plant ranges of varying complexity were formed. Accordingly, the host selection process of the more specialised species *A. proletella* is particularly efficient by consideration of characteristic leaf surface wax stimuli. In contrast, host selection of the extreme generalists *B. tabaci* and *T. vaporariorum* is regulated by simple gustatory stimuli in order to take advantage of the host diversity they are offered. The findings of this research provide the basis for new approaches to optimizing breeding programs for whitefly resistant crops.

Zusammenfassung

Weiße Fliegen sind eine artenreiche Insektenfamilie, die sich durch eine hohe Anpassungsfähigkeit an eine Vielzahl von ökologischen Bedingungen auszeichnet. Infolgedessen zählen sie zu den bedeutendsten Schädlingen, die weltweit erhebliche Schäden an zahlreichen Kultur- und Zierpflanzen verursachen. Mit ihren stechend-saugenden Mundwerkzeugen dringen Weiße Fliegen in das Blattgewebe ihrer Wirte ein und ernähren sich vom Phloemsaft der Pflanzen. Die vorliegende Dissertation umfasst vier Studien und gibt Aufschluss über den Auswahlprozess von Wirtspflanzen durch Weiße Fliegen.

In der ersten Studie wurden in Dual-Choice-Tests die Wirtspräferenzen von *Aleyrodes proletella* (L.), *Bemisia tabaci* (Genn.) und *Trialeurodes vaporariorum* (Westw.) für mehrere Wirtspflanzen bestimmt. Im Anschluss wurden über die Berechnung von Präferenzindizes Wirts-Rangfolgen erstellt, die dann in den folgenden Kapiteln als Referenz herangezogen wurden. Die Wirtsattraktivität gegenüber *A. proletella* nahm über Raps, Grünkohl, Wirsing, blauer Kohlrabi, Blumenkohl, weißer Kohlrabi hin zu Weißkohl ab. Die Wirtspräferenzen von *B. tabaci* und *T. vaporariorum* nahmen in der Reihenfolge Aubergine, Tabak, Tomate, Gurke, Bohne und Paprika ab. Einerseits erweitert diese Studie den Kenntnisstand hinsichtlich des Nahrungsspektrums dieser wirtschaftlich bedeutenden Schädlinge, andererseits unterstreichen die Ergebnisse die Wirtsanpassung der Weißen Fliege.

Die zweite Studie beleuchtete anhand der Aufzeichnung des Probe- und Nahrungsaufnahmeverhaltens von *A. proletella*, *B. tabaci* und *T. vaporariorum* an je zwei Wirtspflanzen potenzielle Quellen der Wirtspflanzenresistenz mittels der *electrical penetration graph* (EPG)-Methode. Alle Arten nutzten längere Probestiche und Wegphasen innerhalb des Blattgewebes auf attraktiveren Wirten. Des Weiteren waren die Probestiche von *A. proletella* vorzeitig unterbrochen und wiesen fehlende Phloem-Phasen auf Wirtspflanzenblättern mit mechanisch entferntem Oberflächenwachs auf. Die Phloem-Phasen von *B. tabaci* und *T. vaporariorum* waren auf weniger bevorzugten Wirtspflanzen verkürzt. Dies lässt darauf schließen, dass Weiße Fliegen anhand der Bewertung mehrerer Pflanzenfaktoren, die sich sowohl in den epidermalen und/oder mesophyllischen Gewebsschichten der Blätter als auch im Phloemsaft der Pflanzen befinden, über die Akzeptanz einer Wirtspflanze entscheiden. Darüber hinaus stellen epikutikuläre Blattwachse einen Schlüsselfaktor im Wirtsselektionsprozess von *A. proletella* dar. Daraus lässt sich die Hypothese ableiten, dass Bestandteile des Blattoberflächenwachses als Stimulanzien wirken, die die Blattpenetration und das Erreichen des Phloems fördern. Die Ergebnisse dieser Studie beleuchten die Anpassung von Weißen Fliegen an ihre jeweiligen Wirtspflanzen.

Das Ziel der dritten Studie war es, die Rolle epikutikulärer Blattwachse mehrerer *Brassica*-Sorten im Wirtsselektionsprozess von *A. proletella* zu identifizieren. Zu diesem Zweck wurden Dual-Choice-Tests an bewachsten und entwachsten Pflanzenblättern sowie mit Parafilm®, der mit verschiedenen Blattwachsextrakten behandelt wurde, durchgeführt. Außerdem wurden lebensgeschichtliche Parameter auf bewachsten und entwachsten Blättern ermittelt und die Nahrungsaufnahmeaktivität von *A. proletella* auf Parafilm® mit und ohne Blattwachsextrakten erfasst. Die Rasterelektronenmikroskopie (REM) wurde zur Visualisierung epikutikulärer Blattwachse auf der Pflanzenoberfläche eingesetzt. Während bewachste Blätter bevorzugt wurden, lösten Blattwachsextrakte das Siedlungsverhalten aus. Im Gegensatz dazu waren die lebensgeschichtlichen Parameter auf entwachsten Blättern beeinträchtigt. Auf Parafilm®, der mit Blattwachsextrakten bevorzugter Wirte behandelt wurde, war zudem die Nahrungsaufnahme erhöht. Da sich die Morphologie der Wachskristalle auf natürlichen Blattoberflächen unterschied, ist zu vermuten, dass sich epikutikuläre Blattwachse verschiedener Wirtspflanzensorten in ihrer chemischen Zusammensetzung unterscheiden. Folglich bewertet *A. proletella* die Eignung von Wirtspflanzen insbesondere anhand der Beschaffenheit der epikutikulären Blattwachse. Schließlich wurde nachgewiesen, dass Blattoberflächenwachse der Wirtspflanzen die Nahrungsaufnahme fördern und als Phagostimulans wirken. Obwohl die Wachsverbindungen, die die Selektion von Wirtspflanzen bestimmen, unbekannt bleiben, bieten diese Erkenntnisse Potenzial für die Züchtung resistenter Kultursorten.

In der vierten Studie wurde der Einfluss von den im Phloemsaft befindlichen freien Aminosäuren auf die Wirtspflanzenselektion von *T. vaporariorum* untersucht. Mittels Flüssigchromatographie mit Massenspektrometrie-Kopplung (LC-MS) wurden die Aminosäureprofile im Phloemsaft von sechs Gemüsekulturen analysiert, die sich hinsichtlich ihrer Wirtspflanzenattraktivität unterschieden. Anschließend wurden schrittweise multiple Regressionen unter Einbezug der relativen Aminosäure-Zusammensetzungen und den zuvor bestimmten Wirtspräferenzen durchgeführt. Um den Beitrag einzelner Aminosäuren in Bezug auf die Wirtswahl zu verifizieren wurden Dual-Choice-Tests auf Saccharosemedien mit und ohne Zusatz einzelner Aminosäuren durchgeführt. Gemäß den multiplen Regressionen waren Glutaminsäure, Threonin, Phenylalanin und Serin die wesentlichsten Aminosäuren, welche die Attraktivität der Wirtspflanzen erklärten. Darüber hinaus beeinflussten essenzielle, aromatische und hydroxylierte Aminosäuregruppen die Wirtspflanzenselektion am meisten. Dem gegenüber konnten Dual-Choice-Tests nachweisen, dass Lysin, Asparagin, Threonin, Valin, Glutamin, Leucin, Tryptophan, Glutaminsäure, Tyrosin, Asparaginsäure, Cystein und Alanin gustatorische Reize ausübten, die die Nahrungspräferenzen auf Saccharosemedien bestimmten. Die Wirkungen einzelner Aminosäuren im Phloemsaft stimmten jedoch nur teilweise mit den *in vitro* gemessenen Wirkungen überein. Neben nicht im Phloem befindlichen Pflanzenfaktoren, die das Wirtswahlverhalten von *T. vaporariorum*

auf natürlichen Wirten bestimmen, könnte die Anwesenheit und Konzentration anderer Verbindungen im Phloem die Wirtsattraktivität zusätzlich beeinflusst haben. Dennoch spielen einzelne Aminosäuren eine aktive Rolle bei der Phagostimulation, während einige Aminosäuren starke hemmende Effekte ausübten. Dies deutet darauf hin, dass die dominante Anwesenheit solcher Aminosäuren die Aufnahme des Phloemsaft reduziert und damit zur Resistenz von Wirtspflanzen gegenüber *T. vaporariorum* beiträgt.

Insgesamt wurde im Rahmen dieser Forschungsarbeit der Auswahlprozess dreier Arten Weißer Fliegen miteinander verglichen, um die ihnen zugrunde liegenden Mechanismen zu identifizieren. Es wird postuliert, dass die beobachteten Wirtsselektionsstrategien das Ergebnis von evolutionären Anpassungen zwischen Weißen Fliegen und ihren Wirtspflanzen sind. Entsprechend der jeweiligen besetzten ökologischen Nische bildeten sich artspezifische Wirtspflanzenspektren von unterschiedlicher Komplexität. Demzufolge gestaltet sich der Wirtsselektionsprozess der spezialisierteren Art *A. proletella* durch die Berücksichtigung charakteristischer Reize, welche von Blattoberflächenwachsen ausgehen, als besonders effizient. Im Gegensatz dazu wird das Wirtswahlverhalten der extremen Generalisten *B. tabaci* und *T. vaporariorum* von einfachen gustatorischen Reizen gesteuert, um die sich ihnen bietende Wirtsvielfalt auszunutzen. Die Ergebnisse dieser Forschung bilden die Grundlage neuer Ansatzpunkte für die Optimierung von Züchtungsprogrammen für Nutzpflanzen, die gegen Weiße Fliegen resistent sind.

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Affidavit

Declaration in lieu of an oath on independent work according to Sec. 18(3) sentence 5 of the University of Hohenheim's Doctoral Regulations for the Faculties of Agricultural Sciences, Natural Sciences, and Business, Economics and Social Sciences

1. The dissertation submitted on the topic

"Morphological and chemical plant properties mediating host plant selection of whiteflies (Hemiptera: Aleyrodidae)"

is work done independently by me.

2. I only used the sources and aids listed and did not make use of any impermissible assistance from third parties. In particular, I marked all content taken word-for-word or paraphrased from other works.

3. I did not use the assistance of a commercial doctoral placement or advising agency.

4. I am aware of the importance of the declaration in lieu of oath and the criminal consequences of false or incomplete declarations in lieu of oath.

I confirm that the declaration above is correct. I declare in lieu of oath that I have declared only the truth to the best of my knowledge and have not omitted anything.

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Place, Date Signature

Curriculum vitae

Name	Nina Sara Stoll
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Professional Career

Since 02/2020	Subject Specialist for Control and Certification ABCERT AG, Esslingen
Since 09/2018	Consultant for Agricultural Investments EBG Capital AG, Zurich
Since 11/2014	Research Associate at the Department of Applied Entomology University of Hohenheim, Stuttgart

University Education

Since 11/2014	Doctoral candidate at the Department of Applied Entomology University of Hohenheim, Stuttgart Doctoral thesis: *Morphological and chemical plant properties mediate host plant selection of whiteflies (Hemiptera: Aleyrodidae)*
10/2011 – 11/2014	Master of Science Crop Sciences, University of Hohenheim Major: Crop Protection Master thesis: *Experiments on host selection and insect-host interaction of* Aleyrodes proletella *L. (Homoptera: Aleyrodidae) on different genotypes of cabbage cultivars*
10/2008 – 10/2011	Bachelor of Science Agricultural Biology, University of Hohenheim Bachelor thesis: *Untersuchungen zur Aufnahme von markiertem Agnique SBO 10® in* Abutilon theophrasti *Medik.,* Sinapis arvensis *L. und* Beta vulgaris *Döll*

Publications

Stoll, N. S and C. P. W. Zebitz (2018): Variability of leaf waxes affect host plant acceptance of *Aleyrodes proletella* (L.) (Hemiptera: Aleyrodidae). Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie, 21:63–66.

Stoll, N. S. and C. P. W. Zebitz (2018): Blattoberflächenwachse von *Brassica*-Arten als mögliches Phagostimulans für Juvenil- und Adultstadien von *Aleyrodes proletella* (L.). Julius-Kühn-Archiv, 461: 540–541.

Participation at scientific conferences

- Entomologentagung 2017, 13. – 16. March 2017 in Freising
- 61. Deutsche Pflanzenschutztagung, 11. – 14. September 2018 in Stuttgart

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Place, Date Signature