Artificial application systems for Thysanoptera – Key to new fields of research

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Chapter I

Introduction

1 General introduction

Artificial diets for insects

The availability of artificial diets for insects is important for a broad spectrum of applications, as it has been succinctly described and summarized by Cohen (2015):

"Insects that are reared on artificial diets are used in many programs – as agents of biological control and sterile insect technologies (Knipling, 1979), as feed for other animals (Versoi & French, 1992), as bioreactors for the production pharmaceuticals and other recombinant proteins (Hughes and Wood, 1998), and as foods for people (DeFoliart, 1999) – and one of their most important uses is in research on virtually all areas of entomology and of other biological sciences.".

The first species of insects reared on an artificial diet was *Calliphora vomitoria* using a medium of peptone, meat extract, starch and minerals by Bogdanow (1908). Since that time, a lot of research was done and many publications focusing on establishing and improving artificial diets were published. However, there are two publications that are outstanding as they established diets that were usable and adaptable for many insect species. Vanderzant et al. (1962) established the use of wheat germs to greatly improve artificial diets. Later Singh (1983) established a diet that can be used and adapted for various insect species. Thus, since the first experiments there were many findings leading to the knowledge that artificial diets have to provide insects with basic substances (water, carbohydrates, amino acids, vitamins, minerals and fatty acids) and that there are specific substances necessary for certain species as growth factors (summarized in Cohen, 2015; Vanderzant, 1974).

For some insect orders like the order Thysanoptera there are only a few publications referring to the long-term application of artificial diets (Kinzer et al., 1972; Lowry et al., 1992) applying an artificial diet for several days without replenishment to large groups of thrips. Since variations in nutrients or added substances could lead to unclear research results, a standardized application method is key to successful studies using artificial diets.

Thysanoptera

Thysanoptera are minute sized insects (1-3 mm) having characteristically fringed wings, a reduced right mandible and a pretarsal arolium (Heming, 1971, 1978; Moritz 1997; Mound et al., 1980).

The order comprises about 6315 extant species known worldwide (ThripsWiki, 2021a) and is divided in the two suborders Tubulifera and Terebrantia. In contrast to tubuliferan species, females of terebrantian species possess an external ovipositor to oviposit their eggs into leaf tissue (Bode, 1975; Heming, 1970; Moritz, 1989, 1997). While the suborder Tubulifera only comprises

one family (Phlaeothripidae), the suborder Terebrantia consists of eight families (Adiheterothripidae, Aeolothripidae, Fauriellidae, Heterothripidae, Melanthripidae, Merothripidae, Thripidae and Uzelothripidae) (Moritz, 2006; Mound, 1997). Yet, most species belong to the families Thripidae (2100 species) (ThripsWiki, 2021b) and Phlaeothripidae (3550 species) (ThripsWiki, 2021c). Most thrips species have a haplo-diploid reproduction mode, being able to reproduce arrhenotokous or thelytokous. The postembryonic development includes two feeding larval stages, a non-feeding propupal and pupal stage (two for Tubulifera) and the adult stage. Generally, species of the order Thysanoptera can be predacious, phytosug or fungivore (Lewis, 1973; Moritz, 2006).

Despite their small size certain species have a significant pest potential. Fewer than 100 species of the order Thysanoptera are economically relevant pest insects in agricultural and ornamental plants (Mound, 1997). Thrips damage plants by feeding and oviposition (Terebrantia) and can additionally be vectors of viruses or other phytopathogens. For the Thysanoptera 16 species are known vectors for various tospoviruses (Ciuffo et al. 2010; Ghosh et al., 2017; Hassani-Mehraban et al., 2010; Jangra et al., 2020; Jones, 2005; Pappu et al., 2009; Riley et al., 2011; Rotenberg, 2015; Ullman, 1997). Like for many other insect pest species the increasing occurrence of pesticide resistance is a growing problem in Thysanopteran species as well, e.g. the resistance in western flower thrips, *Frankliniella occidentalis* (Cloyd, 2016). Thus, finding new control measures is more and more important. However, to find new control measures more knowledge about thrips and their biology is crucial.

To gain this knowledge investigating the impact of substances on thrips by oral application is an important method. Thus, substances like antibiotics (Arakaki et al., 2001; Kumm & Moritz, 2008; Nguyen et al., 2015; van der Kooi & Schwander, 2014), pesticides (Darnell-Crumpton et al., 2018; Huseth et al., 2016; Mautino et al., 2012; Rueda & Shelton, 2003) and lately dsRNA (Andongma et al., 2020; Jahani et al., 2018; Singh et al., 2019; Whitten et al., 2016) haven been applied to thrips orally. However, this was mostly done in short-term application experiments using various different methods. Thus, the establishment of a standardized method allowing a short-term and long-term application from the first instar larvae to the adult thrips, a clear view for the observation of thrips and a separation of thrips and diet for a standardized application would be valuable for future experiments.

Feeding mechanism of Thysanoptera

Thrips unique way of feeding was first described as piercing-sucking by Peterson (1915) and later by many other authors like Moritz (1982) and Chisholm and Lewis (1984) (summarized in Hunter & Ullman, 1992). While the right mandible is greatly reduced, the left mandible forms a blind-

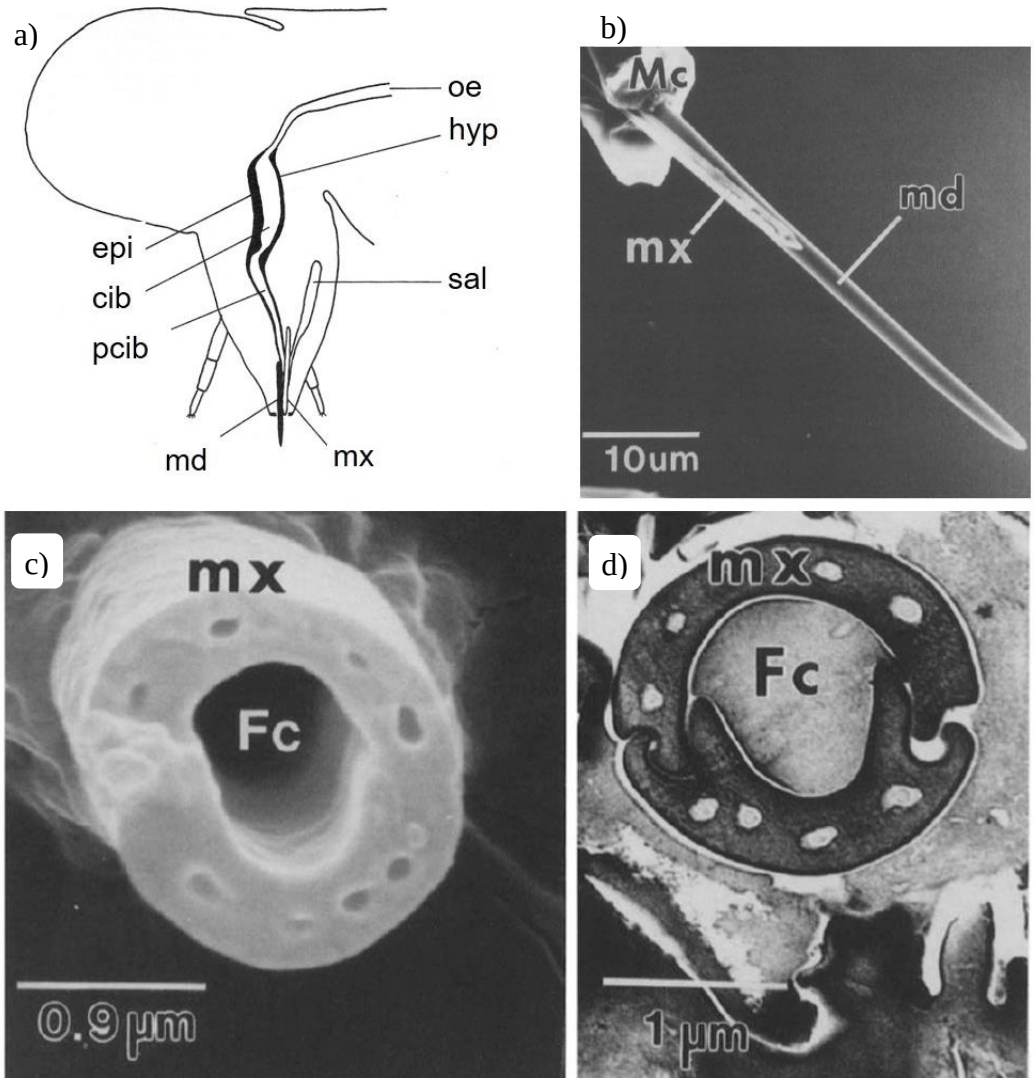

Fig. 1: Overview of thrips mouthcone, maxillary stylets and mandible of *Frankliniella occidentalis*. a) Diagram longitudinal section (after Moritz, 1988; Hunter & Ullmann, 1994; Risler, 1957 in Kirk, 1997) with only the distal parts of the mandible and maxillary stylets being visible. **b)** SEM of tip of mouthcone which has been compressed to show maxillary stylets and left mandible (from Hunter and Ullman 1992). **c)** SEM of maxillary stylets cut to show food canal created when these stylets are interlocked (from Hunter & Ullman, 1992). **d)** TEM of thin section through longitudinal tongue and groove interlocking mechanism of maxillary stylets and food canal between them (from Hunter & Ullman 1992).
cib cibarium, **epi** epipharyngeal sclerite, **Fc** food canal, **hyp** hypopharyngeal sclerite, **Mc** mouthcone, **md** mandible, **mx** maxillary stylet, **oe** oesophagus, **pcib** precibarium, **sal** salivarium

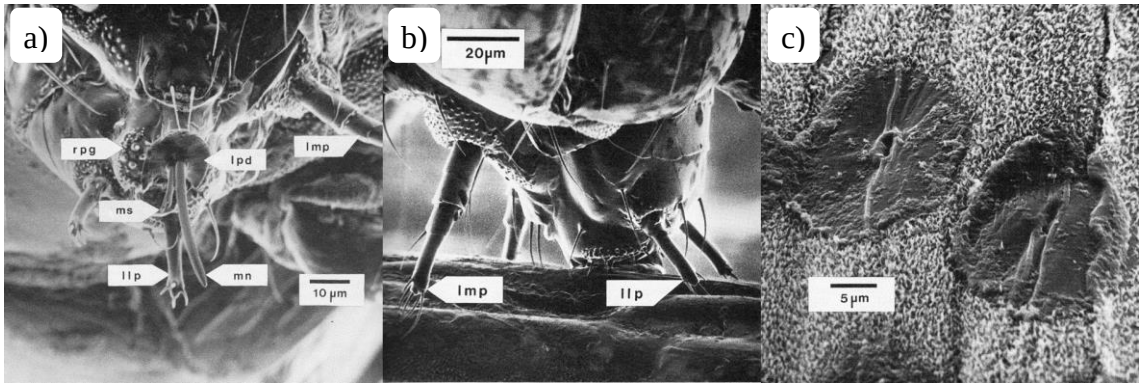

Fig. 2: Overview of thrips mouthcone and feeding mark *Limothrips cerealium*. a) SEM of oblige frontal view of the mouthcone and exserted mouthparts of an adult female (from Kirk, 1997). **b)** SEM of the left side of the mouthcone of an adult female feed on a wheat leaf (from Kirk, 1997). **c)** SEM of marks left on a wheat leaf after feeding of an adulte female (photo: I.F. Chisholm, from Kirk, 1997).
llp left labial palp**, lmp** left maxillary palp, **lpd** labral pad, **mn** mandible, **ms** maxillary stylet, **rpg** right paraglossal lobe

ending stylet (Hunter & Ullman, 1992). The maxillary stylets lie posterior to and on each side of the mandible (Fig. 1 a, b) (Hunter & Ullman, 1992). The distal parts of the maxillary stylets form a food canal by being interlocked in a "tongue and groove" fashion (Fig. 1c, d) (Hunter and Ullman 1992). When feeding, adults and larval thrips press their mouthcone on the substrate surface creating close contact between the labial pad and the surface (Fig 2. a, b, Lewis, 1991). During this close contact, the maxillary and labial palps and paraglossae touch the substrate (Fig. 2b), potentially playing a role in food selection as they have mechano- and chemoreceptory sensilla (Childers & Achor, 1991; Chisholm & Lewis, 1984; Heming, 1978; Hunter & Ullman 1989, 1992; Moritz, 1982, 1988, 1989; Wiesenborn & Morse, 1988; summarized in Kirk, 1997). Then the whole head capsule is thrust downwards and slightly backwards shortening the mouthcone and letting the mandible protrude and pierce the surface of the substrate (Lewis, 1991). Afterwards the mandible is largely withdrawn and the maxillary stylets, which can be moved independently from the mandible and each other (Chisholm & Lewis, 1984; Heming, 1978), are inserted into the cell up to 60 µm (*Limothrips cerealium*, Lewis, 1991). Then the cell content is taken up using the maxillary stylets forming the food canal. However, thrips have no separate saliva canal but the salivary duct inserts where the maxillary stylets meet (Fig. 1a). Thus, thrips are probably not able to feed and pass saliva into the cell at the same time. This hypothesis is supported by results of several authors (Kloft & Ehrhardt, 1959; Kumar et al., 1995; Mitchell et al., 1995; Raman et al., 1991; summarized in Kirk, 1997). The food canal diameter differs between thrips species. While Chisholm and Lewis (1984) described a diameter of 1 µm for *Limothrips cerealium*, there are thrips like *Mecynothrips hardyi* with a food canal with a diameter of 14 µm allowing the uptake of whole fungal spores (Tree et al., 2010). Thus, thrips species have the advantage of being separated from an artificial diet using a thin membrane, which thrips can penetrate and feed through using their maxillary stylets as first shown by Sakimura and Carter (1934).

In order to be able to standardize application of artificial diets, substances and microorganisms in short and long-term experiments, this thesis aim was to establish new methods in thrips cultivation considering their feeding mechanism.

2 Scope of the thesis

This thesis comprises three experimental chapter focusing on the establishment of new methods, which allow a standardized oral application of diets, substances and microorganisms. For most experiments *Frankliniella occidentalis* was used (Chapter II, III & IV) as it can be considered the "gold standard" of thrips species for new method establishment due to its high agility and superior value by means of its pest potential. Additionally in Chapter IV 3.2 *Echinothrips americanus* was

used as feed for *F. occidentalis*, whereas in Chapter IV 3.3 *E. americanus* was used together with *F. occidentalis* and *Thrips tabaci* to show the application of Algae to several thrips species.

Frankliniella occidentalis

The species *Frankliniella occidentalis* or western flower thrips is one of the most important agricultural pests worldwide (Reitz, 2009). Adult females of the western flower thrips are ~1.6 mm in size and can show various colourations ranging from light brownish to dark brownish (Fig. 3). The males adults are smaller (~1.2 mm) and show a pale-yellowish colour. This species reproduces arrhenotokous with females laying up to a total of 228 eggs per adult female (Robb & Parella, 1991). Once endemic to the western North America, *F. occidentalis* started to spread in the late 1970s becoming a global pest (Kirk, 2002; Kirk & Terry, 2003). With more than 240 plant species from 62 different families (Tommasini & Maini, 1995), it shows a wide host range even though not all plant species may support successful breeding (Mound, 2013). Besides being polyphagous regarding host plants, *F. occidentalis* is additionally considered omnivorous, since it can feed as well on mite eggs and white flies (Trichilo & Leigh, 1986; Roda et al., 2000; van Maanen et al., 2012; Zhi et al. 2006), making it a highly adaptive and competitive species. In addition to this, the western flower thrips is a known vector of tospoviruses (current state in: He et al., 2020) significantly increasing its pest potential. Furthermore, the widespread use of pesticides lead to an increasing number of pesticide resistances (Bielza, 2010; Gao et al., 2012; Jensen, 2000; Immaraju et al., 1992).

Order:	Thysanoptera
Suborder:	Terebrantia
Family:	Thripidae
Genus:	*Frankliniella*
Species:	*F. occidentalis* (Pergande, 1895)

Fig. 3: ***Frankliniella occidentalis***, adult female on a leaf of the French bean (*Phaseolus vulgaris*), bar: 500 µm

Echinothrips americanus

Adult poinsettia thrips are brownish-black, about 1.4 mm in size and show a characteristic white band at the wing base (Fig 4). This thrips species reproduces arrhenotokous despite being infected with *Wolbachia* (Chuttke 2015; Dong et al., 2012; Kumm & Moritz, 2008; Zhang & Feng, 2018). Females of *E. americanus* can oviposit between 41 to 86 eggs during their life time (Krueger et

al., 2016). Native to the eastern parts of the US (Stannard, 1968), *E. americanus* started to spread globally from European green-houses (Collins, 1998) to more than 20 European countries (Andjus et al., 2009; Varga & Fedor, 2009; Vierbergen et al., 2006). It continued to Thailand (Mound, 2000), Japan (Itoh et al., 2003), Java of Indonesia (Mound & Ng, 2009), Taiwan, China (Mirab-Balou et al., 2010), Northern Australia (Mound et al., 2013) and the north-eastern US and Canada (Ferguson & Shipp, 2002; Shipp et al., 2001). The polyphagous *E. americanus* is a known pest species of 40 cultivated plant species in the US (Oetting et al., 1993) and shows an increasing number of identified hosts (Vierbergen, 1998; plants from 24 families, Varga et al., 2010; plants from 48 families). The damages of *E. americanus* on plant tissues lead to chlorotic areas with faeces remaining as blackish spots. Thus, *E. americanus* infestations lowers the aesthetic value of its host plant creating its pest potential as it mostly infests ornamental plants like, *Euphorbia milii, Euphorbia pulcherrima, Impatiens spec. and Xanthosoma* spec. (Varga et al., 2010).

Order:	Thysanoptera
Suborder:	Terebrantia
Family:	Thripidae
Genus:	*Echinothrips*
Species:	*E. americanus* Morgan, 1913

Fig. 4: ***Echinothrips americanus***, adult female on a leaf of the French bean (*Phaseolus vulgaris*), bar: 500 µm.

Thrips tabaci

This thrips species is a considerable pest species in the order of Thysanoptera. Adult female thrips of *Thrips tabaci* are ~1.2 mm in size (Fig 5.) and show a pale-yellow to dark-brownish colouration, while males are smaller and yellowish. For this species arrhenotokous and thelytokous reproducing populations are known (e.g. Kendall & Capinera, 1990; Nault et al., 2006; Vierbergen & Ester, 2000). *T. tabaci* shows a high fecundity with a total up to 270 eggs per female (Murai, 2000). Despite being described based on specimens collected in Russia by Lindeman (1889), it is assumed that *T. tabaci* is native to the eastern Mediterranean (Mound & Walker, 1982; Mound, 1997). *T. tabaci* spread throughout the US and southern Canada in the early 1900s (Capinera, 2001) and is now distributed in many European countries, North America, South America, Africa, Asia, and Australia (Mound, 1997). Being polyphagous *T. tabaci* feeds on at least 140 plant species within 40 families (Ananthakrishnan, 1973) but is also known for

feeding on mite eggs (Milne & Walter 1998; Wilson et al., 1996). Furthermore, *T. tabaci* is a vector of Iris yellow spot virus (Cortês et al., 1998; Hsu et al., 2010), Tomato spotted wilt virus (Wijkamp et al., 1995) and Tomato yellow fruit ring virus (Golnaraghi et al., 2007). Like other thrips species, *T. tabaci* shows increasing pesticide resistances (Adesanya et al., 2020; Martin et al., 2003).

Order:	Thysanoptera
Suborder:	Terebrantia
Family:	Thripidae
Genus:	*Thrips*
Species:	*T. tabaci* Lindemann, 1889

Fig. 5: *Thrips tabaci*, adult female on a leaf of the French bean (*Phaseolus vulgaris*), bar: 500 µm

Establishing a standardized application method allowing oral short- and long-term application of diets, substances and microorganisms

The oral application of substances is performed in many publications about Thysanoptera. However, no method allows a standardized long-term application of liquid diets starting from the freshly hatched to the adult thrips using freshly hatched thrips larvae that had no contact with a host plant.

Since the rearing of insects on artificial diets and the availability of an application system for short- and long-term application allow a wide range of new application experiments with an increased reproducibility and accuracy, it is a crucial factor for the future research of Thysanoptera.

Hence, in chapter II newly invented methods that allow the long-term application of liquid artificial diets to Thysanoptera from the freshly hatched larvae to the adult thrips are described. A method for a time-efficient application to groups and a method for single thrips application were established. With application to single thrips allowing specific observations on individual level, preventing group related influences like cannibalism. Furthermore, these methods provided a clear view of the thrips and the possibility of a daily replenishing of diet and cleaning to prevent the contamination with bacteria or fungi.

Impact of colours on artificial setups

Colours are an important visual cue for insects (Chittka et al., 2014; Lunau, 2014; Stavenga, 2002). However, for Thysanoptera the importance of colours was mostly researched by means of its influence on efficiency of control measure like sticky traps (Brødsgaard, 1989; Broughton & Harrison, 2012; Harman et al., 2007; Pobozniak, 2020). Beside sticky traps, colouring agents were used as tool to dye solutions and proof the uptake by checking thrips gut for this specific coloration (Mautino et al., 2012; Nguyen et al., 2015; Rueda & Shelton, 2003; Shelton et al., 2003). For other insects colours are known to have an influence on the choice of food (Latty & Trueblood, 2020) and oviposition sites (Degen & Städler, 1997; Del Solar, 1979; Markheiser et al., 2018; Panigrahi et al.; 2014). This influence may have additional implication in artificial surroundings by means of an increased acceptance of artificial food sources or oviposition sides.

Therefore, in Chapter III a multiple choice test was conducted testing for a preference of adult female *F. occidentalis* for dyed oviposition sites. Additionally, different dyed diets were tested in a dual-choice test for *F. occidentalis* larvae.

Methods for a standardized application of microorganisms

The research reports about the role of microorganisms and their impact on insects significantly increased in the last decades showing that there is a wide range of interaction between microorganisms and their insect hosts (Douglas, 2015). Furthermore, due to new microbiological techniques microorganisms can be modified allowing new experiments like the RNAi method (Whitten et al., 2016). Oral ingestion is an important way of the transmission of microorganisms, naturally and artificially. Thus, finding a reliable standardized method for an oral application of microorganisms for thrips is a vital step for future experiments. Besides artificial application setups, there can be the necessity to examine naturally occurring phenomena like the horizontal transmission of bacteria like *Wolbachia* (Ahmed et al., 2015). *Wolbachia* infections have been shown in several thrips (Arakaki et al., 2001; Kumm & Moritz, 2008; Nguyen et al., 2015; Saurav et al., 2016), but it has not been found in *F. occidentalis* (Jeyaprakash & Hoy, 2000; Kumm & Moritz, 2008, 2010).

Hence, in Chapter IV a method was established to feed pro-/pupae of a *Wolbachia*-infected population of *E. americanus* to larvae of *F. occidentalis* testing for the occurrence of an interspecific horizontal transmission.

Furthermore, methods for an upside down application of the unicellular algae *Chlamydomonas reinhardtii* to single and groups of thrips was established allowing the application of poorly or non-soluble substances and microorganisms to thrips.

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Chapter II

Application of artificial diets under specific conditions

1 Summary

The application of diets to insects is an important tool for basic insect, pest and agriculture research and for applied science. However, no standardized method for the application of liquid diets over all developmental stages to Thysanopteran species has been described so far. Thus, two methods were established that, for the first time, allow the complete development from egg to adult thrips inside the setup for single thrips (Single micro insect application system - SMIAS) or groups of thrips (Group micro insect application system - GMIAS) through use of artificial diets. As larvae hatch in the setups, the food uptake on the hostplant by the early first instar larva was obviated. While the GMIAS is a method that can be used for an efficient application to groups of thrips, the SMIAS allows to examine thrips development on individual level. Therefore, the SMIAS was used to test six diets (D_{1C}, D_{2C}, D_{2W}, D_{1S}, D_{2S}, D_{3S} ($Diet_{Recipe\text{-}nr|Protein\text{-}source}$)) on *Frankliniella occidentalis* differing in the source of vitamin (D_{1C} – D_{2C}), the concentration of wheat germ and protein (D_{2S} – D_{3S}) and the source of protein (D_{2C} (Casein) – D_{2W} (Wheat peptone) – D_{2S} (Soy peptone)). Four diets allowed thrips to develop to the adult stage (D_{1C}, D_{1S}, D_{2S}, D_{3S}). Fed with those diets, male thrips developed faster than female, a pattern which was not found in the control. In contrast, the diet D_{2C} allowed no development at all and D_{2W} only allowed thrips to develop to the second instar larva. Furthermore, none of the artificial diets allowed thrips to develop as fast as the control reared on *Phaseolus vulgaris*. Nonetheless adult thrips were successfully reared using the previously described diets (D_{1C}, D_{1S}, D_{2S}, D_{3S}). Hence, these methods can be used for applying artificial diets including various substances to thrips and may be a first step to establishing a standardized method that allows a wide range of application experiments and lead to new knowledge about Thysanopteran species.

2 Introduction

Artificial setups for thrips rearing in general

The controlled rearing of insects in artificial setups is fundamental for research purposes. Depending on the aim of a study the complexity of setups can vary widely. In general for insect rearing in artificial setups there are three crucial factors: food, water and an adequate relative humidity. To provide these factors in artificial setups is easily realisable for a lot of insect species. However, things become more complicate when it comes to insects like Thysanoptera species. Their small size makes it more difficult to create an escape-proof surrounding. Furthermore, the necessary relative humidity is based on the microclimate of its typical habitat and varies between species. Larvae of the globally distributed species *Frankliniella occidentalis* that normally feed on the leaves of the host plant, need a relative humidity above 80% (Steiner et al., 2011). Over the last decades several suitable setups and techniques have been invented and established for thrips rearing or testing. The complexity of these various setups differs, depending on their research purpose. For the maintenance of a stock culture or a mass rearing of a species, rearing on host plants in a greenhouse can be performed (Bailey, 1932; Beavers & Ewart, 1971; Rivnay, 1935; Sakimura, 1961; Şengoca & Gerlach, 1983; Tanigoshi & Nishio-Wong, 1981; summarized by Loomans & Murai, 1997). Furthermore, in a smaller sized approach, host plants can be kept in acrylic/plastic cages (Brødsgaard, 1993; van Dijken et al., 1994; Mollema et al., 1990; Nugaliyadde & Heinrichs, 1984; Steiner & Goodwin, 1998). For the regulation of humidity those cages have ventilation apertures which are covered with nylon mesh/gauze to prevent thrips from escaping. However, if a better view upon thrips is vital for observations of developmental stage or impact of specific substances, other setups are necessary.

One approach is the use of a "sandwich cage" (Loomans & Murai, 1997). The basic idea is to have an acrylic glass plate as top and bottom layer while flexible materials like rubber foam can be used as a middle layer and barrier, under which an excised leaf is placed. This cage has been described by Munger (1942) for life-time studies of *Scirthothrips citri* and has been modified by Tashiro (1967) to a self-watering and by Morse et al. (1986) to a ventilated cage. Even though this cage is mostly used for pesticide toxicity tests (Brødsgaard, 1994; Grasselly et al., 1991; Morse et al., 1986), it can be used for a variety of purposes like, virus acquisition tests (Wijkamp & Peters, 1993), for behavioural studies (Loomans et al., 1992) or larval development.

Another approach for rearing thrips on excised leaves uses glass tubes or acrylic glass cylinders. This method contains two units. The glass tube or acrylic cylinder with an excised leave or a whole plant in it, which is kept in place by a flexible material like cotton wool around the stem and cotton wool or gauze at the top of the cylinder/tube for ventilation. The second unit contains

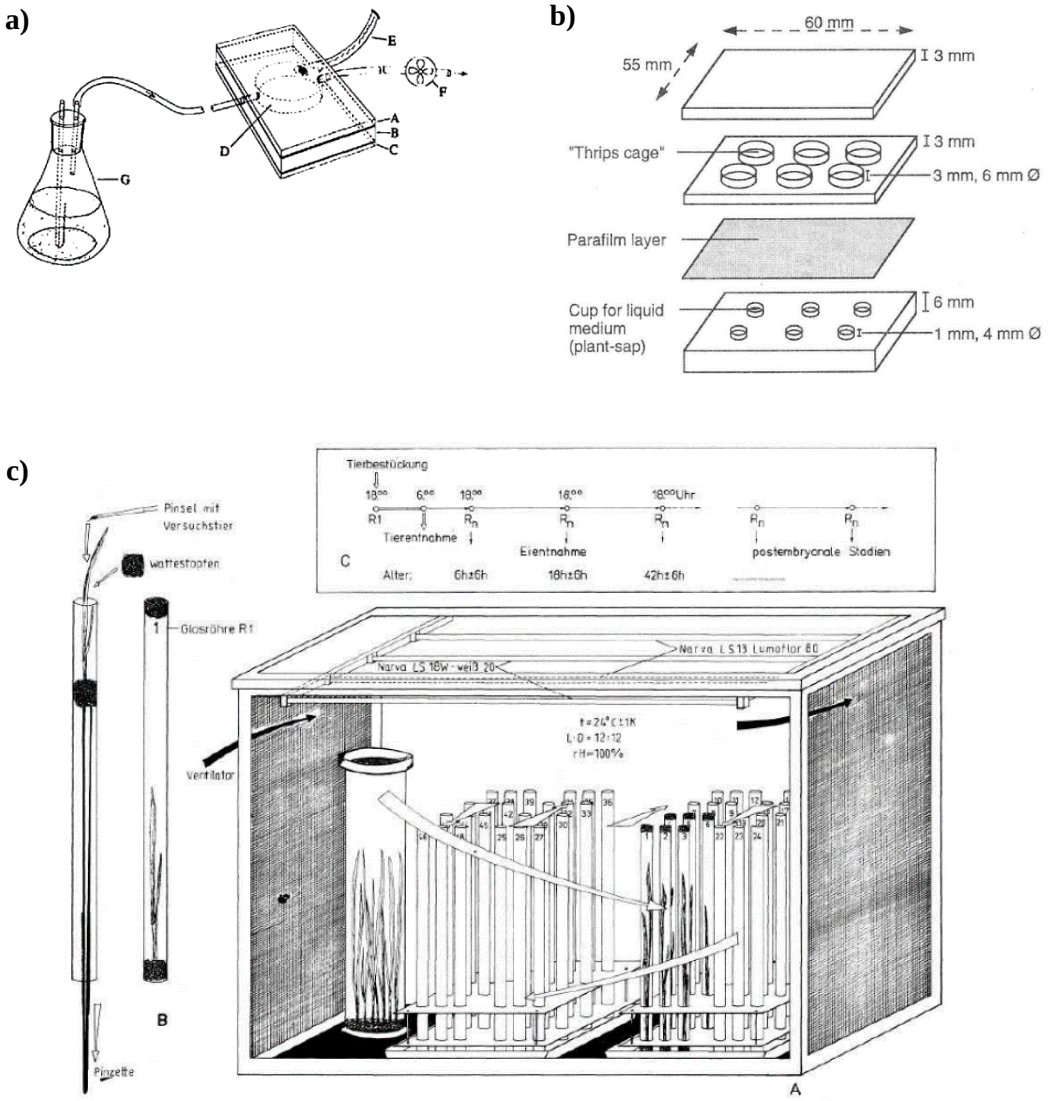


Fig.1: Rearing thrips in different setups. a) "Sandwich Cage" (Brødsgaard, 1994), b) „Sandwich Cage" (de Jager, 1995) c) Glass tube rearing (Moritz, 1988).

water supplying the excised leaf or the plant (Bailey, 1932; Beavers and Ewart, 1971; Koch, 1978; Köppa, 1970; Lewis, 1973; Moritz, 1988; Parella and Horsburgh, 1978; Rivnay, 1935).

Apart from those types of cages that use excised or whole plants, there is the method of using leaf discs floating on water or a nutrient solution in a petri dish or similar container. This method was used for several thrips species (*Caliothrips insulars*, *Selenothrips rubrocinctus*, *Dinurothrips hookeri*, *Heliothrips haemorrhoidalis*, *Selenothrips rubrocinctus* (Callan, 1947), *Aptinothrips*, *Chirothrips* and *Limothrips* (Lewis, 1973)). In later studies on *Scolothrips*, Şengonca and Gerlach (1983) modified this method to the "leaf-island". For this leaf discs with diameter of 25 mm are put on a layer of moist filter paper in a petri dish with a diameter of 55 mm. Ventilation is achieved through a central hole of 25 mm in the petri dish lid, which is covered with gauze. This kind of method was used with some alterations by several authors (Brødsgaard, 1993; Brodeur &

Cloutier, 1992; Doane et al., 1995; Gaum et al., 1994; Hall et al., 1993; Mollema et al., 1990, 1993; Samsøe-Petersen, 1983; Wang & Chu, 1986).

Yet another idea was the separation of the food source and the thrips by using an artificial membrane. The first experiment was conducted by Sakimura and Carter (1934). They used fish skin as membrane to apply 3% sucrose solution to *Thrips tabaci*. Day and Irzykiewicz (1954) used a plastic membrane for *Thrips tabaci* and *Thrips imaginis*. Later Laughlin (1971) used Parafilm for *Hercinothrips femoralis*, *Thrips tabaci* and *Thrips imaginis* as well as *Thrips australis*. Additionally, he demonstrated that female adult thrips (Terebrantia) were capable of laying eggs through the Parafilm using their ovipositor. This method was then used for fecundity studies with pollen as main nutrition source (Kirk, 1985; Kudo, 1971; Teulon & Penman, 1990). A few years later, de Jager (1995) used a setup following the sandwich cage for applying a diet through Parafilm. This was achieved by covering a plastic plate with cups for liquid medium with stretched Parafilm, placing a middle plate with 6 holes on it and finally covering everything with a top plate (see Fig. 1 b).

Artificial diets for Thysanoptera

Together with finding a suitable setup, a diet that allows for full development of the thrips is crucial as well for long-term application experiments. Vanderzant et al. (1962) and Singh (1983) developed formulas that, with minor modifications, are usable for a large variety of insects. Thus, Kinzer et al. (1972) were able to rear *Frankliniella fusca* from larva to the adult stage with a solid artificial diet (modified after Vanderzant et al. (1962). De Jager (1995) used liquid diet modified after Singh (1983) to feed *Frankliniella occidentalis* larvae for 3 days. Another successful composition of a liquid diet was defined by Mittler & Dadd (1962) for the aphid *Myzus persicae*. This specific diet allowed them to have 20 successive generations by only applying that artificial diet through a sealing film (Dadd & Mittler, 1966). Considering this, a new formula was defined that allows thrips of our test species to develop from one day old first instar larva to the adult stage.

Current use of artificial diets

In the last five years artificial diets were used for several purposes. Jones et al. (2017) tested the susceptibility of *Thrips tabaci* to various insecticides. Those were added to a diet of cabbage leaves, which was applied to the thrips. Even RNA can be applied by diet to thrips. This was shown by Singh et al. (2019) who used a sucrose diet to apply RNA to *Thrips tabaci*. Another field of interest in thrips research is the impact of gut bacteria on thrips species where a liquid

diet containing antibiotics is often a key tool used in experiment setups. Van der Koi and Schwander (2014) showed how certain life history traits change due to loss of infection. They put groups of 5 female thrips of 4 *Aptinothrips* species into a 1.5 ml tube together with a paper towel soaked in grass juice containing 50 mg/ml tetracycline. This treatment was able to eliminate *Wolbachia* infection, allowing thelytokous *Aptinothrips rufus* to produce male offspring again. Also, Nguyen et al. (2015) applied a solution of 50% w/v honey (*Apis mellifera*) and distilled water via soaked cotton wool soaked to *Heliothrips haemorrhoidalis*. As antibiotics they added rifampicin and tetracycline hydrochloride in different concentrations. By also using neutral red in the diet solution they were able to see that females ingested the diet. However, there are no publications using a defined diet that allows the thrips to develop fully, therefore making it impossible to apply substances over a complete life time. Furthermore, there is no method described for single thrips rearing even though it would be useful and necessary to observe the impact of substances on single thrips.

Aim of the study

As literature shows artificial diets for Thysanoptera are mostly used incidental as a tool for applying substances via barely defined diets which are then applied by various methods. Thus, establishing a standardized application method and a defined diet that can be used in any experimental setup could have a huge impact on the research field of Thysanoptera. Hence, an artificial diet together with specific setups for thrips rearing under constant conditions were defined, which enables a standardized application of substances over the whole lifecycle of thrips.

To have setups that may be used in wide range of experiments, a well-defined artificial diet was established allowing development from larval hatch to adult thrips and a setup that allows rearing of single thrips and groups of thrips with the following characteristics:

- Ventilation to set a specific rel. humidity

- Size as small as possible for an easy localisation of thrips and as big as necessary to prevent negative side effects

- A clear vision upon the thrips for observation

- Simple, reliable, daily exchange of the diet

- Escape proof/little to no handling of thrips

3 Material and methods

3.1 Thrips rearing

The main population of *Frankliniella occidentalis* is reared in acrylic glass cages (50 cm x 50 cm x 50 cm) on French bean (*Phaseolus vulgaris* L.) and chrysanthemum (*Chrysanthemum* spec.) (T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8 , light on: 6:00 AM MET)). Identification of few random adult females was performed with several adults from the main population using prepared slides and the identification software „ThripsID – Pest thrips of the world." (Moritz et al. 2004).

3.2 Application of artificial diets to separated thrips – *Single Micro Insect Application System* (SMIAS)

Artificial diet

Considering the formulas used by De Jager (1995), Mittler & Dadd (1962), Singh (1983) and Vanderzant et al. (1962), a new formula was invented (Tab. 1).

Tab. 1: Formula of the used diet.

Ingredient	Quantity	Ingredient	Quantity
dH_2O	100 ml	Wheat germ	1 g
Tween 80	100 mg	Yeast extract	2 g
Lecithin	120 mg	Choline chloride	100 mg
Cholesterol	20 mg	Meso-inositol	100 mg
Linoleic acid methyl ester	100 mg	Ascorbic acid	1 g
Casein	3 g	Sucrose	8 g
Wesson's Salt	2 g	Patent blue	500 mg
Pollen	1 g	KOH/HCl	pH 6

Diet preparation

As a first step dH_2O was filled into a closable bottle and was heated to 55 °C ± 5 K. Then the Tween 80 and the lecithin were given into the dH_2O. Following this, cholesterol and linoleic acid methyl ester were added. The bottle was closed and shaken thoroughly for an even dispersion. Then the addition of Wesson's salt, the casein, yeast extract, cold-rolled (liquid nitrogen) pollen, cold-rolled wheat germ, choline chloride and meso-inositol followed and the pH was set to 6 by using HCl and KOH. This solution was then sterilized by autoclaving. To prevent a Maillard reaction, sucrose as a carbohydrate source was given into the solution after sterilization together

with ascorbic acid and Patent Blue V. Then the diet was aliquoted into 1.5 ml tubes and stored at -20 °C until usage. Diets were used for one day and leftovers were discarded to guarantee a constant quality of the used diets.

Thrips eggs

The rearing setup that should be invented, was supposed to show that it allows the thrips to develop from the egg to the adult stage. Therefore, greater amounts of thrips eggs were necessary. To avoid time consuming preparation of plant material to obtain eggs, an artificial setup was used. For this purpose, modified sandwich cages (see Chapter III, 2.1) were used allowing adult female thrips to oviposit eggs through Parafilm in dH_2O dyed with Quinoline Yellow (0.5%). After oviposition the eggs were collected using a pipette and incubated on agar 1.4% (w/v in dH_2O) in petri dishes.

Setup – Single Micro Insect Application System (SMIAS)

For rearing single thrips from the egg to the adult stage, a method was invented using modified pipette tips and 96-well-pcr-plates. For this 0.1-10 µl pipette tips (PP, colourless, Standard MIKRO, Carl Roth GmbH, Germany) were cut horizontally at 24.5 mm (top to bottom, Fig. 2, 2). Then the modified tip was cut a second time diagonally from the middle bottom to 21.5 mm (Fig. 2, 3). The top of the pipette was sealed with cotton wool, allowing ventilation of the tips inside (Fig. 2, 4). For the placement of the thrips eggs agar-active charcoal pillows were made. Active charcoal (0.8 g) was given in 100 ml hot 0.8% agar (w/v in dH_2O). A 3 µl droplet was placed in the bottom opening of the modified pipette tip (MPT) using a micropipette (0.1-10 µl pipette tip). The active charcoal lowers the gel point of the agar and thus makes it easier to pipette it. Furthermore, the charcoal makes it easier to find the pillow again. For the transfer of 72 h ±12 h old eggs, a thin layer of water was created by carefully pouring dH_2O on the previous incubated eggs allowing the transfer of eggs with a pipette. Then eggs with embryos in the red eye stadium were transferred from the incubation-agar-petri-dish to the agar pillow in the MPT using a micropipette (1-20 µl) (Fig. 2, 4). Depending on rel. humidity the pillows dried out and were rehydrated using dH_2O. Afterwards the bottom opening was sealed with two times stretched sealing film (Parafilm ®, Bemis, USA). For attaching the Parafilm to the MPT, the Parafilm was stretched to one side and wrapped around the bottom part with one layer. Then the Parafilm was pressed firmly onto the tip part in front of the opening. The lose part of the Parafilm was stretched away from the tip and firmly pressed on the tip opening. Next, a 96-well-pcr-plate was prepared by piercing a small hole to each well for pressure compensation (Fig. 2, c). Then the outer wells were filled with 20 µl of diet (Fig. 2, 5) and the MPTs are gently put into the wells until they are

stuck (Fig. 2, 6). To ensure identical light conditions only the outer wells of the 96-well-pcr-plate were used (Fig. 2, 7). Then black, opaque duct tape was attached to a lid of a 12 well-cell-culture-plate and the 96-well-plate was then placed on the lid. Thus, just light from the sides was shining in to the setup allowing the thrips to choose between different light regimes in the setup. Afterwards, the setup was stored in a climatic chamber (T: 23 °C ± 1 K, rel. humidity: 80%, LD = 16:8, light on: 6:00 AM MET). The lower part of the MPT was cleaned on a daily basis. For the cleaning the sealing film was removed. Thrips tended to stay at the opening of the MPT. Gently pushing the thrips with a brush led to it running upwards. Afterwards a dH_2O soaked brush was held against the opening. Capillary force soaked the dH_2O upwards the

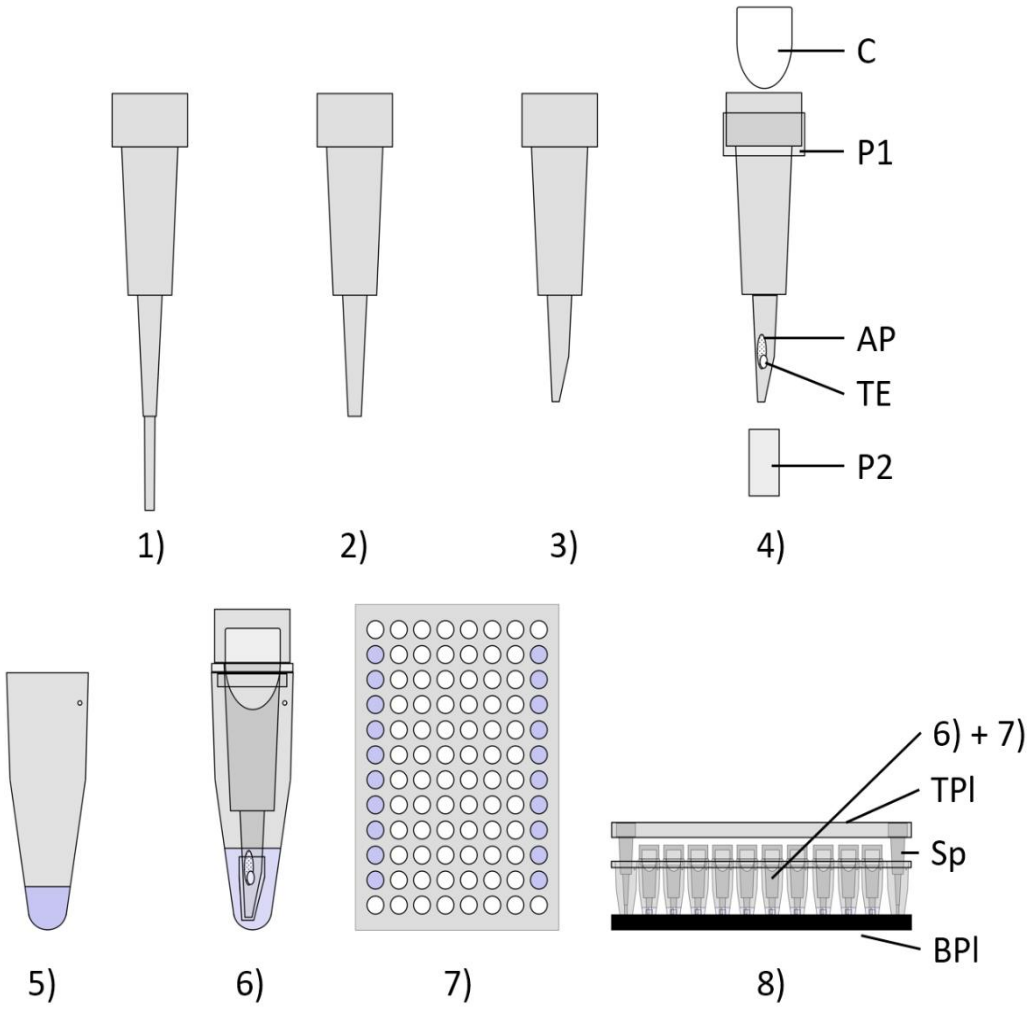


Fig. 2: Preparation of the Single Micro Insect Application System (SMIAS). 1) Original pipette tip (0.1-10 µl). 2) The pipette tip is cut horizontally at 24.5 mm (top to bottom). 3) The pipette tip is cut a second time diagonally from the middle of the lower end to 21.5 mm (top to bottom). 4) Cotton wool (C) is pushed into the tip. Parafilm (P1) is wrapped around the tip part which connects with the upper part of a PCR-well. An agar pillow is placed inside the tip and a 4 day old *Frankliniella occidentalis* egg is placed on it. Then the bottom part of the tip is closed using a double stretched Parafilm. 5) PCR-wells are punctured to allow pressure compensation and are then filled with 20 µl of diet. 6) The prepared pipette tip (4) is placed into the PCR-well (5). 7) Top view of a 96-well-pcr-plate. Light blue marked wells show the positioning of prepared tips. 8) Assembled SMIAS. As a top plate (TPl) a lid of 12-well-cellplate is used to cover the prepared tips with non-modified pipette tips (SP) as spacers. Black duct tape is attached to a second lid, which is then used as bottom plate (BPl).

MPT. Then the MPT was placed on a towel, so that the dH_2O was soaked out of the MPT. The same procedure was carried out with EtOH 70% and then with dH_2O again. Then a new layer of Parafilm was placed on the opening. Thus, the lower inside of the MPT was sterilized every day. While doing this, the position of the thrips was checked regularly to prevent escaping or damaging the thrips. After this procedure, new 96-well-pcr-plates are filled with fresh diet. The MPTs were then put into the wells and the setup was placed into the climatic chamber. Thrips were checked on a daily basis for developmental stage and survival for a maximum of 23 days. For the valuation of the method, hatch rate, loss due to incorrect preparation and loss due to handling were recorded. For this experiment, three replications with each 20 thrips per diet were conducted.

Setup – oviposition attachment for the Single Micro Insect Application System (SMIAS-O)

If male and female adult thrips occurred, they were allowed to mate on Parafilm placed on 1.4% agar in a well of a 12-well-Greiner-plate. Female adult thrips were placed into new MPTs and an oviposition container (OC) was placed on top of the MPT instead of cotton wool (Fig. 3, 2). Then the MPT was placed into a PCR-well filled with diet (Fig. 3, 4), the OC was filled with dH_2O dyed with Quinoline Yellow (0.5%) and sealed with double stretched Parafilm. As control 20 adult thrips raised on French bean were placed in the MPT together with pollen before sealing it and the PCR-wells were filled with dH_2O. The SMIAS-O was then placed in a climatic chamber (T: 23 °C ± 1 K, rel. humidity: 80%, LD = 16:8, light on: 6:00 AM MET) and the number of eggs was checked every day for four days.

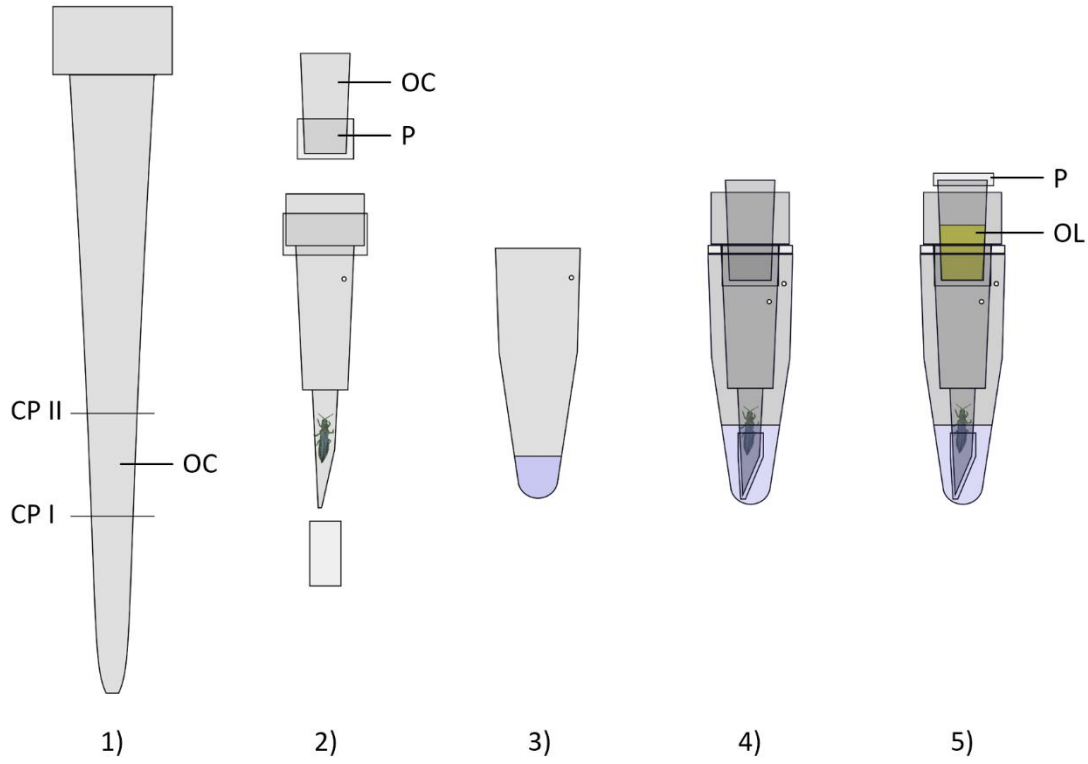


Fig. 3: Preparation of an oviposition attachment for the Single Micro Insect Application System (SMIAS-O). 1) A pipette tip (100-1000 µl) is cut at 37 mm (top to bottom, CP I) and at 25 mm (top to bottom, CP II). The part that is cut out is used as oviposition container (OC). 2) The OC is covered with a double stretched layer of Parafilm and placed into the top of a modified pipette tip (Fig. 2, 4)). After placing an adult female into the MPT the bottom part is sealed with a double stretched Parafilm. 3) A PCR-well is punctured to allow pressure compensation and is then filled with 20 µl of diet. 4) The prepared tip is placed into the PCR-well. 5) An oviposition liquid (dyed dH_2O) is filled into the OC and the OC is sealed with double stretched Parafilm (P).

3.3 Application of artificial diets to groups of thrips – *Group Micro Insect Application System* (GMIAS)

Artificial diet

See 3.2.

Diet preparation

See 3.2.

Thrips eggs

See 3.2.

Setup – Group Micro Insect Application System (GMIAS)

For the group rearing of thrips with artificial diets a specific type of sandwich cage was invented, in which the artificial diet is applied via removable containers. Acrylic plates were used as top and bottom layer and foam rubber (with a cut out) was used as middle layer. Two big holes (11 mm diameter) for the diet containers and 4 ventilation holes (4 mm diameter) were drilled in the bottom acrylic plate (Fig. 3, c).

The ventilation holes were covered with gauze. As containers 1.5 ml tubes were used and cut at the 400 µl mark. Then the now open side of the tube was covered with two times stretched Parafilm. Afterwards a ring of Parafilm was wrapped around the tube to close possible gaps between the acrylic glass and the tube. The cap of the tube was pierced with a needle to allow pressure differences to be compensated.

Then 30 thrips eggs were placed on a layer of 1.4% agar on the bottom plate. The foam rubber and top plate were put on the bottom plate and were kept together by binder clips. The ventilation holes were covered with Parafilm to keep the agar moist. One of the containers was filled with artificial diet and closed with the regular cap. After closing, the hole in the cap was sealed using Parafilm to prevent evaporation. Then the cage was placed in a climatic chamber (T: 23 °C ± 1 K, rel. humidity: 80%, LD = 16:8, light on: 6:00 AM MET).

The next day the empty container was filled with artificial diet while the other container was emptied and left empty overnight. That way thrips larvae will migrate from one container to the other. The next day the empty container was exchanged with a newly prepared container. This container was then filled with diet and the other container was emptied.

This rotation was done every day to remove containers with feces on its Parafilm. Larvae were checked for their developmental stage and survival on a daily basis. Furthermore, for the valuation of the method hatch rate, loss due to incorrect preparation and loss due to handling were recorded. For this experiment, three replications with each 20 thrips were conducted.

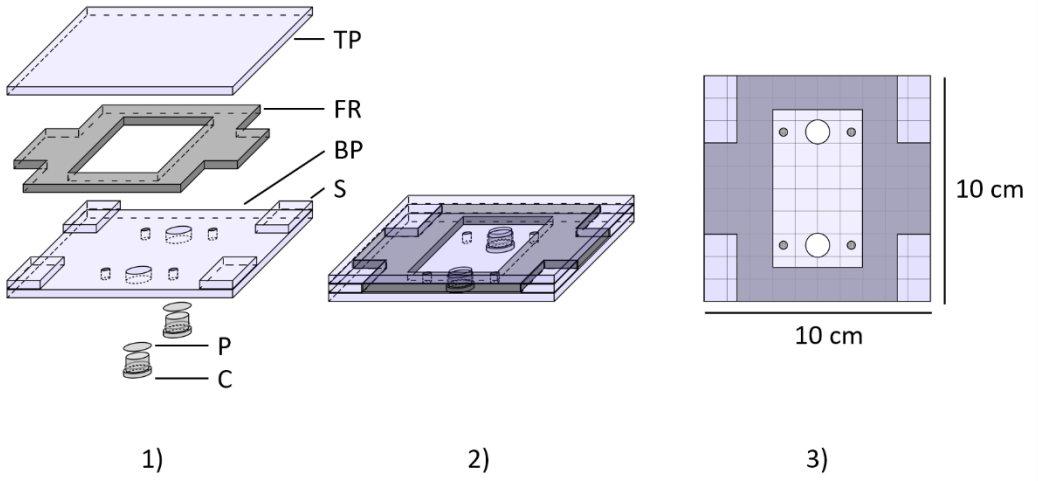


Fig. 4: Preparation of the Group Micro Insect Application System (GMIAS). 1) Exploded view. The top plate (TP) is placed on a layer of foam rubber (FR, 5 mm) and the bottom plat (BP). Spacers (S, 4 mm) are attached to the bottom plate to prevent the foam rubber from getting compressed. Containers (C) sealed with double stretched Parafilm (P) are put into the bottom plate. 2) Assembled GMIAS. To keep the GMIAS assembled book clips are placed at every spacer (not shown) 3) Top view. The plate is modified by drilling two holes (11 mm diameter) in the centre for the positioning of containers. Four small holes (4 mm diameter) are drilled and covered with gauze for ventilation.

3.4 Application of various artificial diets to thrips using SMIAS

Artificial diet

To investigate if various protein sources have different impacts on thrips development, soy peptone, wheat peptone and casein were used as protein source. For this experiment, the formula of the diet used in 3.1 was changed by removing yeast extract and adding Vanderzant's vitamin mixture after autoclaving. As a reference, the results of the diet of chapter 3.1 were used.

Diet abbreviations

Casein containing diet, first formula (3.2) – D_{1C}

Casein containing diet, second formula (3.4) – D_{2C}

Soy peptone containing diet, second formula (3.4) – D_{1S}

Soy peptone containing diet, second formula (3.4) – D_{2S}

Soy peptone containing diet, third formula (3.4) – D_{3S}

Wheat peptone containing diet, second formula (3.4) – D_{2W}

Tab. 2: Formula of the diets D_{2X}.

Ingredient	Quantity	Ingredient	Quantity
dH_2O	100 ml	Wheat germ	1 g
Tween 80	100 mg	Choline chloride	100 mg
Lecithin	120 mg	Meso-inositol	100 mg
Cholesterol	20 mg	Ascorbic acid	1 g
Linoleic acid methyl ester	100 mg	Vanderzant's vitamin mixture	2 g
Protein source	3 g	Sucrose	8 g
Wesson's salt	2 g	Patent blue	500 mg
Pollen	1 g	KOH/HCl	pH 6

Tab. 3: Formula of the diets D_{3S}.

Ingredient	Quantity	Ingredient	Quantity
dH_2O	100 ml	Choline chloride	100 mg
Tween 80	100 mg	Meso-inositol	100 mg
Lecithin	120 mg	Ascorbic acid	1 g
Cholesterol	20 mg	Vanderzant's vitamin mixture	2 g
Linoleic acid methyl ester	100 mg	Sucrose	8 g
Protein source	4 g	Patent blue	500 mg
Wesson's salt	2 g	KOH/HCl	pH 6
Wheat germ	4 g		

Tab. 4: Differences of the diet formulas.

Ingredient	Formula 1	Formula 2	Formula 3
Yeast extract	2 %	-	-
Protein source	3 %	3 %	4 %
Vanderzant's vitamin mixture	-	2 %	2 %
Pollen	1 %	1 %	-
Wheat germ	1 %	1 %	4 %

Diet preparation

The first solution of diet that is sterilized by autoclaving was prepared as in Chapter 3.1. After sterilization it was put together with Vanderzant's vitamin mixture, ascorbic acid and Patent Blue V. Then the diet was aliquoted into 1.5 ml tubes, stored at -20 °C until usage. Diets were used for one day and leftovers were discarded to guarantee constant quality of the used diets.

Thrips eggs

See 3.2.

Setup – Single Micro Insect Application System (SMIAS)

For the setup preparation see 3.2.

Thrips development and survival was controlled every day. If adult thrips occurred, they were placed in MPTs prepared for oviposition (Fig. 3). When male and female adults were available they were allowed to mate on a layer of Parafilm and were transferred to MPTS (see 3.2).

As control and reference for the developmental time on a host plant, single thrips were reared from the egg to adult on leaf discs of French bean in 12 well-cell-culture-plates (see 3.1). For this, 12-well-Greiner-plates (Greiner, CELLSTAR® multiwell culture plates, 12 wells) were filled with 1.4% agar (dH_2O, w/v) and French bean leaf discs (13 mm diameter) were placed on the agar. Then an agar-pillow was put on the leaf disc and one egg was placed on it. The Greiner-plates were closed with glass lids and sealed with Parafilm. The plates were transferred and incubated in the climatic chamber (Sanyo). The developmental stage of every thrips was documented every 24 h. For this experiment, three replications with each 20 thrips per diet were conducted.

3.5 Statistical analysis

Survival analysis

For the statistical analysis of survival time of the preadult thrips the Kaplan-Meier-Survival-Analysis (WinStat® (R. Fitch Software, Bad Krozingen)) was used. The preadult survival of the thrips fed with the different diets (D_{1C}, D_{2C}, D_{2W}, D_{1S}, D_{2S}, D_{3S}) was analyzed. For the analysis of the six groups Bonferroni correction was used ($p< (1- \alpha)/n$, ($n=6$, $\alpha=0.95$, $P< 0.0083$). Thrips that died due to handling or as a result of incorrect set up preparation, thrips that escaped or thrips that emerged to the imago were considered as error.

Development time

The developmental time of thrips reared using the SMIAS with different diets was analysed using WinStat®. Only thrips reaching the adult stage were used for the analysis. First data showed differences between male and female development in one group (same diet). Therefore, both sexes were analysed in one group for significant differences and then one sex of a group was analysed with the same sex of the control group. The data was tested for normal distribution using Shapiro-Wilk-Test. In normally distributed data cases, the t-test was used. If the data was not normally distributed, it was analysed with the U-Test (Mann-Whitney). As for each diet the two sexes of thrips fed with diet and the two sexes of the control were analysed, Bonferroni correction was performed ($p< (1- \alpha)/n$, ($n=4$, $\alpha=0.95$, $P< 0.0125$).

To screen for significant differences between the diets the same procedure was performed with thrips of the same sex of the different diets (Females D_{1C}, D_{1S}, D_{2S}, D_{3S}; Males D_{1C}, D_{1S}, D_{2S}, D_{3S}) and Bonferroni correction was used ($p< (1- \alpha)/n$, ($n=4$, $\alpha=0.95$, $P< 0.0125$).

4 Results

4.1 SMIAS & GMIAS setups

Single Micro Insect Application System (SMIAS)

The new established method allowed thrips to hatch inside of the modified pipette tips. Due to the restricted area for thrips locomotion and the transparent pipette thrips were easily to spot (Fig. 5a) and could be checked for their developmental stage quickly as their exuviae are very prominent in that surrounding (Fig. 5b). After one day of application the amount of faeces produced by one thrips were visible (Fig. 5c) as proof of the intake of diets by the thrips.

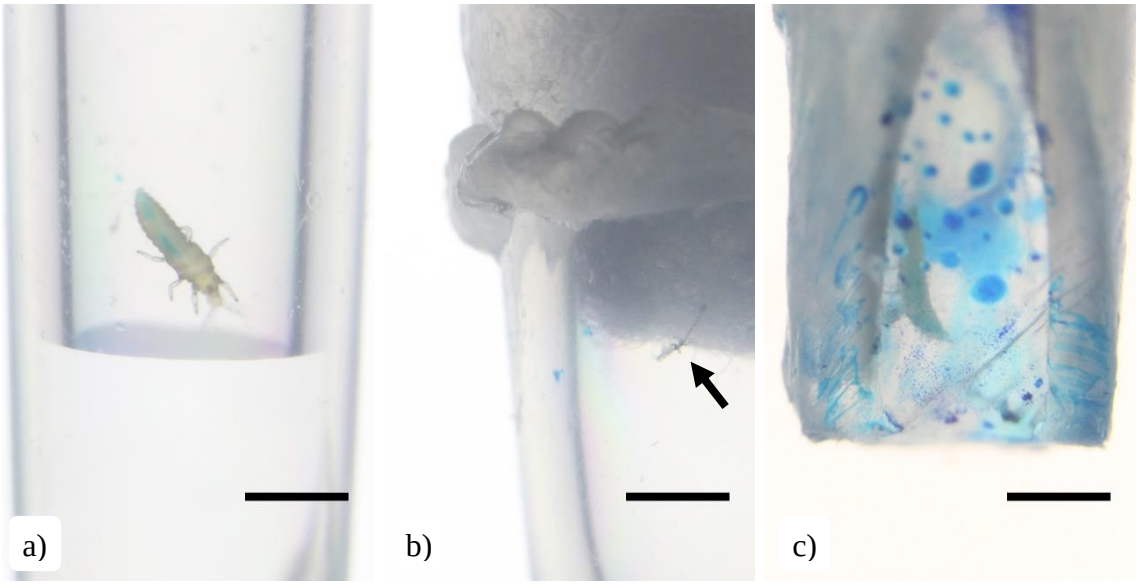


Fig. 5: SMIAS. a) Thrips larva inside the modified pipette tip. Distilled water is used to prevent it from escaping through the bottom opening. **b)** Prominently visible exuvia (arrow) close to the cotton wool plug. **c)** Bottom opening the modified pipette tip sealed with Parafilm after one day of application with larva observable through the Parafilm. Larva's feces (blue droplets) indicate the uptake of the diet. Bar: 500 µm.

Group Micro Insect Application System (GMIAS)

In this setup 90% of thrips successfully hatched inside the setup. Thrips were easy to spot and developmental stage of the thrips was easily identified by thrips morphology. The daily exchange of containers was simple to execute and almost all thrips swapped from the old empty container to the new filled container after one day, so that no thrips escaped or were harmed during the exchange process.

Thrips fed with the diet D_{1C} were able to develop to the adult stage (12%). While the mortality was low in the first four days (8.3%) it increased till day 10 (70.5%) (Fig. 6a). Aggressive behaviour (piercing other thrips) and cannibalism was observed especially for the second instar larvae. Only the cuticle of dead thrips were found.

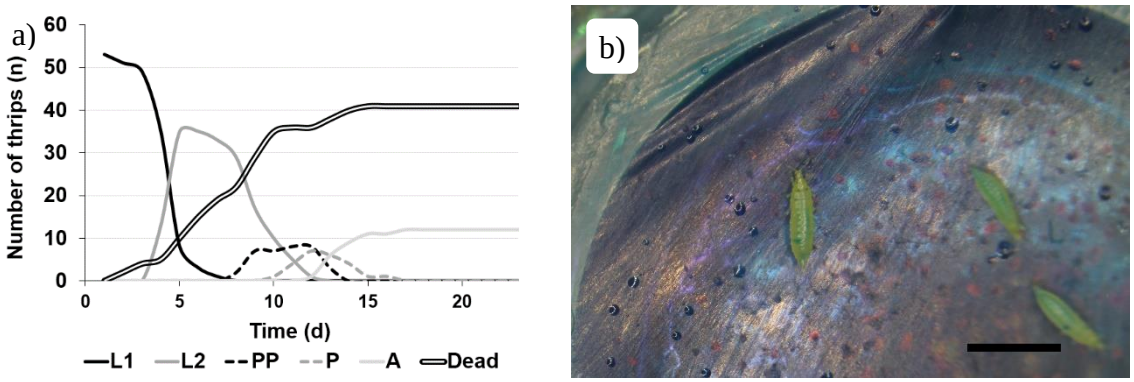


Fig. 6: Development of thrips fed with in the GMIAS. a) Number of thrips in different developmental stages. **b)** Second instar larvae feeding on the Parafilm layer of a container. Bar: 1 mm.
L1 First instar larva, **L2** Second instar larva, **PP** Propupa, **P** Pupa, **A** Adult.

4.2 Application of different diets using SMIAS

D_{1C}

Thrips fed with the diet D_{1C} were able to develop to the adult stage. The development time of male (D_{1C}M) and female (D_{1C}F) thrips showed a significant difference (Fig. 7b, Mann-Whitney U-test, U = -3.27, P =0.001). This significant difference did not occur in the control (Fig. 7). Thus, all developmental stages of both sexes were compared. There were no significant differences in the preadult stages between male and female thrips fed with D_{1C}. The development time of each sex and stage was compared with the development time of the same sex and development time of the control (ConF, ConM). Thrips of both sexes fed whith D_{1C} needed significantly more time in every developmental stage compared to the control, besides the propupa (Mann-Whitney U-test/t-Test, P <0.0125) (Fig 7b).

D_{2C}

Thrips fed with the diet D_{2C} showed no development. After six days of application thrips still were small first instar larvae (Fig. 8b). Thrips were found dead frequently after 8 days of application and often appeared shrunken one or two days before death.

D_{2W}

Thrips fed with diet D_{2W} showed no development to the propupal stage. The second instar larvae appeared vital and well fed (Fig. 9b). Mortality increased after 14 days. Twelve second instar larvae survived until the 23th day and showed no sign of being close to propupal stage like stopping to feed.

D_{1S}

Thrips fed with the diet D_{1S} were able to develop to the adult stage. Female thrips ($D_{1S}F$) developed significantly slower compared to male thrips ($D_{1S}M$) (Fig. 10b, Mann-Whitney U-test, U = -4.9, P =9.42E-07). A further significant difference appeared in the second instar larvae (Fig. 10b, Mann-Whitney U-test, U = -4.4, P =1.25E-05). The females showed significantly longer development times in all developmental stages compared to the female control group (Fig. 10b, Mann-Whitney U-test, P <0.0125). The male thrips fed with D_{1S} took significantly longer to develop in the larval stages and in total compared to the male control (Fig. 10b, Mann-Whitney U-test, P <0.0125).

D_{2S}

Thrips reared with the diet D_{2S} developed to the adult stage. In this group there was no significant difference in female ($D_{2S}F$) and male ($D_{2S}M$) total development time (Fig. 11b). However female pupal stage took significantly longer compared to male thrips (Fig. 11b, Mann-Whitney U-test, U = -2.9, P =0.004). Compared to the control female thrips developed significantly slower in the larval stages, the pupal stage and in total (Fig. 11b, Mann-Whitney U-test, P <0.0125). Whereas the male thrips development time only significantly differed in the larval stages and in total (Fig. 11b, Mann-Whitney U-test, P <0.0125). As seen before in the group fed with D_{2W} in this group nine second instar larvae were observed that did not develop till the 23th day.

D_{3S}

The diet D_{3S} allowed thrips fed with it to develop to the adult stage. Male thrips developed significantly faster compared to female thrips (Fig. 12b, Mann-Whitney U-test, U = -5.28, P =1.26E-07). The male thrips' second larval stage (Fig. 12b, Mann-Whitney U-test, U = -4.74, P =2.11E-07) and pupal stage (Fig. 12b, Mann-Whitney U-test, U = -3.64, P =0.0003) were significantly shorter compared to the female thrips. As the only group male thrips fed with D_{3S} development time did not differ significantly in the first larval stage compared to the control. However, significant differences occurred in the second larval stage and in total (Fig. 12b, Mann-Whitney U-test, P <0.0125). Comparing the female thrips with the control, those fed with D_{3S} needed significantly more time in the larval stages, the pupal stage and in total (Fig. 12b, Mann-Whitney U-test, P <0.0125).

Female development - D_{1C}, D_{1S}, D_{2S}, D_{3S}

The total development time of female thrips fed with D_{2S} was significantly longer compared to all other groups (Fig. 13a, Mann-Whitney U-test, P <0.0125). Thrips females fed with D_{1C}

developed significantly faster compared to thrips females fed with D_{1S} (Fig. 13a, Mann-Whitney U-test, U =-2.73, P =0.006).

In the first larval stage the thrips fed with D_{3S} developed significantly faster compared to the other groups, while the thrips fed with D_{2S} took significantly longer to (Fig. 13a, Mann-Whitney U-test, P <0.0125).

In the second larval stage the development time of the thrips fed with D_{2S} was significantly slower compared to the other groups (Fig. 13a, Mann-Whitney U-test, P <0.0125).

In the propupal stage there was no significant difference between the different groups. However, female thrips fed with D_{2S} showed a significantly faster development in the pupal stage compared to the thrips fed with D_{3S} (Fig. 13a, Mann-Whitney U-test, U =-2.84, P =0.0045).

Male development - D_{1C}, D_{1S}, D_{2S}, D_{3S}

The total development time of male thrips fed with D_{2S} was significantly longer, whereas the total development time of thrips fed with D_{3S} was significantly shorter compared to the other groups. (Fig. 13b, Mann-Whitney U-test, P <0.0125). The same effect was found for the first larval stage (Fig. 13b, Mann-Whitney U-test, P <0.0125). Male thrips fed with D_{2S} showed a significantly slower development in the second larval stage compared to the other groups. (Fig. 13b, Mann-Whitney U-test, P <0.0125). In the propupal and pupal stages the developmental times of the different groups did not differ significantly.

Survival - D_{1C}, D_{1S}, D_{2S}, D_{3S}

While the survival probability in the groups fed with diets that supported the development to the adult stage (D_{1C}, D_{1S}, D_{2S}, D_{3S}) was above 0.75 (Fig .14a), the survival probability of thrips fed with D_{2C} and D_{2W} differed significantly (Fig. 14b).

Adult thrips - D_{1C}, D_{1S}, D_{2S}, D_{3S}

Thrips fed with the different diets that allowed the development to the adult stage all showed a colourless phenotype more than three days after the hatch to the adult stage (Fig. 15). However, they were able to mate and showed a normal behaviour and movement. After transferring the adult female thrips to SMIAS-O cages no oviposition could be observed. Females of the control group laid an average of 2.9 eggs per day.

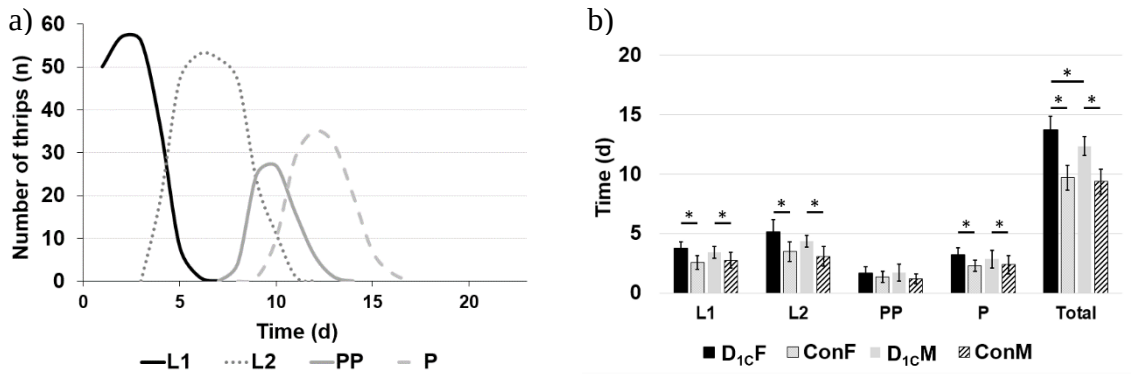


Fig. 7: Development of thrips fed with D_{1C}. a) Number of thrips in different developmental stages. **b)** Average development time in different developmental stages.
L1 First instar larva, **L2** Second instar larva, **PP** Propupa, **P** Pupa.

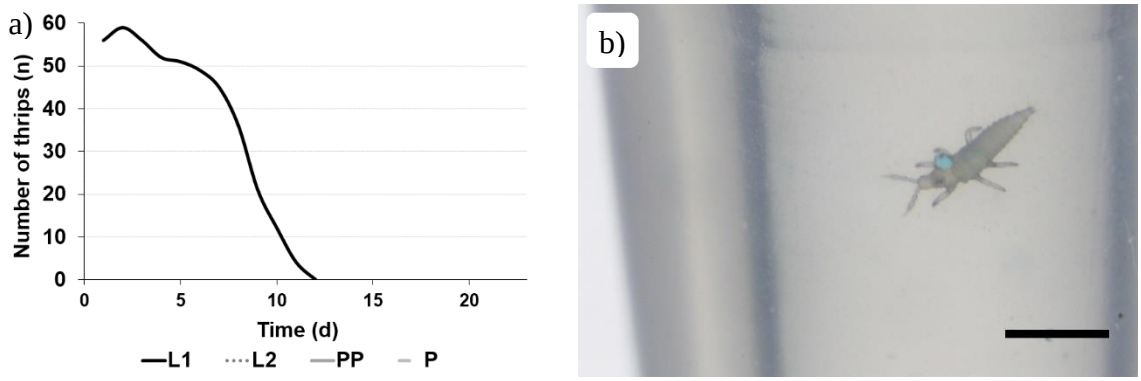


Fig. 8: Development of thrips fed with D_{2C}. a) Number of thrips in different developmental stages. **b)** Thrips larva after 6 days of application. Bar: 500 µm.
L1 First instar larva, **L2** Second instar larva, **PP** Propupa, **P** Pupa.

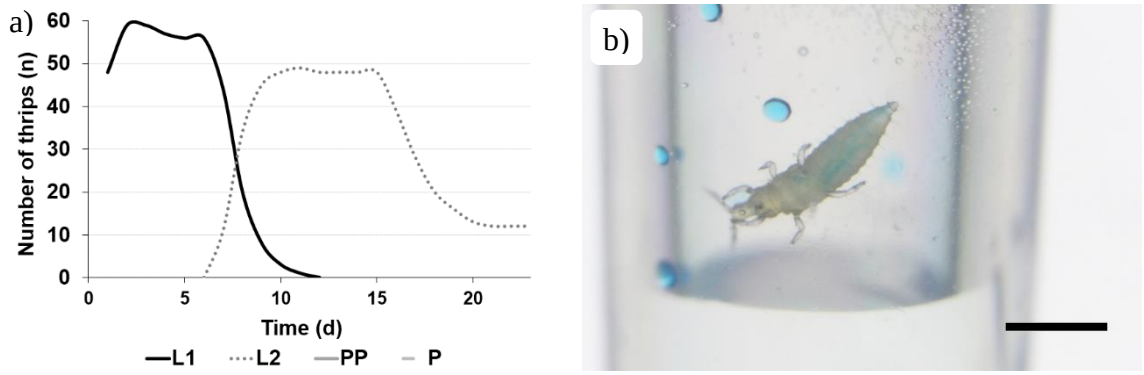


Fig. 9: Development of thrips fed with D_{2W}. a) Number of thrips in different developmental stages. **b)** Thrips larva after 9 days of application. Bar: 500 µm.
L1 First instar larva, **L2** Second instar larva, **PP** Propupa, **P** Pupa.

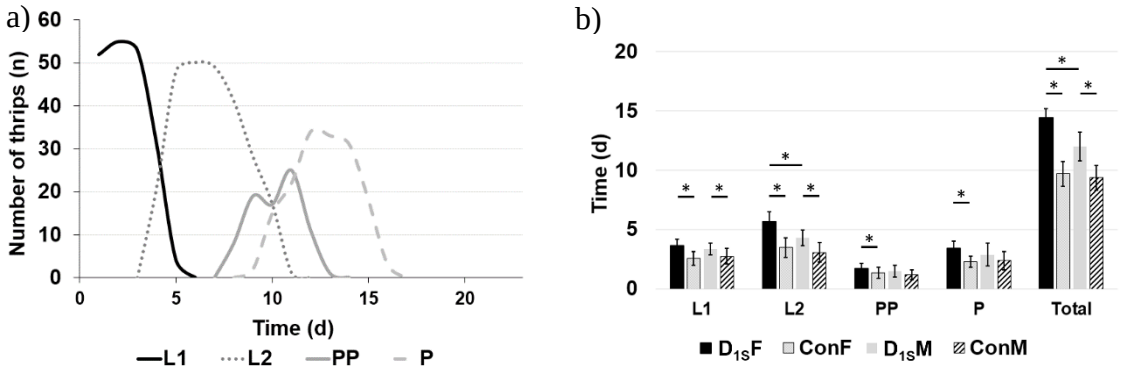


Fig. 10: Development of thrips fed with D_{1S}. a) Number of thrips in different developmental stages. **b)** Average development time in different developmental stages.
L1 First instar larva, **L2** Second instar larva, **PP** Propupa, **P** Pupa.

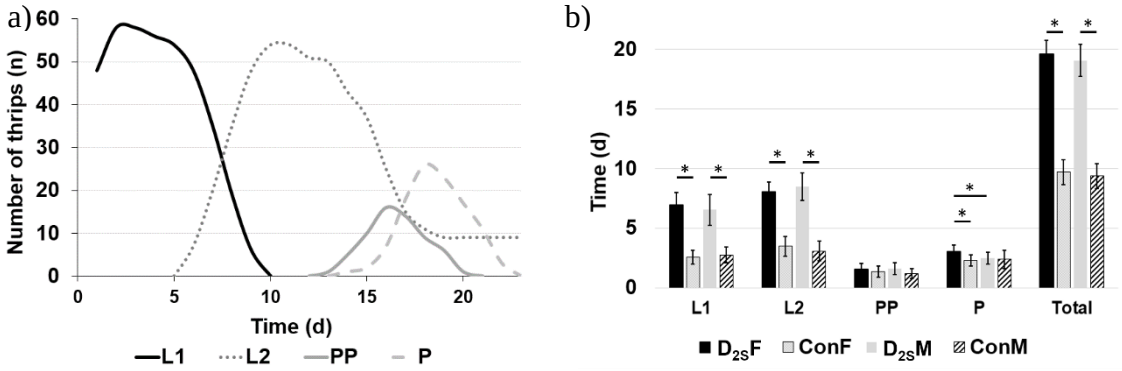


Fig. 11: Development of thrips fed with D_{2S}. a) Number of thrips in different developmental stages. **b)** Average development time in different developmental stages.
L1 First instar larva, **L2** Second instar larva, **PP** Propupa, **P** Pupa.

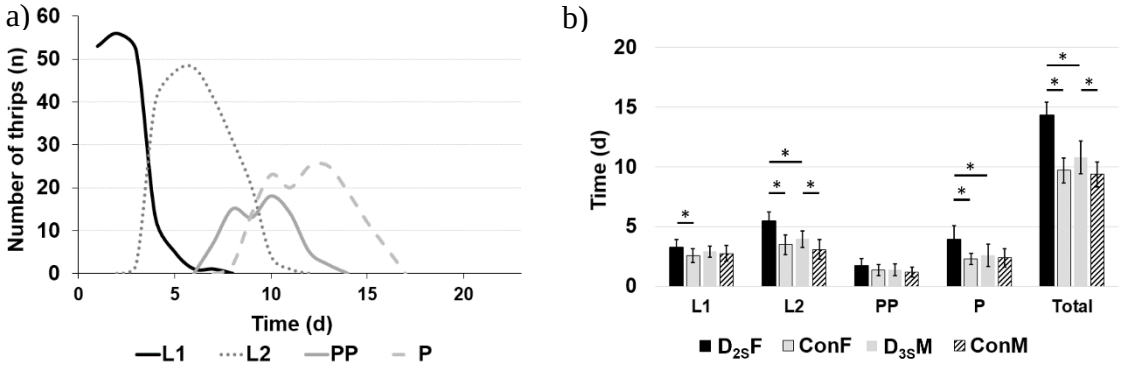


Fig. 12: Development of thrips fed with D_{3S}. a) Number of thrips in different developmental stages. **b)** Average development time in different developmental stages.
L1 First instar larva, **L2** Second instar larva, **PP** Propupa, **P** Pupa.

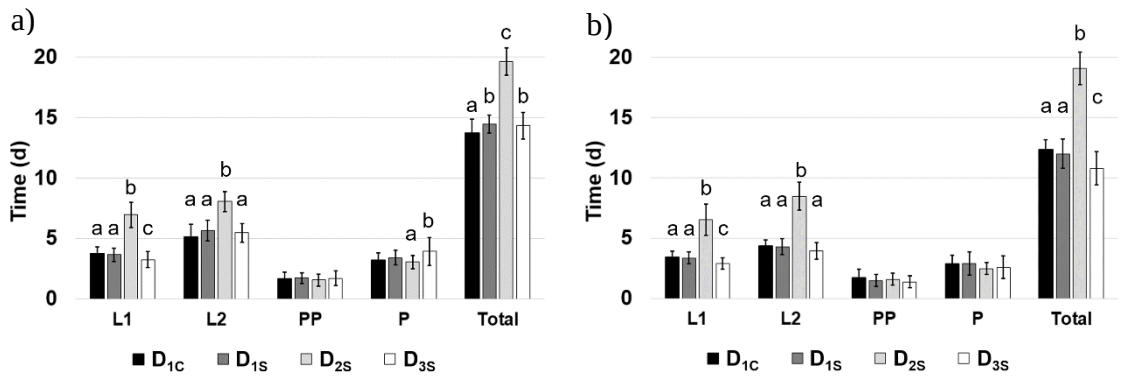
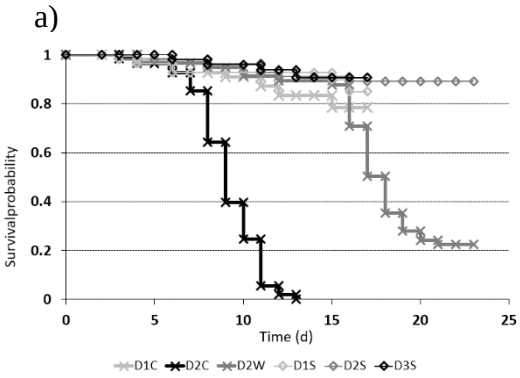


Fig. 13: Average development times of thrips fed with different diets. a) Female thrips. **b)** Male Thrips. Letters indicate for significant differences.
L1 First instar larva, **L2** Second instar larva, **PP** Propupa, **P** Pupa.



b)

	D1C	D2C	D2W	D1S	D2S	D3S
D1C	x	SG	n.s.	n.s.	n.s.	n.s.
D2C	3.64E-20	x	SG	SG	SG	SG
D2W	n.s.	9.19E-23	x	n.s.	SG	n.s.
D1S	n.s.	7.19E-22	n.s.	x	n.s.	n.s.
D2S	n.s.	1.27E-24	7.86E-11	n.s.	x	n.s.
D3S	n.s.	1.79E-22	n.s.	n.s.	n.s.	x

Fig. 14: Survival analysis (Kaplan-Meier). a) Survival probability depending on the applied diet. **b)** Table showing significant differences. **SG** Significant difference

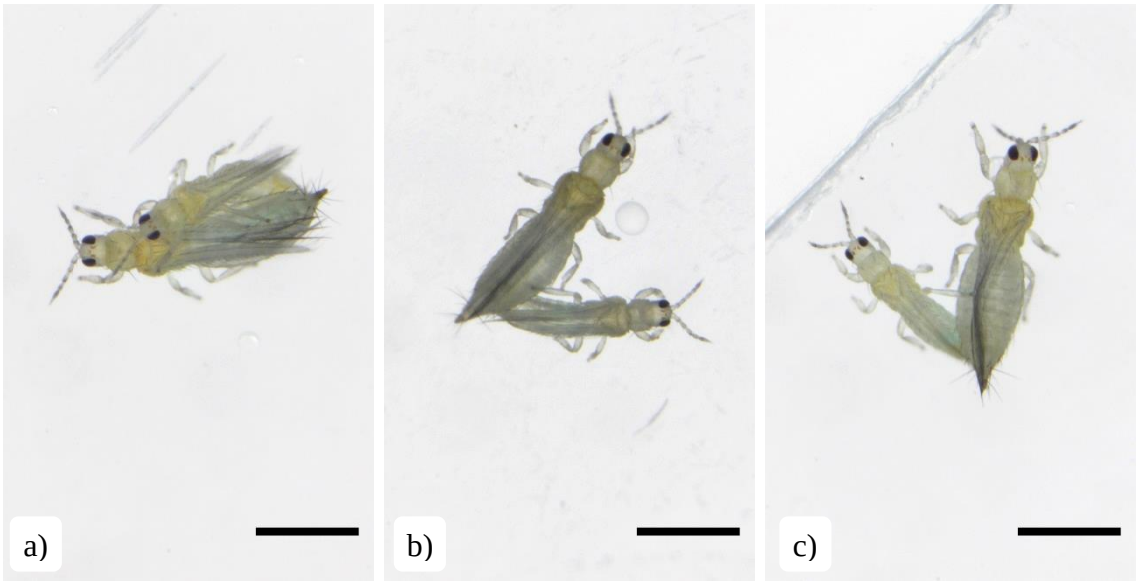


Fig. 15: Mating adult thrips. a) Adult thrips that were raised with D_{1C}. **b)** Adult thrips that were raised with D_{1S}. **c)** Adult thrips that were raised with D_{3S}. Bar: 500 µm.

5 Discussion

SMIAS & GMIAS Setups

The life cycle of thrips was observable in both systems (SMIAS & GMIAS). Both methods showed small numbers in preparation failures and loss due to handling. Furthermore, both methods allowed embryonic development and successful hatching of thrips inside of the setups. Thus, both methods can be considered as reliable for application experiments. So far only Kinzer et al. (1972) described a method to rear thrips from larvae to adult thrips using the tobacco thrips *Frankliniella fusca*. For this method gravid adult females were allowed to oviposit in host plant tissue. The larvae that hatched on that tissue were then shaken into a cup that was filled with a solid wheat germ diet (containing agar, modified after Vanderzant et al., 1962) with no membrane for separating thrips from the diet. The larvae were left in the cup until they emerged to adult thrips. This method was successfully used by Lowry et al. (1992). Another method of applying a fluid diet via an artificial membrane de Jager (1995) used a sandwich-like cage for observing the impact of different diets (modified after Singh, 1983) on *Frankliniella occidentalis* larvae. After gravid adult females were allowed to oviposit in host plant tissue, 0-1 d old first instar larvae coming from those plants tissues were used for the bioassay. Thrips larvae had to be transferred after 2 days as the quality of the medium decreased. In contrast to those methods, both of the methods described here have the advantage that thrips eggs are used to introduce thrips to the setups. Thus, the possibility of first instar larvae feeding on the host plant prior to dietary experiments was eliminated. Furthermore, the diets are renewable with little effort on a daily basis and are separated via an artificial membrane decreasing the risk of contamination or decay of a diet without the necessity of moving thrips. Still, both methods are designed for two different purposes. Referred to number of thrips used the SMIAS is more time consuming than the GMIAS, but allows a precise documentation of thrips life-time-tables on individual level. The GMIAS however allows a time efficient application to large numbers of thrips while still giving the opportunity to monitor thrips for behaviour or some life-time-traits.

GMIAS Development and Mortality

Thrips reared in the GMIAS using the diet D_{1C} developed to the adult stage. Still, just 12% of thrips were able to do so. The mortality started to increase as soon as thrips started to develop to the second instar larva. Aggressive and cannibalistic behaviour was observed when larvae were late especially for second instar larvae. Additionally, only the cuticle of dead thrips could be found showing that cannibalism took place before or after the thrips died. So far cannibalism is only described for *Scolothrips longicornis* (Thysanoptera: Thripidae) (Farazmand et al., 2014). However, despite mostly being referred to as polyphagous species, *Frankliniella occidentalis* has

been described as omnivorous due to feeding on mite eggs by several authors (Agrawal et al., 1999; Martini et al., 2015; Roda et al., 2000; Trichilo & Leigh, 1986). Martini et al. (2015) showed that larvae of *F. occidentalis* show a non-random search behaviour for chemical cues from spider mite webbing, indicating that omnivory might be genetically determined for this species. Additionally, there might be more group related factors like competition or thrips disturbing each other while feeding. To see if those factors have a noticeable impact on survival the density of test thrips could be decreased. This could be done by modifying the GMIAS cage by adding more holes and containers and thus adding more feeding surface or by decreasing the number of tested thrips.

SMIAS-Sex depending difference in development time

In the groups fed with the diets D_{1C}, D_{1S} and D_{3S} female thrips developed significantly slower than male thrips, whereas the control showed no significant difference in development time. Krueger et al. (2016) showed that in *Echinothrips americanus* the development time of thrips of the same sex differed significantly due to the rearing conditions of the mother. Still, no significant difference was found between both sexes with mothers reared under the same condition. Jarosik and Honek (2007) analyzed insects of 132 populations of 122 insect species from 11 orders. They found that the mean effect indicates that males develop significantly faster. For Thysanoptera they showed just a marginally significance ($P < 0.1$). However, it should be noted that the rate of development in Jarosik and Honek's (2007) study as well as in this and Krueger et al.'s (2016) study was analyzed by a control interval of one day, which might be a too long interval for fast developing Thysanopteran species to find a highly significant difference. The data analysis of Teder (2014) also indicates that for species showing sexual size dimorphism, like it occurs in the order of Thysanopteran e.g. *Frankliniella occidentalis*, *Echinothrips americanus*, the larger sex has a prolonged development time, mainly in the larval stages. Thus, the diets that allowed the complete development with a minor delay might be insufficient for the females. As those might have another need for different metabolites compared to males due to their larger size and their different inner organs. Lease and Wolf (2011) analyzed the lipid content of arthropods and were able to show, that female insects generally have a higher lipid content compared to males. Dutkowski (1973) showed that there is a sexual difference in lipolytic activity in *Galleria melonella* (Lepidoptera) with females releasing significantly more free fatty acids compared to males in the pupal stage, supporting the proceeding vitellogenesis in the egg cells. Hence, it might be possible that fatty acids are the limiting factor in the diets, leading to a significantly prolonged development time for the female thrips, as they need more time to accumulate sufficient amounts of fatty acids.

SMIAS-Artificial diets

Using the SMIAS showed that there are significant differences of the development and developmental time between thrips fed with different diets. Thrips fed with D_{1C} developed from first instar larva to adult thrips in 13 days while thrips fed with D_{2C} showed no development at all and a significantly lower survival probability. One major change from the diet D_{1C} to the diet D_{2C} was replacing yeast extract with Vanderzant's vitamin mixture as source of vitamins. As yeast extract additionally contains proteins (about 50% of dry weight) the different effects on thrips might be caused by differences in diet composition in vitamins, amino acids and protein level. However, since Vanderzant's vitamin mixture is especially composed for insects' nutritional needs providing all necessary vitamins it seems unlikely that the change in vitamins lead to the observed differences. Thus, it is more likely that the main impact is caused by the changes in protein/amino acid composition and total protein level between these two diets. Broadway & Duffey (1986) tested the impact of artificial diets with differing concentrations of casein on larval growth of *Heliothis zea* (Lepidoptera: Noctuidae) and *Spodoptera exigua* (Lepidoptera: Noctuidae). They were able to show that growth of *H. zea* larvae was observable for very low concentrations of casein (≤ 0.6%) in the diet, while the optimum growth occurred at 1.2%. For *S. exigua* casein concentrations higher than 0.6% were required for growth and the optimum was found at ≥ 1.2%. Kingsolver and Woods (1998) showed that caterpillars of *Manduca sexta* (Lepidoptera: Sphingidae) had significantly higher consumption and frass production rates on low-protein diets compared with high-protein diets. Simpson and Abisgold (1985) found the same behaviour in *Locusta migratoria* (Orthoptera: Acrididae). When two diets with different protein concentrations (28% and 14% dry weight) were applied, locusts ate more of the low protein concentration diets. But nevertheless the insects fed with the low level protein diet absorbed only 72% of the nitrogen compared to the locusts fed with the high level protein diet. Blanco et al. (2009) used diets differing in their protein concentrations (soybean flour and wheat germ) on *Heliothis virescens* (Lepidoptera: Noctuidae). They were able to show that if the protein concentration were lowered from 2.51% to 2.15% the net reproductive rate of the first generation was significantly lower. Furthermore, in the second generation, the net reproductive rate, development time, percent female survivorship, fertility, intrinsic rate of increase, finite rate of increase and female longevity were significantly lower in both the 2.15% and 2.26% protein diets. For the diet with a protein concentration of 2.05% survival rate of immatures to the adult stage was 1% in the first generation. Hence, thrips might as well be able to compensate minor changes in protein concentration by an increased uptake of the diet while major changes lead to a decrease of life-traits. For more specific conclusions about the impact of protein level further experiments are necessary. However, the consumption and frass production of thrips is difficult to measure (Wiesenborn & Morse, 1985). Thus, feeding thrips a diet of the same composition but with varying concentrations of the same protein source using the SMIAS and examine the impact on development time might help to understand the role of protein level for thrips development.

Furthermore, the results showed that the development and the developmental time differs significantly when using different amino acid sources. While thrips fed with D_{2C} showed no development, thrips fed with D_{2W} developed to the second instar larva but didn't show any further development in 23 d. Thrips fed with D_{2S} however, showed a shorter development time to the second instar larva compared to D_{2W} and developed to the adult thrips in about 19 d. Those findings indicate that besides protein concentration the protein source has a great impact on development of thrips. Cooper and Schal (1992) fed a defined artificial diet with differing protein sources to nymphs of *Blattella germanica* (Blattodea: Ectobiidae). The diet containing milk proteins like casein supported development poorly compared to meat and plant proteins. Soybean protein supported development better than all other highly purified proteins including vitamin-free casein. The results of a food utilization study during the last instar revealed that consumption rates varied between females fed the soybean and casein based diets. However, approximate digestibility, efficiency of conversion of digested food and the efficiency of conversion of ingested food did not vary significantly between the two dietary treatments. Differential development of females fed the two diets was attributed to differences in stage-specific consumption rates and the poorer quality of casein as a source of protein for development in this species. In addition to these findings Lee et al. (2008) showed that the protein sources can have an impact on cuticular melanisation, immune function, survival and growth by feeding a diet containing high-quality casein and another diet containing low-quality zein as protein source to larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae). Larvae fed with the high-quality protein diet showed a higher survival and faster growth compared to larvae fed with the low-quality protein diet. Furthermore, they were able to show that protein-quality had little effect on ingestion rates, but that post-ingestive utilization of nitrogen was reduced for larvae on the low-quality protein diet. Thus, despite the amount of protein uptake, the source of protein seems to be an important factor despite the impact of a single protein source may differ between species. Those findings support the results of our experiment. Furthermore, Ferkovich and Shapiro (2004) examined the effects of diets having proteins and lipids from insect source (extracts from *Plodia interpunctella* (Lepidoptera: Pyralidae) eggs) and non-insect sources (bovine serum albumin, chicken liver, beef liver, and chicken egg albumin) on the oviposition rate of *Orius insidiosus* (Hemiptera: Anthocoridae). The *P. interpunctella* egg diet increased egg production of *O. insidiosus* significantly compared to diets with proteins and lipids from non-insect sources. Those results imply that maybe in general the protein source of a diet should be as close to the natural food as possible. As the host plants *Frankliniella occidentalis* are dicotyledons, this hypothesis would be supported by our results that *F. occidentalis* larvae development was best when soy peptone was used as a single protein source. However, no diet allowed a development time close to the control reared on French bean, *Phaseolus vulgaris*. This may be caused by nutritional differences, but another aspect is the potential uptake of microorganisms found in or

on leave tissue. Gawande et al. (2019) found plant origin endophytes such as Actinomyces, Microbacterium, and Burkholderia in the microbiome of *T. tabaci*.

Furthermore, caution is necessary regarding contamination of the diet with microorganisms. In a preliminary experiment, using an insufficient diet and method for single thrips rearing diet application, it was noticed that there was one thrips larva that reached the second instar larva stage after a few days and appeared well fed, while the rest of thrips (~40) were small first instar larvae. When that second instar larva was observed using a stereomicroscope it was found that a fungal mycelium grew in the thrips' well, through the Parafilm, probably taking resources from the diet. Whereas all other larvae had a blue gut due to the Patent Blue V in the diet, this second instar larva had a transparent appearing gut lumen. Due to this anomalous happening observation of this thrips was continued and it was found feeding on the fungi's hyphae. Feeding on fungal hyphae is known for species of the subfamily Phlaeothripinae (Mound, 2002) but not for Terebrantian species. In awareness that this larva's observed behaviour was a single occurrence under specific conditions, it still has to be emphasized that this happening may underline the potential omnivory of certain thrips species being an additional aspect explaining their great success in spreading worldwide and adapting to new surroundings or hosts.

Adult thrips

All adult thrips raised with the different diets showed no darker coloration and had a pale appearance. Hochkirch et al. (2008) showed that the substrate colour has an influence on the morphology type of two ground-hopper species, *Tetrix subulata* and *Tetrix ceperoi*. Despite that there were shifts in frequency of colour morphs on a specific substrate, still different colour morphs were found on the substrate. Thus, it is unlikely that none of the adult thrips reared in a transparent surrounding showed a colouration. Lee et al. (2008) showed that the dietary quality can have an impact on the cuticular melanisation by feeding casein-supplemented and zein-supplemented diets to *Spodoptera littoralis*. However, since *F. occidentalis* larvae were fed with diets having different amino acid sources (casein, soy peptone) and all adults showed no colouration, secondary plant metabolites may play a role in the melanisation of *F. occidentalis*. Furthermore, the adult females reared and fed with artificial diet did not lay eggs. A finding which was also made by Kinzer et al. (1972) and Lowry et al (1992). Thus, further experiments have to be conducted to find the lacking ingredients that are necessary to allow thrips to oviposit.

Perspective

So far, there has been no method to conduct dietary and application experiments for Thysanopteran species on individual level. For most recent application experiments various methods have been used for short-term (24h – 48h) applications of different active substances

like antibiotics (de Vries et al., 2012; Kumm & Moritz, 2008; van der Kooi & Schwander, 2014), RNA (Singh et al., 2019) and pesticides (Huseth et al., 2016). With the SMIAS, a new method is established. Due to the single rearing, impacts of different diets and substances can be examined on individual level and thus it allows a wide range of new experiments in the fields of basic research as well as in the field of applied science. The GMIAS can be used to rear larger groups of thrips under constant conditions with little effort. Both systems may be established as new standardized methods for single and group application experiments in the studies of Thysanoptera and other sucking-piercing micro insects (Hemiptera).

Furthermore, the establishment of different diets supporting the development from the freshly hatched first instar larvae to the adult thrips for *F. occidentalis*, allows new application experiments of substances over all postembryonic ontogenetic stages and new dietary experiments for a better understanding of Thysanopteran nutritional needs.

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7 Supplementary data

Development time and rates SMIAS (4.1). Overview data show the total number of thrips (n), rates of successful hatching (in relation to n), thrips reaching the different developmental stages (in relation to n) and the loss due to preparation failure.

Tab. 1: Control - Thrips reared on French bean. Overview of experimental data and average duration developmental stages.

Overview		Developmental	Female		Male	
		stage	Mean	SD	Mean	SD
n	60	L1	2.57 d	±0.58 d	2.75 d	±0.66 d
Hatch rate	0.98	L2	3.48 d	±0.83 d	3.06 d	±0.83 d
L2	0.83	PP	1.35 d	±0.48 d	1.19 d	±0.39 d
PP	0.75	P	2.30 d	±0.46 d	2.38 d	±0.78 d
P	0.67	Total	9.70 d	±1.04 d	9.38 d	±1.05 d
A	0.63					
Loss	0.00					

Tab. 2: Thrips fed with D_{1C}. Overview (left) of experimental data and average duration of developmental stages (right).

Overview		Developmental	Female		Male	
		stage	Mean	SD	Mean	SD
n	60	L1	3.75 d	±0.57 d	3.43 d	±0.49 d
Hatch rate	0.97	L2	5.10 d	±1.05 d	4.36 d	±0.48 d
L2	0.88	PP	1.64 d	±0.59 d	1.71 d	±0.70 d
PP	0.80	P	3.18 d	±0.60 d	2.86 d	±0.74 d
P	0.77	Total	13.68 d	±1.17 d	12.36 d	±0.81 d
A	0.70					
Loss	0.08					

Tab. 3: Thrips fed with D_{2W}. Overview (left) of experimental data and average duration of developmental stages (right).

Overview		Developmental	Female		Male	
		stage	Mean	SD	Mean	SD
n	60	L1	7.06 d	±1.18 d	-	-
Hatch rate	0.98	L2	-	-	-	-
L2	0.88	PP	-	-	-	-
PP	-	P	-	-	-	-
P	-	Total	-	-	-	-
A	-					
Loss	0.10					

Tab. 4: Thrips fed with D_{1S}. Overview (left) of experimental data and average duration of developmental stages (right).

Overview		Developmental stage	Female		Male	
			Mean	SD	Mean	SD
n	60	L1	3.66 d	±0.54 d	3.35 d	±0.48 d
Hatch rate	0.95	L2	5.66 d	±0.84 d	4.29 d	±0.67 d
L2	0.87	PP	1.72 d	±0.45 d	1.47 d	±0.50 d
PP	0.83	P	3.41 d	±0.62 d	2.88 d	±0.96 d
P	0.83	Total	14.45 d	±0.72 d	12.00 d	±1.24 d
A	0.77					
Loss	0.10					

Tab. 5: Thrips fed with D_{2S}. Overview (left) of experimental data and average duration of developmental stages (right).

Overview		Developmental stage	Female		Male	
			Mean	SD	Mean	SD
n	60	L1	6.96 d	±1.04 d	6.53 d	±1.31 d
Hatch rate	0.97	L2	8.04 d	±0.81 d	8.47 d	±1.15 d
L2	0.90	PP	1.57 d	±0.50 d	1.60 d	±0.49 d
PP	0.67	P	3.04 d	±0.55 d	2.47 d	±0.50 d
P	0.65	Total	19.61 d	±1.13 d	19.07 d	±1.34 d
A	0.63					
Loss	0.10					

Tab. 6: Thrips fed with D_{3S}. Overview (left) of experimental data and average duration of developmental stages (right).

Overview		Developmental stage	Female		Male	
			Mean	SD	Mean	SD
n	60	L1	3.25 d	±0.66 d	2.89 d	±0.45 d
Hatch rate	0.98	L2	5.46 d	±0.76 d	3.95 d	±0.69 d
L2	0.83	PP	1.71 d	±0.61 d	1.37 d	±0.48 d
PP	0.77	P	3.92 d	±1.15 d	2.58 d	±0.94 d
P	0.75	Total	14.33 d	±1.11 d	10.79 d	±1.36 d
A	0.72					
Loss	0.18					

Chapter III

Impact of specific dyes on artificial applications to thrips

1 Summary

Visual cues can have important effects on insects' behaviour. For the western flower thrips (*Frankliniella occidentalis*) the attractiveness of several colours is known for the use of sticky traps. Due to increasing numbers of pesticide resistances and the environmental risks, alternative control measures for thrips pests are necessary. For the finding or enhancing of new control measures, colour may play an important role. Thus, for a possible control of the offspring, it was investigated, if a preference of *F. occidentalis* adult thrips for coloured oviposition sites and larvae for dyed food sources is observable. For the experiments two variations of GMIAS setups were used. For the oviposition site preference a multiple choice modified GMIAS setup was used that offered four oviposition sites containing a non-dyed (dH_2O) and three differently dyed dH_2O (Quinoline Yellow, Green S, Patent Blue V). Female adult thrips showed a significant preference for placing eggs in dyed solutions instead of the non-dyed control. However, there was no significant difference between the different dyed solutions.

To examine the larvae's behaviour in a food choice experiment a dual choice setup, offering a non-dyed diet and a dyed diet (Quinoline Yellow, Green S, Patent Blue V), was used. The larvae showed a significant preference for the non-dyed food source when tested together with diet dyed with Patent Blue V. There was no preference when testing Quinoline Yellow and Greens S.

2 Introduction

Some species of the order Thysanoptera are economically important pests of agricultural and ornamental plants. Like for many other pest insects the numbers of pesticide resistances increase. For the western flower thrips (*Frankliniella occidentalis*), one of the economically most important pest thrips (Kirk, 2002; Reitz, 2009), several pesticide resistances have been reported for various pesticides (Bielza 2008; Herron & James, 2005; Jensen, 2000). Furthermore, negative impacts of pesticide on non-target insects, environment and public health have been heavily discussed in the recent years. Thus, the finding and invention of new pest control strategies is necessary. A potential strategy is the control of offspring numbers by influencing oviposition and larvae. For this, however, the understanding of a species' biology is crucial. One main approach is the manipulation of insects' behaviour. This is heavily influenced by visual cues and their attractant or repellent effects on insects. For Thysanoptera coloured sticky traps are a common way of using visual cues to attract different species to a glutinous surface (Brødsgaard, 1989; Childers & Brecht, 1996; Teulon & Penman, 1992; Ranamukhaarachchi & Wickramarachchi, 2007). Still, there are a lot of factors besides the coloration which influence the efficiency of those traps (summarized in Hoddle et al., 2002). Another approach of using visual cues is the cultivation of specific host plant breeds, which show a colouration that makes them less attractive to certain thrips species (Bergh & Le Blanc, 1997; Diaz-Montano et al., 2012). To find new methods for thrips pest control in terms of manipulation by visual cues laboratory studies are necessary. Thus, for laboratory studies methods like the Murai cage (Murai & Ishii, 1982), allowing females to oviposit through a layer of Parafilm into a solution to test oviposition rates under different conditions, have been established. However, when this method was used by different authors (de Vries et al., 2006; Gerritsen et al., 2005; Teulon, 1992) the impact of colouration of the oviposition substrate/liquid on the oviposition was not considered. Thus, we wanted to test if the adult thrips of *F. occidentalis* show a preference to a specifically dyed solution for oviposition.

Additionally, another widely used method that often uses colorants is the application of diets containing substances like antibiotics for symbiont or pest interaction research. To proof the uptake by thrips Nguyen et al. (2015) added neutral red as dye to the diet. For pesticide assays Rueda and Shelton (2003), Shelton et al. (2003) and Mautino et al. (2012) used different food colorants for a 10% sucrose feeding solution. Methylene blue was used by Andongma et al. (2020) and Whitten et al. (2016) for a bacteria solution and by de Vries et al. (2001) for an antibiotic solution. However, despite possibly having further implications on finding new control measurements, the impact of coloration in artificial laboratory systems for thrips behaviour was not considered so far and has not been established in a methodically congruent way. Thus we wanted to examine if thrips larvae of *F. occidentalis* show a preference for a specifically colorized diet.

3 Material and methods

3.1 Thrips rearing

The main population of *Frankliniella occidentalis* was reared in acrylic glass cages (50 cm x 50 cm x 50 cm) on French bean (*Phaseolus vulgaris* L.) and chrysanthemum (*Chrysanthemum* spec.) (T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8, light on: 6:00 AM MET). Identification of few random adult females was performed with several adults from the main population using prepared slides and the identification software "ThripsID – Pest thrips of the world." (Moritz et al. 2004).

3.2 Dye preference for oviposition

GMIAS-O

For the experiment modified GMIAS cages (see Chapter II 3.3, GMIAS) were used. Acrylic plates were used as top and bottom layer with foam rubber in between (Fig. 1). The bottom plate was additionally modified by drilling four additional holes (11 mm diameter) into it. For this experiment we used two different types of containers. The containers (1.5 ml reaction tubes cut at the 400 µl mark), which were placed in the outer two positions, were modified by heat-fixing fly screen to it. Then pollen (Blütenpollen, mildlich-süß, Naturprodukte Lembcke GbR, Faulenrost, Germany) as food source was placed into the container on the fly screen. The cap of the container was pierced with a needle for ventilation. Then cotton wool was pressed into the cap forming a dense plug. This cotton plug prevents thrips from escaping through the ventilation hole and it soaks up thrips faeces impeding mould growth on the pollen. The cut sides of the four inner placed containers were covered with double stretched Parafilm. Afterwards a ring of Parafilm was wrapped around the container to close possible gaps between the acrylic glass and the container. Adult female thrips were cooled down on ice. For one cage 25 random aged females were placed on a small piece of filter paper (5 mm x 10 mm). After placing all adults on the paper, it was transferred to the middle of the bottom plate using a moist brush. Then the cage was closed by putting the top plate and the foam rubber (5 mm) on the bottom plate and adjusting four binder clips (32 mm, SBS®, Schlößer Baustoffe GbR, Wittlich, Germany) at the sides where the spacers were placed. The inner containers were filled with dyed dH_2O (dye 0.5% (w/v), dyes: Quinoline Yellow, Patent Blue V, Green S, Control: dH_20) and one was filled with dH_2O as control. The position of each dye in each replica were alternated. Three replicas were conducted. As control two different set ups were used. As no-dye-control all containers were filled with dH_2O and as a dye-control all containers were filled with Quinoline Yellow solution. After filling the containers, the pressure compensation hole in the cap was sealed with a small and thin layer of stretched Parafilm to prevent evaporation. The cages were then placed into a climatic chamber (Sanyo, T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8, light on: 6:00 AM MET). After 24 h the Parafilm on the container caps was removed, the cap was opened and the eggs in every container were

counted, noted and removed with a micropipette (20 µl). Afterwards the rest of the solution was removed and refilled with fresh solution using a pipette. The caps were sealed with Parafilm again and the cages were placed in the climatic chamber.

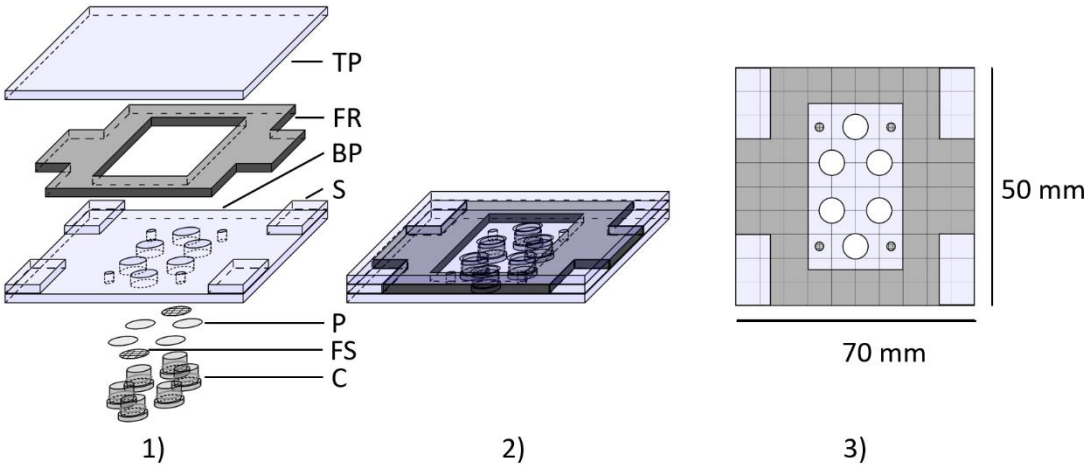

Fig. 1: Preparation of GMIAS-O. A modification of the Group Micro Insect Application System (GMIAS) for oviposition experiments.
1) Exploded view. **2)** Assembled view. **3)** Top view with graphically added 5 mm x 5 mm squares. **TP** Top plate; **FR** Foam rubber; **BP** Bottom plate; **S** Spacer; **P** Parafilm; **FS** Fly screen; **C** Container.

3.3 Dye preference for artificial diets

To test thrips larvae for colour preferences in artificial diets we used small sandwich cages and a self-made camera system, allowing the camera to shoot pictures at eight positions every 15 minutes in a closed, opaque, illuminated box.

GMIAS-S

For the sandwich cages two acrylic glass plates (4 mm, 7 cm x 7cm) and rubber foam were used. For each trial conducted 8 cages were recorded at the same time (see Fig. 3). For the application of diets two holes (8 mm in diameter) were drilled into the bottom plate. Additionally, for the placement on the alignment plate four 5 mm holes were drilled in each corner of the bottom plates (see Fig. 2 and Fig. 3). Twice stretched Parafilm was placed on the central holes on one side of the bottom plate. After placing the diet into the hole, it was sealed with twice stretched Parafilm. A layer of foam rubber (1 mm) with a 33 mm hole in the middle was placed on the bottom plate (Fig 2). *Frankliniella occidentalis* larvae were put on ice on a moist piece of filter paper (5 mm x

5 mm) until eight pieces of filter paper were prepared with 32 random aged larvae. One filter paper was transferred to the middle of one prepared bottom plate and the cage was completed by placing the top plate on the foam rubber and adjusting two fold-back clips (25 mm) at the sides.

Artificial diets and dyes

For this experiment the diet D_{1C} (Chapter II 3.2) without Patent Blue V was used. The diet was boiled and 1% agar and the specific dye was added (Concentration: 0.5%, Dyes: Quinoline Yellow, Patent Blue V, Green S, Control: no dye). Then each diet was aliquoted in 0.65 ml tubes and stored at -20 °C. For each set of trials, a fresh diet was used. The solid diet was removed out of the tube by piercing the end of the tube and gently shaking the diet out of the tube. Then the solid diet cylinder was cut 5 mm in length and placed into one of the central hole of bottom plate. Every dyed diet was tested together with control. Additionally, one trial was conducted using control diet in both holes. For each dye/control and control/control 6 replications with 32 larvae were performed.

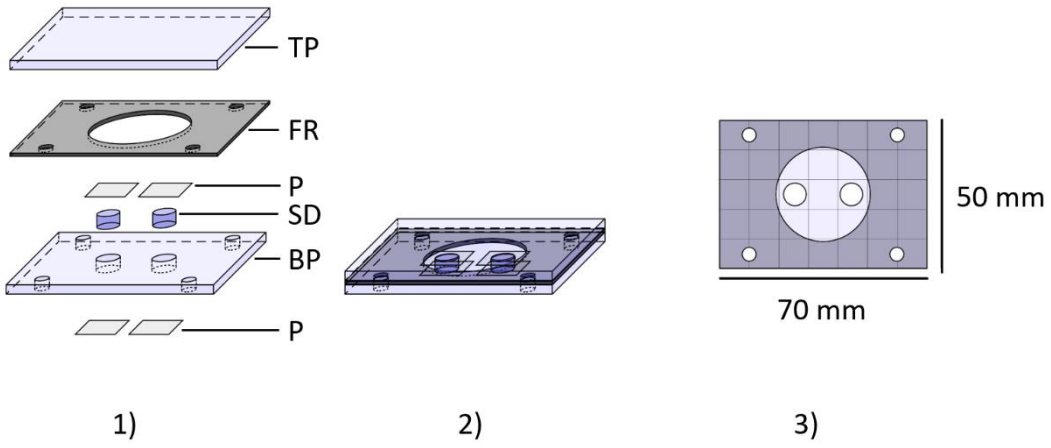

Fig. 2: Preparation of GMIAS-S. A smaller cage following the idea of the Group Micro Insect Application System (GMIAS) for specific application experiments. **1)** Exploded view. **2)** Assembled view. **3)** Top view with graphically added 5 mm x 5 mm squares. **TP** Top plate; **FR** Foam rubber; **BP** Bottom plate; **P** Parafilm; **SD** Solid diet.

Camera system

For the documentation of thrips positioning during the experiment a self-build camera chamber system (thankfully planned, built and programmed by Grit Herter) was used. The walls of the chamber were build using an outer layer of transparent acrylic glass (2 mm), a middle layer of black rubber sheet (1 mm) and an inner layer of white, opaque acrylic glass. The camera (Camera body: Lumix G DMC-GX80KEGK, Panasonic; Lens: H-HS030E Lumix G - 30 mm f/2.8 ASPH, OIS, Panasonic) was moving on an x- and y-axis and was taking pictures on every position (cage) (Fig. 3). To hold the cages in the right position an acrylic glass plate was prepared. Screws (30 mm length) were fixed to the plate fitting to the outer holes of the cage bottom plates (Fig. 2). Pictures were taken every 15 min for 225 min. Afterwards the number of thrips on a feeding area (Parafilm layer with diet under it) was counted on every picture. Thrips larvae were counted when the head was above the feeding area allowing the uptake of diet.

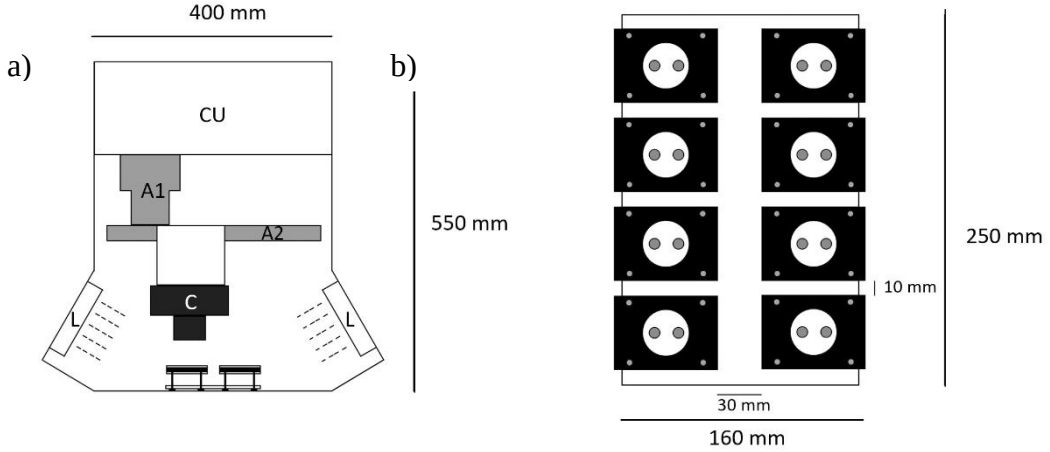

Fig. 3: Camera system for the recording of thrips larvae preference.
a) Camera system and chamber, front view. **b)** Alignment plate with GMIAS-S cages on it. **A1** Axis 1; **A2** Axis 2; **CU** Computing Unit; **C** Camera; **L** Light source.

3.4 Statistics

The data of the trial were sorted by the specific dye (Quinoline Yellow, Patent Blue V, Green S, and Control). For the controls, using only dH_2O and only Quinoline Yellow solution, the data was sorted by the position of each container (1, 2, 3, and 4). The number of egg per day of each group (dye or position) was analysed using the Friedman test (WinStat® (R. Fitch Software, Bad Krozingen, $p<0.05$). When the p-value was below 0.05 the Wilcoxon test was used as post-hoc test. As four groups were analysed Bonferroni correction ($p< (1- \alpha)/n$, ($n=4$, $\alpha=0.95$, $P< 0.0125$) was used.

The data of one dye and its control were analysed using the Wilcoxon test (WinStat®, $P< 0.05$) for every single point of time.

4 Results

4.1 Dye preference for oviposition

The GMIAS-O allowed a time efficient and simple counting and removal of eggs and refilling of the used dyed solutions. Adult thrips can be observed through the top plate, the bottom plate or the Parafilm of opened containers (Fig. 4).

Adult female thrips of *F. occidentalis* laid eggs into all solutions from the first day. The experiment group, where three differently dyed solutions were used (Quinoline Yellow, Patent Blue V, Green S, Control: dH_20) showed that the amount of eggs laid per day in dyed solutions is significantly higher compared to the non-dyed control (Wilcoxon test, P <0.0025, Z= -3.06; Fig. 5a), but no significant difference between the dyed solutions was observed (Wilcoxon test, P >0.28, Z> -1.06). When all containers are filled with the Quinoline Yellow solution (Control I) or with the non-dyed control (Control II), there is no significant difference between the different containers (Fig. 5b, 5c; Control I, Friedman test, P =0.21; Control II, Friedman test, P =0.56). The amount of eggs laid per day and set-up (experiment, control I and control II) showed a significant difference for the first day with the group of non-dyed control laying less eggs compared to the experiment group (Fig. 5d, unpaired t-test, P =0.02) and as well significantly less eggs compared to the control I group with the Quinoline Yellow solution (Fig. 5d, unpaired t-test, P =0.0001). The number of eggs laid at day 2, 3 and 4 were not significantly different comparing the groups.

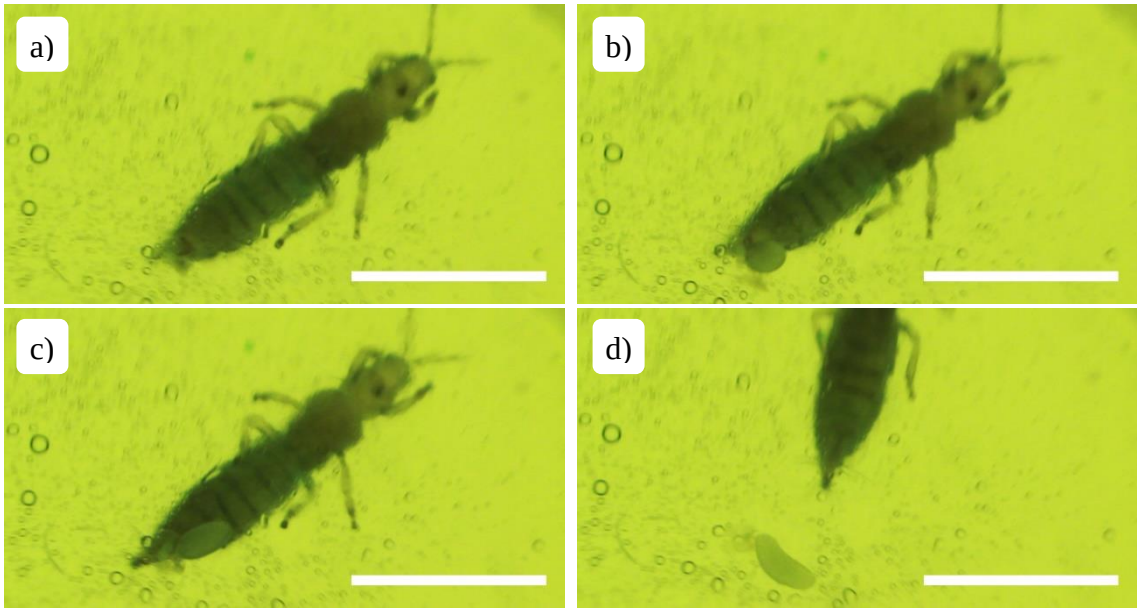

Fig. 4: Oviposition in GMIAS-O.
a) The ovipositor is pierced through the Parafilm of a container filled with dyed solution (Quinoline Yellow). **b)** The egg is pushed through the ovipositor. **c)** The egg is placed into the dyed solution. **d)** The female leaves after successful oviposition. Bar: 750 µm

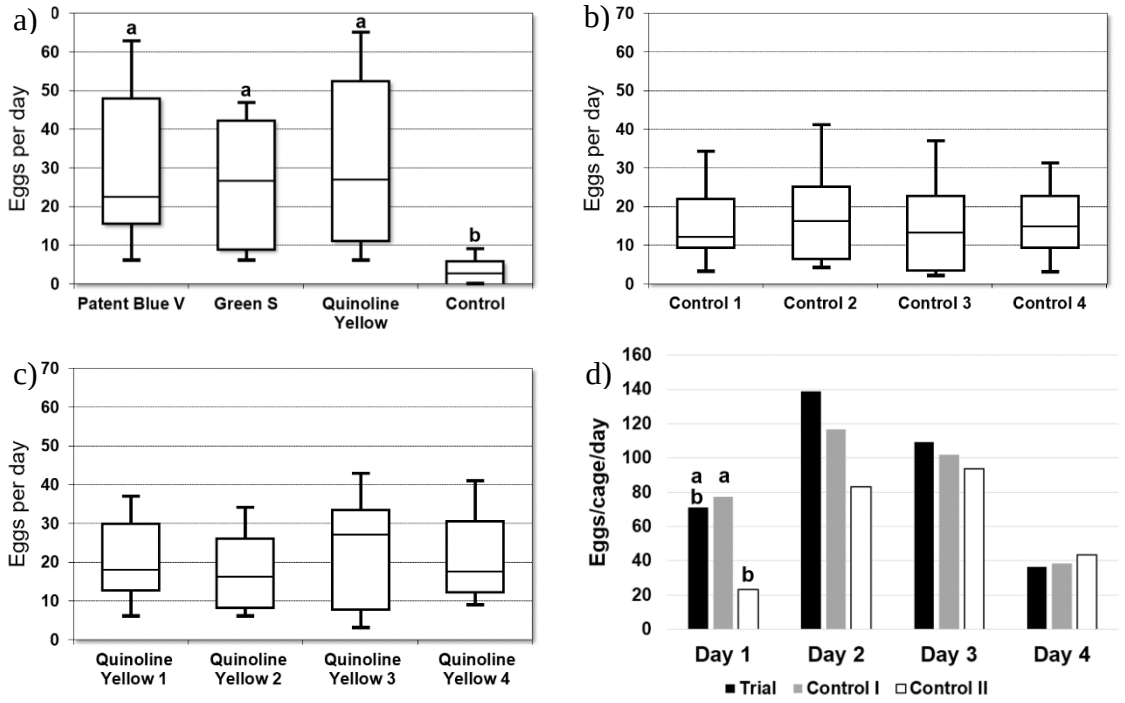

Fig. 5: Data oviposition in GMIAS-O.
a) Trial, eggs laid per day per dye. Letters indicate significant difference (P<0.0125) **b)** Control I (Quinoline Yellow in dH_2O), eggs per day per container position. **c)** Control II (dH_2O), eggs per day per container. **d)** Total number of eggs laid per trial/control per day. Letters indicate significant difference (P<0.016).

4.2 Preference of larvae for dyed artificial diets

The larvae started moving towards the feeding areas within 1 min of the beginning of the experiment. After 45 min the number of thrips per feeding area only showed minor changes between the chosen intervals (Fig 6. a-d). Thrips larvae significantly preferred the non-dyed control diet over the dyed with Patent blue V at most of the given points of time (Wilcoxon test, P <0.05, Fig. 6e). For the other dyed diets and the control diets there were no significant differences.

When the number of thrips per diet/control at the first captured points of time is compared to the mean value for each Trial per dye there were only minor changes in the distribution pattern (Fig. 7 a-c). Thrips offered diet dyed with Patent Blue V preferred the non-dyed control diet in four trials (Fig. 7a T1, T2, T4, T5), with one trial out of the five having a more even distribution (Fig. 7a T3). For Quinoline Yellow there were two trials with larvae tending to the dyed diet (Fig. 7b T1, T4), three trials with larvae tending to non-dyed control diet (Fig. 7b T3, T5, T6), and one trial with larvae showing an even distribution (Fig. 7b T2). When Green S dyed diet was offered to thrips larvae a tendency to the non-dyed control was observable in three trials (Fig. 7c T1, T5, T6). In two trials larvae preferred the Green S dyed diet (Fig. 7c T2, T4) and in one trial there was a more even distribution (Fig. 7c T3).

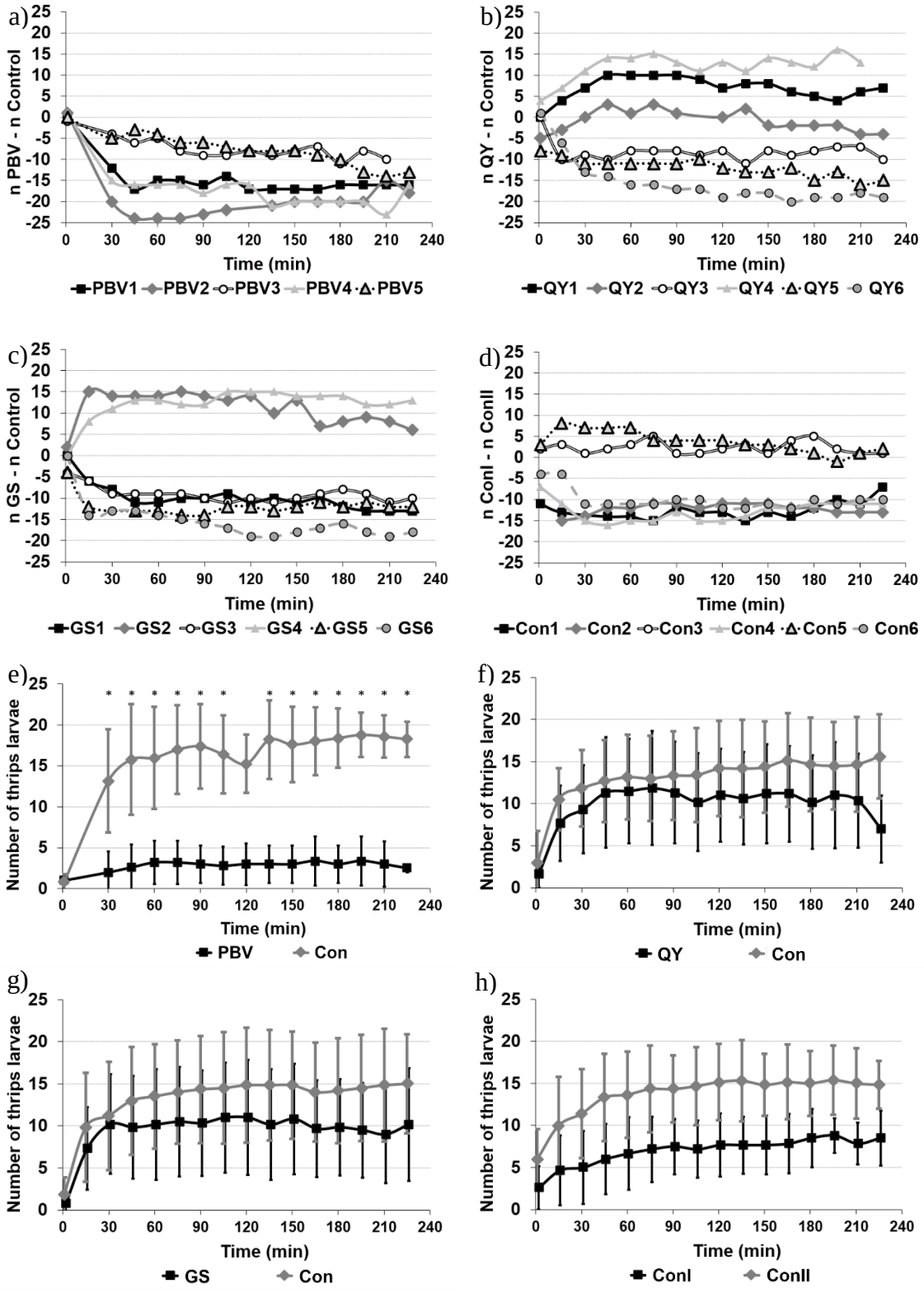

Fig. 6: Distribution of thrips larvae in dye preference experiment.

a)-d) Difference between the number of thrips located on the dyed diet and the control for all dyes and single trials (DyeTrialnumber). **e)-h)** Mean values of points of time of all trials per dye. * indicate significant differences.

GS Green S; **PBV** Patent Blue V; **QY** Quinoline Yellow; **Con** Control

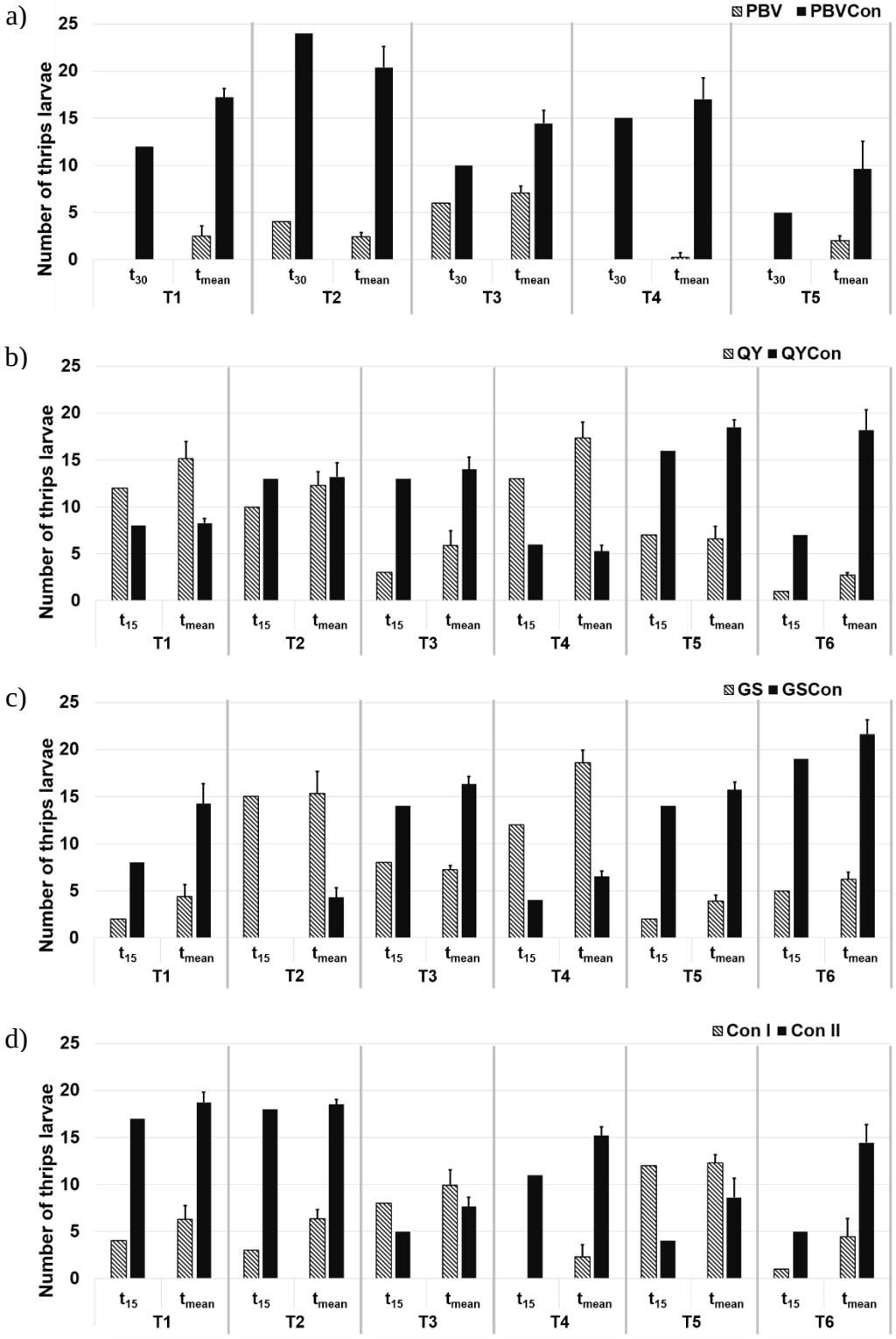

Fig. 7: Comparison oh thrips larvae distribution between $t_{15/30}$ compared to t_{mean}.
a)-d) Number of thrips at the dyed diet or control diet at t_{15} (t_{30} for PBV as there were no data available at t_{15}) compared to the mean value of all points of time (t_{mean}) for each trial (T) of a dye.
GS Green S; **PBV** Patent Blue V; **QY** Quinoline Yellow; **Con** Control

5 Discussion

Colour preference and oviposition

Adult females *F. occidentalis* preferred dyed solutions for oviposition over the non-dyed control. Female thrips normally oviposit their eggs into plant tissue of host plants. Brødsgaard (1989) tested 20 differently coloured sticky traps and found a specific blue shade to be the most attractive for *F. occidentalis*. Vernon and Gillespie (1990) (and Gillespie & Vernon, 1990) showed that adults of *Frankliniella occidentalis* were attracted to sticky traps that were bright blue, violet, yellow and white, colours often found in host plants of *F. occidentalis* (Roses, Strawberries, Chrysanthemum, et cetera). Based on the electrophysiological study of Matteson et al. (1992) *F. occidentalis* has two spectral efficiency peaks, one in the ultra violet and one in the green range. However, Stukenberg et al. (2020) used LEDs and identified blue and green as two separate attractive ranges for adult *F. occidentalis*, while both ranges mixed were less attractive. While blue and green were most attractive, UV only was moderately attractive. Thus, Stukenberg et al. (2020) suggested a trichromatic photoreceptor setup for *F. occidentalis*. Furthermore, Stukenberg et al. (2020) showed that when only the green-yellow range (colours from cyan to amber) was investigated, the yellow LED (579 nm) was the second most attractive following green (547 nm). While those findings were made for flying thrips, Bergh and Le Blanc (1997) tested petal disks of different cultivars of miniature rose (*Rosa chinensis* 'minima') for oviposition preference of *F. occidentalis* and found no difference in colour preference when testing petal disks of preferred cultivars pairwise. The results of those studies match with results of this study showing a clear preference to the colours blue, green and yellow, despite there being no significant difference between the colours itself.

Furthermore, the effect that females placed in control setups with four containers filled with non-dyed dH_2O showed less oviposition at the first day suggests colour as essential for their orientation in means of finding a suitable oviposition site. DeKogel and Koschier (2002) conducted a wind tunnel experiment in a Y-tube with adult female *F. occidentalis* allowing thrips to use their vision and olfaction. When open chrysanthemum flowers and buds were presented thrips preferred open flowers. But when flowers and buds were covered with a perforated hood there was no preference of thrips detectable. DeKogel and Koschier (2002) concluded that colour is a dominant factor for thrips orientation towards flowers. Thus, when only containers filled with dH_2O are presented to thrips, it takes longer for them to recognize those as oviposition site.

With the results in mind, the GMIAS-O allows for a wide range of new experiments. The oviposition containers can be used to test substances in the oviposition solution that improve or inhibit oviposition like bacterial toxins (Gerritsen et al., 2005). Additionally, experiments can be conducted to investigate if thrips can discriminate between containers filled with toxins or any desired substances and a control solution. As the GMIAS-O allows a clear view on thrips such an

experiment cannot only be conducted by checking the oviposition rate but by video analysis too. Moreover, when the oviposition solution is not manipulated, the used Parafilm can be treated with (volatile) substances instead of leaf discs (Koschier & Sedy, 2001; Sedy & Koschier, 2003), this makes screening for new repellent or attractant volatile detergents possible without dependence on a certain plant species. Mainali and Lim (2011) showed that most thrips responded to the combination of p-anisaldehyde and artificial flower compared to probes only having the olfactory or the visual cue. Thus, using additional olfactory cues may increase oviposition site recognition of thrips. In addition, the GMIAS-O allows the application of artificial diets, when pollen containing containers are exchanged with additional containers sealed with Parafilm. Thus, the effect of artificial diets and substances added to the diet can be tested while presenting oviposition containers with dyed solutions, which greatly support oviposition as shown in this study.

Colour preference in larvae

When larvae were given the choice between a dyed diet and a non-dyed diet, the only significant difference found was larvae preferring the non-dyed diet over the diet dyed with Patent Blue V. Despite not being dyed, due to its ingredients the control has a specific colouration. As this difference in preference was not found for diets dyed with Quinoline Yellow and Green S, the coloration of the diet itself might be closer to the natural food source of larvae like plant leaves or pollen compared to the Patent Blue V dyed diet.

However, the single trials of the different dyed diets showed a variety of thrips' distribution ranging from a tendency to the dyed or the non-dyed diet or a similar distribution. For all different distribution patterns of thrips larvae there were only minor changes 15 min after the start of the trial, implying that larvae mostly tend to stay and feed at one site, as soon as they found a suitable food source. In contrast to adult thrips, little is known about thrips larvae's behaviour regarding colour perception. While colour perception is important for adult female *F. occidentalis* to find new host plants for oviposition, the ability of colour discrimination may only play a minor role for larvae. Kiers et al. (2000) showed that for cucumber most eggs of *F. occidentalis* are laid in leaves and only few first instar larvae can be found in the flowers of the plant later. This indicates that larvae have no need for a distinction or preference of certain colours, as they feed on the plant tissue close to their egg. Thus, thrips larvae might primary orientate by olfactory cues for finding a nutrition source, not only produced by the host itself but other thrips as well. De Vries et al. (2006) showed that young *F. occidentalis* larvae preferred to feed on leaves that were grazed by other thrips before and concluded that the factors for this preference may be the physical leaf damage or odours produced by the plant or bacteria. Thus, when the first larvae of the dyed diet experiment starting to feed and to drop faeces, this might have influenced others through olfactory cues caused by their gut bacteria or pheromones produced by the thrips larvae themselves.

Different pheromones are known for adult thrips. Teerling et al. (1993) identified an alarm pheromone produced by *F. occidentalis* larvae, but no aggregation pheromones have been identified for thrips larvae so far.

Perspective

The results of this study showed that besides knowing species-appropriate abiotic factors like temperature, humidity and light regime, the importance of visual cues like the colouration of oviposition sites or diet should be considered as those could have an important impact of thrips acceptance of the artificial surroundings. Especially, as those preferences could differ from thrips species to thrips species and therefore oviposition and feeding experiments may show effects that are more linked to a missing recognition by thrips as negative effects by food or oviposition site/substrate. This shows that the importance of artificial setups will increase as they are important tools for a very specific and standardized application of substances and diets allowing new findings in the field of basic research and applied science.

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7 Supplementary data

Tab. 1: Oviposition of *Frankliniella occidentalis* in GMIAS-O (experiment group).

Trial 1	Day	1	2	3	4
	Patent Blue V	9	27	28	15
	Green S	34	43	44	22
	Quinoline Yellow	31	65	63	17
	Control	5	4	6	0
Trial 2	Day	1	2	3	4
	Patent Blue V	17	53	51	18
	Green S	7	47	31	9
	Quinoline Yellow	23	12	10	6
	Control	1	9	2	0
Trial 3	Day	1	2	3	4
	Patent Blue V	17	63	39	6
	Green S	9	40	11	6
	Quinoline Yellow	53	51	43	11
	Control	7	3	0	0

Tab. 2: Oviposition of *Frankliniella occidentalis* in GMIAS-O (control I group).

Trial 1	Day	1	2	3	4
	Quinoline Yellow	16	30	24	6
	Quinoline Yellow	31	22	27	11
	Quinoline Yellow	26	34	32	9
	Quinoline Yellow	9	31	29	12
Trial 2	Day	1	2	3	4
	Quinoline Yellow	32	37	29	14
	Quinoline Yellow	16	34	22	6
	Quinoline Yellow	7	28	32	6
	Quinoline Yellow	25	41	41	13
Trial 3	Day	1	2	3	4
	Quinoline Yellow	19	12	17	11
	Quinoline Yellow	6	23	13	7
	Quinoline Yellow	35	43	22	3
	Quinoline Yellow	10	15	18	17

Tab. 3: Oviposition of *Frankliniella occidentalis* in GMIAS-O (control II group).

Trial 1	Day	1	2	3	4
	Control	3	12	10	14
	Control	4	26	41	18
	Control	3	28	37	2
	Control	9	13	9	24
Trial 2	Day	1	2	3	4
	Control	11	24	34	12
	Control	6	28	18	10
	Control	8	22	21	4
	Control	3	19	31	13
Trial 3	Day	1	2	3	4
	Control	8	25	14	9
	Control	7	14	22	5
	Control	3	23	18	2
	Control	5	16	26	17

Chapter IV

Application of microorganisms to thrips

1 Summary

For a better understanding and new approaches in pest control of thrips the application of microorganisms is an important factor, as those can function as a vehicle for specific substances or as thrips pathogen.

Thrips additionally can function as vector of plant pathogenic viruses like Tospoviruses. However, for those thrips like *Frankliniella occidentalis* no infection with *Wolbachia* was found leading to the hypothesis that thrips can't be infected with Tospoviruses and *Wolbachia* at the same time. Thus, six different methods were tested to feed *F. occidentalis* larvae with *Echinothrips americanus* pro-/pupae from a *Wolbachia*-infected population. The sixth method showed the highest consumption rate of *E. americanus* pro-/pupae and allowed second instar larvae to develop to the adult stage by feeding *E. americanus* pro-/pupa only. This method was used to rear thrips (late second instar larvae and adults) to proof the uptake of *Wolbachia* by PCR and *in situ* PCR. The PCR results showed signals of the *wsp* gene for the *Wolbachia* symbiont in several *Frankliniella occidentalis* larvae. With the *in situ* PCR signals in different tissues of adult females and larvae were found. However, some signals were found in adults and larvae of the control.

Furthermore, an efficient method for the application of the algae *Chlamydomonas reinhardtii* was established, allowing the application of a genetically modifiable organism to thrips. *C. reinhardtii* was applied to three different thrips species (*Echinothrips americanus*, *Frankliniella occidentalis*, *Thrips tabaci*) after feeding them with 5% sucrose solution (in H_2O) for one day. The uptake was proven by microscopic examination (DIC and UV). While the control thrips had no coloured gut lumen, thrips that *C. reinhardtii* suspension was applied to showed a bright greenish (DIC)/ reddish (UV) gut lumen indicating the uptake of chlorophyll.

2 Introduction

In the order Thysanoptera there are some species that are global pests in agriculture and horticulture. Due to their fast development and high fecundity populations can increase dramatically. The use of insecticides lead to an increasing number of pesticide resistances in thrips species like *Frankliniella occidentalis* making it more and more difficult to control infestations. In order to face this problem finding new measures is a key factor. For this, one important approach could be the manipulation of pest species.

A way of naturally occurring manipulation are bacteria influencing their insect host. Many bacteria are known to influence the fitness of their host. Gram-negative, intracellular bacteria like *Wolbachia* are estimated to be widely spread in arthropods (Zug & Hammerstein, 2015). Infections with *Wolbachia* are known to prevent or influence the infection of the host with certain viruses. Teixeira et al. (2008) showed that a *Wolbachia* infection of *Drosophila melanogaster* lead to an increased resistance against Drosophila C virus. Hedges et al. (2008) additionally tested cricket paralysis and Flock House virus on *D. melanogaster* infected with *Wolbachia* and showed a protective effect compared to *D. melanogaster* not infected with *Wolbachia*. For mosquitoes the influences of *Wolbachia* infections on virus transmission were shown in several studies (summarized in Rainey et al., 2014). Few species of the order Thysanoptera are known vectors of phytopathogenic tospoviruses. The family Thripidae comprises of more than 1700 species and only 14 thrips species are reported to transmit tospoviruses (Riley et al., 2011). Five of those 14 species were reported to be infected with *Wolbachia* (*Frankliniella fusca* (Jeyaprakash & Hoy, 2000), *Frankliniella intonsa* (Luo et al., 2015), *Frankliniella schultzei* (Sintupachee et al., 2006), *Thrips palmi* (Saurav et al., 2016), and *Thrips tabaci* (Gawande et al., 2019). However, the influence of a *Wolbachia* infection on the vector function of thrips for tospoviruses were not examined so far. Furthermore, one of the most important and the best researched pest thrips *Frankliniella occidentalis* was not found to be infected with *Wolbachia* so far (Jeyaprakash & Hoy, 2000; Kumm & Moritz, 2008, 2010). *Wolbachia* are maternally transmitted but can be horizontally transferred as well (Baldo et al., 2008; Brown & Lloyd, 2016; Huigens et al., 2004; Schilthuizen & Stouthamer, 1997; Vavre et al., 1999). Thus, it was the goal to develop a setup that allows the horizontal transfer *Wolbachia* from thrips of an infected *Echinothrips americanus* population to *Frankliniella occidentalis* larvae to be able to research the influence of *Wolbachia* on *F. occidentalis*.

Furthermore, a new way of artificial manipulation, is the application of RNA interfering with the host RNA, leading to a silencing effect of targeted genes. For thrips Badillo-Vargas et al. (2015) described a method to inject RNA into the hemocoel of adult *F. occidentalis*. Jahani et al. (2018) and Singh et al. (2019) fed dsRNA to thrips via sucrose-water solutions. As an alternative way to apply the RNA Han et al. (2019) used a disc feeding method, while Whitten et al. (2016) and

Andongma et al. (2020) applied genetically modified symbiotic gut bacteria, producing RNA, to thrips. As this bacterium may be thrips specific and have further effects on thrips as it is a symbiont, the application of the genetic model organism *Chlamydomonas reinhardtii* may be an alternative to an application system allowing to apply a wide range of artificial molecules to various thrips species. *C. reinhardtii* is a unicellular green algae for that a wide range of mutant strains exist. Jilge (2016) showed that the application of *C. reinhardtii* to *F. occidentalis* adults is possible under certain conditions. However, the setup for the application of algae was inefficient and time consuming. Thus, in this experiment the goal was to establish an efficient application setup allowing the application of *C. reinhardtii* to larvae and adults of *Echinothrips americanus*, *T. tabaci* and *F. occidentalis*.

3 Material and methods

3.1 Thrips rearing

The main populations of the thrips species *Echinothrips americanus*, *Frankliniella occidentalis* and *Thrips tabaci* were reared in acrylic glass cages (50 cm x 50 cm x 50 cm) (T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8, light on: 6:00 AM MET). *E. americanus* was given cotton (*Gossypium* spec.) and hibiscus (*Hibiscus* spec.), *F. occidentalis* French bean (*Phaseolus vulgaris* L.) and chrysanthemum (*Chrysanthemum* spec.) and *T. tabaci* was reared on chives (*Allium schoenoprasum*) and leek (*Allium ampeloprasum*). Identification of few random adult females was performed with prepared slides and the identification software "ThripsID – Pest thrips of the world." (Moritz et al., 2004).

For the application of *Wolbachia* to *F. occidentalis*, adult females of *Echinothrips americanus* were held on *Phaseolus vulgaris* leaves that were placed in 1.4 % agar in transparent 500 ml plastic boxes for oviposition. The adult females were transferred to new boxes every 3 to 4 days. The boxes were kept in a climatic chamber (T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8, light on: 6:00 AM MET). When the offspring inside those boxes developed to pro-/pupa they were used for the *Wolbachia* application experiment. *Frankliniella occidentalis* larvae were reared by allowing the oviposition of adult females to oviposit in *Phaseolus vulgaris* leaf discs placed on 2 ml 1.4% agar (w/v in dH_2O) in 12well-Greiner-plates. After placing the females on the leaf discs the plates were closed with a glass lid, sealed with Parafilm and placed in climatic chamber (T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8, light on: 6:00 AM MET).

3.2 Application of *Wolbachia*

Application

The application of *Wolbachia* was tested in six different experimental setups. In all setups *Echinothrips americanus* propupae and pupae were offered as food to *Frankliniella occidentalis* larvae. Thrips were checked for the number of pro-/pupae consumed. Consumption of pro-/pupae was defined, when the pro-/pupa was shrunken or just the cuticle was left.

Setup I – First instar larva and pro-/pupa

For easier handling of thrips only the three wells on each side of a 12 well-Greiner-plates (Greiner, CELLSTAR® multiwell culture plates, 12 wells) were filled with 2 ml 1.4% agar (w/v in dH_2O). A green acrylic plate (green, 20 mm x 20 mm) was placed in each well. A first instar lava of

F. occidentalis and one pro-/pupa of *E. americanus* were placed together in one well. The Greiner-plate was closed with a glass lid, sealed using Parafilm and placed in a climatic chamber (T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8, light on: 6:00 AM MET). Two replications with each 12 larvae were conducted.

Setup II – First instar larva and paralyzed pro-/pupa

A 12 well-Greiner-plate was prepared (see Trial I). The *E. americanus* pro-/pupae were squished using a thin preparation needle. The right pressure leads to a paralysation-like condition of the pro/-pupae making it incapable of defending itself by swinging the abdominal segments. The pro-/pupae were replaced by new prepared pro-/pupae after one day. The Greiner-plate was closed with a glass lid, sealed using Parafilm and placed in a climatic chamber (T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8, light on: 6:00 AM MET). Two replications with each 12 larvae were conducted.

Setup III – Second instar larva and paralyzed pro-/pupa

A 12 well-Greiner-plate was prepared (see Trial I). The *E. americanus* pro-/pupae were treated like described for Trial II. Early, intermediate and late second instar larvae of *F. occidentalis* were used (0-24 h, 25-48 h and 49-72 h after hatching from the first to the second larval stage). The pro-/pupae were replaced by new prepared pro-/pupae after one day. The Greiner-plate was closed with a glass lid, sealed using Parafilm and placed in a climatic chamber (T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8, light on: 6:00 AM MET). For each larvae condition two replications with each 12 larvae were conducted.

Setup IV – Second instar larva and two paralyzed pro-/pupae

A 12 well-Greiner-plate was prepared (see Trial I). Then two paralyzed pro-/pupae were offered to one intermediate second instar larva (25-48 h after hatching from the first to the second larval stage). The pro-/pupae were replaced by new prepared pro-/pupae after one day. The Greiner-plate was closed with a glass lid, sealed using Parafilm and placed in a climatic chamber (T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8, light on: 6:00 AM MET). Two replications with each 12 larvae were conducted.

Setup V – Two second instar larvae and two paralyzed pro-/pupae

A 12 well-Greiner-plate was prepared (see Trial I). Two paralyzed pro-/pupae were offered to two intermediate second instar larvae (25-48 h after hatching from the first to the second larval

stage). The pro-/pupae were replaced by new prepared pro-/pupae after one day. The Greiner-plate was closed with a glass lid, sealed using Parafilm and placed in a climatic chamber (T: 23 °C ± 1 K, rel. humidity: 50%, LD = 16:8, light on: 6:00 AM MET). Two replications with each 12 larvae were conducted.

Setup VI – 10 second instar larvae and 10 paralyzed pro-/pupae

A plastic petri dish was filled with 10 ml 1.4% agar (w/v in dH_2O) (Fig. 1). When the agar was cooled down and solid a 33 mm disc of non-stretched Parafilm was placed on the agar. A plastic petri dish with a hole was placed on the bottom plastic petri dish and tightened by wrapping Parafilm around the sides of the two petri dishes. Ten intermediate second instar larvae (25-48 h after hatching from the first to the second larval stage) and ten 10 paralyzed pro-/pupae were cooled on ice and placed on two moist pieces of filter paper. The thrips were introduced to the arena by transferring the moist filter papers through the hole in the top petri dish. Afterwards the hole was closed with double stretched Parafilm. The pro-/pupae were replaced by 10 new prepared pro-/pupae every day for 4 days. Thrips were additionally checked for developmental stage on a daily basis. Six replications with each 10 larvae were conducted.

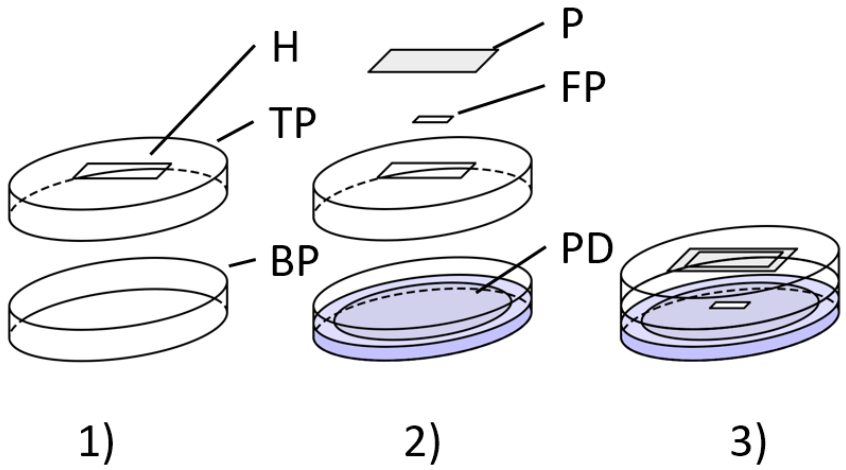

Fig. 1: ***Frankliniella occidentalis-Echinothrips americanus*** **feeding arena. 1)** Two plastic petri dishes of the same size are used. A hole (H) was cut into the top petri dish (TP). **2)** The bottom petri dish (BP) was filled with 10 ml 1.4% agar (w/v in dH_2O). A disc (PD, 33 mm) out of non-stretched Parafilm was placed on the agar. The TP was placed on the BP and sealed with Parafilm. Thrips were placed on filter paper pieces (FP) and transferred into the cage via the hole (H). The hole was sealed with Parafilm (P). **3)** Assembled cage.

Molecular and in situ hybridisation detection

For the detection of a transmission of *Wolbachia* from *E. americanus* to *F. occidentalis* the trial 6 setup was used. DNA of *F. occidentalis* larvae was extracted after one day and three days of the feeding experiment. The DNA was extracted from single larvae and pooled groups (2 and 4 larvae

together). For the in situ hybridisation thrips reared in the trial 6 setup were used 48 h after the start of the experiment and adult thrips 148 h after the start of the experiment. As control thrips from the main population reared on French bean (*P. vulgaris*) were used. Two separated *in situ* preparations were conducted. In first preparation thrips from the experiment and control thrips were used. As the results of the first *in situ* hybridisation showed signals in the control samples indicating a *Wolbachia* infection, control samples that already were embedded in the year 2017 (February) were additionally used.

(*In situ* set 1: Experiment: 7 larvae, 11 adult females; Control: 12 larvae, 12 adult females. *In situ* set 2: Experiment: 9 larvae 12 adult females; Control: 12 larvae, 12 adult females; Control (embedded 2017) 16 adult females.)

DNA extraction

For the DNA extraction of F. occidentalis larvae and adults a modified variety of the "100 fly method" after Roberts (1998) was used. After the application of *Echinothrips americanus* pro-/pupae, larvae and adult thrips of *F. occidentalis* were transferred to a 1.5 ml reaction tube filled with 50 µl extraction buffer. The tube was put into liquid nitrogen for at least 30 s. The frozen samples were taken out of the liquid nitrogen and ground using a plastic pestle. When the sample was thoroughly ground the tube was closed and placed on ice until the next step. All samples were placed into a heating block (70 °C) for 15 min. Tubes were then put on ice immediately. To each sample 15 µl freezing potassium acetate was added and the solutions were mixed using a vortex mixer. The samples were incubated on ice for 15 min and thereafter centrifuged by 16,000 rpm for 15 min. The supernatant was transferred to a new reaction tube to which 225 µl EtOH (100 %) and 31.5 µl 3 M sodium acetate (4 °C) were added. The tube was then placed on a vortex mixer. For the precipitation of DNA, the mix was incubated for 30 min to 60 min at -80 °C followed by centrifugation at 16,000 rpm for 15 min. The supernatant was discarded. The pellet was washed with 100 µl 70 % EtOH and centrifuged at 16,000 rpm for 5 min. The supernatant was discarded and the pellet was heat-dried at 45 °C for 5 min. The pellet was solved in 16 µl ddH_2O and incubated at 45 °C for 10 min. When not used immediately the samples were stored at -20 °C.

PCR

For the PCR 2 µl of template DNA and 48 µl of master mix were given into a 0.5 ml reaction tube (Tab. 1). The PCR was performed following the given program (Tab. 2). The used primer pair was 81F/691R (Tab. 3, Zhou et al., 1998).

Gel electrophoresis

The results of PCR were visualized with gel electrophoresis. A 2% agarose gel (1% TBE-Puffer) containing ethidium bromide was filled with 10 µl PCR product mixed with 2 µl loading buffer. As marker Gene Ruler™ (100 bp Thermo Scientific, Waltham) was used. The gel electrophoresis was performed over 2 h with 120 V (250 mA, 150 W). Gels were inspected using UV light (gel documentation system DIAS-III (SERVA)), GelScan V6.0.)

Tab. 1: PCR master mix.

Reagent	Quantity	Reagent	Quantity
10 x *Taq* -buffer	5 µl	Primer 81F 20 pmol	1.5 µl
$MgCl_2$ 25 mM	4 µl	Primer 691R 20 pmol	1.5 µl
dNTPs 25 mM	0.4 µl	*Taq* -polymerase	0.2 µl
		ddH_2O	35.4 µl

Tab. 2: PCR program.

Program	Temperature	Duration	Cycles
Initial Denaturation	94 °C	4 min	1
Denaturation	94 °C	1 min	
Annealing	58 °C	1 min	35
Elongation	72 °C	1 min	
Final Elongation	72 °C	7 min	1
End	10 °C		

Tab. 3. Primer pair for the *wsp* gene.

Primer name	Sequence 5′ → 3′	Primer concentration
81F	TGGTCCAATAAGTGATGAAGA	20 pmol
691R	AAAAATTAAACGCTACTCCA	20 pmol

In situ hybridisation

The *in situ* hybridisation method was modified after Morel et al. (2001) for paraffin sections (Chuttke, 2015).

However, the in situ PCR process has an impact on the tissue quality including contrast, sharpness and cell structure of the final sections.

Gauze was heat-fixed to small tubes made from cut plastic Pasteur-pipettes. Thrips samples were placed into those modified tubes and fixed in 4 % paraformaldehyde and 0.25 % glutardialdehyde in 0.1 M Soerensen's buffer (pH 7.2) for 3 h. Afterwards, the samples were rinsed four times with 0.1 M Soerensen's buffer (pH 7.2). The samples were left in fresh 0.1 M Soerensen's buffer (pH 7.2) over night at 4 °C. The next day the samples were dehydrated via a graded ethyl alcohol series following isopropyl alcohol as intermediate for embedding the samples in paraffin (Tab.

4). After mounting the samples on wooden cubes sections were cut using a microtome (thickness of 6 µm). The sections were fixed onto Polysine® coated slides (Thermo Scientific, Menzel-Gläser, Braunschweig) using dH_2O. When the sections were dry, deparaffinization and dehydration were performed using xylol, isopropyl alcohol and a descending ethyl alcohol series (Tab. 5).

Tab. 4: Scheme of ethanol series for dehydration and following paraffinization.

Chemicals	Temperature	Concentration	Duration
Ethyl alcohol	RT	10%	15 min
Ethyl alcohol	RT	30%	15 min
Ethyl alcohol	RT	50%	15 min
Ethyl alcohol	RT	70%	1 h
Ethyl alcohol	RT	80%	1 h
Ethyl alcohol	RT	90%	1 h
Ethyl alcohol	RT	100%	1 h
Isopropyl alcohol I	RT	100%	2 h
Isopropyl alcohol II	RT	100%	24 h
Isopropyl alcohol III	RT	100%	24 h
Isopropyl alcohol : Paraffin	61 °C	1:1	24 h
Paraffin I	61 °C	100%	24 h
Paraffin II	61 °C	100%	24 h
Paraffin III	61 °C	100%	24 h

Tab. 5: Rehydration scheme.

Chemicals	Concentration	Duration
Xylol	100%	2
Isopropyl alcohol	100%	2
Ethyl alcohol	100%	1
Ethyl alcohol	90%	1
Ethyl alcohol	80%	1
Ethyl alcohol	75%	1
Ethyl alcohol	50%	1
Ethyl alcohol	30%	1

The slides were washed with 9% sodium chloride for 5 min and 0.1 M phosphate buffer (pH 7.2) for additional 5 min. A container was filled with 100 ml TRIS-HCl/$CaCl_2$ buffer and placed in a heating chamber (37 °C). Once the buffer was heated up to 37 °C, the slides were placed in the container and proteinase K (2.5 µl, concentration: 5 µg/ml) was added. After 15 min the enzymatic reaction was stopped by transferring the slides into fresh TRIS-HCl/$CaCl_2$ buffer for 2 min. Then the slides were washed with 0.1 M phosphate buffer (pH 7.2) for 5 min. For the post fixation the slides were given into 4% paraformaldehyde for 5 min following three sets of washing with 0.1 M phosphate buffer (pH 7.2) for 5 min and additional 2 min in 9% sodium chloride. The dehydration of sections was performed with an ascending ethyl alcohol series (30%, 50%, 75%,

80%, 90%, 100%, 2 min per concentration) and following drying process at room temperature for 60 min. Gene Frames ® (Thermo Scientific, Menzel-Gläser, Braunschweig) were placed onto the slides surrounding the sections. For the PCR-mixture the primer pair for the *fts-Z* gene (Tab. 7, Holden et al. 1993) was used. The PCR-mixture (Tab. 8) was mixed without *Taq*-polymerase (Thermo Scientific, Maxima Hot Start *Taq*-DNA-Polymerase) and heated to 82 °C for 5 min. The slides were placed into the master cycler with *in situ* adapters and preheated to 82 °C. Then the *Taq*-polymerase was added to the PCR-mixture, each frame was filled with 45 µl of the PCR-mixture, the reaction chambers were closed with coverslips and the PCR-program was started (Tab. 9). When the PCR-program was finished the coverslips were removed and the slides were washed with 0.1 M phosphate buffer (pH 7.2) for 5 min. Then 4% paraformaldehyde was used for a second postfixation for 10 min at 4° C. The slides were washed with 0.1 M phosphate buffer (pH 7.2) for 5 min and 2 min of washing with 9% sodium chloride. After that, the slides were transferred to 95% ethyl alcohol and 100% ethyl alcohol for 2 min. The slides were then dried for 60 min.

Tab. 7: Primer pair for the *fts-Z* gene.

Primer name	Sequence 5´ → 3´	Amplicon
494F	CCGTATGCCGATTGCAGAGCTTG	700 bp
1262R	TCGCCATGAGTATTCACTTGGCT	

Tab. 8: Master-mix *in situ* PCR for one frame.

Component	Volume
Taq -buffer (10 x)	15 µl
$MgCl_2$ (25 mM)	12 µl
dUTPs (10 mM)	0.09 µl
dTTPs (10 mM)	1.71 µl
dATPs (10 mM)	1.8 µl
dGTPs (10 mM)	1.8µl
dCTPs (10 mM)	1.8µl
forward/reverse Primer	3 µl
Taq -polymerase	0.6 µl
ddH_2O	49.2 µl

Tab. 9: Program of the *in situ* PCR.

Program	Temperature	Duration	Cycles
Initial denaturation	82 °C	3 min	1
	82 °C	hold	-
Denaturation	95 °C	1 min	
Annealing	55 °C	1 min	25
Extension	72 °C	1 min	
Final end	72 °C	5 min	-
	4 °C	10 min	-
End	4 °C		-

The slides were washed in TRIS-HCl/NaCl buffer for 10 min. Each frame was filled with 120 µl blocking buffer for 30 min. Then the slides were washed with TRIS-HCl/NaCl buffer for 10 min three times. Buffer remains were removed using filter paper. The anti-digoxigenin primary antibody (Roche Diagnostics, Penzberg, Germany) was diluted 1:250 in TRIS-HCl/NaCl. Then, 60 µl were given on the slides and incubated for 75 min in a moist chamber. Afterwards, 120 µl of Fab fragments (1:5000 diluted in TRIS-HCl/NaCl) from an anti-digoxigenin antibody, conjugated with alkaline phosphatase (Roche Diagnostics, Germany) were incubated for 75 min on slides in a moist chamber. Then the slides were washed three times for 10 min again in TRIS-HCl/NaCl.

Nitro blue tetrazolium salt (NBT) and 5-bromo-4-chloro-indolyl phosphate (BCIP) react with the alkaline phosphatase producing a brownish-red signal localizing the *Wolbachia* DNA. BCIP and NBT solutions were diluted in dimethylformamide. For the final detection solution 3.5 µl BCIP and 4.5 µl NBT were added to 992 µl TRIS-HCl/NaCl/$MgCl_2$ buffer. The frames were filled with 100 µl detection solution and incubated for 120 min at 37 °C in the dark. To stop the reaction, the slides were washed in dH_2O for 5 min. Afterwards, the frames were removed and the sections were mounted in mowiol and sterile coverslips were adjusted on top.

3.3 Application of algae

Algae rearing

The *Chlamydomonas reinhardtii* mutant CC-400 was transferred from YA medium to 50 ml TAP-medium. After two weeks of incubation (T: 23 °C ± 1 K, rel. humidity: 30%, LD = 16:8, light on: 6:00 AM MET) 1 ml of culture was transferred into new Erlenmeyer flasks with 50 ml of TAP-medium every week. One week old cultures (OD_{750}: 0.689) were used for the application experiments.

Application

For the application to thrips 5 g of sucrose was added to 10 ml of one-week old *C. reinhardtii* culture (TAP medium). The sucrose functions as a phagostimulant and improves the uptake of liquid/algae by thrips (Jilge 2016). As a control 5 g of sucrose was added to 10 ml of TAP medium. After adding the sucrose, five 1 ml aliquots of the *C. reinhardtii* culture and the control were centrifuged for 60 s at 1.800 rpm. After this 800 µl of the supernatant was carefully removed with a micropipette without moving the algae pellet. Then the pellets were resuspended in the 200 µl of solution left over and added into one 1.5 ml tube. Larvae and adult thrips of the species *Echinothrips americanus*, *Frankliniella occidentalis* and *Thrips tabaci* were fed with a 5% sucrose solution (dH_2O) for one day. The next day the algae suspension was applied. For a first

approach the suspension was applied to *F. occidentalis* using the SMIAS (Chapter II), SMIAS-UDA (Fig. 2) and GMIAS-S-C (Fig. 3) (GMIAS-S see Chapter III). As the GMIAS-S-C was the most time efficient approach, it was additionally used to apply the algae suspension to *E. americanus* and *T. tabaci*.

For the application with SMIAS the 4th step was performed differently (Chapter II, Fig. 2, step 4). The bottom of the modified pipette tip was sealed with double stretched Parafilm. The thrips was placed into the inner lower part of the modified pipette tip using a fine moist brush and the top part was closed using the cotton plug. Then the modified pipette tip was placed in a well of 96 well-PCR-plate filled with 20 µl algae suspension. For this setup 10 larvae of *F. occidentalis* were tested. Two replications were conducted.

For the application via SMIAS-UDA (Fig. 2) thrips were placed in a PCR well with a fine moist brush and the well was then immediately closed with the one-sided-sealed modified pipette tip. Next 15 µl of algae suspension was filled in the modified pipette tip and the top part was sealed using Parafilm. For this setup 10 larvae of *F. occidentalis* were tested. The test was replicated a second time.

The GMIAS-S-C was prepared like described in Chapter III (Fig. 2 GMIAS-S). As modification, containers (0.65 ml tubes cut at 4 mm) were added to the setup. With the addition of containers to the GMIAS-S method was established allowing long-term application experiments for smaller groups for future experiments following the application mechanism of the original GMIAS. For one GMIAS-S-C cage, 10 larvae were placed together with 10 adult females (5 for *F. occidentalis*). For each species two replications were done.

All setups were placed into a climatic chamber (T: 23 °C ± 1 K, rel. humidity: 80%, LD = 16:8, light on: 6:00 AM MET).

Visual examination

To proof the uptake of algae thrips were placed in 70% glycerine solution (in PBS pH 6.5) on a slide. Then the slide was covered with a cover slip and the thrips' gut was checked for the uptake of algae with a microscope using light microscopy (DIC) and fluorescence microscopy (UV-light).

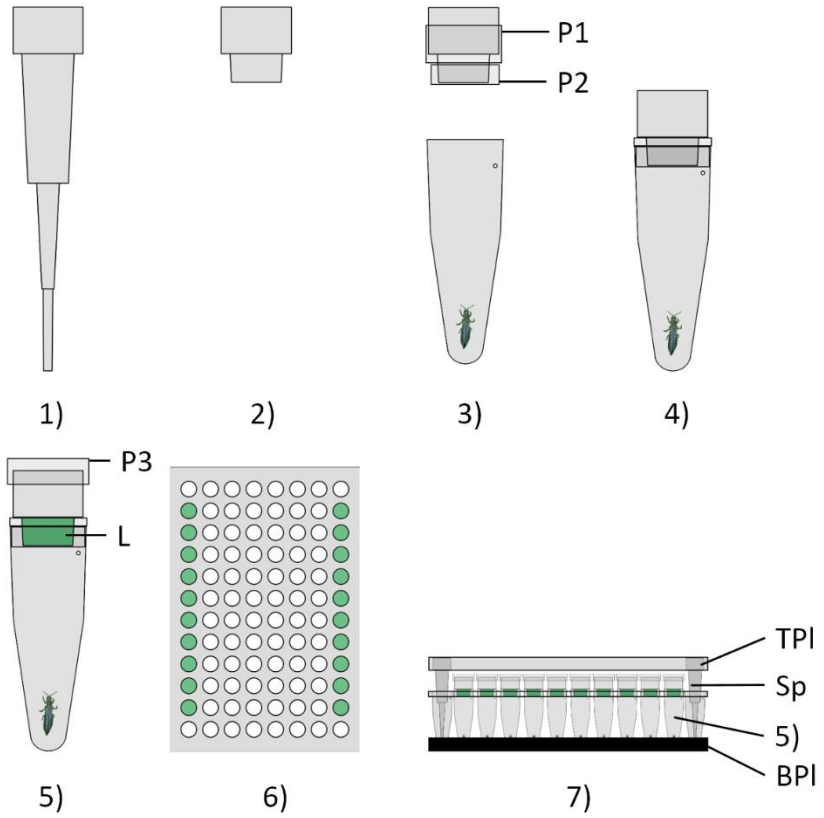

Fig. 2: SMIAS-UDA, upside-down-application. 1) Original pipette tip (0.1-10 µl). **2)** The pipette tip is cut horizontally at 8 mm (top to bottom). **3)** The lower opening of the modified pipette tip (MPT) is sealed with double stretched Parafilm (P1). Parafilm (P2) is wrapped around the MPT for a tight fit between the MPT and the PCR-well. The thrips is placed into the PCR-well. **4)** The MPT is placed into the PCR-well. **5)** The algae suspension is filled into the MPT. The upper opening is then closed with stretched Parafilm. **6)** Top view of a 96-well-pcr-plate Green marked wells show the positioning of prepared tips. **7)** Assembled SMIAS. As a top plate (TPl) a lid of 12-well-cell-plate is used to cover the prepared tips with non-modified pipette tips (Sp) as spacers. Black duct tape is attached to a second lid, which is then used as bottom plate (BPl).

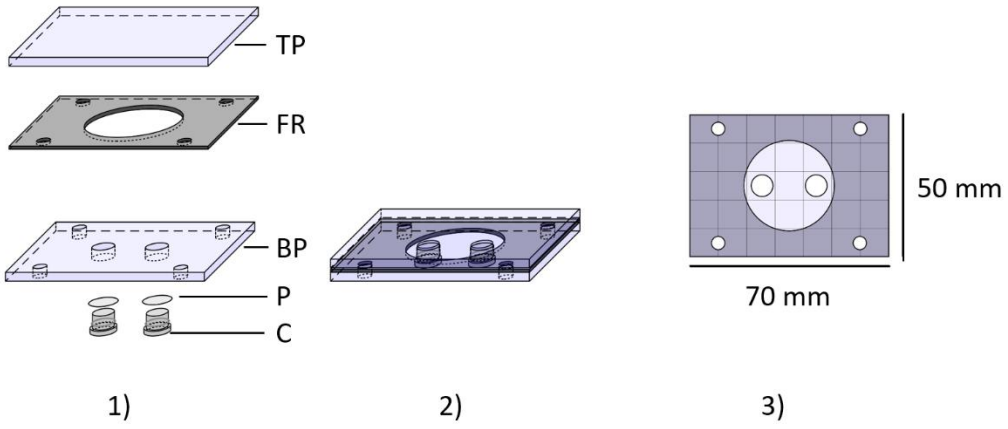

Fig. 3: Preparation of GMIAS-S-C. A smaller cage following the idea of the Group Micro Insect Application System (GMIAS) for specific application experiments using containers**. 1)** Exploded view. **2)** Assembled view. **3)** Top view with graphically added 5 mm x 5 mm squares. **TP** Top plate; **FR** Foam rubber; **BP** Bottom plate; **P** Parafilm; **C** Container.

4 Results

4.1 Application of *Wolbachia*

Feeding Frankliniella occidentalis larvae with Echinothrips americanus pro-/pupa

The various experimental setups lead to different consumptions of *Echinothrips americanus* pro-/pupae (Tab. 10). In the first trial the first instar larvae of *Frankliniella occidentalis* did not consume any of the pro-/pupae. After one day the larvae were mostly found on the wall of the well or at the glass lid. In the second trial two first instar larvae consumed a paralyzed pro-/pupa. Only the cuticle of the pro-/pupa was left over (Fig. 4b). Most larvae were found at the walls of the well or at the glass lid. In the third trial 8 pro-/pupae were consumed by early second instar larvae, 18 by intermediate second instar larvae and 9 by late second instar larvae. The larvae were observable feeding on pro-/pupa (Fig. 4a). While some intermediate larvae aggressively started feeding shortly after the beginning of the experiment despite of having a greenish abdomen, most of the late and early larvae were found at the walls of the well or the glass lid. In the fourth trial two paralyzed pro-/pupae were placed together with the intermediate second instar larvae and 27 pro-/pupae were consumed after 48 h. When in trial five two intermediate larvae were placed in a well together with two paralyzed pro-/pupae the consumption increased to 69 out of 80 offered pro-/pupae. Some thrips larvae feeding on a pro-/pupae showed aggressive behaviour towards approaching other larva by hitting the other larvae with their flicking abdomen or piercing it with their maxillary stylets. Few larvae were observed feeding on the same pro-/pupae without aggressive behaviour (Fig. 4c). In the sixth trial 105 pro-/pupae were consumed in 48 h.

Tab. 10: Results of the feeding experiments after 48 h.

Trial	N	*F. occidentalis*	*E. americanus*	*E. americanus* consumed
I	20	First instar larva (1)	Pro-/pupa (1)	0/40
II	21	First instar larva (1)	Pro-/pupa (1) (paralyzed)	2/42
III	21	Second instar larva (1) (early)	Pro-/pupa (1) (paralyzed)	8/42
III	20	Second instar larva (1) (intermediate)	Pro-/pupa (1) (paralyzed)	18/40
III	22	Second instar larva (1) (late)	Pro-/pupa (1) (paralyzed)	9/44
IV	21	Second instar larva (1) (intermediate)	Pro-/pupa (2) (paralyzed)	27/84
V	20	Second instar larva (2) (intermediate)	Pro-/pupa (2) (paralyzed)	69/80
VI	60	Second instar larva (10) (intermediate)	Pro-/pupa (10) (paralyzed)	105/120

Intermediate second star larvae fed with pro-/pupae for several days were able to develop to adult thrips (Fig. 5).

When feeding on the pro-/pupae thrips showed no specific location on the pro-/pupae that was preferred for piercing it. Larvae were observed piercing and sucking at the wing sheaths (Fig. 3a), the legs (Fig. 4d, e) or the abdomen (Fig. 4f, g, h). The process leads to a steadily shrinking pro-/pupae (Fig. 4g) while the uptake of the pro-/pupae's haemolymph and tissues was visible in larvae by their repulsive movement of the digestive system.

E. americanus pro-/pupae that were not paralyzed due to incorrect execution of the squishing process stayed unharmed by the *F. occidentalis* larvae.

Molecular biological detection

Signals of the determined size of the *wsp* gene amplicon (590-632 bp) were detected in pooled probes of *Frankliniella occidentalis* larvae fed with *Echinothrips americanus* pro-/pupae for 24 h (Fig.6, 4L-1, 4L-3). For single probes signals with a size of 590-632 bp were detectable in larva fed for 24 h (Fig.7, 1dL-1, 1dL-5, 1dL-6) and in larva fed for 72 h (Fig. 7, 3dL-1, 3dL-4). There was no signal found in control thrips.

In situ hybridisation

Single signals and cluster of signals of *Wolbachia* DNA were found in several larvae and adult thrips that were fed with *E. americanus* pro-/pupae during their second larval stage. However, signals were sporadically found in larvae and adults of the new control thrips too, while there were no signals found in the samples from 2017.

Signals in larvae were located in the gut epithelium (Fig. 8b), gut tissue (Fig. 8c), coxa (Fig. 8d) and in the subcuticular tissue (Fig 8e). In the control larvae, signals were found in the gut tissue (Fig. 8f) and fat body (Fig. 8g).

In adult female thrips, there were multiple single signals and clusters in eggs that were ready for oviposition (Fig. 9b, c). Further signals were found in the thorax musculature (Fig. 9d) and in the fat body (Fig. 9e). The adult female thrips of the control showed signals of *Wolbachia* as well. The signals were located in the fat body (Fig. 9f, g), eggs ready for oviposition (Fig. 9h) and thorax musculature (Fig. 9i).

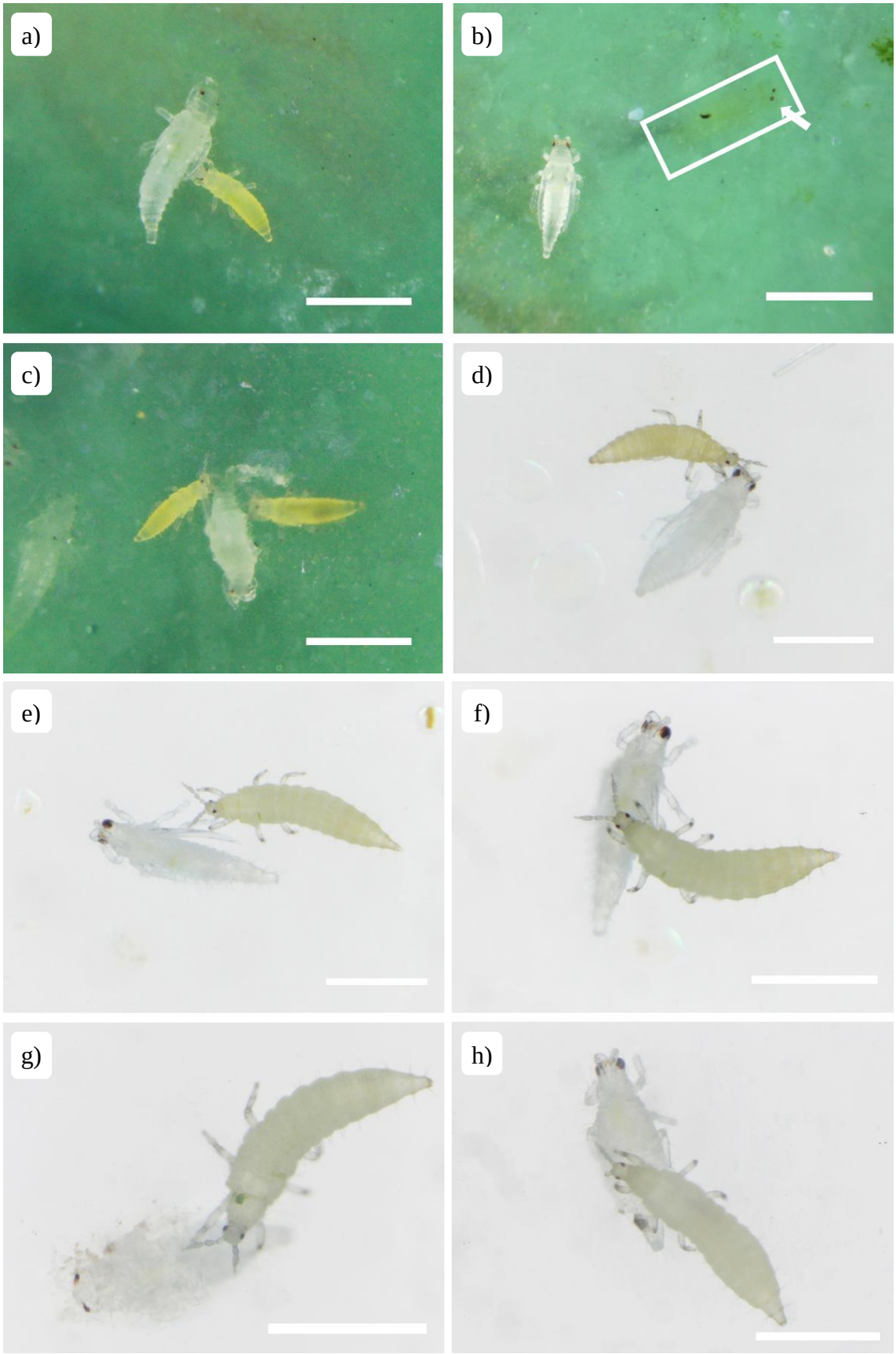

Fig. 4: ***Frankliniella occidentalis*** **larvae feeding on** ***Echinothrips americanus*** **pro-/pupa. a)** Trial 3, intermediate second instar larva feeding on a paralyzed propupa. **b)** Trial 2, pupa besides a cuticle of a pupa of the day before (marked area). **c)** Trial 5, two intermediate second instar larvae feeding on a shrunken propupa. **d)-h)** Trial 6, second instar larvae feeding on pupae. Bar: a)-e) 1 mm, f)-h) 750 µm

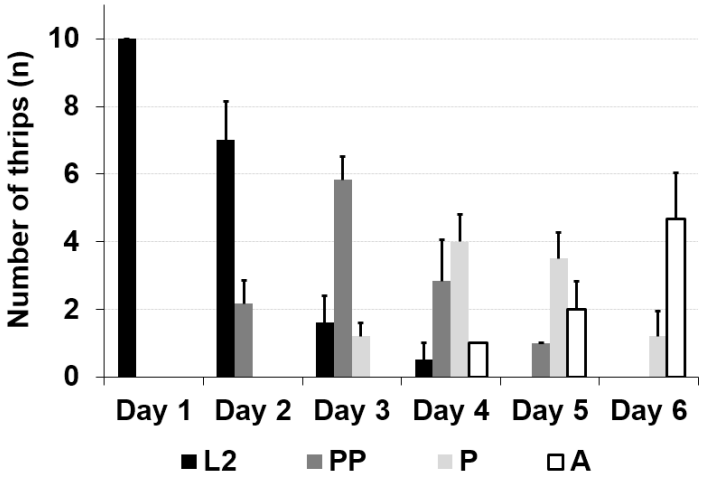

Fig. 5: Development of thrips (*F. occidentalis*) fed with paralyzed *Echinothrips americanus* pro-/pupae. **L2** Second instar larva, **PP** Propupa, **P** Pupa, **A** Adult thrips.

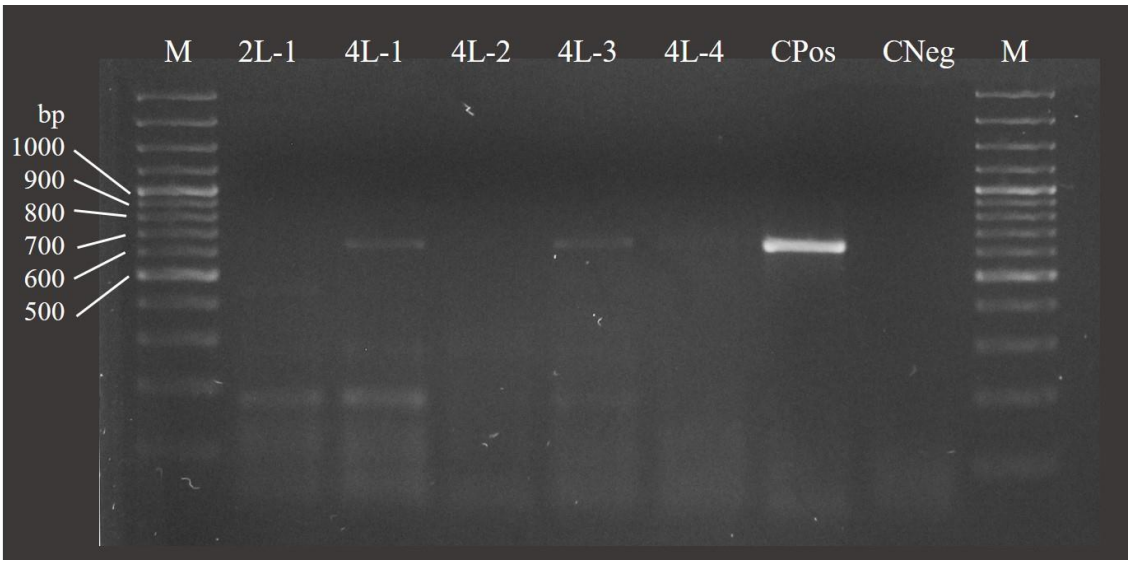

Fig. 6: PCR results of larvae (*F. occidentalis*) fed with *Echinothrips americanus* pro-/pupae for 24h (pooled probes). Primer pair: 81F/691R. Amplicon size: 590-632 bp. **M** Marker, **2L-X** Two larvae pooled, **4L-X** Four larvae pooled, **Cpos** Positive control, **CNeg** Negative Control.

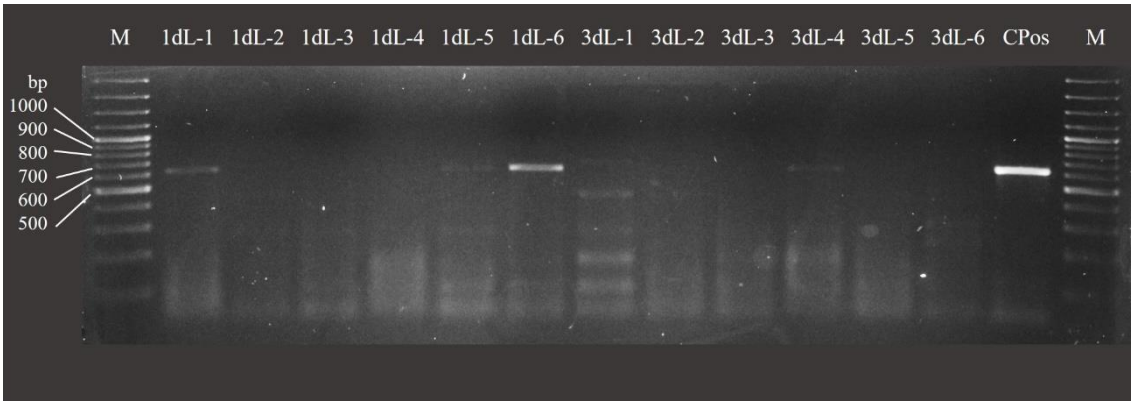

Fig. 7: PCR results of larvae (*F. occidentalis*) fed with *Echinothrips americanus* pro-/pupae for 24h/72h. Primer pair: 81F/691R. Amplicon size: 590-632 bp. **M** Marker, **1dL-X** Larva fed for 24h, **3dL-X** Larva fed for 72h, **Cpos** Positive control.

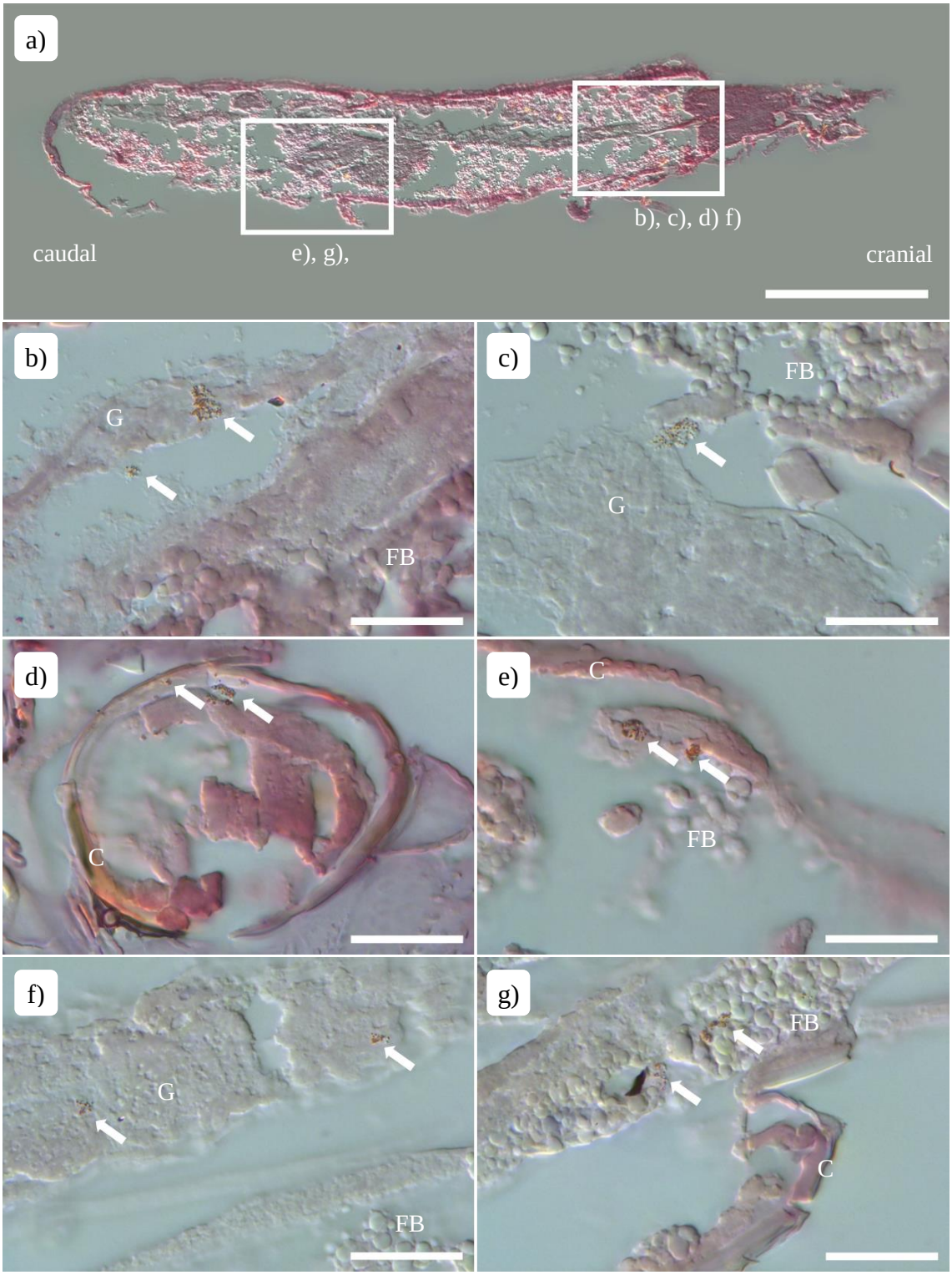

Fig. 8: *In situ* hybridisation, *Frankliniella occidentalis* larvae. Larvae fed with *Echinothrips americanus* pro-/pupae (b-e) and larvae reared on French bean (f & g). **a)** Overview. **b)** Gut epithelium with a cluster of *Wolbachia* signals (arrow). **c)** Gut tissue with clusters *Wolbachia* signals. **d)** Coxa with one single signal and a cluster of signals of *Wolbachia*. **e)** Subcuticular tissue with two clusters of signals of *Wolbachia*. **f)** Gut tissue with two clusters of signals of *Wolbachia*. **g)** Fat body with multiple single signals and one cluster of signals of *Wolbachia*. Bar: a) 100 µm; b)-g) 20 µm. **C** Cuticle, **FB** Fat Body, **G** Gut

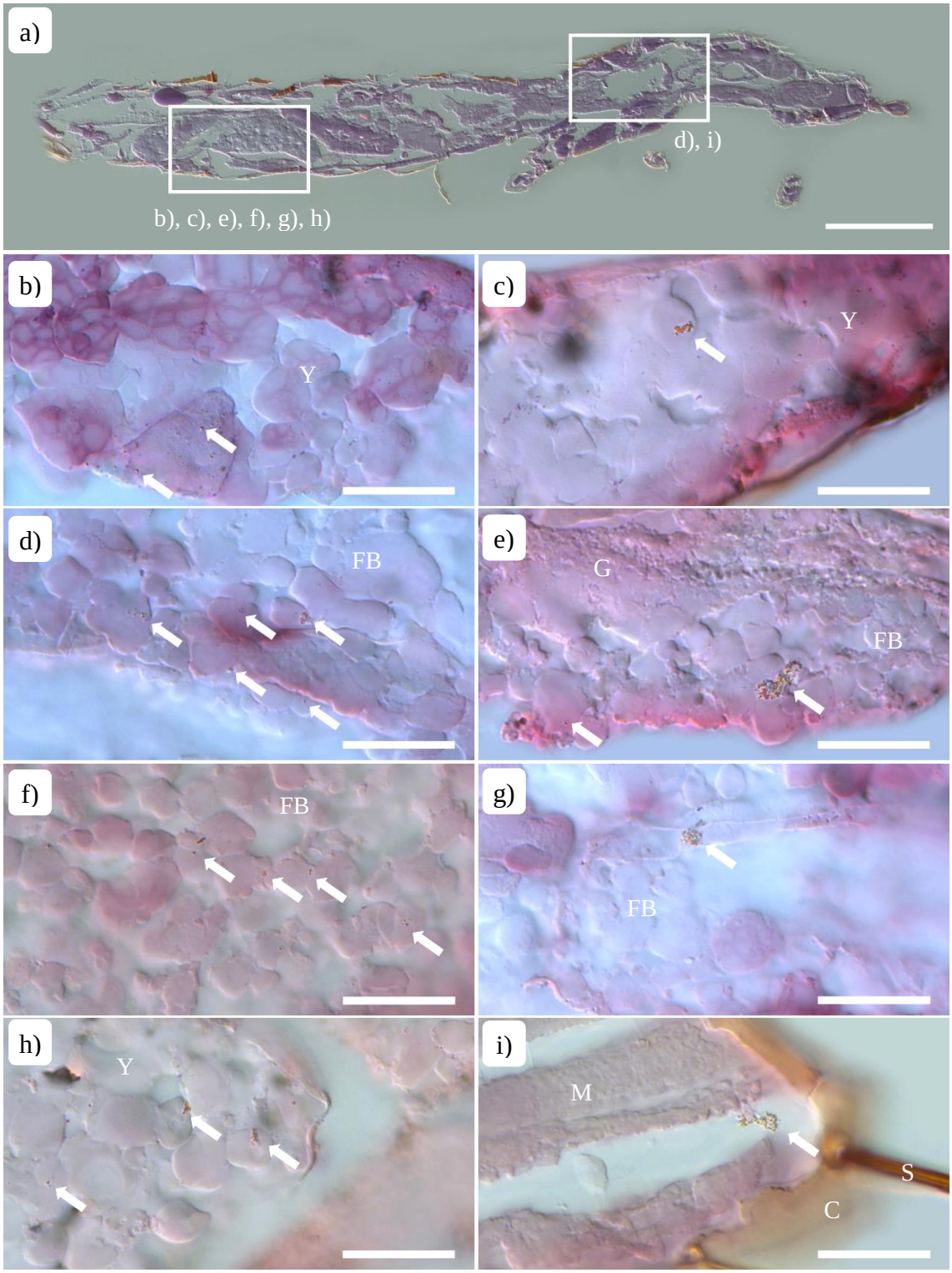

Fig. 9: *In situ* hybridisation, *Frankliniella occidentalis* adult. Adult females raised by feeding *Echinothrips americanus* pro-/pupae to the larval stages of *F. occidentalis* (b-e)) and adult control females raised on French bean (f-i). **a)** Overview sagittal. **b)** Egg with multiple single *Wolbachia* signals. **c)** Egg with a cluster of *Wolbachia* signals (arrow). **d)** Fat body with single *Wolbachia* signals. **e)** Fat body with one cluster of signals and one single signal of *Wolbachia*. **f)** Fat body with multiple single signals and of *Wolbachia*. **g)** Fat body with one cluster of signals. **h)** Egg with multiple single *Wolbachia* signals. **i)** Thorax musculature with a cluster *Wolbachia* signals. Bar: a) 100 µm; b)-i) 20 µm. **C** Cuticle, **FB** Fat body, **G** Gut, **M** Muscle, **S** Seta

4.2 Application of algae

Different application methods (SMIAS, SMIAS-UDA and GMIAS-S-C)

When the *Chlamydomonas reinhardtii* suspension was applied to thrips by the SMIAS the faeces or the gut lumen of thrips were transparent one day after the application start. When the algae suspension in the SMIAS was inspected, the algae were not dispersed in the suspension, but formed a pellet on the bottom of the PCR-plate-well.

Application with the SMIAS-UDA and the GMIAS-S-C lead to greenish faeces (Fig. 10a) and gut lumen (Fig. 10c) of thrips fed with the algae suspension one day after application start with exception of late second instar larvae showing no colouration of the gut lumen. After one day the algae formed a thick layer at the Parafilm at the bottom of modified pipette tip of the SMIAS-UDA and the containers of the GMIAS-S-C.

The gut lumen of adult thrips was barely visible depending on their cuticle colouration. While the gut lumen of light brownish *F. occidentalis* and *T. tabaci* adults was clearly visible, for dark brownish adults of *F. occidentalis* and *T. tabaci* and all adults of the dark brownish to blackish species of *E. americanus* the gut lumen was only faintly visible through the lighter intersegmental skin.

Microscopic examination

The uptake of the *C. reinhardtii* suspension was visible using light (DIC) and fluorescence (UV) microscopy. Larvae of *E. americanus* (Fig. 11a-d), *F. occidentalis* (Fig. 10) and *T. tabaci* (Fig. 12a-d) showed a green (DIC)/red (UV) gut lumen, while the larvae of the control group showed no sign of chlorophyll uptake.

Adult *E. americanus* were opened at the distal thorax/proximal abdomen and the two halves were pulled apart to expose the gut allowing clear vision. The gut of *T. tabaci* adults was visible through the cuticle, thus the thrips were not opened. Adults fed with algae suspension showed a coloured gut lumen, whereas the control showed no specific coloration (*E. americanus*, Fig. 11e-h), (*T. tabaci*, Fig. 12e-h). Because of a small number of individuals and a poor preparation, no pictures of adult *F. occidentalis* were taken.

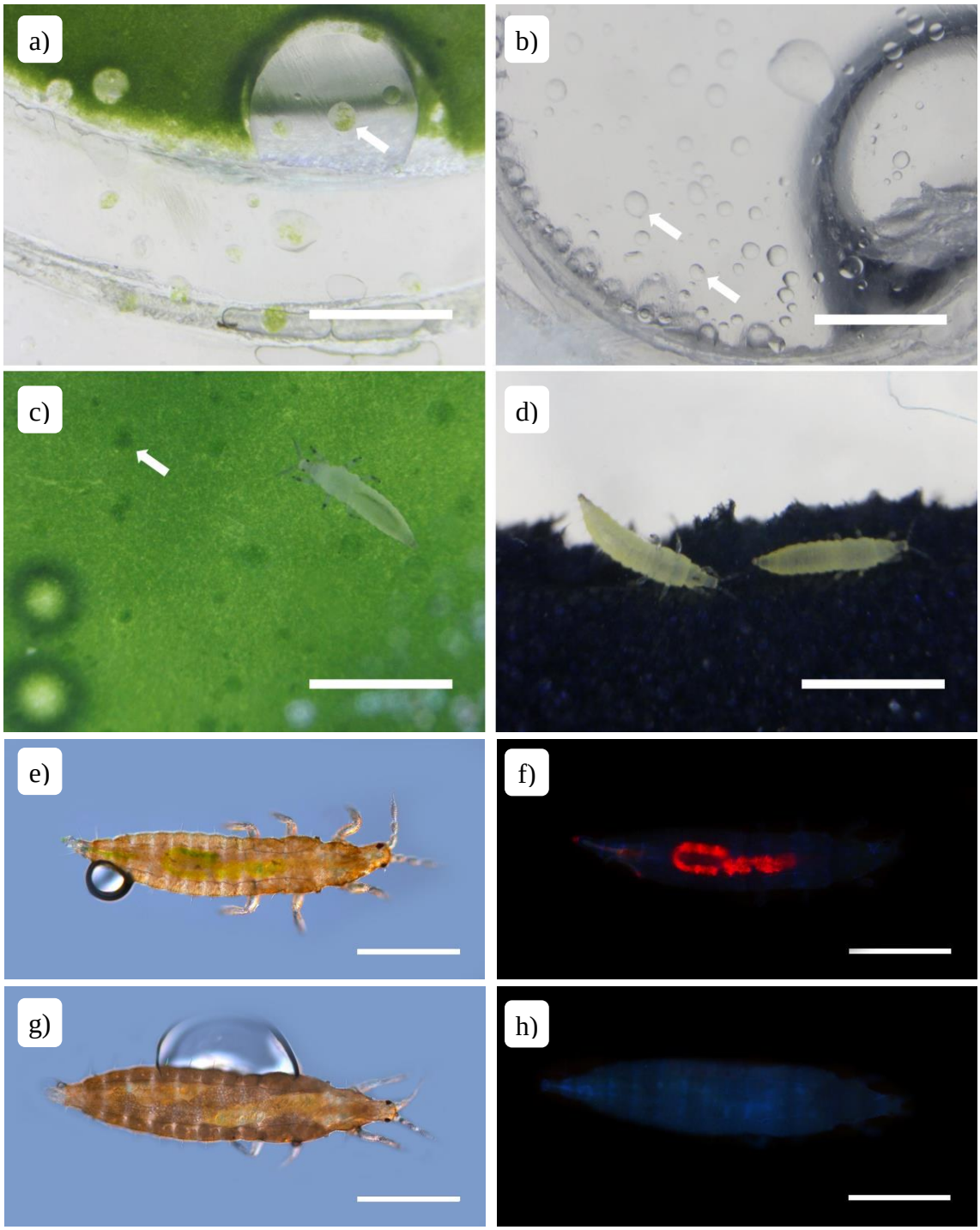

Fig. 10: ***Chlamydomonas reinhardtii*** **application to** ***Frankliniella occidentalis*** **larvae. a)** Fluid thrips faeces (arrows) one day after application start containing greenish inclusions. **b)** Thrips faeces of the control group showing no evidence of algae uptake. **c)** Larvae feeding on the algae suspension showing a green gut lumen. **d)** Larvae of the control group. The gut shows no sign of chlorophyll uptake. **e)** Larva fed with algae suspension (DIC). **f)** Larva fed with algae suspension (UV). **g)** Larva fed with control solution (DIC). **h)** Larva fed with control solution (UV).Bar: a), b) 2 mm; c), d) 1 mm. e)-h) 200 µm

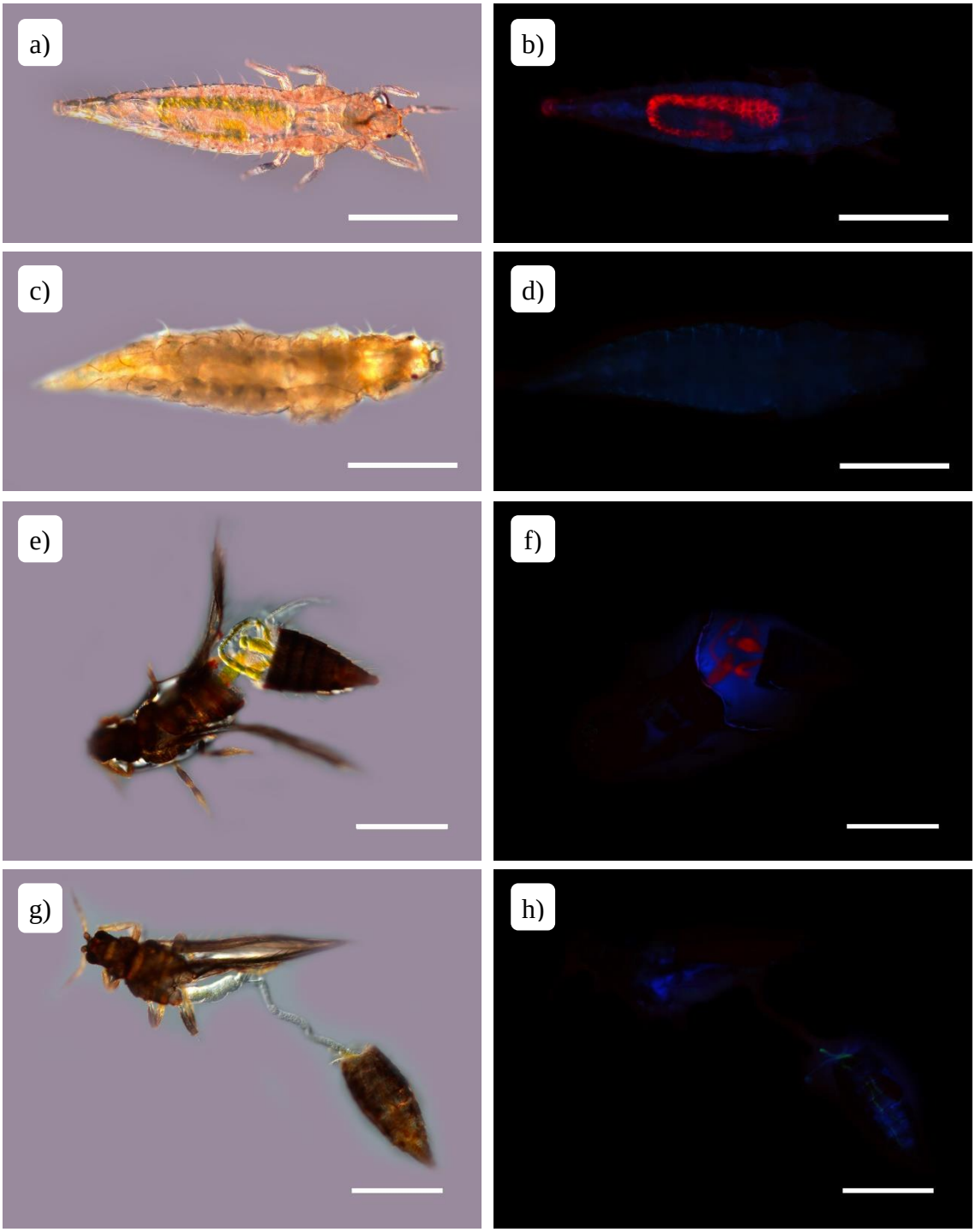

Fig. 11: ***Chlamydomonas reinhardtii*** **application to** ***Echinothrips americanus*****. a)** Larva fed with algae suspension (DIC). **b)** Larva fed with algae suspension (UV). **c)** Larva fed with control solution (DIC). **d)** Larva fed with control solution (UV). **e)** Adult thrips fed with algae suspension (DIC). **f)** Adult thrips fed with algae suspension (UV). **g)** Adult thrips fed with control solution (DIC). **h)** Adult thrips fed with control solution (UV). Bar: 200 µm.

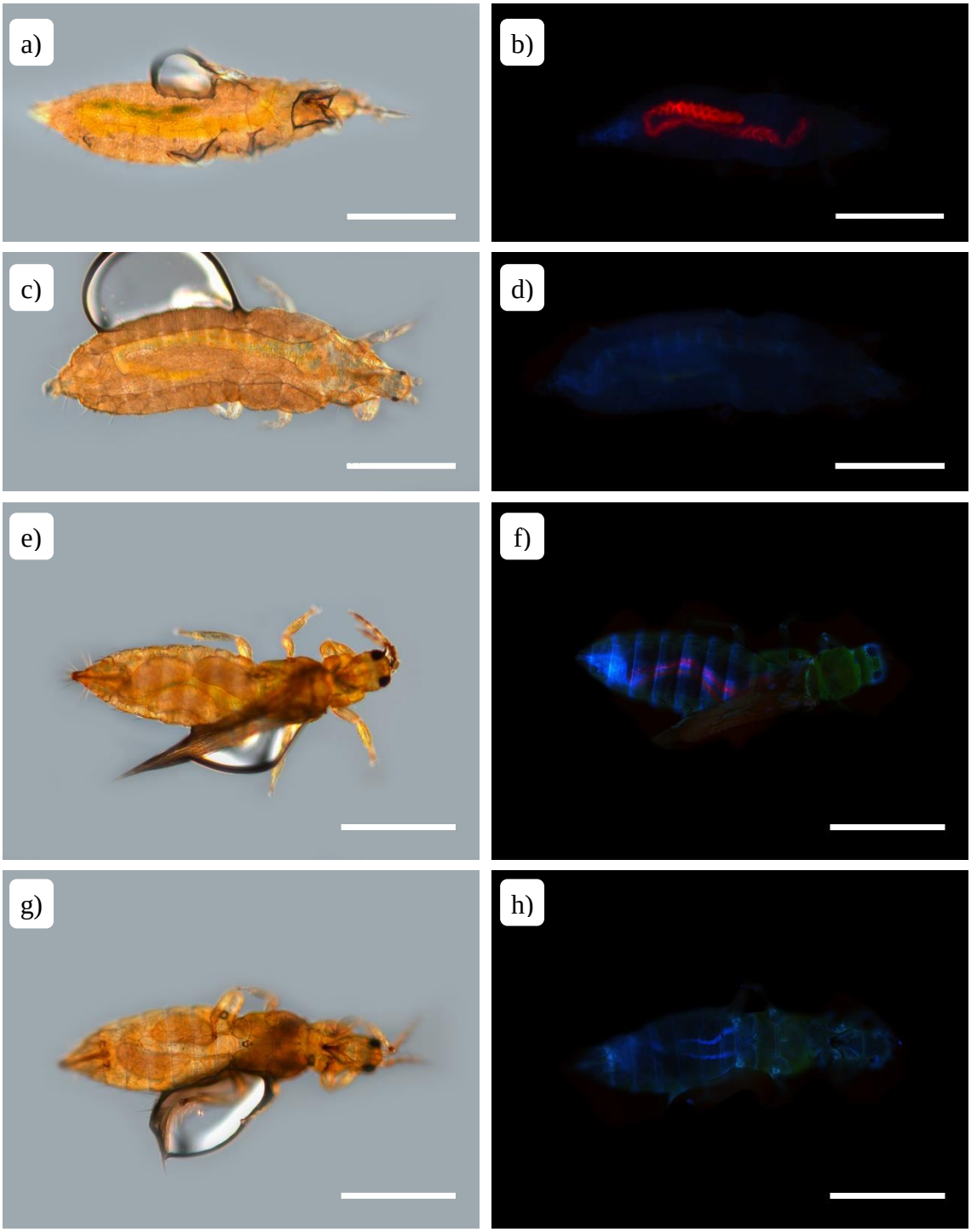

Fig. 12: ***Chlamydomonas reinhardtii*** **application to** ***Thrips tabaci*****. a)** Larva fed with algae suspension (DIC). **b)** Larva fed with algae suspension (UV). **c)** Larva fed with control solution (DIC). **d)** Larva fed with control solution (UV). **e)** Adult thrips fed with algae suspension (DIC). **f)** Adult thrips fed with algae suspension (UV). **g)** Adult thrips fed with control solution (DIC). **h)** Adult thrips fed with control solution (UV). Bar: 200 µm.

5 Discussion

Frankliniella occidentalis larvae feeding on Echinothrips americanus pro-/pupae

Under specific conditions, *Frankliniella occidentalis* larvae showed an omnivorous feeding behaviour. While first instar larvae and early second instar larvae barely fed on *Echinothrips americanus* pro-/pupae, second instar larvae 24 to 48h after the hatch to the second instar larvae steadily consumed pro-/pupae. Furthermore, *E. americanus* pro-/pupae that were not paralyzed by mistake stayed unharmed, showing that the paralysation method was necessary to increase the larvae's acceptance of pro-/pupae as food source.

Trichilo and Leigh (1986) showed that larvae and adult females of the *F. occidentalis* have a functional predatory response to increased densities of spider mite eggs. However, adult female thrips had faster rate of egg discovery compared to larvae. They described *F. occidentalis* as an omnivorous opportunist. The feeding on mite eggs has been described for *Frankliniella schultzei* and *Thrips tabaci* too (Wilson et al., 1996). Moreover, they found a similar behaviour regarding the mite egg consumption rate of first and second instar larvae of *F. schultzei* with first instar larvae showing a much lower consumption. Additionally, *Frankliniella tritici* is known to feed on alfalfa weevil eggs (Barney et al., 1979). Agrawal et al. (1999) showed that when resistance in host plants is induced, *F. occidentalis* shifts their feeding behaviour by lowering herbivory and increasing predatory by consuming half of the amount of plant material and twice the number of mite eggs compared to non-induced control plants. Furthermore, Agrawal and Klein (2000) showed a preference behaviour of *F. occidentalis* for treated mite eggs. Mite eggs derived from mites reared on resistance induced cotton plants were less attractive compared to mite eggs derived from mites reared on non-induced cotton plants. Stafford-Banks et al. (2014) showed that tospovirus-infected female thrips become more predaceous in response to pathogen infection. Furthermore, van Maanen et al. (2012) showed that *F. occidentalis* feeds on whitefly crawlers. Thus, omnivory seems to be a natural trait for *F. occidentalis* that can be greatly affected in several ways. Yet, the predation part of the described omnivory is just linked to the uptake of immobile spider mite eggs.

F. occidentalis feeding on other thrips is only known from feeding on conspecifics. The occurrence of cannibalism in *F. occidentalis* have been reported in several publications (Arthurs & Heinz, 2002; Broadbent et al., 2003; Jahani et al., 2018; Uzun et al., 2015) and is described for several other thrips species like *Aelothrips intermedius* (Uzun et al., 2015), *Androthrips flavipes* (Varadarasan & Ananthakrishnan, 1981), *Elaphrothrips tuberculatus* (Crespi, 1990), *Haplothrips victoriensis* (Le, 2010) and three predatory thrips species of the genus *Scolothrips* (*S. longicornis* (El-Basha, 2015; Farazmand et al., 2014), *S. sexmaculatus* (Coville & Allen, 1977; Gilstrap et al., 1976) *S. takahashii* (Liu et al., 2020)). Farazmand et al. (2014) and Liu et al. (2020) showed that the rate of cannibalism of *S. longicornis* and *S. takahashii* depends on the availability of prey as

it is increasing in the absence of non-conspecific prey. Hence, feeding on conspecifics seems to be an important behavioural trait for obligate and facultative predacious omnivory thrips to compensate times of low availability of the primary food source. As described by Richardson et al. (2010) cannibalism in non-carnivorous insects can play an important role in regulating host plant-insect relationships. Furthermore, it can improve growth rate, survivorship, vigour, longevity, and fecundity and is not solely occurring due to laboratory setups lacking nutritional needs but is more a natural phenomenon increasing adaptivity.

Thus, despite of indications that predacious behaviour is natural to *F. occidentalis*, this is the first time that the feeding of *F. occidentalis* on another thrips species is shown. However, due to the very specific conditions that were necessary for *F. occidentalis* larvae to feed on *E. americanus* pro-/pupae, *F. occidentalis* larvae should be less considered as predators in the sense of a predator actively fighting with competitive prey but more like scavengers, which occasionally consume ill or weaker thrips and immobile prey like mite eggs or less mobile prey like whitefly crawlers.

Additionally, the experiments placing two larvae in one well lead to a higher consumption rate and aggressive behaviour was observable. While most publications about intraspecific aggressive competition are linked to competing adult male thrips (*F. occidentalis* (Terry & Dyreson, 1996), *Hoplothrips karnyi* (Crespi, 1988), *Thrips tabaci* (Woldemelak, 2020), only Bhuyain and Lim (2019) investigated the intra- und interspecific competition for food of *F. occidentalis* and *Frankliniella intonsa*. They showed that bouts of confrontation as reaction of competition for food occurred for second instar larvae and adult females inter- and intraspecifically. Adult females of *F. occidentalis* showed 2.6 times more bouts of confrontation compared to *F. intonsa* when competing for pollen or 2.5 times more when competing for honey intraspecifically. Thus, *F. occidentalis* might in general be the more aggressive species leading to a higher chance of observing aggressive behaviour. However, as the difference in food uptake between single thrips and a group thrips was not investigated, the effect of an increased consumption of *E. americanus* pro-/pupae shown in this remains a first finding that has to be examined by further studies.

Horizontal transmission of Wolbachia

Since in most cases only the cuticles of the pro-/pupae were left over by *F. occidentalis* larvae, chances were high that larvae took up tissues containing *Wolbachia*. The *wsp* gene of the *Wolbachia* symbiont was detected using PCR in pooled and non-pooled samples of *F. occidentalis* larva feeding on *E. americanus* pro-/pupae for 24 h and 72 h. No signals were detected in the control.

After the *in situ* hybridisation, signals were detected in several larvae and adult females of *F. occidentalis* after being fed with *E. americanus* pro-/pupae. However, sporadically signals were also found in larvae and adults of the control. Signals were found in somatic and

reproductive tissues. Nevertheless in the old control samples (from 2017), no signals were found. When the main population was checked for other arthropods as potential source of *Wolbachia* infection, predatory mites and sciarid flies were found for which some species are known to be infected with *Wolbachia* (predatory mites (Schütte & Dicke, 2008), sciarid fly (Tao et al., 2015)). This indicates that a horizontal transmission event may already have occurred in the *F. occidentalis* main population after 2017 leading to low level infections that are not detectable with standard PCR. Thus, feeding *F. occidentalis* larvae with *E. americanus* pro-/pupae may lead to higher infection rates and more *Wolbachia* in thrips tissue, allowing a detection using standard PCR and showing more signals in *in situ* samples. Maybe more signals can even be detected when using the more sensitive long PCR (Jeyaprakash & Hoy, 2000) or a DNA isolation method that is more specialized on bacterial endosymbiont DNA compared to the 100-fly method after Roberts (1998). Nevertheless, these findings imply that a horizontal transmission of *Wolbachia* is possible for *F. occidentalis*.

First indications of a horizontal transmission of *Wolbachia* were found by Schilthuizen and Stouthamer (1997). They performed comparative molecular phylogenetics on 20 parthenogenetic *Trichogramma* cultures and their *Wolbachia*, finding a phylogenetic distribution of *Wolbachia* that implies to be a result of occasional horizontal transmission events. Vavre et al. (1999) detected *Wolbachia* infections in four of five common European species of hymenopteran parasitoids of frugivorous *Drosophila* species. The similarities of the analysed *wsp* gene of the hosts and parasites greatly supported the hypothesis of natural occurring horizontal transmission. Heath et al. (1999) were the first to experimentally show that the horizontal transmission between distant insect species is possible. They were able to detect *Wolbachia* in *Wolbachia*-free parasitic wasps (*Leptopilina boulardi*) after parasiting *Wolbachia*-infected *Drosophila simulans*. Furthermore, *Wolbachia* were found in the progeny of the new *Wolbachia* host showing that a horizontal transmission can be the beginning of a vertical transmission, what supports the finding of signals in eggs ready for oviposition in adult female *F. occidentalis*. Huigens et al (2004) showed, that horizontal transmission, besides interspecific distant species, occurs in close related species in the genus of *Trichogramma*. Still, in their study intraspecific horizontal transmission was the more successful when compared to the interspecific transfer. That horizontal transmission of *Wolbachia* can occur in a wide range of taxonomic relationships was shown by Ahmed et al. (2016). Their results revealed interspecific, intergeneric, interfamilial, and interordinal horizontal transmission of *Wolbachia* strains in Lepidopteran species. Furthermore, Pattabhiramaiah et al., (2011) found evidence that the horizontal transfer of *Wolbachia* additionally occurs between different classes of arthropods by finding identical sequences of the *Wolbachia* of a honeybee subspecies *Apis mellifera carnica* and its ectoparasite *Varroa destructor*. Thus, the horizontal transmission between species of different taxa levels of arthropods seems to be a natural phenomenon with a frequency that still has to be investigated. Besides the horizontal transmission

from arthropod to arthropod Kolasa et al. (2017) and Li et al. (2017) showed that the horizontal transmission by infected leave tissue should be considered a significant way of transmission as well. Kolasa et al (2017) detected *Wolbachia* DNA in leaf tissue of host plants exploited by the leaf beetles of the genus *Crioceris*. Furthermore, Li et al. (2017) showed that *Wolbachia* can persist in cotton plant leaves at least 50 days after infection by *Wolbachia* positive whiteflies (*Bemisia tabaci*). Additionally, when *Wolbachia* negative whiteflies were introduced to infected plant leaves the majority became infected and started transmitting *Wolbachia* vertically.

Still, despite several potential transmission ways *F. occidentalis* were not tested positive for *Wolbachia* DNA using PCR so far (Kumm & Moritz 2008, 2010; additional several regular tests of the main population of the working group, unpublished).

One reason may be the choice of primers for the PCR. Sintupachee et al. (2006) detected *Wolbachia* DNA in *Frankliniella schultzei* using the primers for the *ftsZ* gene but were not able to detect it using the primers for the *wsp* gene, despite modifying the PCR process. Thus, the choice of primers is crucial due to genetic variety of *Wolbachia* supergroups as shown by Simoes et al. (2011). Hence, the differing results in the PCR with no *Wolbachia* positive *F. occidentalis* controls and positive control thrips found with the *in situ* hybridisation, may be linked to an infection of the control population with a *Wolbachia* group, which is not detectable with the used primers for the *wsp* gene (PCR) but with the primers for the *ftsZ* gene (*in situ* hybridisation).

However, besides methodical reasons there can be biological reasons that there has been no *Wolbachia* infection reported for *F. occidentalis* so far. Faria et al., (2016) showed that when *Wolbachia* negative larvae and adults of *Drosophila melanogaster* were fed with a homogenate made from *Wolbachia* positive adults/embryos no *Wolbachia* infection were found later in the fed *D. melanogaster* implying that unknown factors may influence the success of horizontal transmission. In fact, for vertical transmission of *Wolbachia* the influence of temperature as an abiotic factor is known (Hurst et al., 2001; Mouton et al., 2007). Moreover, the more important role may be the already existing microbiome of the host insect. Hughes et al. (2014) used naturally *Wolbachia* uninfected *Anopheles* mosquitos to examine the effect of the host's microbiome on the horizontal transmission via intrathoracic microinjection. While some *Wolbachia* strains invaded the germ line of *Anapholes gambiae* resulting in a poor vertical transmission, the infection of *Anopheles stephensi* lead to massive post blood meal mortality preventing vertical transmission of *Wolbachia*. When mosquitos were treated with antibiotics a perfect vertical transmission was observable. De Vries et al. (2001) examined the gut microbiome of *F. occidentalis*. While there was no evidence for vertical transmission, the gut bacteria showed unlimited growth during the larval stages. Additionally, all larvae of the second instar stage were infected with high numbers of bacteria. While the number of bacteria declined in the propupal and pupal stage, it increased again in adult thrips. Thus, the gut microbiome, together with thrips

only occasionally consuming other arthropods and abiotic factors like inconsistent temperature may prevent a horizontal or consistent vertical transmission of *Wolbachia*, while consistent laboratory conditions and thrips as solely food source may support the transmission.

Furthermore, *Wolbachia* infections were detected in the two species *Frankliniella intonsa* (Lou et al., 2015), *Frankliniella schultzei* (Sintupachee et al., 2006) closely related to *F. occidentalis* implying that under certain conditions the *Wolbachia* infection of species of that genus may occur.

However, establishing this method, allowing a reliable application of *E. americanus* pro-/pupae to *F. occidentalis* larvae, is just a first step for the examination of the potential horizontal and vertical transmission of *Wolbachia* in *F. occidentalis*. Further studies are necessary regarding persistence of the infection, vertical transmission and potential implications of the infection for the vector function of *F. occidentalis* for tospoviruses or life history traits of the host.

Application of the unicellular algae Chlamydomonas reinhardtii

The unicellular algae *Chlamydomonas reinhardtii* was applied to *F. occidentalis* using three different methods. When the SMIAS method was used there was no proof of an uptake of the algae by thrips. As the used strain of *C. reinhardtii* is unlikely to develop flagella, it formed a pellet on the bottom of wells of the 96 well-PCR plate, which could not be reached by the thrips. In contrast to the SMIAS, the SMIAS-UDA and the GMIAS-S-C supported the uptake of *C. reinhardtii*, as the algae suspension was applied upside down. Thus, for poorly soluble or insoluble application substances or organisms those setups are recommendable and furthermore allow single application (SMIAS-UDA) and group application (GMIAS-S-C) experiments.

With the GMIAS-S-C the uptake of *C. reinhardtii* was proven for *Echinothrips americanus*, *F. occidentalis* and *Thrips tabaci* 24h after the start of application. Thus, two application methods were established allowing a time efficient and standardized application of algae complementing the first methods of earlier experiments (Jilge, 2016) leading to the first reliable application methods described so far allowing a short or continuous long-term application of unicellular algae to Thysanoptera. With *C. reinhardtii* as a well-researched genetic model organism this method provides a high potential tool for the oral application of genetically engineered molecules like proteins or RNA. Furthermore, these methods could be used as a standardized method for the application of other microorganisms like bacteria for RNA (Andongma et al., 2020; Whitten et al., 2016). Even if the application of genetically modified symbionts has several positive aspects compared to other RNA application methods (Whitten & Dyson, 2017), the unknown potential side effects of those additional symbionts on the host and the risk of undeliberate release or transfer, may make the application of dsRNA via *C. reinhardtii* or a naked dsRNA containing diet, which can be renewed on a daily base, to a safer and more specific alternative.

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7 Supplementary data

DNA-Extraction and gel electrophoresis

Tab. 1: Extraction buffer modified 100 fly method.

Component	Volume
0.1 M TRIS	2.5 ml
0.1 M NaCl	2.5 ml
0.05 EDTA	5 ml
0.2 M Sucrose	5 ml
0.5% SDS (w/v)	2.5 ml
dH_2O	37.5 ml
pH	9.2 (25 °C)

Tab. 2: (6x) Loading buffer gel electrophoresis.

Component	Volume/weight
Glycerol	6 ml
Bromophenol blue	0.05 g
Xylene cyanol	0.05 g
dH_2O	14 ml

In situ hybridisation

Tab. 3: 0.1 M Soerensen's phosphate buffer (pH 7.2).

Component	Volume
0.2 M Na_2HPO_4	72 ml
0.2 M NaH_2PO_4	28 ml
	ad 200 ml dH_2O

Tab. 4: TRIS-HCl/NaCl buffer pH (7.6).

Component	Volume
TRIS	20 mM
NaCl	300 mM
	ad ddH_2O

Tab. 5: TRIS-HCl/NaCl/$MgCl_2$ buffer (pH 9.5).

Component	Volume
TRIS-HCl	20 mM
NaCl	300 mM
$MgCl_2$	50 mM
	ad ddH_2O

Tab. 7: Blocking buffer (10 ml).

Component	Volume/weight
1 M TRIS	0.062 ml
5 M NaCl	0.15 ml
Albumin	0.0125 g

Chlamydomonas reinhardtii cultivation medium

Tab. 8: TAP medium (1 L, pH 7.0).

Component	Volume
1 M TRIS base	20 ml
Phosphate buffer II	1 ml
Solution A	10 ml
Hutner's trace elements	1 ml
Glacial acetic acid	1 ml

Tab. 9: TAP medium - phosphate buffer II (100 ml).

Component	Volume/weight
K_2HPO_4	10.8 g
KH_2PO_4	5.6 g
	ad 100 ml dH_2O

Tab. 10: TAP medium – solution A (500 ml).

Component	Volume
NH_4Cl	20 g
$MgSO_4$-$7H_2O$	5 g
$CaCl_2$-$2H_2O$	2.5 g

Chapter V

Discussion

1 General discussion

Finding a method for a long-term application of diets to Thysanoptera

The rearing of insects on artificial diets is an important technique in means of scientific experiments and other purposes (Cohen, 2015). Artificial diets that allow the development of insects from the first instar larvae to the adult insect can be used for a wide range of experiments for basic research as well as for applied science purposes. Additionally, for insect species that are economically relevant pests paired with a limited knowledge of their biology, artificial diets can be a key factor for finding new control measures. The order Thysanoptera has several economically relevant pest species, like *Frankliniella occidentalis*. However, in spite of their economically relevance there is still no standardized method allowing an efficient long-term application (from first instar larva to the adult thrips) of diets for Thysanoptera. Thus, in this thesis a new method was established – the micro insect application system (MIAS). It is the first system allowing a highly standardized long-term application of diets to single thrips (SMIAS –single micro insect application system) or groups of thrips (GMIAS –group micro insect application system). So far the rearing of thrips in artificial surroundings on an artificial diet was only performed by Kinzer et al. (1972) and Lowry et al. (1992), who used the method after Kinzer et al. (1972). They reared *Frankliniella fusca* on an artificial diet after Vanderzant et al. (1962) in 1-oz medicine cups containing agar. For this, larvae hatched on peanut plants were shaken into the medicine cups. The diet was not renewed during the experiment as it retained adequate freshness during a thrips generation. However, Kinzer et al. (1972) used two preservative agents (potassium sorbate, methyl parahydroxybenzoate (summarized in: Lewis, 1997)) in that solid diet. In preliminary studies of this work, these agents didn't show an effective prevention of microbial growth for liquid artificial diets. Sikorowski and Lawrence (1994) summarized many ways of microbial contamination. Microorganisms are omnipresent and can be introduced to the experimental setup by the ambient air, ingredients of the diet, the scientist or the insect itself. Keeping a diet and the experimental setup sterile is still possible but time consuming and cost-intensive. Thus, the SMIAS and GMIAS supports a regular renewing of the liquid diet and cleaning or removing of the feeding site without the necessity of handling the thrips or transfer them to a newly prepared setup.

Furthermore, for an observation of the diets' impact on thrips a clear view is crucial. A good material that provides a clear view is glass, which can be perfectly used for observing thrips like done by Kirk (1987), Olaniran & Kirk (2012) and Barney et al. (1979) using microscope slides and cover slips. However, glass is a material that is more difficult to modify and is less stable compared to transparent synthetic materials like polystyrene or polymethyl methacrylate (acrylic glass). Therefore, for the application setups synthetic materials were used. As thrips are between

1-3 mm in size, the setups have to be smoothly closable, as thrips can escape through the smallest gaps. Thus, the economical relevant and very mobile and active thrips species *Frankliniella occidentalis* was the perfect choice to proof the functionality of our setups.

Frankliniella occidentalis an opportunistic omnivore

The western flower thrips *Frankliniella occidentalis* is often referred to as "polyphagous" (Bielza, 2008; de Vries et al., 2001; Kirk & Terry, 2003; Koschier et al., 2000; Reitz, 2009; Wimmer et al., 2008) referring to its mainly phytosugous feeding behaviour. However, the horizontal transmission of *Wolbachia* experiment in chapter IV showed the omnivorous character of *F. occidentalis*. Second instar larvae of *F. occidentalis* were easily reared with "paralyzed" *Echinothrips americanus* propupae and pupae without providing parts of a host plant or pollen. In fact, several authors refer to *F. occidentalis* as omnivore (Agrawal et al., 1999; Agrawal & Klein, 2000; Janssen et al., 2003; Pappas et al., 2018; Pickett et al., 1988; Spence et al., 2014; Zhi et al., 2006) as it additionally feeds on mite eggs (Roda et al., 2000; Trichilo & Leigh 1986) and crawlers of the whitefly (van Maanen et al., 2012). Besides feeding on other insects, the occurrence of cannibalism in *F. occidentalis* is described in various publications (Arthurs & Heinz, 2002; Broadbent et al., 2003; Jahani et al., 2018; Uzun et al., 2015). Additionally, as mentioned in chapter II, in preliminary experiments a *F. occidentalis* larvae was observed feeding on a fungi hyphae, performing better, in means of size and development to the second instar stage, compared to the other larvae indicating that the omnivory of *F. occidentalis* may be more versatile than described so far. Furthermore, there are a few more thrips species that are described as omnivore as they were found to feed on other arthropod eggs (*Frankliniella tritici* (Barney et al., 1979)), *Frankliniella schultzei* and *Thrips tabaci* (Wilson et al., 1996), species that also are considered as pest thrips.

With their maxillary stylets allowing their piercing-sucking feeding mechanism, thysanopteran species are having a versatile tool to get access to food sources. Thus, the range of possible food sources used by specific thrips species should be investigated and considered for dietary experiments, as it might have further implications for nutritional needs, when a thrips is an opportunistic omnivore or an obligate herbivore.

The rearing of single thrips and group of thrips with artificial diets

The possibility of rearing single individuals allows to investigate the nutritional needs or the impact of substances applied through diets without several group related factors like competition, cannibalism or the disturbance of feeding by conspecifics, making it a key factor for a detailed, precise analysis. Single thrips rearing combined with using eggs to introduce the thrips to the setup allows conducting long time application experiments for precise life time tables. Observing thrips' developmental times and survival in order to create life time tables is an important method to examine the influence of several factors on thrips, like the influence of host plants (Gaum et al., 1994; van Rijn et al., 1995; Zhang et al 2007), additional food sources (Milne & Walter, 1997; Soleiman Nejadian et al., 2009; Trichillo & Leigh, 1988), the impact of the rearing situation of the parental generation (Krueger et al., 2016, 2020; Li et al., 2015), the effect of viruses (de Angelis et al., 1993; Inoue & Sakurai, 2006; Ogada et al., 2013) or abiotic factors (Murai, 2000; Pakyari et al., 2011). However, for experiments examining different factors than the impact of different host plants, an artificial diet of consistent quality can be used for a more standardized food source, as the nutritional values of plant parts may vary due to the different experimental abiotic factors like light, humidity or temperature.

While the rearing of single thrips is a very precise method for examining effects on thrips, it is too time consuming for rearing large numbers of thrips. Thus, for the rearing of *Wolbachia*-free strains of thrips or as a standardized method for group application experiments the GMIAS could be used, as antibiotic treatments of thrips were conducted differently by most authors (Arakaki, 2001; Kumm & Moritz, 2008, Nguyen et al., 2015, 2017; van der Kooi and Schwander, 2014). Furthermore, it can be used as an additional method to de Vries et al. (2004) for creating aposymbiotic strains of thrips. In addition to that, the GMIAS could support rearing males for a sterile insect technique in Thysanoptera, competing with natural non-sterile males for integrated pest management of crops. For other insects the sterile insect technique proofed to be a valid method for integrated pest management (Dyck et al., 2006; Vreysen et al., 2007). Nonetheless, for a consequent rearing on artificial diets many factors have to be considered (Cohen et al., 1999, Sørensen et al., 2012).

Still, besides an appropriate setup, crucial for the artificial rearing of thrips is a diet suitable for thrips species allowing the development of thrips from larva to adult stage.

Artificial diets for Thysanoptera

There are many established artificial diets for various insects from different orders and literature for the formulation and improvement of diets (Cohen, 2015; Singh, 1977; Vanderzant, 1974). Despite being a versatile tool for the research of insect species, only Kinzer et al. (1972) and Lowry et al. (1992) reared thrips on an artificial diet. They used a solid artificial diet after Vanderzant et al. (1962) to rear *Frankliniella fusca* without separating thrips from the diet. However, for this thesis we wanted to use liquid diets for easier handling when separating thrips from the diet by an artificial membrane. Thus, we established 6 different diets varying in the source of amino acid (casein, soy peptone, wheat peptone, yeast extract) and the concentration of different ingredients. When casein, soy peptone and wheat peptone were used as the main amino acid source, only thrips larvae reared on soy peptone developed to adults, while there was no development and a higher mortality when using casein, indicating that at least for the chosen concentrations the choice of amino acid source has a great impact on the suitability of a diet. The importance of the availability of artificial diets can be seen when looking at the European corn borer (*Ostrinia nubilalis*; Order: Lepidoptera), a main pest insect for maize. In 1942 Bottger conducted experiments using a synthetic medium for nutrition studies for *O. nubilalis*. Seven years later Beck et al. (1949) modified the diet of Bottger and successfully reared *O. nubilalis* with it. Becton et al. (1962) showed that larvae of *O. nubilalis* can be continuously reared with the artificial diet. Later, in 1963 an easy to prepare and relatively cheap diet was developed by modifying the diet of Becton et al. (1962) and successfully used for the mass rearing of *O. nubilalis* (Guthrie et al., 1965). After that, Lewis and Raun (1966) and Lewis and Lynch (1969) adapted the diet of Vanderzant et al. (1962) for *O. nubilalis* becoming the standard diet for the rearing of *O. nubilalis* (Andow & Stodola, 2001). Still, the procedure established by Guthrie et al. (1965) was used by several authors for various studies over the years, for example for studies of *O. nubilalis* resistances to *Bacillus thuringiensis* toxins (Alves et al., 2006; Crespo et al., 2009; Siquiera et al., 2004) or metabolism studies (Durham et al., 2002) or behavioural studies (Loughner & Brindley, 1971). Thus, successful establishment and application of an artificial diet can lead to an improved and highly standardized diet and rearing procedure. This in turn can be a vantage point for further publications and experiments following the initial newly established diet. Thus, the successful methods found in this thesis might be the starting point for a similar development for species of the order Thysanoptera, which might develop faster, as there is a wide range of literature for establishing and improving diets.

However, despite the amount of literature about artificial diets for insects, there are factors, for which the role in the insects' acceptance for artificial diets remain largely unknown, even though, like for colours, their importance is known for host plant selection for herbivore insects (Bernays & Chapman, 1994).

Importance of colour for oviposition and feeding site recognition

The preference of dyed dH_2O for oviposition was investigated for adult females of *F. occidentalis* using the GMIAS-O (GMIAS adapted for oviposition). The females showed a significant preference for dyed dH_2O over non-dyed dH_2O and a significantly increased oviposition at the first day of experiment. However, there was no significant difference between the used dyes (Quinoline Yellow, Greens S and Patent Blue V). Thus, the colouration itself may be a key factor for oviposition side recognition of adult females of *F. occidentalis*. So far the use of artificially coloured surface or liquids in experiments with Thysanoptera is mostly linked to the attractiveness of sticky traps (Brødsgaard, 1989; Childers & Brecht, 1996; Hoddle et al., 2002; Ranamukhaarachchi & Wickramarachchi, 2007; Teulon & Penman, 1992) or for liquids as proof of uptake (Andongma et al., 2020; de Vries et al., 2001; Mautino et al., 2012, Rueda & Shelton, 2003; Shelton et al., 2003; Whitten et al., 2016). In contrast to the findings of the experiment of this study, Kiers et al. (2000) found when comparing colours in host plants that *F. occidentalis* females tend to preferably oviposit in leaves (38.40 ± 5.27) and petioles (10.50 ± 2.23) compared to flowers (2.10 ± 0.75) of cucumber plants. It should be noted that those findings do not have to be related to the colour of the plant parts, but may be linked to their nutritional value or to other factors like olfactory cues. De Kogel et al. (1997) showed that on leaf discs of cucumber the reproduction of *F. occidentalis* was highest on apical leaves and lowest on basal leaves and that in dual-choice essays, females preferred younger over older leaves for oviposition indicating that thrips are able to prefer and to discriminate plant parts independently from their colour. Despite flowers being less attractive for oviposition for *F. occidentalis* the within-plant distribution of adult *F. occidentalis* and other flower thrips show a great preference for flowers (Hansen et al., 2003; Reay-Jones et al., 2017). This preference is probably linked to the availability of pollen, which have a positive effect on thrips (Kirk, 1985; Trichilo & Leigh 1988; Zhi et al., 2005). Thus, these findings support the result, that there was no preference of female thrips between the different colours as all dyes were solved in dH_2O, indicating that having a colour in general supports the recognition as oviposition side whereas the composition of the liquid might be more important for a preference between the coloured liquids. Still, this might be different for other thrips that are not specialized on flowers and more abundant on leaves than on flowers.

Furthermore, the results of this study showed that dyed diets (Green S, Patent Blue V, Quinoline Yellow) are not more attractive for *F. occidentalis* larvae, compared to non-dyed diets (pale brownish), but can be even less attractive with larvae preferring the non-dyed diet over the diet dyed with Patent Blue V. Thus, colours may play a minor role in food preference for thrips larvae. Reay-Jones et al. (2017) showed that while adults are mostly present in flowers, immature flower thrips are more common on seedlings with being 11.3- and 11.2-fold more abundant on seedlings than the average of all other plant parts analysed. In addition to that, when comparing the

abundance of larvae in flowers Reitz (2002) found out, that larvae are more present in lower canopy flowers, compared to upper canopy flowers suggesting a qualitative difference in resources within the plant parts may be the causation. Hence, colour seems to be important for adult flower thrips for finding host plants and pollen as extra food source. But when it comes to the preference for oviposition or feeding sites for larvae, a general coloration seems helpful for the recognition of the site, while for preference the nutritional values or olfactory cues might be of greater importance. Additionally, colouration may play a bigger role for thrips that are more specialized on one or a few host plants in comparison to *F. occidentalis* as polyphagous omnivore.

Thus, a colouration should at least be considered when using otherwise transparent liquids as it may lead to a faster recognition by thrips. This might be helpful for short-term application experiments using substances or microorganisms to gain a maximum uptake of the solution by thrips.

Application of bacteria and other microorganisms to thrips

The importance of microorganisms in means of symbiosis, pathogenicity and vectoring are of increasing relevance, for basic research purpose and for the pest management of Thysanoptera. Crucial for experiments with microorganisms is the standardized application to the target organism. In this thesis two microorganisms were applied to thrips, *Wolbachia* and *Chlamydomonas reinhardtii*. While the unicellular algae *C. reinhardtii* was applied in a modified liquid culture medium via an artificial setup (GMIAS-S-C) to *Echinothrips americanus*, *F. occidentalis* and *Thrips tabaci*, the intracellular bacterium *Wolbachia* was applied using paralyzed *E. americanus* pro-/pupae as host of *Wolbachia* to *F. occidentalis* larvae. The successful application of *C. reinhardtii* was shown through a greenish colouration of the gut lumen of thrips. The artificial application of microorganisms to thrips was conducted in only a few publications. Uma and Prabhuraj (2010) infected *Thrips palmi* with nematode-associated *Photorhabdus luminescens*, while Whitten et al. (2016) or Andongma et al. (2020) applied a genetically modified gut symbiont to *F. occidentalis*. Besides the artificial application, de Vries et al. (2001) examined the growth and transmission of gut bacteria in *F. occidentalis*, the ability of *F. occidentalis* symbiotic gut bacteria (*Erwinia* sp.) to affect the transmission of tospovirus (de Vries et al., 2012) and *F. occidentalis* preference for thrips-damaged leaves over fresh leaves enabling the uptake of symbiotic gut bacteria (de Vries et al., 2006). In addition to that, new studies examined the microbiome of thrips (Dickey et al., 2014; Gawande et al., 2019; Kaczmarczyk et al., 2018), potentially leading to new experimental ideas of trying to cause shifts in the microbiome by manipulating the thrips via the application of different bacteria.

Additionally, the MIAS-methods can be used for application studies of viruses to Thysanoptera like tospoviruses (Moritz et al., 2004; Riley et al., 2011). However, if the bacteria or virus can't be cultured in liquid medium feeding other arthropods as vessel can be an option for predatory or omnivore thrips species.

Importance and perspective of the MIAS in the field of Thysanoptera studies

The SMIAS and GMIAS combined with the established diets allowing the development from the freshly hatched larva to the adult thrips and the application of the genetically modifiable unicellular algae *C. reinhardtii* allow a wide range of new experiments.

Most artificial diets used in experiments for Thysanoptera so far were only applied for a few days or to a specific developmental stage. Short-term application experiments are often linked to the application of substances and the used diets are seen as a vehicle. Hence, mostly simple solutions made of sucrose or honey mixed with water are used and thus may not support survival and fitness to a maximum due to the lack of nutritional necessities (Bhuyain & Lim, 2020; Gerritsen et al., 2005; Mautino et al., 2012; Rueda, 2000; Rueda & Shelton, 2003). An artificial diet specific for Thysanoptera might be helpful to support survival and fitness, when substances like antibiotics are applied. Additionally they may support long-term application of antibiotics possibly leading to *Wolbachia*-free thrips, as short-term applications seem to be not efficient for eradicating all *Wolbachia* from thrips (Arakaki et al. 2001; Kumm & Moritz, 2008). Furthermore, antibiotics have been applied in various ways by different authors (Arakaki et al. 2001; Kumm & Moritz, 2008; Nguyen et al., 2015, 2017; van der Kooi & Schwander 2014). The MIAS may have the potential to become the standard method for the application of substance leading to more comparable studies.

In addition to this with the SMIAS-O and GMIAS-O two setups were created allowing new experiments for reproductive-oviposition studies. The fecundity and thus oviposition of insects is an important fitness factor, which is especially important for pest insects. Hence, the examination of the impact of substances or microorganisms on fecundity has been and still is researched for various insect species (Bovornnanthadej et al., 2014; Catchot et al., 2020; Ramanaidu & Cutler, 2013; Sadeghi et al., 2006; Valizadeh et al., 2013; van Randen & Roitberg, 1998). There are also various publications that examine the impact of substances or diet quality on the fecundity of Thysanopteran species (Annadurai & Noble Morrison, 1987; Kirk, 1985; Pakyari & Enkegaard, 2013; Steinbach et al., 2012; Trichilo & Leigh, 1988; Ugine et al., 2006). In contrast to the Murai cage (Murai & Ishii, 1982), a method used for artificial oviposition by various authors (Gerritsen

et al., 2005; Nagata et al., 1997; de Vries et al., 2001, 2004, 2006), the SMIAS-O and GMIAS-O allows the simultaneous use of separated feeding and oviposition sites offering new opportunities for potential experimental setups.

Together with further modifications for feeding poorly soluble substances or genetically modifiable microorganisms (SMIAS-UDA) and the opportunity of using different shapes, sizes and number of containers (GMIAS-S, GMIAS-S-C, GMIAS-O), highly versatile methods have been established with the potential to have a great influence on the future research in the field of Thysanoptera and additionally may be adaptable for other piercing-sucking micro insects of the close related order Hemiptera.

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Chapter VI

Summary/Zusammenfassung

1 Summary

Artificial diets and their application methods are important tools for many insect species, especially pest insects as those can lead to new findings allowing new control measures. However, for Thysanoptera no diets and methods are established that allow a long-term application and observation of potential impacts of applied substances and diets. Thus, the main focus of this thesis was to find and establish application methods that allow a standardized application of artificial diets, substances and microorganisms in order to manipulate thrips under consistent conditions with a clear view on the thrips. *Frankliniella occidentalis* was used for the establishing of methods (Chapter II, III & IV) as it's one of the most important and most difficult to handle thrips species worldwide. Additionally, in chapter IV *Echinothrips americanus* and *Thrips tabaci* were used.

Single Micro Insect Application System (SMIAS) and Group Micro Insect Application System (GMIAS)

There are many different published methods for the application of diets or solutions to thrips. However, so far only a few of those methods have been used in different publications and are only needed for applying solutions to thrips for 24-72 hours. Furthermore no liquid diet was published so far allowing thrips to develop from first instar larvae to adult making a long-term application experiment realizable. Thus, two methods for the long-term application of diets to thrips were established. Both methods allow the complete development from egg to adult thrips inside the setup. One setup was made for the rearing of single thrips (Single micro insect application system - SMIAS) allowing the observation of impacts by applying various diets on individual level without group related factors. For an efficient application to groups of thrips the GMIAS (Group micro insect application system) was established. With exchangeable containers, it allows the rearing of a group of thrips without moving thrips. Both methods allow the hatch of first instar larvae inside the setups obviating food uptake on the host plant. The SMIAS was used to test six different diets of which four allowed thrips to develop from first instar larvae to adult thrips.

Impact of specific dyes on artificial applications to thrips

Different colours are known to attract adult thrips. However, the effect of colours in artificial setups (application and oviposition experiments) has not been examined so far. Thus, variations of the GMIAS-setup were used to test the preference of oviposition of *Frankliniella occidentalis*

for three different dyes (Green S, Quinoline Yellow, Patent Blue V, multiple choice) and larvae's preference for dyed diets (dual choice). Adult thrips showed a preference for dyed solutions for oviposition compared to the non-dyed control. However, there was no significant difference between the dyes. Thrips larvae preferred non-dyed diet over diet dyed with Patent Blue V. For the diets dyed with Green S and Quinoline Yellow there was no significant difference. Further research into colour and scent with use of the GMIAS might be able to find more distinct differences.

Application of microorganisms to thrips

As more and more is known about the possibilities of manipulation of insects by microorganisms, the standardized application of those, especially for Thysanoptera species, is an important factor. Therefore, a method was established to apply *Wolbachia* to *Frankliniella occidentalis* and the algae *Chlamydomonas reinhardtii* to *Echinothrips americanus*, *F. occidentalis* and *Thrips tabaci*. For the application of *Wolbachia* paralyzed *E. americanus* pro-/pupae were fed to *F. occidentalis* larvae in a feeding arena. The *wsp* gene for *Wolbachia* symbionts was detected in *F. occidentalis* using PCR and *in situ* PCR. However, single signals of *Wolbachia* DNA by in situ PCR were found in some of the controls as well.

For the application of *C. reinhardtii* three different setups were used (SMIAS, a SMIAS variation and a GMIAS variation). As proof of uptake of *C. reinhardtii* by thrips gut lumen was checked for coloration (greenish (DIC), reddish (UV)). The uptake was proven for all species in the SMIAS variation and the GMIAS variation.

2 Zusammenfassung

Methoden zur künstlichen Applikation von Nährlösungen und Wirkstoffen ermöglichen eine Vielzahl an Experimenten. Besonders in Anbetracht steigender Pestizidresistenzen bei Schadinsekten, wie z.B. einigen Arten der Thysanoptera können diese einen wichtigen Ansatz zur Findung neuer Bekämpfungsstrategien darstellen. Allerdings sind für Thysanopteren keine Nährlösungen oder Methoden etabliert, die eine Langzeitapplikation sowie die Beobachtung möglicher Auswirkungen von verabreichten Substanzen oder Nährlösungen erlauben. Daher lag das Hauptaugenmerk dieser Arbeit auf der Entwicklung und Etablierung von Applikationsmethoden, die eine standardisierte Applikation von künstlichen Nährlösungen, Wirkstoffe und Mikroorganismen erlaubt, um Thripse bei gleichbleibenden Bedingungen und guter Sicht auf den Thrips zu manipulieren. Für alle Experimente wurde *Frankliniella occidentalis* als Testspezies verwendet, da es einer der wichtigsten und am ökonomisch relevantesten Thrips-Arten weltweit ist.

Single Micro Insect Application System (SMIAS) und Group Micro Insect Application System (GMIAS)

Es gibt verschiedene publizierte Methoden für die Applikation von Nährlösungen oder Lösungen an Thripse. Allerdings wurden bisher nur wenige Methoden publiziert, die für eine Verabreichung von Lösungen an Thripse für mehr als 48 h konzipiert sind. Weiterhin wurde bisher keine flüssige Nährlösung beschrieben, die die Entwicklung vom ersten Larvenstadium bis zum Imago ermöglicht, welche Langzeitapplikationsexperimente realisierbar macht. Daher wurden zwei Methoden für die Langzeitapplikation von Nährlösungen an Thripse etabliert. Beide Methoden erlauben die komplette Entwicklung vom Ei zum Imago in den Aufbauten. Ein Aufbau wurde für die Anzucht vereinzelter Versuchstiere entworfen (Single micro insect application system - SMIAS). Diese Methode ermöglicht die Beobachtung von Auswirkungen verschiedener Nährlösungen auf individueller Ebene ohne gruppenbedingte Faktoren. Für eine effiziente Verabreichung an Gruppen von Thripsen wurde das GMIAS (Group micro insect application system) entwickelt. Mit auswechselbaren Container ermöglicht es die Anzucht von Gruppen, ohne dass die Thripse umgesetzt werden müssen. Beide Methoden erlauben den Schlupf der Versuchstiere aus dem Ei innerhalb des Aufbaus. Dadurch kann eine Nahrungsaufnahme auf den Wirtspflanzen der Versuchstiere ausgeschlossen werden. Das SMIAS wurde genutzt, um sechs verschiedene Nährlösungen zu testen, von denen vier eine Entwicklung vom Ei bis zum Imago ermöglichen.

Auswirkungen spezifischer Farbstoffe auf die künstliche Applikation an Thripse

Es ist bekannt, dass verschiedene Farben eine attraktive Wirkung auf Thripse haben. Allerdings ist der Effekt von Farben in künstlichen Aufbauten (Applikations- und Ovipositionsexperimente) bisher nicht untersucht. Daher wurden Variationen des GMIAS-Aufbaus genutzt, um die Präferenz von *Frankliniella occidentalis* Weibchen bezüglich drei verschiedenen Farbstoffen (multiple-choice) sowie von Larven bezüglich der Färbung von Nährlösungen (dual-choice) zu untersuchen. Adulte Weibchen zeigten für die Oviposition eine Präferenz zu gefärbten Lösungen verglichen mit der nichtgefärbten Kontrolle. Allerdings zeigte sich kein signifikanter Unterschied zwischen den einzelnen Farbstoffen. Die Thripslarven bevorzugten die nichtgefärbte Nährlösungen gegenüber der mit Patentblau V gefärbten Nährlösung. Bei den mit Lissamingrün B und Chinolingelb gefärbten Nährlösungen gab es keine signifikanten Unterschiede als Futterquelle.

Applikation von Mikroorganismen an Thripse

Da immer mehr über die Möglichkeiten der Manipulation durch Mikroorganismen bei Insekten bekannt wird, ist auch die standardisierte Applikation dieser, besonders für Thysanopteren, ein wichtiger Faktor. Daher wurde eine Methode etabliert um Wolbachien an *Frankliniella occidentalis* und die Alge *Chlamydomonas reinhardtii* an *Echinothrips americanus*, *F. occidentalis* und *Thrips tabaci* zu verabreichen. Für die Verabreichung von Wolbachien wurden paralysierte *E. americanus* Pro-/Puppen an *F. occidentalis* Larven in einer Arena verfüttert. Das *wsp*-Gen der Wolbachien konnte durch PCR und *in-situ*-Hybridisierung in *F. occidentalis* nachgewiesen werden. Allerdings wurden auch Signale bei der *in-situ*-Hybridisierung in Kontrolltieren entdeckt.

Für die Applikation von *C. reinhardtii* wurden drei verschiedene Aufbauten verwendet (SMIAS, a SMIAS-Variation und eine GMIAS-Variation). Als Nachweis der Aufnahme von *C. reinhardtii* durch die Thripse wurde das Darmlumen auf eine Färbung hin untersucht (grünlich (DIC), rötlich (UV). Die Aufnahme konnte für alle Thripsarten bei Verwendung der SMIAS-Variation und der GMIAS-Variation nachgewiesen werden.

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Statement of authorship

Hereby, I declare on my honour, that this document has been composed by myself and without additional references or tools than that given. This complete work or in a similar form has not been submitted for any other degree.

Further, I certify that I have no degree or have tried to acquire, except my Bachelor of Science in biology at the University of Halle and Master of Science in biology at the University of Halle.

Eigenständigkeitserklärung

Hiermit erkläre ich ehrenwörtlich, dass ich die vorliegende Dissertation selbstständig angefertigt und keine anderen als die angegebenen Quellen oder Hilfsmittel verwendet habe. Diese Dissertation wurde bisher weder in dieser noch in ähnlicher Form in einem anderen Prüfungsverfahren vorgelegt.

Ich erkläre weiterhin, dass ich außer meinem Bachelor of Science in Biologie an der Universität Halle und dem Master of Science in Biologie an der Universität Halle keine akademischen Grade erworben oder zu erwerben versucht habe.

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