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MASTER'S THESIS

"Laboratory trials to investigate potential repellent and oviposition deterrent effects of selected essential oils on adult *Drosophila suzukii* L."

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Statutory Declaration

I hereby declare that I am the sole author of this work. No assistance other than that permitted has been used. All quotes and concepts taken from unpublished sources, published literature or the internet in wording or in basic content have been identified as such. This written work has not yet been submitted in any part.

Date	Signature

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Table of content

1.Introduction

Drosophila suzukii Matsumura (Drosophilidae: Diptera), commonly named Spotted-wing Drosophila (SWD), is an invasive species from Asia that is spreading across the globe and causing worldwide tremendous economic losses in soft- and stone-fruit production (Mazzi et al., 2017). SWD is a highly polyphagus pest that is able to survive low temperatures during winter in the northern hemisphere. Unlike other Drosophilids, SWDs prefer late ripening or ripe thin-skinned fruits as oviposition sites (Cini et al., 2012), which makes pest management with insecticides close to harvest to minimize the SWD population difficult, due to the required minimum waiting period between the application of the plant protection products and the harvest. Apart from that the range of available effective plant protection substances against *D. suzukii* in Austria is limited, as currently only Spinosad® is authorized. Therefore alternative methods and tools for the control of *D. suzukii* got into focus. Measures such as extensive sanitation (i.e. removal of dropped fruits from the ground), the use of physical barriers (netting of whole plants), mass trapping of *D. suzukii* with attractant substances or the release of antagonists are already used or under development but with mixed results (Walsh et al., 2011; Haye et al., 2016).

Another option could be the application of deterrent substances to prevent *D. suzukii* flies from feeding or to oviposit into the fruits with the objective to establish eventually a push-pull system. In this context essential oils have been described to contain chemical compounds with insecticidal and repellent properties like monoterpenoids, sesquiterpenes and alcohols (Sathantriphop et al., 2015; Wallingford et al. 2015; Lee, 2018).

The present master thesis had the goal to identify essential oils as potential repellent and/or oviposition deterrent substances for *D. suzukii*.

Therefore the following research questions were formulated and were addressed in a systematic extensive literature search and in laboratory trials:

1. Which essential oils have a potential repellent and/or oviposition deterrent effect on *D. suzukii*?
2. Which methods can be used to determine a repellent and/or oviposition deterrent effect on *D. suzukii*?
3. Which size of repellent and/or oviposition deterrent effect exhibit the selected essential oils on adult *D. suzukii* in the laboratory?

2. Systematic extensive literature search (ELS)

2.1 Materials and methods

2.1.1 Materials

2.1.1.1 Description of the used sources for ELS

2.1.1.1.1 Scientific literature

Records of scientific literature were first retrieved from electronic databases OVID and BOKU:Litsearch. Both databases were tested in a pre-test phase for accuracy and number of recorded hits. OVID provided more accurate records. Therefore it was decided to continue the systematic extensive literature search only with the OVID database. OVID database includes records and full-text journals from AGRICOLA, CAB Abstracts, OVID Medline and Books@Ovid.

2.1.1.1.2 Grey literature

No grey literature was included into the literature search.

2.1.1.1.3 Language restrictions

Only records in English and German language were considered for the ELS.

2.1.2 Methods

2.1.2.1 Search strategies

Search strategies were designed to answer following review questions:

1. Which essential oils have a potential repellent and/or oviposition deterrent effect on *D. suzukii*?
2. Which methods can be used to determine a repellent and/or oviposition deterrent effect on *D. suzukii* in the laboratory?

All search terms for review question 1 included common terms for repellency, subject-related variations and the taxonomic group of pest (family, order or species). For review question 2 all search terms included common nomenclature for “method”, synonyms and subject-related variations as well as the taxonomic group of pest (family, order or species). The search terms

were combined to search term sets. For the formation of the search terms truncations and different Boolean operators (AND, OR, NOT) were used.

2.1.2.1.1 Limits applied to the search

The extensive literature search was only limited by language restrictions.

2.1.2.2 Precise search strategy for electronic database search

2.1.2.2.1 Search terms: pre-search

To optimize the search strategy a pre-search with following search terms was conducted for the review questions:

Search terms review question 1: “Which essential oils have a potential repellent and/or oviposition deterrent effect on *D. suzukii*?”

Search term 1: (Dipter* AND repell*)
Search term 2: (Dipter* AND repell* AND oviposition*)
Search term 3: (Dipter* AND repell* AND oviposition* AND deterrent*)
Search term 4: (Drosoph* AND repell*)
Search term 5: (Drosoph* AND repell* AND oviposition*)
Search term 6: (Drosoph* AND repell* AND oviposition* AND deterrent*)
Search term 7: (Drosoph* AND suzukii AND repell*)
Search term 8: (Drosoph* AND suzukii AND repell* AND oviposition*)
Search term 9: (Drosoph* AND suzukii AND repell* AND oviposition* AND deterrent*)

Search terms review question 2: “Which methods can be used to determine a repellent and/or oviposition deterrent effect on *D. suzukii*?”

Search term 10: (test* OR method OR bioassay*)
Search term 11: (semi-field* OR lab*)

Tab. 1: Search strategy

Search terms		Search term set
1: (Dipter* AND repell*) 10: (test* OR method OR bioassay*) 11: (semi-field* OR lab*)	->	1.1.: (Dipter* AND repell*) AND (test* OR method OR bioassay*) AND (semi-field* OR lab*)

Search term sets:

Set 1.1.: (Dipter* AND repell*) AND (test* OR method OR bioassay*) AND (semi-field* OR lab*)
Set 1.2.: (Dipter* AND repell* AND oviposition*) AND (test* OR method* OR bioassay*) AND (semi-field* OR lab*)
Set 1.3.: (Dipter* AND repell* AND oviposition* AND deterrent*) AND (test* OR method* OR bioassay*) AND (semi-field* OR lab*)
Set 2.1.: (Drosoph* AND repell*) AND (test* OR method* OR bioassay*) AND (semi-field* OR lab*)
Set 2.2.: (Drosoph* AND repell* AND oviposition) AND (test* OR method* OR bioassay*) AND (semi-field* OR lab*)
Set 2.3.: (Drosoph* AND repell* AND oviposition AND deterrent*) AND (test* OR method* OR bioassay*) AND (semi-field* OR lab*)
Set 3.1.: (Drosoph* AND suzukii AND repell*) AND (test* OR method OR bioassay*) AND (semi-field* OR lab*)
Set 3.2.: (Drosoph* AND suzukii* AND repell* AND oviposition*) AND (test* OR method* OR bioassay)
Set 3.3.: (Drosoph* AND suzukii* AND repell* AND oviposition* AND deterrent*) AND (test* OR method* OR bioassay)

2.1.2.2.2 Search terms: main search

Based on the results of the pre-search the potential test substances were chosen and search terms were modified to optimize the search strategy. Advanced search terms were a combination of the scientific name of the test organism (taxonomical level: order), the chosen test substance and the term for repellent.

Search terms:
Search term 12: (neem* AND dipter* AND repell*)
Search term 13: (celery* AND dipter* AND repell*)
Search term 14: (patchouli* AND dipter* AND repell*)
Search term 15: (catnip* AND dipter* AND repell*)

2.1.2.3 Set-up of the EndNote libraries

For management of the search records a library was set up in EndNote (version X 8.2.). Search results were collected in the group set “Test substances”, which consisted of four groups – one for each test substance (Fig.1). Records from the literature search in the Ovid Database were

deduplicated, transferred to their specific group in the EndNote library and checked again manually for duplicates. Literature entries were examined for completeness and missing information was added if necessary and possible.

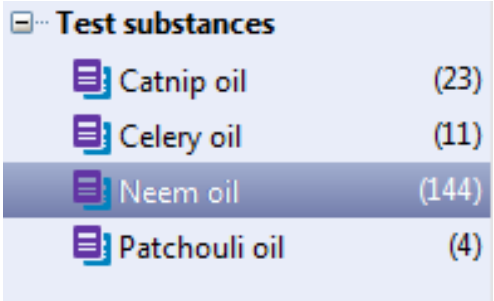

Fig. 1: Group set and groups with number of records in EndNote Library

2.1.2.4 The selection process/selection criteria

To evaluate the collected records in the EndNote Library, a rating system was established. Five levels of relevance were defined (1 - 5 stars) and applied for the references (Tab.2).

Tab. 2: Rating system for EndNote Library

*	Irrelevant
**	Incomplete
***	Partially relevant
****	Relevant
*****	Very relevant

Criteria for rating system (Fig. 2 – 6):

1-star: Irrelevant
Record is not about repellency and/or oviposition deterrence of the specific test substance or related compounds or insects of the order Diptera.

2-stars: Incomplete
Record missed crucial formal aspects, e.g. keywords or abstract.

3-stars: Partially relevant
Record contains information about repellency and/or oviposition deterrence of the specific test substance or related compounds regarding insects of the order Diptera of any development stage.

4-stars: Relevant

Record contains information about repellency and/or oviposition deterrence of the specific test substance or related compounds regarding insects of the order Diptera of the appropriate development stage.

5-stars: Very relevant

Record contains information about repellency and/or oviposition deterrence of the specific test substance or related compounds regarding insects of the family Drosophilidae of the appropriate development stage.

Author	Year	Title	Rating
Zuber, M.; Zingg, D.; Wyss, E.	1997	Biological control of cherry fruit fly Rhagoletis cerasi - new prospects? [German]	★
Weintraub, P. G.; Arazi, Y.; Horowi...	1996	Management of insect pests in celery and potato crops by pneumatic removal	★
Verma, J. S.; Amith, Nath	2003	Effect of neem based pesticide on the egg-laying by fruit flies (Tephritidae: Diptera)	★
Steffens, R. J.; Schmutterer, H.	1982	The effect of a crude methanolic neem (Azadirachta indica) seed kernel extract on metamorphosis and quality of adult...	★

Fig. 2: Detail of references rated with 1-star in EndNote Library

Author	Year	Title	Rating
Seye, F.; Ndiaye, M.	2008	Compatibility between Aspergillus clavatus (Hyphomycetes) and neem oil (Azadirachta indica) against the vector mos...	★★
Raghvani, K. L.; Juneja, R. P.; Parm...	2010	IPM modules with farmer.s practice against pest complex of pearl millet	★★

Fig. 3: Detail of references rated with 2-stars in EndNote Library

Author	Year	Title	Rating
Ramesh, T.; Devi, N. K. A.; Manoh...	2006	Efficacy of selected plant extracts on the larvicidal and repellentaction against Culex quinquefasciatus (Diptera: Culicid...	★★★
Partoutomo, S.; Sukarsih,; Satria, E...	1998	Development of an in vivo assay technique as a tool for measuring protective immune responses after vaccination aga...	★★★
Bezzar-Bendjazia, R.; Kilani-Morak...	2017	Azadirachtin induced larval avoidance and antifeeding by disruption of food intake and digestive enzymes in Drosophi...	★★★

Fig. 4: Detail of references rated with 3-stars in EndNote Library

Author	Year	Title	Rating
Zhu, J. W. J.; Dunlap, C. A.; Behle, ...	2010	Repellency of a wax-based catnip-oil formulation against stable flies	★★★★
Valecha, N.; Ansari, M. A.; Prabhu, ...	1996	Preliminary evaluation of safety aspects of neem oil in kerosene lamp	★★★★
Tuetun, B.; Choochote, W.; Rattan...	2004	Mosquito repellency of the seeds of celery (Apium graveolens L.)	★★★★
Subbarayudu, B.; Indira, S.	2007	Integrated pest management for the shoot fly (Atherigona soccata) in sorghum (Sorghum bicolor) in Andhra Pradesh	★★★★

Fig. 5: Detail of references rated with 4-stars in EndNote Library

Author	Year	Title	Rating
Kilani-Morakchi, S.; Bezzar-Bendja...	2017	Preimaginal exposure to azadirachtin affects food selection and digestive enzymes in adults of Drosophila melanogast...	★★★★★

Fig. 6: Detail of reference rated with 5-stars in EndNote Library

2.1.2.4.1 Reasons for exclusion of records

Records were excluded if they were not written in one of the pre-defined search languages English or German. Incomplete references that lacked essential parts, such as abstracts or keywords, were also excluded for formality reasons.

2.1.2.4.2 Reasons for inclusion of records

References were included if they provided information about any mode of deterrent action (repellency and/or oviposition deterrence) of chosen test substances regarding members of the order Diptera.

2.2 Results

The Ovid Database provided in total 182 records for the four applied search terms of the main search (search terms 12 – 15). Only 0.6% of the records fulfilled all criteria for a 5-stars rating. The vast majority of the references qualified for a 4-stars rating (69.2%). 1.7% were graded a 3-stars rating and 1.1% a 2-stars rating. 27% of all found records were rated 1-star (Tab. 3). The amount of search hits differed widely between the search terms. For example, search term 12 was responsible for 79.1% of all search hits, while search term 14 was only for 2.2%. The quality of the search records also varied. 95.7% of all records of search term 15 qualified at least for a 4-stars rating, compared to 63.6% of search term 13 (Tab. 3).

Tab. 3: Number of records for each group in EndNote Library

Search terms	**Ratings**					**Total (n)**
	5-stars	**4-stars**	**3-stars**	**2-stars**	**1-star**	
12: (neem* AND dipter* AND repell*)	1	93	3	2	45	144
13: (celery* AND dipter* AND repell*)	-	7	-	-	4	11
14: (patchouli* AND dipter* AND repell*)	-	4	-	-	-	4
15: (catnip* AND dipter* AND repell*)	-	22	-	-	1	23
Total (n)	1	126	3	2	50	182

3. Laboratory Trials

3.1 Materials and methods

3.1.1 Materials

3.1.1.1 Test organism *Drosophila suzukii*

3.1.1.1.1 Taxonomical classification

Drosophila suzukii (Drosophilidae: Diptera) was first described in 1931 by Matsumura (Kanzawa, 1935) and is known under the common name Spotted-wing Drosophila (SWD) (Tab. 4).

Tab 4: Taxonomy of *D. suzukii* (EPPO, 2018)

Kingdom	Metazoa
Phylum	Arthropoda
Subphylum	Hexapoda
Class	Insecta
Order	Diptera
Family	Drosophilidae
Genus	Drosophila
Species	*Drosophila suzukii*

3.1.1.1.2 Morphology

SWDs are drosophilid flies with red eyes and short antennae with branched arista. Adult flies are 2 - 3 mm long, 0.8 - 1 mm wide with a wingspan of 5 - 6.5 mm. They have a pale to yellowish brown thorax with black transverse stripes on the abdomen (Cini et al., 2012). SWD adults show sexual dimorphism. The males have black spots on the tip of each wing, which is the name giving characteristic for “Spotted-wing Drosophila”, and two sex combs on the foretarsi (on the first and second tarsal segment) (Anfora et al., 2012) (Fig. 7, 8).

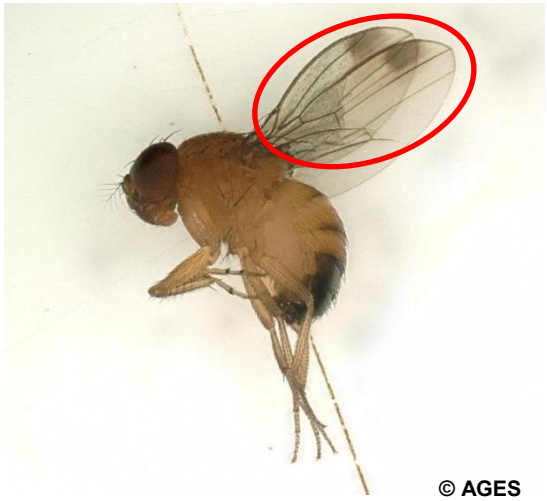

Fig. 7: Male Spotted-wing Drosophila with typical black spots on tips of wings

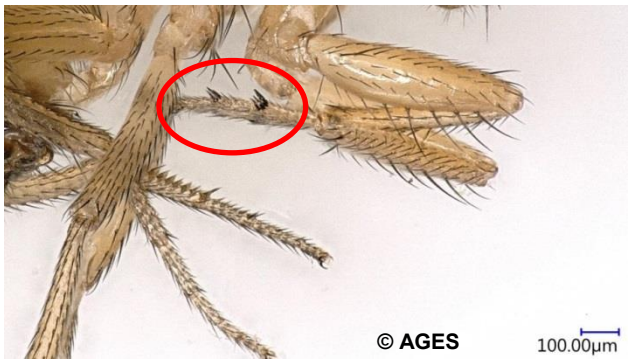

Fig. 8: Black combs on foretarsi of male SWD

Female *D. suzukii* are slightly bigger than the males and have a strongly serrated and sclerotized ovipositor (Fig. 9), which enables the females to penetrate most thin-skinned fruits (Walsh et al., 2011).

Fig. 9: Ovipositor of female SWD

D. suzukii adults are able to adapt physically to low temperatures during winter, which results in a winter-morph phenotype as most cold-tolerant life stage, displayed by bigger and darker flies with longer wings, occuring in autumn (Dalton et al., 2011; Hamby et al., 2016).

Eggs of *D. suzukii* (0.6 mm long, 0.2 mm wide) are translucent, glossy white and have two respiratory filaments on one side of the egg (Beers et al., 2010) (Fig. 10). Larvae are 0.6 mm long at larval instar 1 and can grow up to 4 mm (larval instar 3) (Walsh et al., 2011). They are milky white to lucent and cylindrical with black mouth hooks at the front (Beers et al., 2010; Walsh et al., 2011) (Fig. 11). Pupae are yellowish brown with soft skin at start, which turns dark brown and hard during further development (Beers et al., 2010; Walsh et al., 2011) (Fig. 12).

Fig. 10: Ovipositor of female SWD with egg (Beers et al., 2010)

Fig. 11: SWD larva (Beers et al., 2010)

Fig. 12: SWD pupa (Beers et al., 2010)

3.1.1.1.3 Host plants

D. suzukii is a highly polyphagous invasive species. It infests a wide range of soft-skinned wild, ornamental and cultivated plants and is able to feed and develop on it through nearly all seasons (Tab. 5) (Lee et al., 2015; Poyet et al., 2015; Briem et al., 2016; Kenis et al., 2016). For Europe more than 80 different host plant species of *D. suzukii* are described (Kenis et al., 2016) (Tab. A1). The host spectrum includes cultivated fruits of economic importance such as soft fruits (*Rubus* spp.: e.g. raspberries, strawberries, blackberries, blueberries), stone fruits (*Prunus* spp.: e.g. cherries, plums, peaches) and grapes (*Vitis* spp.) (Lee et al., 2011, 2015; Poyet et al., 2015; Briem et al., 2016; Kenis et al., 2016). While crop plants in monocultures enable the *D. suzukii* population to grow fast during summer, ornamental and wild plants are suitable hosts for overwintering habitats (e.g. *Lonicera nitida, Hippophae rhamnoides*) and reinfestion in spring (e. g. *Aucuba japonicum*, *Viscum album*) (Cini et al., 2012; Klick et al., 2012). Additionally they can serve as shelter in summer and autumn against e.g. adverse climatic conditions and plant protection treatments in cultivated crops (e.g. *Cornus sericea*, *Lonicera xylosteum, Taxus baccata*) (Lee et al., 2015; Cini et al., 2012; Poyet et al., 2015;

Kenis et al., 2016). Another important factor for the spread of *D. suzukii* is the ability to develop in host plants despite the influence of toxic organic substances such as alkaloids, terpenoids or glycosides. It is still unknown how exactly *D. suzukii* larvae are coping with those substances, but it can be assumed that they own specific enzymes for biochemical detoxification (Poyet et al., 2015).

Tab. 5: Reported host plant families for *D.suzukii*

Host plant families	**Reported number of suitable species**	**Source**
Actinidiaceae	2	Lee et al. (2015) Kenis et al. (2016)
Adoxaceae	7	Kenis et al. (2016) Lee et al. (2015) Mitsui et al. (2010) Poyet et al. (2015)
Annonaceae	1	Rauleder (2015)
Araceae	2	Kenis et al. (2016) Poyet et al. (2015)
Asparagaceae	1	Kenis et al. (2016)
Berberidaceae	3	Lee et al. (2015) Poyet et al. (2015) Rauleder (2015)
Buxaceae	1	Lee et al. (2015)
Caprifoliaceae	11	Kenis et al. (2016) Lee et al. (2015) Poyet et al. (2015) Rauleder (2015)
Cornaceae	11	Kenis et al. (2016) Lee et al. (2015) Mitsui et al. (2010)
Dioscoreaceae	1	Kenis et al. (2016)
Ebenaceae	1	Kanzawa (1935, 1939)
Elaeagnaceae	3	Kanzawa (1939) Lee et al. (2015)
Ericaceae	11	Kenis et al. (2016) Lee et al. (2015) Mitsui et al. (2010) Rauleder (2015)
Grossulariaceae	2	Poyet et al. (2015)
Lauraceae	1	Lee et al. (2015)
Melanthiaceae	1	Kenis et al. (2016)
Moraceae	6	Lee et al. (2015) Mitsui et al. (2010)
Myrtaceae	1	Lee et al. (2015)
Phytolaccaceae	2	Kenis et al. (2016) Lee et al. (2015)
Rhamnaceae	4	Lee et al. (2015) Kenis et al. (2016)

Host plant families (contin.)	Reported number of suitable species (contin.)	Source (contin.)
Rosaceae	58	Kanzawa (1935, 1939) Kenis et al. (2016) Lee et al. (2015) Mitsui et al. (2010) Poyet et al. (2015) Rauleder (2015)
Rutaceae	2	Lee et al. (2015)
Santalaceae	1	Poyet et al. (2015)
Solanaceae	7	Arno et al. (2012) Kenis et al. (2016) Lee et al. (2015) Poyet et al. (2015)
Spiraeoideae	1	Poyet et al. (2015)
Taxaceae	2	Mitsui et al. (2010) Poyet et al. (2015)
Thymelaeaceae	1	Kenis et al. (2016)
Vitaceae	2	Kenis et al. (2016) Lee et al. (2015)

3.1.1.1.4 Biology (Reproduction biology)

D. suzukii has four developmental stages: egg, larval, pupal, adult. Larvae hatch 2 - 72 hours after oviposition inside the fruit. There are three larval instars which last in total 3 - 13 days and the pupal stage with 4 - 15 days (Kanzawa, 1939; Anfora et al., 2012). Usually the whole development cycle is completed inside the fruit. Nevertheless, leaving of the third larval stage and finishing pupal developing outside the fruit is possible, leading to an additional contamination risk. The duration of the developmental cycle from egg to egg-laying female is temperature dependent and can be completed in 8 days at 21.1 °C respectively 12 - 15 days at 18.3 °C (Walsh et al., 2011). During the vegetation period adult SWDs reach maturity 1 - 2 days after emergence and immediately start mating (Anfora et al., 2012). SWDs mate during the whole day with a peak close to dawn (Lin et al., 2014). Depending on temperature, oviposition takes place from March to November in the northern hemisphere (Zerulla et al., 2015; Hamby et al., 2016). Female SWDs lay 1 - 3 eggs per fruit and can infest up to 7 - 16 fruits per day (Anfora et al., 2012). 7 - 15 generations can occur per year, which results in a total lifetime fecundity of the females of 200 - 600 eggs (Beers et al., 2010; Anfora et al., 2012, Hamby et al., 2016) (Fig. 13). Experiments with cherries and blueberries showed that there was a significantly higher fecundity at 18 to 22 °C, compared to lower (14 °C) or higher temperatures (>26 °C) (Tochen et al., 2014). Higher humidity (94% RH) and constant temperature (22 °C) also resulted in higher fecundity (Tochen et al., 2016) than in experiments

with lower humidity. The lifespan of an adult *D. suzukii* is usually 3 - 9 weeks, but SWDs that overwinter can live for many months (Beers et al., 2010).

Studies indicate that *D. suzukii* flies overwinter as winter-morph adults in safe shelters, e.g under leaf litter or agricultural structures and feed on tree saps, nectars and yeast (Dalton et al., 2011; Coop and Dreves, 2013; Rossi-Staccioni et al., 2016, Zhai et al., 2016). There is evidence that *D. suzukii* exhibit a reproductive diapause under short-day conditions (8L:16D) and temperatures below 10 °C (Mitsui et al., 2010; Tochen et al, 2014; Zhai et al., 2016).

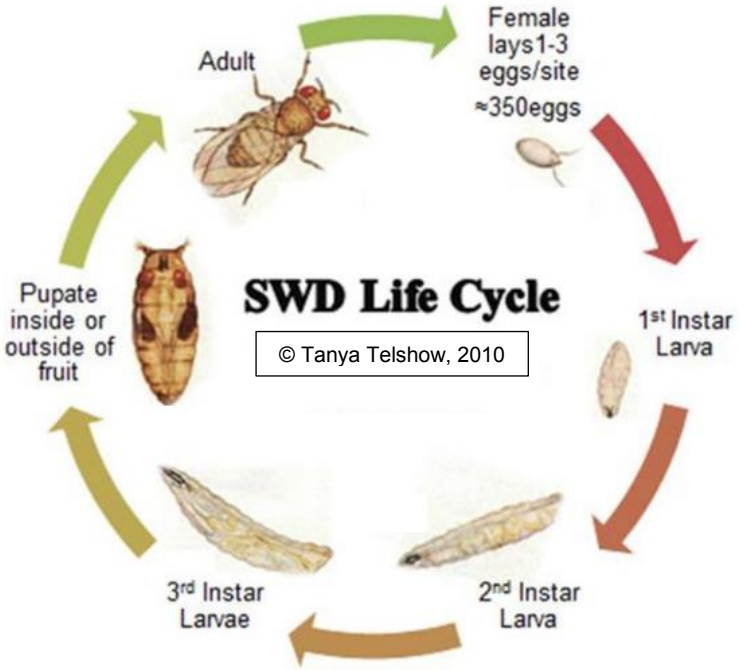

Fig. 13: Life cycle of SWD (Coop and Dreves, 2013)

3.1.1.1.5 Damage and economic importance

In contrast to most Drosophila species that feed on overripe fruits, *D. suzukii* is one of the few drosophilids which is able to feed and lay their eggs on healthy ripening fruits that are still hanging on the plant (Cini et al., 2012). Main damages are caused by oviposition of female SWDs and larval feeding on the fruit pulp inside the fruit (Fig. 14). For oviposition females cut a hole in the fruit skin with their serrated ovipositor where they lay their eggs underneath the skin, leaving small lesions which act as gateways for secondary infections (Baker et al., 2010; Beers et al., 2010; Anfora et al., 2012; Cini et al., 2012).

Fig. 14: *D. suzukii* flies on raspberries

The oviposition site shows a collapsed appearance that expands with further larval development and feeding activity (Beers et al., 2010) (Fig. 15). Mature larval stages additionally cut breathing holes in the fruit skin promoting faster decay and additional losses (Beers et al., 2010; Anfora et al., 2012). Although there is a clear preference for soft-skinned fruits with high soluble sugar content (e.g. raspberries, strawberries), *D. suzukii* is also able to penetrate hard-skinned fruits, such as apples, if they provide entries like wounds or damages (Kenis et al., 2016). Damages caused by *D. suzukii* result in severe quality losses and unmarketable fruits. Evaluation of the economic losses for growers is difficult and depends on several direct and indirect factors. Besides the obvious damages on the fruits, there are several additional costs occurring for the growers such as surveillance, pest management activities (e.g. increased amount of insecticides, netting of the orchards), sanitation or disposal of infested fruits (Mazzi et al., 2017).

Fig. 15: *D. suzukii* larvae in raspberry

Depending on different grades of infestion, the economic losses in sweet cherry production in Switzerland ranged from 24.000 CHF/ha (21.000 EUR) (low infestion rate of <20% at harvest with following disposal measures of infested fruits) to 71.000 CHF/ha (62.000 EUR) if infested fruits are found at delivery, which would lead to total neglection by the retailers (Mazzi et al., 2017). In California, Oregon and Washington an economic yield loss of 40% for blueberries, 50% for cranberries, 33% for cherries and 20% for strawberries was estimated for 2008 (Bolda et al., 2010). Production losses by *D. suzukii* in these three states reached 511 million USD annually (Bolda et al., 2010). In Brazil, a potential economic loss (based on expected yield losses) of about 30 million USD was estimated for the production of peaches and figs (Benito et al., 2016). An evaluation for the production of strawberries, raspberries, blueberries, blackberries and cherries in the Trento province in northern Italy resulted in an economic loss of 500.000 EUR in 2010 and 3 million EUR in 2011 caused by *D. suzukii* (De Ros et al., 2013).

3.1.1.1.6 Geographical distribution

D. suzukii is an invasive pest from Asia, which is distributed from China, Taiwan and North-/South Korea to Thailand, Russia and India (Kang and Moon, 1968; Sidorenko, 1992; Cini et al., 2012) (Fig. 16). It was first described 1916 in Japan by Kanzawa (1935), but there is still ongoing discussion if it is originating from Japan (Cini et al., 2012). In 2008 *D. suzukii* was first detected in California (USA) (Hauser, 2011) from where it spread along the west coast to Mexico, British Columbia and Canada and eventually along the east coast to Florida (Calabria et al, 2012; Cini et al., 2012). In 2014 *D. suzukii* reached the South American continent with findings in Brazil (Depra et al., 2014) (Fig. 16).

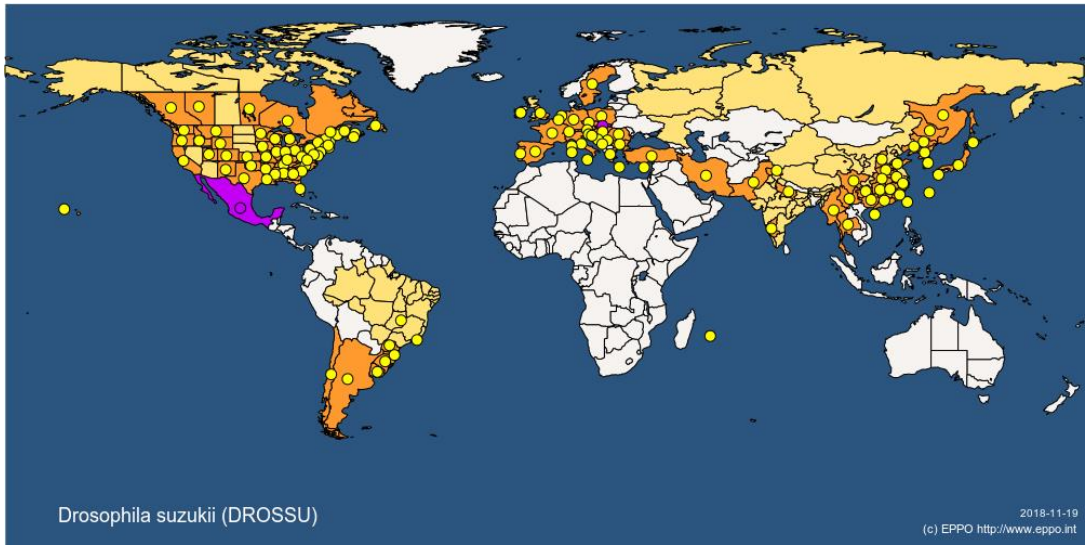

Fig. 16: Worldwide distribution of *D. suzukii* (yellow dots=present; purple dots=transient) (EPPO, 2018)

In Europe *D.suzukii* was first recorded 2008 in Spain (Calabria et al., 2012). One year later trap catches were reported from France and Northern Italy (Grassi et al, 2011; Calabria et al., 2012). From 2010 - 2011 *D. suzukii* spread across Europe with findings in Switzerland (Baroffio and Fisher, 2011), Austria (Lethmayer, 2011), Germany (Vogt et al., 2011), Belgium (Cini et al., 2012) and later in Hungary (Lengyel et al., 2015) and Ukraine (Lavrinienko et al., 2017) (Fig. 17).

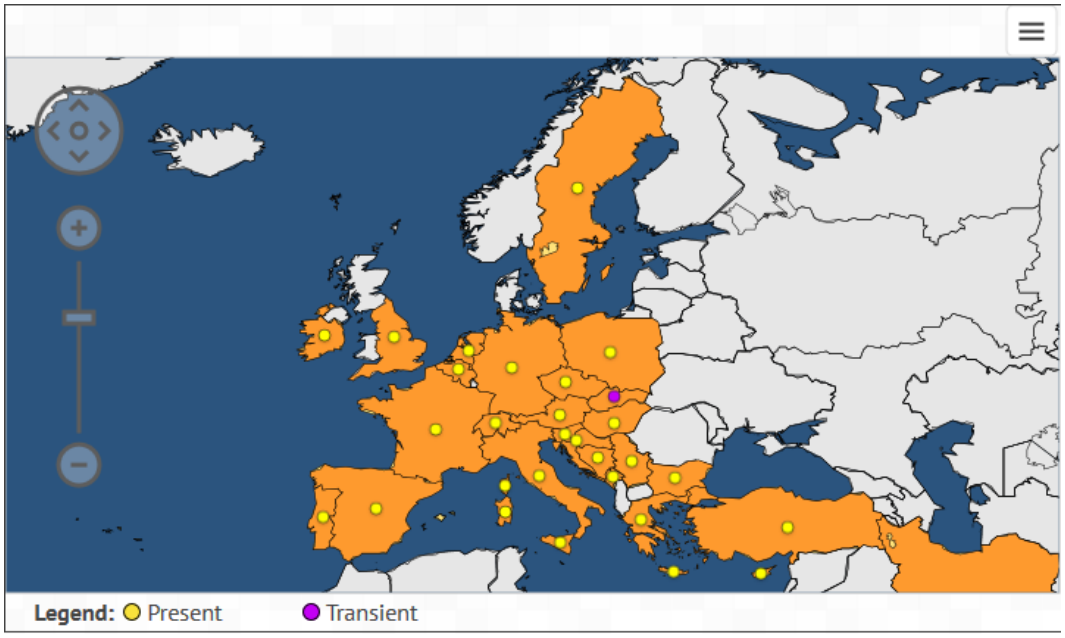

Fig. 17: Distribution of *D. suzukii* in Europe (EPPO, 2018)

3.1.1.2 Test substances

For the selection of the four test substances (Tab. 6) the six criteria as listed below were applied:
A test substance should

- exhibit a deterrent (repellent) effect on oviposition for Diptera
- belong to the essential oils
- be an untested substance for SWD
- be harmless for human health
- be suitable for practical use
- be cost-efficient for practical use

Tab. 6: Selected test substances for laboratory trials and the reported insecticidal and repellent activity of the essential oils and their main biologically active compounds on selected species

Test substances-common name	CAS-Nr.	Source	Purity	Solvent	Insecticidal against	Repellent against
Celery oil	8015-90-5	Sigma-Aldrich, Austria	100%	Acetone	*Aedes spp.*[1,2,3]	*Aedes spp.*[2,3,4] *Anopheles spp.*[4] *Culex spp.*[4] *Mansonia spp.*[4]
Patchouli oil	8014-09-3	Sigma-Aldrich, Austria	100%	Hexane	*Aedes spp.*[5] *Anopheles spp.*[5] *Blattela spp.*[6] *Amitermes spp.*[7] *Camponotus spp.*[8] *Myzus spp.*[9] *Musca spp.*[10]	*Aedes spp.*[11,12] *Anopheles spp.*[12] *Blattela spp.*[6] *Amitermes spp.*[7] *Camponotus spp.*[8] *Myzus spp.*[9]
Catnip oil	8023-84-5	Aromaland, Germany	100%	Hexane		*Aedes spp.*[13,14] *Anopheles spp.*[13,15,16] *Culex spp.*[15] *Musca spp.*[17] *Blattella spp.*[17]
Neem oil	8002-65-1	Naissance, UK	100%	Hexane	*Aedes spp.*[18] *Anopheles spp.*[19] *Culex spp.*[19]	*Aedes spp.*[18] *Bactrocera spp.*[20] *Melanotus spp.*[21]

1: Chaiyasit et al. (2006), 2: Choochote et al. (2004), 3: Kumar et al. (2014), 4: Tuetun et al. (2005), 5: Gokulakrishnan et al. (2013), 6: Liu et al. (2015), 7: Bacci et al. (2015), 8: Albuquerque et al. (2013), 9: Chen et al. (2017), 10: Pavel (2008), 11: Jantan and Zaki (1999), 12: Trongtokit et al. (2005), 13: Bernier et al. (2005), 14: Chauhan et al. (2005), 15: Amer and Mehlhorn (2006), 16: Menger et al. (2014), 17: Schultz et al. (2004), 18: Benelli et al. (2014), 19: Dua et al. (2009), 20: Chen et al. (1996), 21: Cherry and Nuessly (2010)

3.1.1.2.1 Celery oil

Celery, *Apium graveolens* L., is a member of the Apiaceae family. It is native to Europe and Asia where it is grown for its roots, petioles, leaves and seeds (Peter, 2012). Besides its consumption as vegetable, celery is also used for pharmaceuticals, perfumes and medicines (Momin et al., 2000). The oil is extracted from the crude seeds, usually by steam distillation (Chaiyasit et al., 2006; Kumar et al., 2014), ethanol extraction (Choochote et al., 2004), hexane extraction (Momin et al., 2000; Tuetun et al., 2005, 2008) or hydro-distillation (Khalil et al., 2018). The main compounds are limonene, p-mentha-2, 8-dien-1-ol, p-mentha-8(9)-en-1,2diol, 3-n-butylphthalide, sedanolide and selinene (Zheng et al., 1993; Momin et al., 2000; Momin and Nair, 2002; Tuetun et al., 2008). Celery oil has a strong repellent effect against a broad range of mosquito species belonging to various genera, including *Aedes*, *Anopheles*, *Armigeres*, *Culex* and *Mansonia* as well as an ovicidal, larvicidal and adulticidal effect against *Aedes aegypti* L. (Momin et al., 2000; Choochote et al., 2004; Tuetun et al., 2005, 2008, 2009; Warikoo et al., 2011; Kumar et al., 2014).

3.1.1.2.2 Patchouli oil

Pogostemon cablin (Blanco) Benth., commonly named patchouli, is a species of the genus *Pogostemon*. It belongs to the Lamiaceae, a family well known for their species with medicinal properties (Gokulakrishnan et al., 2013). *P. cablin* is a hardy perennial herb, which reaches heights of 1.2 m and prefers hot and humid climate (Swamy et al. 2015). Patchouli has his origins in the Philippines, but grows wild in many South Asian countries (Swamy et al., 2015). Due to its various applications for the food, perfume and pharmaceutical industry, it is presently cultivated around the globe from Brazil, Malaysia and Pakistan to China (Gokulakrishnan et al., 2013; Swamy et al., 2015). The essential oils of patchouli are accumulated in the glandular trichomes (Guo et al., 2013). There are various extraction methods for obtaining patchouli oil, i. e. supercritical carbon dioxide extraction (Donelian et al., 2009), molecular distillation (Hu et al., 2004) or microwave assisted extraction (Kusuma et al., 2017), but generally, steam or fractional distillation of the aerial plant parts is used (Swamy et al., 2015). The main components of the essential oil are β-patchoulene, α-guaiene, γ-patchoulene, α-bulnesene, patchouli alcohol, pogostone, caryophyllene and seychellene (Albuquerque et al., 2013; Gokulakrishnan et al., 2013; Bacci et al., 2015; Liu et al., 2015; Kusuma et al., 2017). The essential oil and its major active constituents, α- and β-patchoulene, pogostone and patchoulol (Swamy et al., 2015), exhibited high insecticidal and repellent activity against various mosquitoes species, i. e. *Aedes aegypti*, *Anopheles dirus* Peyton and Harrison, *Anopheles*

stephensi Liston, *Culex quinquefasciatus* Say (Jantan and Zaki, 1999; Trongtokit et al., 2005; Gokulakrishnan et al., 2013; Widawati et al., 2015;). There is also an insecticidal and repellent effect against German cockroaches (*Blattella germanica* (L.)) (Liu et al., 2015), termites (*Amitermes cf. amifer* Silvestri and *Microcerotermes indistinctus* Mathews) (Bacci et al., 2015), ants (*Camponotus melanoticus* Emery*, Camponotus novograndensis, Dorymyrmex thoracicus* Santschi) (Albuquerque et al., 2013), aphid (*Myzus persicae* Sulzer (Chen et al., 2017) and the house fly, *Musca domestica* L. (Pavela, 2008).

3.1.1.2.3 Catnip oil

Nepeta cataria L., commonly known as catmint or catnip, is a species of the genus *Nepeta* in the Lamiaceae family. *N. cataria* is a robust herbaceous plant that grows perennial (Herron, 2001). It is native to Europe, the Middle East and Asia, but widely cultivated in temperate and tropical zones from N-America to New Zealand (Hawke, 2007). Besides its most popular applications, as a stimulant for felines and an herb, it is used in traditional medicine for the treatment of various conditions such as headaches, inflammations and colds. The oil is usually extracted from the aerial parts by steam distillation (Peterson et al., 2002; Schultz et al., 2004; Patience et al., 2018) or hydro distillation (Zomorodian et al., 2013). The major constituents of catnip oil are nepetalactones (Z,E-nepetalactone, E-Z-nepetalactone), β-caryophyllene, 1,8-cineole, trans-β-ocimene, limonene, nerol and citronellol (Schultz et al., 2004; Sparks et al., 2017; Patience et al., 2018; Salehi et al., 2018). The main active components are the isomers of nepetalactone, Z,E- and E,Z-nepetalactone and nepetalactol (Chauhan et al., 2012, Ricci et al., 2010). There is a well-documented repellency of catnip oil and its main component nepetalactone against various mosquitoes species, including *Aedes*, *Anopheles* and *Culex* (Bernier et al., 2005; Chauhan et al., 2005; Amer and Mehlhorn, 2006; Feaster et al., 2009; Menger et al., 2014; Sathantriphop et al., 2015; Patience et al., 2018). Based on that, there have been approaches to establish a push-pull system against *Aedes aegypti* (Menger et al., 2014; Obermayr et al., 2015). Spatial repellency was also reported against *Musca domestica* (Schultz et al., 2004), the German cockroach *Blattella germanica* (Schultz et al., 2004), as well as the house dust mites, *Dermatophagoides pteronyssinus* Bogdanov and *D. farinae* Hughes (Khan et al., 2012). Additionally, there is an antifeedancy effect against the stable fly, *Stomoxy calcitrans* L. (Zhu et al., 2012) and the horn fly, *Haematobia irritans* L. (Zhu et al., 2015) reported.

3.1.1.2.4 Neem oil

Neem oil is extracted from the neem tree, *Azadirachta indica* Juss. (Family: Meliaceae), a fast growing evergreen tree native to the Indian subcontinent. Primary source of the oil are the seeds, but other plant parts, such as leaves and stems, are used as well for extraction (Nicoletti et al., 2012). The essential oil is normally gained by cold-pressing, but there are other extraction methods such as extraction with diethylether (Chen et al., 1996), ethanol or hexane (Liauw et al., 2008) and microwave assisted extraction (Nde et al., 2015). Neem oil contains nearly 100 biologically active compounds (Campos et al., 2016). The major constituents are triterpenes (limonids), with azadirachtin as the most important one for most of the commercial products. Other compounds are nimbin, nimbidin, nimbolides meliantriol, fatty acids (oleic, stearic, and palmitic) and salannin (Silva et al., 2007; Nicoletti et al., 2012; Campos et al., 2016). Neem oil and his compounds have a versatile spectrum of action against numerous arthropods, such as insecticidal activity, i. e. on *Aedes albopictus* Skuse (Benelli et al., 2014), *Anopheles stepehensi* and *Culex quinqufasciatus* (Shanmugasundaram et al., 2008), antifeedancy, growth deregulation, affecting hormone functions in juvenile stages, i. e. in *Drosophila melanogaster* Meigen (Boulahbel et al., 2015) and *Aedes aegypti* (Mitchell et al., 1997), reducing fertility in *Anopheles stephensi* (Lucantoni et al., 2006) and *Mononychellus tanajoa* Bondar (Silva et al., 2013), oviposition and repelling deterrent effects on *Aedes albopictus* (Benelli et al., 2014), the oriental fruit fly, *Bactrocera dorsalis* Hendel (Chen et al., 1996) and wireworms, *Melanotus communis* Gyllenhal (Cherry and Nuessly, 2010).

3.1.1.3 Test fruits

Untreated raspberries (*Rubus idaeus* L. 'Autumn bliss') were used as test fruits for the experiments. Ripe fruits (BBCH 89-897) (Schmid et al., 2001) were harvested at a fortnight interval (14.09.2017; 29.09.2017) from a self-picking, organically managed raspberry orchard in Dobersberg, Austria, which was considered as free from *D. suzukii* infestation. Approximately 4000 raspberries were collected randomly throughout the whole orchard. After harvesting fruits were stored in plastic boxes, transferred to laboratory, frozen at -34 °C and stored at this temperature. To ensure that the rate of infested fruits used in the laboratory trials was 5% at max., 120 randomly picked raspberries were analyzed under the stereomicroscope (Tab. A2) for the presence of Drosophilid eggs or larvae. As only 1 Drosophila larva was found, the test fruits were considered to be free from *D. suzukii* and could be used in the trials.

3.1.1.4 Test Units

For the conduct of the No-Choice-Test a modified version of the test unit according to Wallingford et al. (2017) was chosen. A modified Hesler-Plate (Wallingford et al., 2017), that allows the passively emission of volatiles, was used as main unit for releasing the test substances and presenting the test fruits to the flies. The Hesler-Plate (Fig. 18) consists of a Petri dish (Tab. A2; polystyrene; Ø 90 mm; 25 mm height) with a perforated lid and a cotton dental wick (Tab. A2; Ø 10 mm; 10 mm height) in the middle of the bottom of the Petri dish. Every lid had 157 equally distributed holes (1 hole/5 mm). The holes were burned with a laser into the lid (Dr. Bohrer Lasertec GmbH, Neusiedl am See, Austria). On top of the lid 3 test fruits (raspberries) were placed centered in a triangle shape (20 - 30 mm distance to each other).

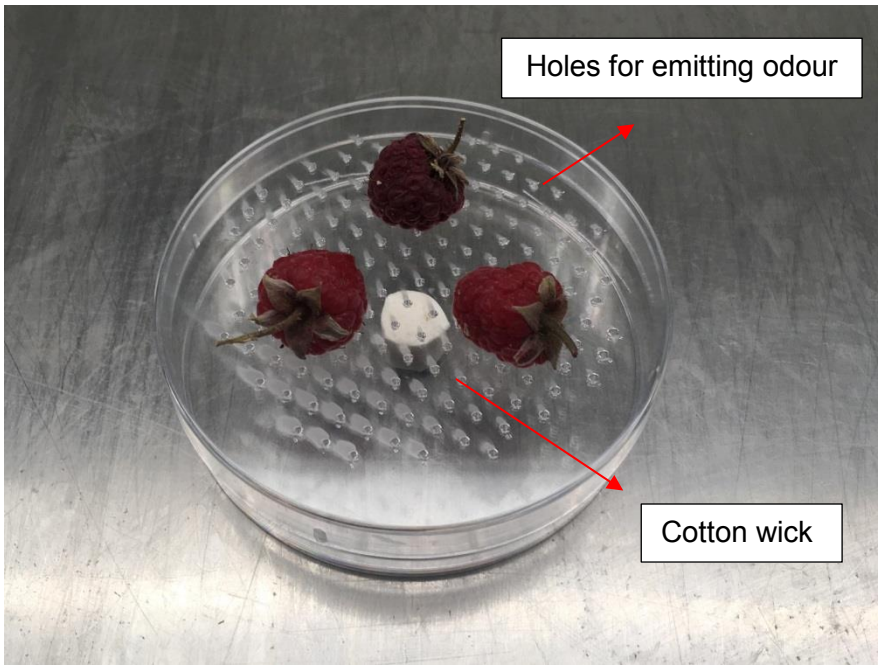

Fig. 18: Test unit: Modified Hesler-plate with perforated lid and test fruits (raspberries)

The Hesler-Plate was placed in the middle of the test arena. Test arena was a polypropylene box (Fig. 19) (Tab. A2; 40x34x17 cm). A 30x22 cm rectangular window was cut in the lid and covered with curtain tissue (Tab. A2; mesh size: 0.3 mm) for ventilation. The tissue was attached with a hot glue gun (Tab. A2) to the lid. Test organisms were released by placing a plastic tube with 7 flies (5♀/2♂), sealed by a foam stopper, in the front left corner of the test arena.

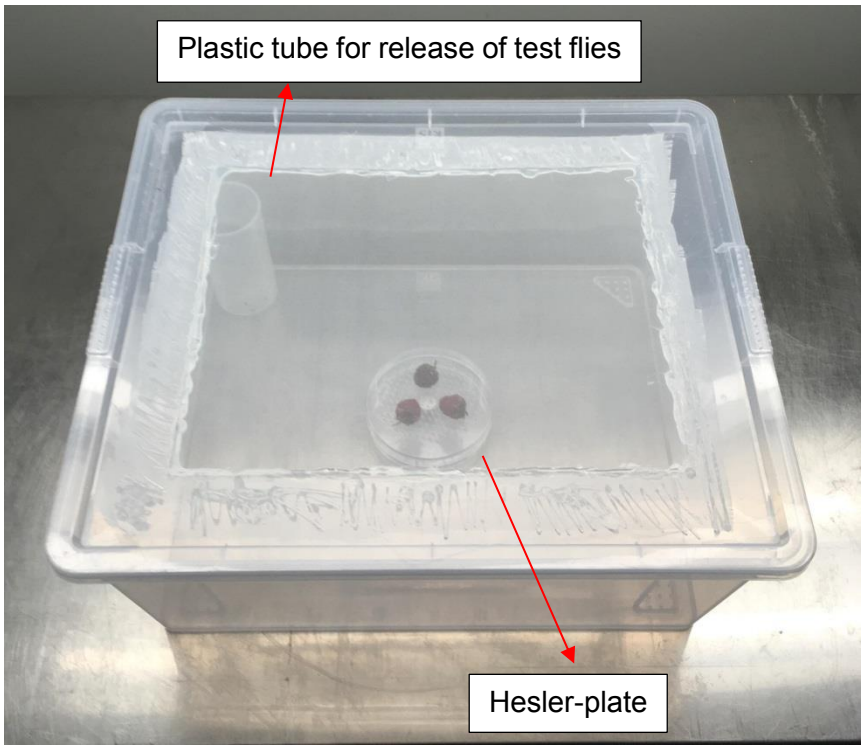

Fig. 19: Test arena for No-Choice-Test-assay

Prior to the experiments, Hesler-plates were washed with soap and drowned in ethanol (96%) for 24 hours to remove any odours. Test arenas were washed with soap, dried and wiped out with ethanol (96%) after every trial. Hesler-plates and cotton dental wicks were used only once per bioassay and thrown away afterwards. Plastic tubes were washed in the dishwasher at 80°C for 40 minutes.

3.1.2 Methods

3.1.2.1 Test design / Experimental set-up

To measure the influence of volatile chemicals on the behaviour of *D. suzukii* a No-Choice-Test-assay experimental set-up was used. The four essential oils patchouli oil, celery oil, catnip oil and neem oil were tested in two concentrations. For the control group only solvents, depending on solubility of the test substances either hexane or acetone, were used. Per treatment 500 µl of the specific liquid were applied to a cotton wick (Tab. 7).

Bioassays were conducted in climate controlled walk-in chambers at 23 ± 1 °C, 80 ± 5% relative humidity, and a photoperiod of 16:8 h (L:D;Tab. A2). The light period started at midnight and ended at 16:00.

Tab. 7: Test design

Test subtances	Treatment (v/v)	N Trial series	n replicates per trial series	Total n replicates	Test dates
Celery oil	Control (acetone)	3	5	15	01.03.- 08.03.2018
	1%	3	5	15	
	10%	3	5	15	
Catnip oil	Control (hexane)	3	5	15	09.04. - 16.04. 2018
	1%	3	5	15	
	10%	3	5	15	
Patchouli oil	Control (hexane)	3	5	15	23.04. - 03.05. 2018
	1%	3	5	15	
	10%	3	5	15	
Neem oil	Control (hexane)	3	5	15	07.06. - 18.06. 2018
	1%	3	5	15	
	10%	3	5	15	

3.1.2.2 Rearings

Rearing started in early 2016 with SWDs from an existing colony[1]. In October 2017 new flies were added from another colony[2] to refresh the genetic material of the population. For the rearing flies were kept in two separate polypropylene boxes (38x29x16.5cm; 20 L volume), with lids ventilated through rectangular holes (18x10 cm) covered with curtain material (Tab. A2; mesh size: 0.03 mm). For the handling of the rearings, additional holes (18x10 cm) were cut into the front and covered with sleeves of curtain material to create a closable opening (Fig. 20). Rearing boxes were maintained in a climate-controlled chamber at 23 ± 1 °C, 80 ± 5% rel. humidity and a photoperiod of 16L: 8D from 00:00h until 16:00h.

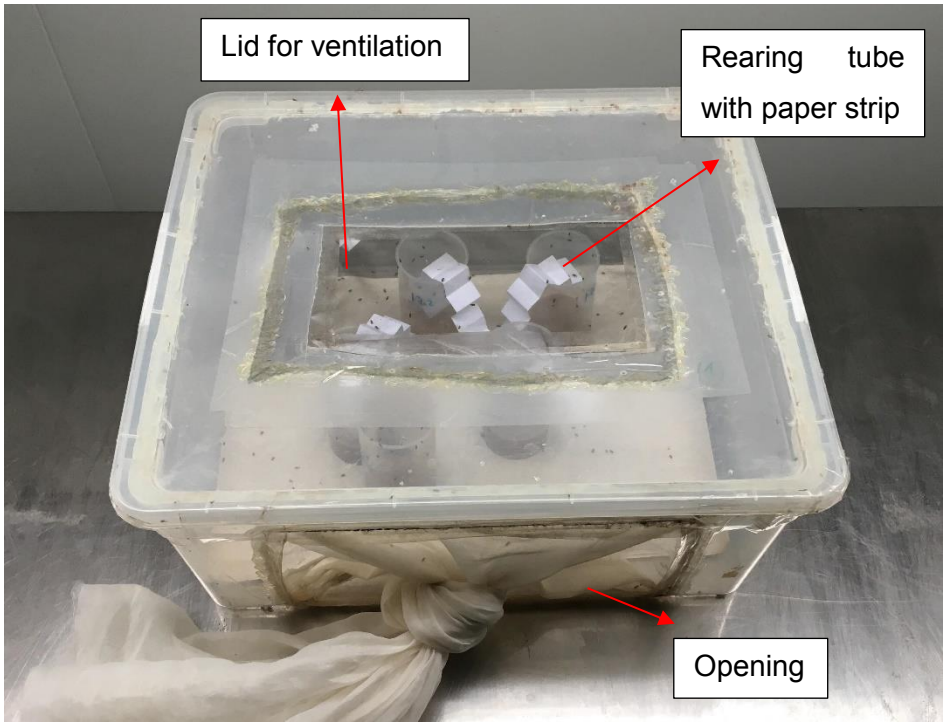

Fig. 20: Rearing box and rearing plastic tubes

For nutrition a modified Drosophila diet (Yoon, 1985) based on mashed potato flakes was used (Tab. 8). Nutrition was presented in polyproyplene tubes (Tab. A2; Ø 50; 100 mm height). Dry ingredients were mixed and 8.3 g were added to every tube. Afterwards 40 ml of the mixed liquid ingredients were applied and stirred until well blended. Every 3 - 4 days (Monday + Thursday) tubes were changed and five fresh tubes per box were supplied. To support the mobility of the flies a folded paper strip was added to every tube. Removed tubes were closed with foam stoppers (Ø 50 mm; 25 mm height) and kept under the same climate-controlled

[1] HBLA Klosterneuburg
[2] KOB – Kompetenzzentrum Obstbau-Bodensee, Ravensburg-Bavendorf, Germany

conditions for further development. Flies emerged 11 - 14 days after removal from rearing box. Once per week newly emerged flies were added to the rearing boxes to maintain the population. For the experiments adult SWDs were moved 1 - 3 days after they emerged to polypropylene tubes with fresh media and were held there additional 7 days for maturation (total age: 8 - 10 d) before they were used in bioassays.

Tab. 8: Ingredients of *D. suzukii* diet (modified after Yoon, 1985)

Dry ingredients	**Amount per tube**
Mashed potato mix	6.70 g
Yeast (dried brewer's yeast)	1.70 g
Methyl 4-hydroxybenzoate	0.17 g
Liquid ingredients	
Canned pineapple juice	20 ml
Apple vinegar	20 ml

3.1.2.3 Conduct of trial

Preparations for the bioassays started on the test day by placing 50 frozen raspberries into a glass Petri dish (Ø 100 mm, 25 mm height) for 2 hours at room temperature until they were completely unfrozen. The test substance was removed from the refrigerator to acclimatize to room temperature. 15 plastic boxes (test arenas) and Hesler-plates (test units) were placed in the climate-controlled walk-in chambers (5 per chamber). One by one, 120 matured *D. suzukii* flies (8 - 10 d) were removed from rearing plastic tubes with an exhaustor (Leybold) (Fig. 21), individually transferred to polystyrol tubes (Tab. A2; 14x100 mm; 10 ml) and sealed with foam stoppers. Flies were checked for their gender under the stereomicroscope (Tab. A2; magnification: 0.8 - 1.2x) and separated into female and male. Afterwards 5 female and 2 male flies were moved into one empty rearing plastic tube, which was also sealed with a foam stopper. In total, 15 plastic tubes with 7 flies each were prepared per bioassay.

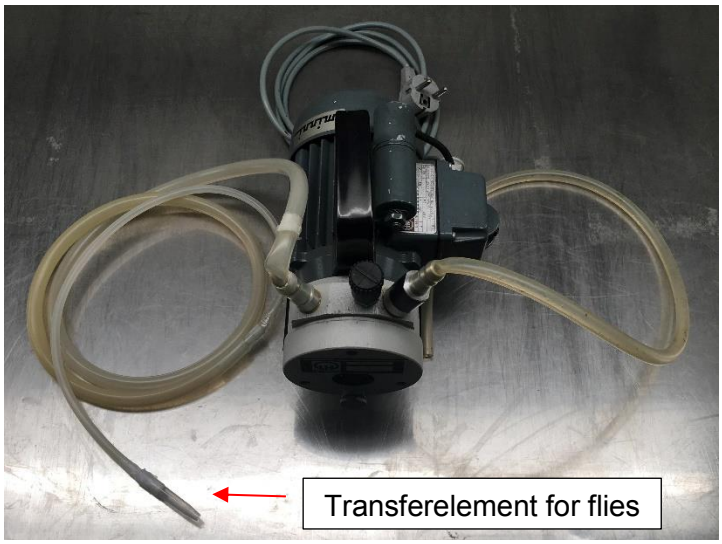

Fig. 21: Exhaustor for transfer of test flies

Test substances were prepared in the flow box. Essential oils were first diluted with solvent to a 10% (v/v) solution in an Eppendorf tube (5 ml). To ensure uniform mixing, it was treated in the Vortex for 30 seconds. Afterwards 300 µl were extracted to another Eppendorf tube and filled up with solvent till it reached a 1% solution. A treatment in the Vortex followed. For the extraction of the liquids pipettes (Tab. A2; 5000 + 1000 µl) and pipettes tips (Polystyrol, 5000 + 1000 µl) were used. Test substances were kept sealed in the Eppendorf tubes until the beginning of the experiment to avoid evaporation.

Main trials started between 15:00 and 16:00 at the end of the light phase when oviposition was peaking (Lin et al., 2014). In the middle of every Hesler-Plate a cotton dental wick was placed with a sterilized tweezer and 500 µl of each specific treatment was applied. After five minutes of evaporation the perforated lid of the Petri dish was closed and 3 raspberries were put on it in the centre in a triangle shaped form. Then the Hesler-plates were placed in the centre of the test arenas. A rearing polystyrol tube with 7 flies was positioned in the front left corner of each test arena. The foam stopper was removed and the lid of the test arena was closed and sealed with sticky tape to avoid the flies from escaping. To avoid olfactory interference, every treatment was conducted in a separate climate-controlled chamber. After 24 hours flies were extracted from the test arena with the exhaustor. Dead flies were recorded. Raspberries were treated with a 0.01% solution of the antifungal Nipagin (Methyl 4-hydroxybenzoate), dried with paper towels and returned in the test arena to allow larvae further developing. 3 days later raspberries were transferred individually to polypropylene cups (Tab. A2; Ø 35x38 mm) and frozen at -16 °C until further analyzing. Larvae that remained on the lid of the Petri dish were recorded for later (Fig. 22).

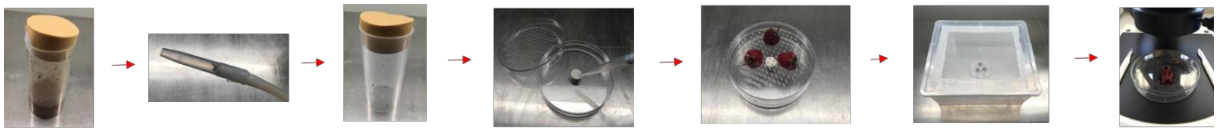

Fig. 22: Experimental process: Flies in rearing tube (a), flies in transferelement of exhaustor (b), flies in empty rearing tube for transfer in test arena (c), application of test substance on cotton wick (d), Hesler-plate with arranged test fruits (e), complete test arena and start of experiment (f), dissecting test fruits for counting of *D. suzukii* larvae.

3.1.2.4 Evaluation

3.1.2.4.1 Microscopical evaluation

Frozen raspberries were unfrozen at room temperature. Afterwards a single raspberry was placed in a Petri dish (glass; Ø 150 mm), the stem was removed and the berry teared up with 2 scalpels until it was a flat mass. 2 droplets of water were applied to moisture the texture. Under the stereomicroscope (Tab. A2; magnification: 0.8 - 1.2x) tissue and stem were systematically searched for larvae. Larvae of all development stages were counted equally. Eggs were not considered.

3.1.2.4.2 Data analysis

For statistical analysis the mean number of *D. suzukii* larvae per test fruit in the treatments and trial series were compared for significant differences (SPSS Statistics, version 22). After an analysis with a non-parametic test for independent samples with (Kruskal-Wallis-Test) to assure the equal distribution of the data for all treatments in all trial series, the mean values were compared with an ONEWAY ANOVA (Bonferroni-Test) if homogeneity of variances was not significantly different (Levene-Test).

3.2 Results

3.2.1 Laboratory trials (No-Choice-Test)

Except for neem oil, none of the tested essential oils demonstrated a repellent and/or oviposition deterrent effect against SWDs (Fig. 23). Celery oil did not show a repellent effect on *D. suzukii* flies, neither in the 1% nor 10% treatment. With 31.6 larvae per test fruit the control group (acetone) had slightly more larvae than the 1% (mean: 26.6) and 10% (mean: 27.5) treatment, which was in range of the standard deviation. No repellent properties were also determined for catnip oil. Although the average amount of larvae per test fruit was in general higher than with celery oil (control: 39.4; 1%: 42.3; 10%: 42.0), there were no significant differences between the control group (hexane) compared to the treatmens with the test substance.

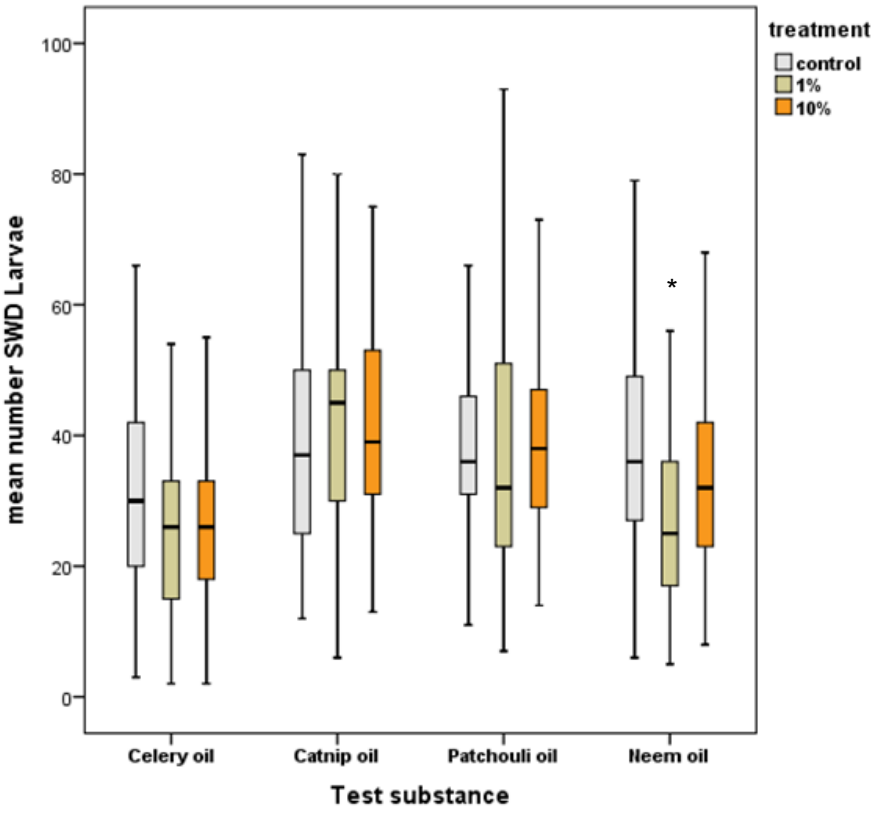

Fig. 23: Mean numbers of *D. suzukii* larvae per test fruit at different treatments (n=45). Asterisks indicate that significantly less larvae were found compared to control group (α=0.05).

Similar to catnip oil, patchouli oil also showed higher total average numbers of larvae per test fruit than celery oil (control: 40.4; 1%: 37.4; 10%: 39.3) but no oviposition deterrent effect on *D. suzukii* flies. For neem oil significantly less larvae were found in raspberries of the 1% treatment. The mean number of larvae per test fruit was 38.8 for the control group (hexane) and 27.9 for the 1% treatment, which resulted in a reduction of 28.1% larvae per test fruit. However, at higher concentrations of neem oil (10%) the number of larvae per test fruits (mean: 35.8) increased and repellency of neem oil was no longer observed.

4. Discussion

4.1 Systematic extensive literature search

4.1.1 Method

The systematic extensive literature search was conducted as 2-step process involving a pre-search and a main search applying specific search terms to answer the research questions. The pre-search was used to obtain an overview of the existing literature for repellency and/or oviposition deterrent substances against members of the order diptera and methods to measure them. Based on the results of the pre-search and the selection criteria for potential test substances, the four essential oils celery oil, catnip oil, patchouli oil and neem oil were chosen as test substances for the bioassays. Afterwards a main search was conducted to obtain detailed results on the repellency and/or deterrent potential of the chosen test substances against members of the order Diptera respectively Drosophilids.

4.1.2 Results

The systematic extensive literature search revealed a large variety of methods to test substances for repellency or oviposition deterrence effects against members of the order Diptera. However, most of them were not suitable to answer the research questions of the present master thesis, as they dealt e.g. with repellency against blood-feeding mosquito species. Therefore, more specific search terms focused on methods for testing repellency and/or oviposition deterrence on Drosophilids were applied, which provided appropriate search results on this subject.

Search results of the main search revealed differences in the amount and qualtiy of the search records between the essential oils. Neem oil archieved by far the most search hits with nearly 80% of all records, but over 30 percent of them focused on neem oil and its main active constituent Azadirachtin as a pesticide and not as a repellent and/or oviposition deterrent substance. Therefore they were rated as 1-star records. A similar result was observed for celery oil for which over 36% of the search hits were rated 1-star, because records were about celery as a target plant for various pests and not as a source for repellent substances. The seach records for patchouli oil (100%) and catnip oil (>95%) on the other hand provided a high number of relevant search hits (4-stars rating). Only one search record resulted in a 5-stars rating for Drosophilidae, although *D. melanogaster* is one of the primary model organisms for research on insect olfaction.

There was a high accuracy (70%) regarding the appropriate developmental stage of the test organisms (adults). A reason for that is that most search records focused on repelling disease distributing vectors, mainly various mosquito species that are threatening the human health. Therefore, the adult stage was the primary target for repellent substances in those studies.

4.2 Laboratory trials (No-Choice-Test)

4.2.1 Method

To answer the research question which size of repellent and/or oviposition deterrent effect the selected essential oils exhibit on adult *D. suzukii* in the laboratory a No-Choice-Test-assay was chosen. The No-Choice-Test method applied in the present master thesis was the result both of experimental experiences from pre-trials and of data obtained by the extensive systematic literature search. A No-Choice-Test-assay with separated test arenas and climate chambers for the different treatments (control group, test substances and different concentrations thereof) was chosen to avoid any potential interference of the test organisms. One purpose of the test design was to simulate the conditions on the field. It was assumed that essential oils would be emitted in the field by a dispenser system (e.g. cardboards or active dispensing machines) and not sprayed. For this reason the selected test substances were applied to cotton wicks for passive odour release from the test unit to the test arena and shielded by a lid to avoid any direct contact of *D. suzukii* flies and the test substance. A continuous emission of volatiles of the test substances during the experiments is considered essential for the success of a repellent or oviposition deterrent (Renkema et al., 2016; Stevenson et al., 2018). Another important factor is the amount of applied test substance. Information on this subject varied widely in the literature, depending on the test substance, the test method and mode of application. Therefore, pre-tests (data not shown) with a standard repellent for *D. suzukii* were conducted to figure out the right amount of a test substance to ensure a continuous release over the whole test duration.

4.2.2 Results

The goal of the present master thesis was to identify essential oils with a repellent and/or oviposition deterrent effect on *D. suzukii*. The oils of celery, catnip and patchouli did not demonstrate a deterrent effect at the different test concentrations in the No-Choice-Test-assays. Previously reported repellent and/or oviposition deterrent effects of these essential oils

against other members of the order Diptera (Bernier et al., 2005; Tuetun et al. 2005; Trongtokit et al. 2005; Kumar et al. 2014) could not be observed in the present trials with *D. suzukii*. Only neem oil showed a slight repellent and/or oviposition deterrent effect at a concentration of 1%. This effect was however no longer observed at the higher test concentration of 10%. A reason for that could be the varying sensitivity of olfactory receptor neurons of flies to different concentrations of repellent and/or oviposition deterrent substances. The olfactory neural network of Drosophilidae is a highly sophisticated system, which is still poorly understood. Approximately a thousand olfactory receptor neurons (Shanbhag et al., 1999; Hallem et al., 2006) are responsible for processing millions of odorants and translating them into adequate behaviour. To achieve this complex task, numerous factors such as composition, concentration, ratio, residence time or quantity of an odour and its volatiles have to be evaluated by the olfactory system. Changing one factor of the equation could lead to a complete different result. The concentration of a volatile compound can influence the behaviour of flies in different ways. As a result, odours can be attractive at lower concentrations and deterrent at higher ones - and reverse. In bioassays with *D. melanogaster* ethyl hexanoate and butyl butyrate attracted at lower concentrations and repelled at higher concentrations (Stensmyr et al., 2003). Experiments in a caged greenhouse demonstrated that traps filled with a higher amount of ethyl acetate (55 and 550 µl) caught fewer SWDs flies than traps with less (5.5 µl) (Kleiber et al., 2014). It is assumed that the varying test results of neem oil at different concentrations are based on the same effect of varying behaviour at different concentrations of volatiles.

Neem oil was tested once before (Erland et al., 2015) for repellency against SWD on blueberries with a No-Choice Dip-method, but the results were not conclusive due to the high standard errors which were attributed to the method as being unappropriate. Although the test method applied in this master thesis is fundamently different from Erland et al. (2015), also high variation of data was observed, which are however due to the high variation of the oviposition rate of SWD. Pre-tests on raspberries under comparable climatic conditions as the No-Choice-Test-assays revealed that one female SWD could lay up to 80 eggs per day, while the mean number was only 22.4. One way to address this issue and decrease the variation could have been to increase the number of repetitions. But due to the time necessary for the microscopic evaluation of the test fruits in the No-Choice-Test-assays, an increase in repetitions would not have been manageable. Apart from that, a comparison with relevant studies about the repellency of test substances against *D. suzukii* (Krause Pham and Ray, 2015; Wallingford et al., 2015, 2017; Renkema et al. 2016, 2017) showed that the chosen number of repetitions was considered adequate for these kind of bioassays.

Pre-tests with celery oil, conducted with a Choice-Test-assay (Direct Airborne Repellent-Test), resulted in a repellency against *D. suzukii* at a concentration of 10% and 50%. Therefore, it

was assumed that celery oil could also elicit an oviposition deterrent effect in a No-Choice-Test-assay. But experiments could not confirm this assumption. A reason for that could be the variation of the applied duration of the different bioassays. Pre-tests lasted 30 minutes, while No-Choice-Test-assays were conducted for 24 hours. Additionally, the test units varied in size and volume with a volume of 14 ml for the pre-test polystyrol tubes and 20 L for the No-Choice-test-assay polypropylene boxes respectively. Limited space during the pre-tests could have promoted deterring behaviour. It is possible that neural receptors responsible for detecting repelling odours were saturated after some time by volatiles of celery oil and were no longer able to promote a repellent reaction. Another reason for the lack of an oviposition deterrent effect of celery oil in the No-Choice-Test-assay could be that the attractiveness of the test fruits was stronger than the repelling ability of the test substance. At the pre-tests, test substances were only compared to the control substance (solvent) without any test fruits present. Hence, flies had only to choose between two odours and were not influenced in their decision by an attraction stimulus.

Patchouli and catnip oil were examined for the first time for its repellency and/or oviposition deterrence on *D. suzukii*. However, nepetalactol, a key volatile compound of catnip oil (Ricci et al., 2010), was tested positively for its repellent effect against SWD in Choice-Tests with larvae and oviposition deterrent assays with adults of *D. melanogaster* and *D. suzukii* respectively, as part of a mixture of substances obtained from washes of the Drosophilid parasitoid *Leptopilina boulardi* (Ebrahim et al., 2015). Nepetalactol, which acts as a sexual pheromone for aphids (Goldansaz et al., 2004), iridomyrmecin and actinidin, which are defense compounds of parasitoid wasps against ants (Völkl et al., 1994; Stökl et al., 2012; Weiss et al., 2013) were identified by Ebrahim et al. (2015) as the main active volatile components of the tested mixture. Nepetalactol and the chemically related compound nepetalactone are the main active volatiles of catnip oil (Ricci et al., 2010; Chauhan et al., 2005). Therefore, it was assumed that catnip oil could also elicit a repellent and/or oviposition deterrent response against SWD in the present study, which could however not be confirmed. A reason for that could be that the applied catnip oil volatiles offered a wrong ratio of nepetalactol and nepetalactone to evoke a deterrent effect. Although the portion of nepetalactone isomers in catnip volatiles amounts 70 – 80%, compared to only 0.5% for nepetalactol (Ricci et al., 2010), it was assumed that catnip oil could have a repelling effect on *D. suzukii* flies, as nepetalactone has also been reported to exhibit repellent activity against members of the order Diptera (Zhu et al., 2012; Gkinis et al., 2014). It is also possible that both components of catnip oil deter SWD only in combination with the other volatiles, as it was demonstrated in other trials that it is essential if a substance is presented individually or in a mixture with other substances to (Cha et al., 2012).

4.3 Outlook

During the present study four essential oils were tested for their deterrent or oviposition repellent effect against *D. suzukii* flies with a laboratory No-Choice-Test-assay, which was found to be an appropriate and reliable test method for this purpose. Although neem oil reduced the number of larvae per test fruit by 28.1% at the lower tested concentration of 1%, the observed deterrent effect cannot be considered as effective enough to continue with trials in the field. The other tested essential oils did not result in any deterrent or oviposition repellent effect against *D. suzukii* flies. For future trials it is proposed to work with individual components of essential oils instead with the whole mixture of components. If a new effective test substance was found, it could be the first step to develop a push-pull system against *D. suzukii* in the field.

5. Summary

The objective of the present master thesis was to evaluate essential oils as new potential repellent and/or oviposition deterrent substances against the Spotted-wing Drosophila (*Drosophila suzukii*) to avoid egg laying into fruits of selected host plants. The research questions were addressed in a systematic extensive literature search (ELS) and in laboratory trials. The ELS according to the EFSA guidance document on systematic review methodology (EFSA, 2010) included the electronic database Ovid for scientific literature. Search terms included common terms for repellency, for method and for the taxonomic group of pest as well as subject-related variations. The ELS was conducted in two steps: a pre-search for selecting potential test substances and screening for relevant laboratory test methods and a main search for relevant records regarding the chosen test substances. Based on the results of the ELS pre-search four essential oils with reported repelling effect on members of the order diptera, celery oil, catnip oil, patchouli oil, neem oil, were chosen as test substances. The main search of the ELS for the four chosen essential oils resulted in 182 records. To organize the search records according to their relevance, specific criteria were defined and a rating system was established. Criteria were based on completeness of the record (keywords, abstract), relevance (article is about repellency), and appropriate life stage of the test organisms and taxonomy (test organisms are at least members of the order diptera). From all search records over 70% were rated as very relevant, relevant or partially relevant, while the rest was rated as irrelevant or incomplete. For the laboratory trials a No-Choice-Test-assay using modified Hesler-plates (Wallingford et al., 2017) with three raspberries each as test units was conducted. Test units were placed in ventilated polypropylene boxes as test arenas. Test design comprised three series with five replicates per treatment. Each treatment was applied in two concentrations (1%, 10%) in 500 µl on a cotton wick with either hexane or acetone as control. For the bioassays seven SWDs (5♀/2♂; age: 8 - 10 d) were placed in each test arena. SWDs were removed after ovipositing for 24h, while rapsberries remained for additional three days in the test arena for potential larval development (total: 4d) before they were removed and frozen. For counting of the larvae, raspberries were unfrozen and dissected under the stereomicroscope. All larval stages were considered for analysis.

Test results demonstrated no repellent and/or oviposition deterrent effect of celery oil, catnip oil and patchouli oil on *D. suzukii*. Only neem oil at a concentration of 1% showed a reduction in the number of larvae per fruit (28.1%) compared to the mean number of the control group. However, at a concentration of 10% this effect was no longer observed. The repellent effect of neem oil at a concentration of 1% was not considered effective enough to conduct trials in the

field. The present study suggests to test additional test substances for their repellent and/or oviposition deterrent potential on *D. suzukii*.

6.Zusammenfassung

Das Ziel dieser Masterarbeit war die Abschätzung von ätherischen Ölen als neue, potentielle repellente und/oder ovipositions-deterrente Wirkstoffe für die Kirschessigfliege *(Drosophila suzukii*) zur Vermeidung der Eiablage in Früchte der Wirtspflanzen. Zur Beantwortung der Forschungsfragen wurde eine Literatursuche (systematic extensive literature search: ELS) und Laborversuche durchgeführt. Die ELS erfolgte nach den Richtlinien des „EFSA guidance document on systematic review methodology“ (EFSA, 2010). Für die ELS wurde die elektonische Datenbank für wissenschaftliche Literatur, Ovid, genutzt. Die Suchbegriffe umfassten gängige Bezeichnungen für Repellenz, Methode sowie für die taxonomische Gruppe des Schädlings. Die ELS wurde in zwei Schritten absolviert: eine Vorsuche für die Auswahl potentieller Testsubstanzen und relevanter Testmethoden im Labor sowie eine Hauptsuche zur Selektion relevanter Literatur für die gewählten Testsubstanzen. Basierend auf den Ergebnissen der Vorsuche wurden vier ätherische Öle mit nachgewiesenem repellenten Effekt auf Mitglieder der Ordnung Diptera, nämlich Sellerieöl, Katzenminzenöl, Patchouliöl, Neemöl, als Testsubstanzen ausgewählt. Die ELS-Hauptsuche für die vier Testsubstanzen ergab 182 Sucherergebnisse. Für die Bewertung der Suchergebnisse wurden Bewertungskriterien definiert und ein Rating-System etabliert. Die Bewertungskriterien basierten auf Vollständigkeit des Suchergebnisses (Schlüsselwörter, Zusammenfassung), Relevanz (Publikation thematisiert Repellenz), Taxonomie (Testorganismus ist zumindest Mitglied der Ordnung Diptera) sowie gesuchtes Lebensstadium des Testorganismus. Von allen Suchergebnissen wurden über 70% als sehr relevant, relevant oder teilweise relevant erachtet, während der Rest als irrelevant oder unvollständig galt.

Für die Laborversuche wurde eine No-Choice-Test-Versuchsmethode mit modifizierten Hesler-Platten (Wallingford et al., 2017) (drei Himbeeren/Hesler-Platte) als Testeinheit gewählt. Testeinheiten wurde in der Mitte einer belüfteten Polypropylen-Box (Testarena) platziert. Pro Behandlung wurden fünf Wiederholungen durchgeführt. Jede Testsubstanz wurde in zwei Konzentrationen (1%, 10%) getestet. Für die Applikation wurden jeweils 500µl auf einen Wattebausch pipettiert, die Lösungsmittel Aceton und Hexan fungierten als Kontrolle. Für die Laborversuche wurden sieben Kirschessigfliegen (5♀ / 2♂; Alter: 8 – 10 Tage) in der Testarena platziert. *D. suzukii* Fliegen hatten 24 Stunden Zeit für die Eiablage. Himbeeren wurden drei weitere Tage in der Testarena belassen (Insgesamt: 4 Tage), um potentielle Larvenentwicklung zu ermöglichen. Danach wurden die Testfrüchte ebenfalls entfernt und eingefroren. Für die Zählung der Larven wurden die Himbeeren aufgetaut, unter dem

Stereomikroskop zerteilt und analysiert. Alle Larvenstadien wurden für die Zählung berücksichtigt.

Testergebnisse ergaben keinen repellenten und/oder Ovipositions deterrenten Effekt von Sellerieöl, Katzenminzeöl und Patchouliöl gegen *D. suzukii*. Lediglich Neemöl führte bei einer Konzentration von 1% zu einer Reduktion der Larven pro Testfrucht (28.1%) im Vergleich zum Durchschnittswert der Kontrollgruppe. Dieser Effekt konnte allerdings bei einer Konzentration von 10% nicht mehr festgestellt werden. Der repellente Effekt von Neemöl bei einer Konzentration von 1% wurde als nicht effizient genug erachtet, um weitere Freilandversuche durchzuführen. Die Testung von zusätzlichen Testsubstanzen wird daher empfohlen.

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8. List of figures

9. List of tables

10. Annex

Tab. A1: Reported host plant species for *D. suzukii* (Infestation of host plants were confirmed by observation in the field or by laboratory assays)

Scientific name	Family	Source
Actinidia arguta (Sieb. & Zucc.) Planch. ex Miq. *A. chinensis* Planch.	Actinidiaceae	Lee et al. (2015) Kenis et al. (2016)
Alangium platanifolium (Siebold & Zuccarini) Harms	Cornaceae	Mitsui et al. (2010)
Aucuba japonica Thunberg	Cornaceae	Mitsui et al. (2010)
Amelanchier lamarckii F.G. Schr. *A. ovalis* Medik.	Rosaceae	Kenis et al. (2016) Kenis et al. (2016)
Arbutus unedo L.	Ericaceae	Arno´ et al. (2012)
Aronia melanocarpa (Michx.) Elliott	Rosaceae	Rauleder (2015)
Arum italicum Mill. *A.maculatum*	Araceae	Kenis et al. (2016) Poyet et al. (2015)
Asimina triloba (L.) Dunal	Annonaceae	Rauleder (2015)
Atropa belladonna L.	Solanaceae	Poyet et al. (2015)
Berberis aquifolium Pursh *B. hortensis*	Berberidaceae	Lee et al. (2015) Poyet et al. (2015)
Citrus sinensis L.	Rutaceae	Lee et al. (2015)
Cornus alba L. *C. amomum* Mill. *C. controversa* Hemsl. ex Prain *C. foemina* Mill. *C. kousa* Hance *C. mas* L. *C. racemose Lam.* *C. sanguinea* L. *C. sericeae* L.	Cornaceae	Kenis et al. (2016) Lee et al. (2015) Lee et al. (2015) Mitsui et al. 2010 Lee et al. (2015) Lee et al. (2015) Kenis et al. (2016) Lee et al. (2015) Kenis et al. (2016)
Cotoneaster franchetii Boiss. *C. lacteus* W.W. Smith *C. rehderi* Pojark.	Rosaceae	Kenis et al. (2016) Lee et al. (2015) Kenis et al. (2016)

Scientific name (contin.)	Family (contin.)	Source (contin.)
Crataegus chrysocarpa Ashe	Rosaceae	Kenis et al. (2016)
C. monogyna Jacq.		Kenis et al. (2016)
Daphne mezerum L.	Thymelaeaceae	Kenis et al. (2016)
Diospyros kaki Thunberg	Ebenaceae	Kanzawa (1935, 1939)
Duchesnea indica (Andr.) Focke	Rosaceae	Kenis et al. (2016)
Elaeagnus multiflora Thunb.,	Elaeagnaceae	Kanzawa, 1939
E. umbellate Thunberg		Lee et al. (2015)
Eugenia uniflora L.	Myrtaceae	Lee et al. (2015)
Eriobotrya japonica (Thunb.) Lindley	Rosaceae	Kanzawa 1935
Ficus carica L.	Moraceae	Lee et al. (2015)
Fragaria vesca L.	Rosaceae	Poyet et al. (2015)
F. ananassa L.		Lee et al. (2011)
Frangula alnus Miller	Rhamnaceae	Lee et al. (2015)
F. purshiana (de Candolle) A. Gray		Lee et al. (2015)
Gaultheria adenothrix (Miquel) Maximovich	Ericaceae	Mitsui et al. (2010)
G. shallon Pursh		Lee et al. (2015)
G. wisleyensis Marchant & Airy Shaw		Kenis et al. (2016)
Hippophae rhamnoides L.	Elaeagnaceae	Kenis et al. (2016)
Lindera benzoin (L.) Blume	Lauraceae	Lee et al. (2015)
Lonicera alpigena L.	Caprifoliaceae	Kenis et al. (2016)
L. X bella Zabel		Lee et al. (2015)
L. caerulea L.		Lee et al. (2015)
L. caprifolium L.		Kenis et al. (2016)
L. ferdinandii Franch.		Kenis et al. (2016)
L. kamtschatica Dippel		Rauleder (2015)
L. morrowii A. Gray		Lee et al. (2015)
L. nitidia (Franch.) P.S.Hsu & H.J.Wang		Poyet et al. (2015)
L. tatarica L.		Lee et al. (2015)
L. xylosteum L.		Poyet et al. (2015)
Lyceum barbarum L.	Solanaceae	Kenis et al. (2016)
Mahonia aquifolium (Pursh) Nutt.	Berberidaceae	Rauleder (2015)

Scientific name (contin.)	Family (contin.)	Source (contin.)
Malus baccata Borkh.	Rosaceae	Kenis et al. (2016)
Malus pumila Miller		Kanzawa (1939)
Morus alba L.	Moraceae	Lee et al. (2015)
Morus alba x rubra		Mitsui et al. (2010)
M. australis Poiret *(=bombycis)*		Lee et al. (2015)
M. nigra L.		Mitsui et al. (2010)
M. rubra L.		Lee et al. (2015)
Murray paniculata (L.) Jack	Rutaceae	Lee et al. (2015)
Paris quadrifolia L.	Melanthiaceae	Kenis et al. (2016)
Parthenocissus quinquefolia (L.) Planch.	Vitaceae	Kenis et al. (2016)
Photinia beauverdiana L.	Rosaceae	Kenis et al. (2016)
P. villosa (Thunberg) DC.		Kenis et al. (2016)
P. prunifolia Lindl.		Kenis et al. (2016)
Phytolacca americana L.	Phytolaccaceae	Lee et al. (2015)
P. esculenta Van Houtte		Kenis et al. (2016)
Physalis alkekengi L.	Solanaceae	Poyet et al. (2015)
Prunus avium L.	Rosaceae	Lee et al. (2015)
P. armeniaca L.		Lee et al. (2015)
P. cerasifera Ehrh.		Kenis et al. (2016)
P. cerasus L.		Kanzawa (1939)
P. domestica L.		Kenis et al. (2016)
P. donarium Siebold		Kanzawa (1939)
P. japonica Thunb.		Kanzawa (1935,1939)
P. laurocerasus L.		Lee et al. (2015)
P. lusitanica L.		Lee et al. (2015)
P. mahaleb L.		Kanzawa (1939)
P. nipponica Matsumura		Mitsui et al. (2010)
P. padus L.		Kenis et al. (2016)
P. salicina Lindley *(= triflora)*		Kanzawa (1935, 1939)
P. sargentii Rehder		Kanzawa (19399
P. serotina Ehrh.		Poyet et al. (2014)
P. spinosa L.		Poyet et al. (2015)
P. yedoensis Matsumura		Kanzawa (1935, 1939)

Scientific name (contin.)	Family (contin.)	Source (contin.)
Prunus. virginiana L.	Rosaceae	Lee et al. (2015)
P. x yedeoensis Matsumura		Kanzawa (1935, 1939)
Polygonatum multiflorum (L.) All.	Asparagaceae	Kenis et al. (2016)
Pyracantha sp.	Rosaceae	Kenis et al. (2016)
Pyrus sinensis L.	Spiraeoideae	Poyet et al. (2015)
Rosa acicularis Lindl.	Rosaceae	Kenis et al. (2016)
R. canina L.		Kenis et al. (2016)
R. glauca Pourr.		Kenis et al. (2016)
R. pimpinellifolia L.		Kenis et al. (2016)
R.rugosa Thunb.		Kenis et al. (2016)
Ribes rubrum L.	Grossulariaceae	Poyet et al. (2015)
R. sanguineum Pursh.		Poyet et al. (2015)
R. uva-crispa L.		Lee et al. (2015)
Rubus allegheniensis Porter	Rosaceae	Lee et al. (2015)
R. armeniacus Focke		Lee et al. (2015)
R. bifrons Vest		Lee et al. (2015)
R. caesius L.		Kenis et al. (2016)
R. crataegifolius Bunge		Mitsui et al. 2010
R. fructicosus aggr.		Rauleder (2015)
R. idaeus L.		Rauleder (2015)
R. microphyllus L.f.		Mitsui et al. 2010
R. parvifolius L. (=triphyllus)		Kenis et al. (2016)
R. phoenicolasius Maxim.		Kenis et al. (2016)
R. spectabilis Pursh		Lee et al. (2015)
Rhamnus purshiana DC.	Rhamnaceae	Lee et al. (2015)
R. cathartica L.		Kenis et al. (2016)
Sambucus nigra spp. *cerulean* (Raf.)	Adoxaceae	Lee et al. (2015)
S. ebulus L.		Poyet et al. (2015)
S. nigra L.		Lee et al. (2015)
S. racemose var. *melanocarpa* (A. Gray) McMinn		Lee et al. (2015)
Sarcococca confuse Sealy	Buxaceae	Lee et al. (2015)

Scientific name (contin.)	Family (contin.)	Source (contin.)
Solanum dulcamara L.	Solanaceae	Lee et al. (2015)
S. nigrum L.		Poyet et al. (2015)
S. lycopersicum L.		Lee et al. (2015)
S. villosum Miller (=luteum)		Arno´ et al. 2012
Sorbus aucuparia L.	Rosaceae	Kenis et al. (2016)
S. aria (L.) Crantz		Lee et al. (2015)
S. sitchensis M.Roem.		Lee et al. (2015)
Symphoricarpos albus (L.) S.F.Blake	Caprifoliaceae	Lee et al. (2015)
Tamus (=Dioscorea) communis (L.) Caddick & Wilkin	Dioscoreaceae	Kenis et al. (2016)
Taxus baccata L.	Taxaceae	Poyet et al. (2015)
Torreya nucifera (L.) Siebold & Zuccarini	Taxaceae	Mitsui et al. 2010
V. myrtillus L.	Ericaceae	Kenis et al. (2016)
V. myrtilloides Michx.		Kenis et al. (2016)
V. oldhamii Miq.		Kenis et al. (2016)
V. ovatum Pursh		Lee et al. (2015)
V. praestans lamb.		Kenis et al. (2016)
V. vitis-idaea L.		Lee et al. (2015)
Viburnum dilatatum Thunberg	Adoxaceae	Mitsui et al. 2010
V. lantana L.		Kenis et al. (2016)
V. rhytidophyllum Hemsl.		Kenis et al. (2016)
Viscum album L.	Santalaceae	Poyet et al. (2015)
Vitis vinifera L.	Vitaceae	Lee et al. (2011)

Tab. A2: Materials for the conduct of the bioassays

	Materials
Test fruits:	Stereomicroscope: Zeiss, Stemi 2000-C
Test units:	Hesler-Plate Petri dish: Semadeni, Ostermundigen, Schweiz Dental wick: Rauscher, Vienna, Austria Test arena: KIS C Box, Ormello, Italia Curtain tissue: IKEA Curtain “Teresia”, Vienna, Austria Hot glue gun: Steinel Gluematic 3002; glue: Cristal Glue Sticks Ø11 mm
Test design:	Illumination climate chamber: Philips, True Light, 36W/5500
Rearings:	Curtain material: IKEA Curtain “Teresia”, Vienna, Austria Polypropylene tubes: Semadeni, Italy
Conduct of trial:	Polystyrol tubes: VWR, Austria Stereomicroscope: Zeiss, Stemi SV 11 Pipettes: Gilson, Pipetman Polypropylene cups: VWR, Austria
Analysis:	Stereomicroscope: Zeiss, Stemi SV 11