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Integrated Pest Management of *Drosophila suzukii* (Diptera: Drosophilidae)

Monitoring, insecticide efficacy in cherry and population modelling

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ABSTRACT

Drosophila suzukii, Matsumura 1931, is a fruit fly native to Southeast Asia and has spread across Europe and Northern America since 2008. Due to the female's serrated ovipositor it is able to pierce ripening fruits to lay their eggs. This causes large economic losses in soft and stone fruit all over the world. The unique feeding pattern, egg laying in pre-harvested fruits, fast reproduction and the lack of natural enemies placed *D. suzukii* in a single niche in the western world. This makes controlling *D. suzukii* as a pest organism challenging, even for an integrated pest management (IPM) strategy. The key of an IPM approach is an adequate monitoring of *D. suzukii* in order to know which strategy to use at the right moment. Due to the vast amount of available commercial trap combinations with different lures and drowning solutions it is difficult for growers to know which setup to use. The combination of the New Droso Trap (Biobest) and Super Gasser (Riga), New Droso Trap and NRI Dry lure (National Resource Institute) with water, New Droso Trap and Fruit Fly Attractant (Koppert) and New Droso Trap combined with Dros' Attract (Biobest) showed the best results for monitoring *D. suzukii* in a cherry orchard. At the moment the traps are used to monitor *D. suzukii* in order to know when to apply insecticides to control *D. suzukii*. Because chemical control is currently the most effective strategy available to manage *D. suzukii*, certainly when there is zero tolerance towards the presence of *D. suzukii* in harvested fruit, it is key to know which active ingredients show the most potential and can be used in an IPM strategy in combination with biological control. An array of nine insecticides were tested to establish their efficacy against *D. suzukii*. Cyantraniliprole and λ-cyhalotrhin showed the best control of *D. suzukii* up to 14 days after a single treatment. Deltamethrin and spinosad also showed also a good control of *D. suzukii* up to seven days. These active ingredients do have three different modes of action but more are desired due to the potential susceptibility to resistance as a result of the fast reproduction of *D. suzukii* with to 15 generations per year. Therefore it is important to have multiple active ingredients with different modes of action available to reduce the risk of developing resistance.

In order to have a better understanding of *D. suzukii* as an organism and to learn the population dynamics throughout the year a model can be useful, especially to give better advice to growers on how to control *D. suzukii* infestation in their crops. A first model was established by using temperature depended egg to adult development times, a combination of temperature and age specific egg laying numbers by the female adults and a mortality rate. The model was calculated by comparing these data, which were found in literature, to 15 minutes interval weather data. This raw model was validated to the *D. suzukii* flies caught in the national monitoring of the UK. The predictions of the population model seems to be dependent of the region of the different farms and the starting value of the calculation start. However the starting time seems not to be that important in the range of the first egg laying estimation, established by ovary dissection. The best predictions can be made in region East with a correlation coefficient bigger than 0.88.

DUTCH ABSTRACT

Drosophila suzukii, Matsumura 1931, behoort tot de familie van de Drosophilidae en komt oorspronkelijk uit zuidoost Azië. Sinds 2008 heeft deze fruitvlieg haar introductie gemaakt in de westerse wereld en is er in geslaagd om zich in zes jaar tijd te verspreiden over heel Europa en Noord-Amerika. Door de getande ovipositor zijn vrouwelijke *D. suzukii* vliegen in staat om rijpend fruit te beschadigen door er hun eitjes in af te leggen. Dit zorgt voor grote economische verliezen in zacht en steen fruit over heel de wereld. Het unieke voedingspatroon, de eiafzet voor de oogst van het fruit, de snelle reproductiecyclus en het ontbreken van natuurlijke vijanden hebben ervoor gezorgd dat *D. suzukii* in een niche is terecht gekomen wat haar verspreiding in de hand gewerkt heeft. De bestrijding van dit schadelijk organisme is dan ook een uitdagende opdracht, zelfs met een geïntegreerde bestrijding. De sleutel bij elke geïntegreerde bestrijding is een adequate monitoring van de plaag, zo ook bij *D. suzukii*. Deze monitoring is een hulp bij het kiezen van een juiste strategie om deze toe te passen op het juiste moment met een zo groot mogelijke efficiëntie. Voor telers is het monitoren van *D. suzukii* geen gemakkelijke opgave zeker met een groot aanbod aan vallen en lokstoffen. De bombinatie van de nieuwe Droso val (Biobest) en Super Gasser (Riga), nieuwe Droso val en NRI Dry lure (National Resource Institute) met water, nieuwe Droso val en Frui Fly Attractant (Koppert) en de nieuwe Droso val gecombineerd met Dros'Attract (Biobest) vertoonde de beste resultaten om *D. suzukii* te monitoren in een kersenboomgaard. Deze vallen worden momenteel door telers gebruikt om te weten wanneer insecticiden ingezet moeten worden om *D. suzukii* te bestrijden. Chemische bestrijding is voorlopig nog de meest effectieve manier om *D. suzukii* onder controle te houden, zeker wanneer er in veilingen en winkels een nul tolerantie heerst op de aanwezigheid van schade door *D. suzukii*. Om deze chemische bestrijding verder te optimaliseren en te gebruiken in een geïntegreerde bestrijdingsstrategie in cominatie met biologische middelen, is het belangrijk om te weten welke actieve stoffen het meest effectief zijn en de langste nawerking hebben. Negen actieve stoffen werden in de kersenteelt getest op hun effectiviteit tegen *D. suzukii*. Cyantraniliprole en λ-cyhalotrhin vertoonden de beste bestrijding van *D. suzukii* tot 14 dagen na een enkele behandeling. Deltamethrine en spinosad vertoonden ook een goede controle van *D. suzukii* schade maar de werking verminderde na zeven dagen. Deze vier actieve stoffen behoren samen tot drie verschillende werkingsgroepen. Dit is belangrijk omdat deze parasiet gevoelig is voor resistentie tegen insecticiden omdat *D. suzukii* tot 15 generaties per jaar kan hebben. Het is dus belangrijk om meerdere actieve stoffen met verschillende werkings-mechanismen te combineren om de resistentie van *D. suzukii* niet in de hand te werken.

Om *D. suzukii* als organisme beter te begrijpen en in het bijzonder de verschillende populatie fluctuaties te leren kennen is een model onmisbaar. Zeker om te voorspellen hoe *D. suzukii* in verschillende klimatologische omstandigheden zal reageren. Wanneer er een mogelijkheid bestaat om de fluctuaties in de populatie voorspellen en te weten wanneer de eerste ei afleg plaatsvindt kan dit model geïntegreerd worden in een IPM strategie om *D. suzukii* onder controle te krijgen. Een eerste model kwam tot stand door gebruik te maken van temperatuur gerelateerde ei tot volwassene ontwikkelingstijden, een combinatie van temperatuur en leeftijdsgebonden eiafleg en een temperatuur gebonden sterftecijfer, bepaald in laboratoriumomstandigheden. Het model werd berekend door deze gegevens uit de leteratuur te vergelijken met weerdata, bestaande uit 15 minuten intervallen. Het ruwe model werd vervolgens afgetoetst met behulp van de nationale monitoring data van het Verenigd Koninkrijk. De populatie voorspellingen van het model zijn afhankelijk van de regio waar de fruitkweker is gevestigd en zijn ook afhankelijk van de beginwaarde van de berekeningen al is de startdatum van ondergeschikt belang. De beste voorspellingen kunnen gemaakt worden in de regio oost met een correlatie coëfficiënt groter dan 0.88.

DUTCH ARTICLE

Aziatische fruitvlieg, een bedreiging voor de fruitteelt.

Drosophila suzukii, beter bekend als de Aziatische fruitvlieg is sinds 2011 een recente plaag in de fruitsector. De Aziatische fruitvlieg is eenvoudig te herkennen doordat de volwassen mannetjes een zwarte stip op beide vleugels hebben.

Doordat de Aziatische fruitvlieg haar ei afleg doet in rijpend fruit dat nog aan de boom of struik hangt maakt het een gevreesde parasiet die voor heel wat schade kan zorgen. In het zuiden van Frankrijk en Amerika zijn er in sommige percelen oogstverliezen gerapporteerd van 80 procent. Zo'n vaart heeft het in België nog niet gelopen. Al zorgt dit insect ook bij ons voor heel wat problemen voor telers van zacht- en steenfruit zoals aardbeien, bosbessen, frambozen, braambessen en kersen.

De Aziatische fruitvlieg zorgt voor heel wat uitdagingen voor de huidige bestrijdingsstrategieën, zo ook voor een geïntegreerde aanpak. De huidige sanitaire maatregelen zoals het verwijderen van afgevallen vruchten, de teelt leegplukken op het einde van het seizoen, het niet composteren van fruitafval helpen mee om de plaag te bestrijden. De chemische aanpak is momenteel de meest effectieve al mag de invloed van de andere maatregelen niet onderschat worden. Zonder een goede bedrijfshygiëne laat de chemische controle het eveneens afweten. De biologische bestrijding door nuttige insecten staat momenteel nog niet op punt.

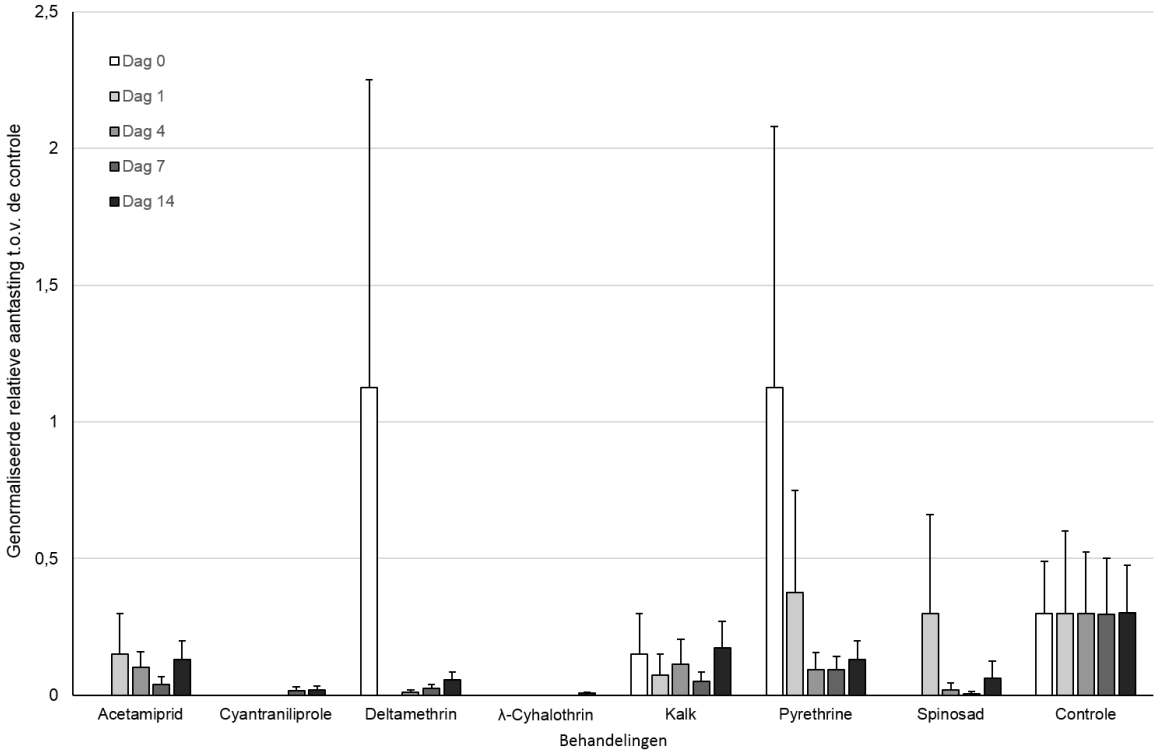
In het kader van de strenge Europese normen ten aanzien van gewas-beschermingsmiddelen wordt er ge-streefd naar een optimaal gebruik van de toegestane middelen. Dit is een hele uitdaging omdat bij een foutief gebruik van gewasbeschermingsmiddelen resistentie in de hand gewerkt wordt. Dit wordt nog eens versterkt door de snelle generatiewissel van de Aziatische fruit-vlieg.

Momenteel zijn alleen de actieve stoffen spinosad (max. 2 toepassingen, 7 dagen wachttijd), lambda-cyhalothrin (max. 2 toepassingen, 7 dagen wachttijd) en dimethoaat (max. 1 toepassing, 28 dagen wachttijd) toegelaten tegen de Aziatische fruitvlieg in de kersenteelt (fytoweb.be). Dit maakt het zeer moeilijk om resistentievorming t voorkomen.

In een kersenboomgaard in de zomer van 2015 aan East Malling Research (East Malling, Kent, UK) zijn er in een veldproef zeven insecticiden getest om hun effectiviteit na een enkele behandeling tegen Aziatische fruitvlieg na te gaan. In deze veldproef werden acetamiprid, cyantraniliprole, lambda-cyhalothrin, pyrethroïden, spinosad en kalk gecombineerd met microkoper, -zink en –mangaan. Niet alle middelen zijn in België erkend voor de Aziatische fruitvlieg.

De rijen van de kersenboomgaard die werden gebruikt tijdens het onderzoek werden overkoepeld door tunnels die aan de zijkanten open waren. De tunnels dienden alleen om de uitspoeling van de insecticidenbehandeling door de regen te vermijden. Elk insecticide werd eenmaal toegepast in een blokken systeem zodat de veldproef zes keer herhaald werd.

De effectiviteit van de insecticiden werd gemeten door op dag 0, dag 1, dag 4, dag 7 en dag 14 na de behandeling telkens 20 vruchten te oogsten. Deze vruchten werden drie weken lang bij 20°C bewaard in plastic dozen met verluchtingsgaten. De dozen werden twee maal per week gecontroleerd op volwassen fruitvliegen die uit gekomen waren. Deze volwassen fruitvliegen werden verwijderd om verdere contaminatie door een nieuwe generatie te vermijden. Na drie weken werden de vruchten vernietigd door ze luchtdicht, bij 15°C te bewaren.



Genormaliseerde relatieve aantasting t.o.v. de controle. Hierdoor wordt de variatie in de infectiedruk op de werking van de actieve stoffen weg gefilterd. Op dag 1 van het experiment waren er twee objecten die een verhoogde infectie door de Aziatische fruitvlieg vertoonde. Deze eitjes zijn waarschijnlijk voor de behandeling gelegd en waren niet volledig geraakt door deltamethrin en pyrethrine.

Lambda-cyhalothrin en cyantraniliprole geven een goede en langdurige controle van de Aziatische fruitvlieg. Ook deltamethrin is een effectief middel maar heeft na zeven dagen al een iets mindere werking. Spinosad heeft ook een goede werking maar heeft volgens deze resultaten niet meteen een shock effect maar geeft wel tot zeven dagen na de toepassing een werking tegen de Aziatische fruitvlieg. Acetamiprid heeft volgens de resultaten wel een werking maar het heeft zeker geen shock effect en heeft ook maar een beperkte werking van zeven dagen. De kalk, gemengd met microkoper, micromangaan en micro-zink, geeft geen eenduidig resultaat en biedt onvoldoende bescherming. Als bijkomend nadeel laat deze kalk-behandeling ook nog een zeer sterk zichtbaar residu achter. Ook de natuur-lijke pyrethroïden bieden weinig be-scherming omdat deze snel door het licht en de hogere temperatuur afgebroken worden.

Alleen lambda-cyhalotrhin en spinosad zijn in België erkend in de kersenteelt maar de andere middelen geven een indicatie welke nog erkend zouden kunnen worden. Deltametrin is wel erkend in het Verenigd Koninkrijk in kersen maar is in België enkel erkend in appel en peer.

Dieter Baets, KU Leuven in samenwerking met East Malling Research en gefinancierd door de Agriculture and Horticulture Development Board (AHDB).

POSTER POPULATION MODEL

KU LEUVEN

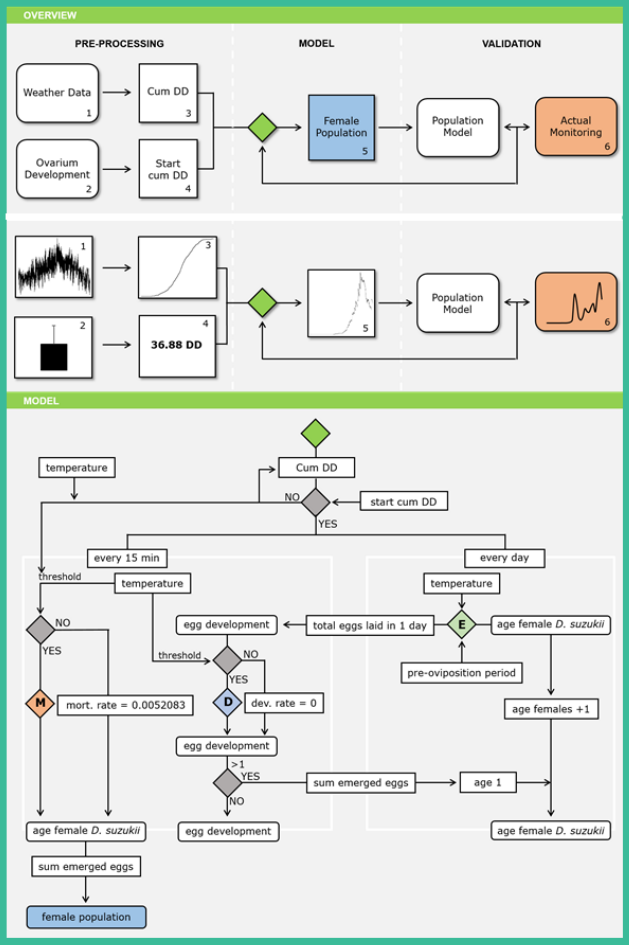
emr east malling research

AHDB HORTICULTURE

Population model of *Drosophila suzukii*

[1]Baets Dieter, [2]Buss David, [2]Fountain Michelle

Drosophila suzukii is since 2008 a new pest in our western world. It affect the soft and stone fruit industry by laying eggs in ripening fruit before harvest. In order to better understand the population dynamics throughout the year a theoretical population model was made to advice growers to control D. suzukii.



Weather data with 15 minute intervals together with the first egg estimation by dissecting female ovaries are the base for this population model. The mortality, development rate and egg laying are derived from literature.
The model is validated with UK's national monitoring and has a correlation coefficient of 0,88 for the region 'East' in the UK.

M $$M(T) = 0.00035\,(T-15)^2 + 0.01$$

Asplen et al., 2015

D $$D(T) = aT(T-T_l)(T_u-T)^{\frac{1}{m}}$$

a = 0,00020 and m = 2,51 Ryan et al., 2016

E $$\begin{cases} \alpha\left[\frac{\gamma+1}{\pi\lambda^{2\gamma+2}}\left(\lambda^2 - ([T-\tau]^2+\delta^2)\right)^{\gamma}\right] 4A \\ 0 \end{cases}$$

Ryan et al., 2016

α = 659.06, γ = 88.53, λ = 52.32,
δ = 6.06, τ = 22.87

$$A(age) = \frac{0.585\ age}{1.0475^{age}}$$

Asplen et al., 2015

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INTRODUCTION

Drosophila suzukii Matsumura, 1931 (Diptera: Drosophilidae) is a recent, highly invasive and polyphagous pest, native to Southeast Asia. The more common name in English speaking countries is Spotted Wing Drosophila or SWD. This name refers to the spots on the wings of male *D. suzukii* flies.

Commonly they are known as vinegar flies along with other Drosophilidae with *Drosphila melanogaster* Meigen, 1830 as the most known member. Recently *D. suzukii* has invaded several western countries. Females lay their eggs in unwounded ripening fruit. This is in contrast with other Drosophila flies depositing their eggs on overripe or rotten fruit. It is therefore a pest of great concern for the production of almost all small fruits, especially cherries, strawberries, blueberries, raspberries and grapes. *D. suzukii* could have a great economic impact because yield losses of over 80% are estimated (Bolda & Goodhue, 2010).

Like most other biological invasions the recent spread of *D. suzukii* is mediated by humankind due to an increase in global trade (Westphal, Browne, MacKinnon, & Noble, 2008). The first successfully invaded country outside the native region of *D. suzukii* was the United States and more specifically Hawaii in the 1980's (Hauser, 2011). More recently in 2008, there were reports of fruit damage by *D. suzukii* in Spain and California (Cini, Ioriatti, & Anfora, 2012; Hauser, 2011).

From attending some workshops and meetings with growers it became clear that they are very careful with this new pest organism and are really afraid of the consequences *D. suzukii* could have on their income. There is already a lot of research done in all sorts of fields but there are still many questions unanswered. Often the information is not readily available or is sometimes contradictory to each other, making it difficult for growers to obtain the necessary knowledge in order to manage the damage done by *D. suzukii*. Due to the high reproduction rate and hiding of the eggs in ripening fruit it is not easy to manage this pest. The only way to manage a *D. suzukii* infestation is to make use of a well-balanced integrated pest management strategy with the use of sanitary measures, chemical and biological control, netting, attract and kill trapping and mass trapping. Comparing different combinations of trap and attractant for monitoring purposes is key to start the development of an IPM strategy, as well as the comparison of the efficacy of different insecticides against *D. suzukii*.

The research in this thesis consists of three parts: a comparison of different monitoring traps, insecticide efficacy evaluation in cherry and population modelling of *D. suzukii*. The comparison of different commercially available monitoring traps is not only key in the development of an IPM strategy but also important to advise growers in their choice of monitoring traps. Following on the monitoring trap comparison, the insecticide efficacy evaluation in cherry gave information on which active ingredients could have a controlling effect on *D. suzukii*. To know which active ingredients do have an effect it is important to minimise insecticide applications without enhancing the resistance against the different insecticide classes. The last part of this thesis consists of a population model of *D. suzukii* with population dynamics throughout the year. This model can be used to better understand *D. suzukii* as an organism and how it behaves on climatological conditions such as the temperature. This information can further be used to help in the development of management strategies.

The trap comparison and insecticide efficacy are funded by the Agriculture and Horticulture Development Board (AHDB), Defra, The Worshipful Company of Fruiterers, British Summer Fruit and The East Malling Trust under project number SF 145 and is led by East Malling Research (EMR) in conjunction with The James Hutton Institute. The last part, the population modelling, is based on the national monitoring data and ovary development samples as part of the same project, as well as weather data provided by Agrii Intelligence.

1 GENERAL CHARACTERISTICS AND IPM STRATEGY

1.1 Introduction

With more than 1500 *Drosophila* species (Brake & Bächli, 2008; Markow & O'Grady, 2006) this genus of flies belonging to the Drosophilidae family is a very large group with subtle visual characteristics, making determination challenging. The general appearance of *D. suzukii* is that of a typical vinegar fly like *Drosophila melanogaster* Meigen, 1830. Both adults have an overall yellow-brown colour with brown lateral bands on the abdomen and red eyes (*Figure 1*). They have also three segmented antennae, a flagellum with a plumose arista which is characteristically forked at the tip. Their size is about two to three millimetres. (Calabria, Máca, Bächli, Serra, & Pascual, 2012). A correct identification is essential to know which management strategy to apply (Hauser, 2011).

Figure 1: ***A.*** *Male D. suzukii with black spots on the wing tips.* ***B.*** *Female D. suzukii. (Photograph by Martin Hauser, California Department of Food and Agriculture)*

1.2 Identification of *D. suzukii*

1.2.1 Male characteristics

Male adults can relatively easily be determined *in situ* by their conspicuous black spots on the wings. The black spot is centred around the subcosta (Sc) and radius (R1) (*Figure 2, A.* and *Figure 1*, *A.*) (Stark, Bonacum, Remsen, & DeSalle, 1999), according to the Comstock-Needham classification system for wings (Wootton, 1979). The black spot is not a unique characteristic of *D. suzukii*, also *Drosophila biarmipes* Malloch, 1924 (Gompel, Prud'homme, Wittkopp, Kassner, & Caroll, 2005; Scott, Carissa, Mark, Jennifer, & Larry, 1997) and *Drosophila subpulchrella* Takamori and Watabe, 2006 (Takamori, Watabe, Fuyama, Zhang, & Aotsuka, 2006) have a distinct black spot on their wings (*Figure 2, B.)*. However *D. biarmipes* is known from India and *D. subpulchrella* has only recently been described in Japan and China (Takamori et al., 2006) and the western part of the Himalayan/Tibetan plateau (Markow & O'Grady, 2006). Very little is known about the spread of both species across western countries (EPPO, 2015). With the recent concerns for infestation by *D. suzukii* and the little knowledge about the two other species most growers take for granted that every *Drosophila* fly they encounter is a *D. suzukii.*

In order to determine with certainty which flies are *D. suzukii* the shape of the sex combs has to be examined. These sex combs are placed on the first and second tarsomere of the front legs. The sex combs of *D. suzukii* are arranged in a single row on both the first and second tarsomere and they are placed in the direction of the leg (*Figure 3*). Whereas the sex combs of *D. biarmipes* are only present on the first tarsomere and they are placed in two separate rows (Hauser, 2011). *D. subpulchrella* has sex combs also arranged in two separate rows (Takamori et al., 2006).

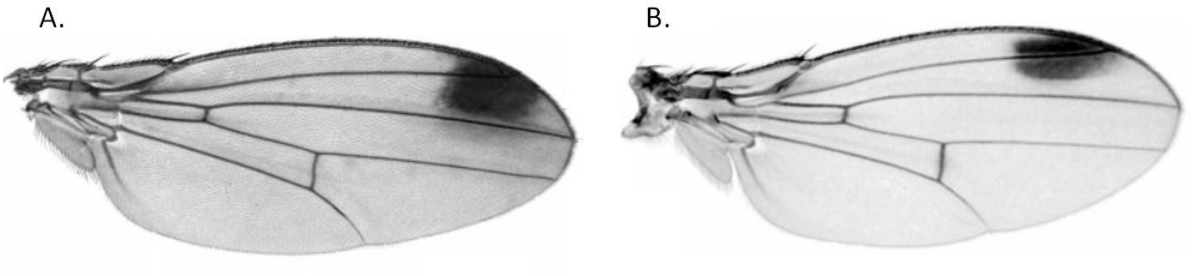

Figure 2: ***A.*** *Male wing with black spot of D. suzukii (Hauser, 2011)* ***B.*** *Male wing with black spot of D. subpulchrella (Takamori et al., 2006)*

In addition, male *D. suzukii* have unbroken brown bands on the top of their abdominal segment (Vlach, 2010). Young males have a not yet fully developed a black spot on their wings. But these spotless males, like the full-grown adults, also have 2 fully developed sex combs on the front feet and have unbroken brown bands on their abdomen (Vlach, 2010). Another characteristic that can give certainty about the species is the examination of the male genetalia in a potassium hydroxide solution (KOH) (Hauser, 2011). This advanced process is very time consuming and will not be done during identifications made by growers.

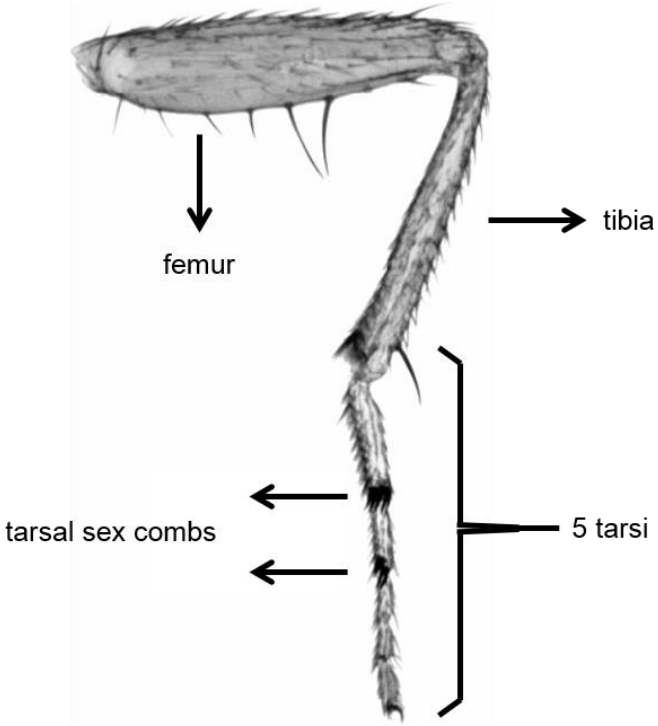

Figure 3: Male D. suzukii front leg with two tarsal sex combs placed on the first and second tarsomere (Hauser, 2011). The sex combs consist out of three to six spikes and the spikes are arranged parallel with the leg (Vlach, 2010).

1.2.2 Female characteristics

Female adults can be determined by their enlarged ovipositor when examined under a microscope. This ovipositor is used by the females to lay eggs in unwounded ripening fruits. The saw-like ovipositor consists of two valvae and has teeth that are much darker than the rest of the ovipositor. These ovipositor valvae contain 30-60 sclerotized teeth which are much stronger and larger than normal teeth that are found on European and North American *Drosophila* species (EPPO, 2013b; Hauser, 2011). These distinct sclerotized teeth are found in the distal half of each valva. Therefore the ovipositor of

D. suzukii is easily distinguishable from other native *Drosophila* species (EPPO, 2013b). For an easier determination of the ovipositor the female flies are placed in ethanol. As a result of the ethanol the ovipositor often extrudes out of the body (Hauser, 2011). Another characteristic that can give certainty about the species is the comparison of the two spermathecae (receptacula seminis) with the ovipositor. A spermathecal is part of the female reproductive tract where it serves as storage for sperm. When the spermathecae are much smaller than the ovipositor (*Figure 4*) in combination with a large ovipositor and its typical sclerotized teeth it can be concluded that the species is *D. suzukii*. The downside for this method is that it is time consuming and the spermathecae are only visible in a KOH solution, similar to the examination of the male genetalia (Hauser, 2011). The method can be useful when there is confusion between *Drosophila immigrans* Sturtevant, 1921, with also a large ovipositor, and *D. suzukii* (*Figure 4*). Not only *D. immigrans* is difficult to distinguish from *D. suzukii* females. *D. biarmipes* and *D. subpulchrella* also have an enlarged ovipositor. However, the shape is different; if the ovipositor is arched or the number of teeth is lower (usually < 25) then it is definitely not *D. suzukii*. *D. biarmipes* and *D. subpulchrella* have not been detected in Europe before 2013 (EPPO, 2013b). Their status is unknown for the last few years (EPPO, 2015).

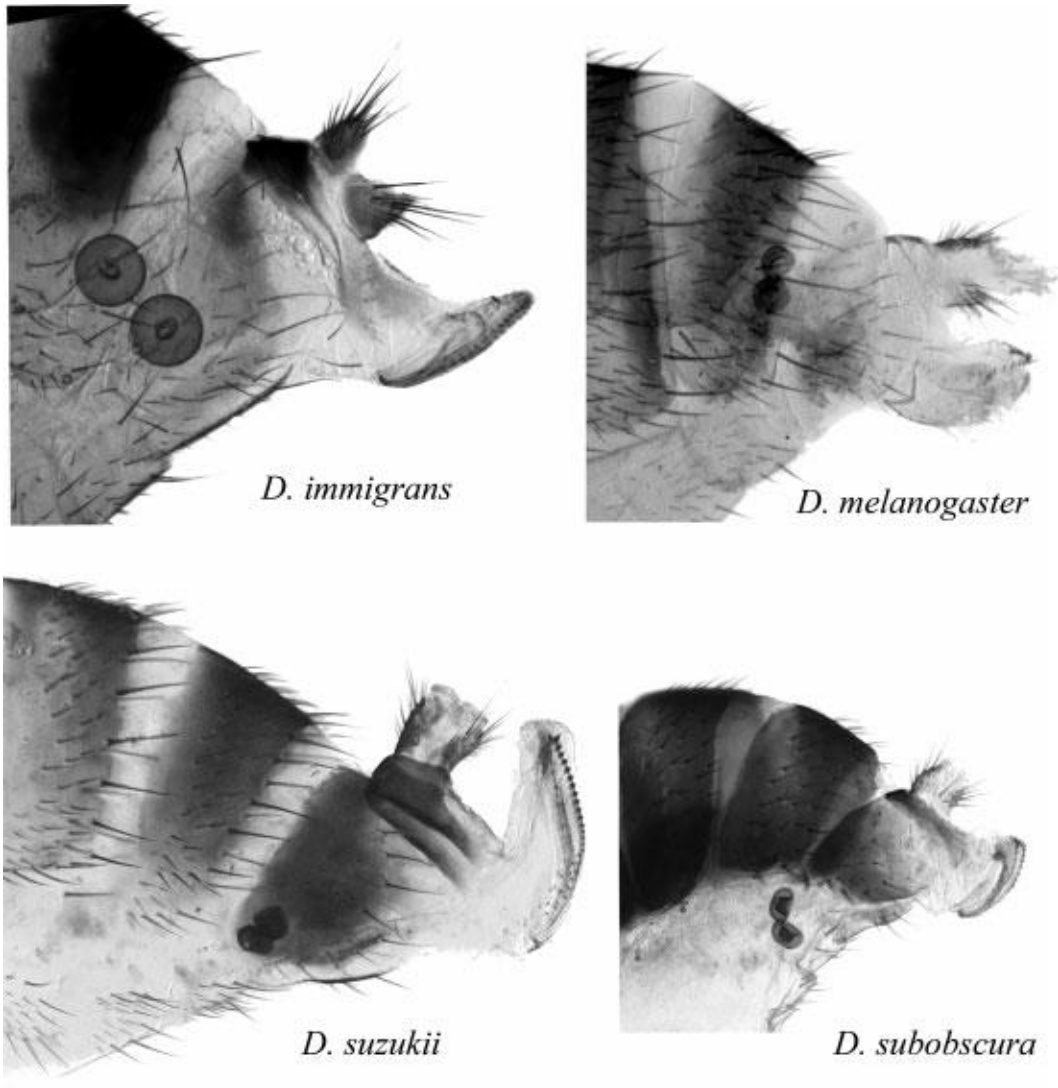

Figure 4: Ovipositor and the two spermathecae for four different Drosophila species. The spermathecae of D. suzukii are small compared to the large ovipositor with sclerotized teeth. Although the ovipositor of D. immigrans has some differences with the ovipositor of D. suzukii the spermathecae can give a better result. The two spermathecae are made visible in a KOH solution. (Hauser, 2011)

Female *D. suzukii* flies also have unbroken brown bands on the abdominal segments (*Figure 1, B.*) with no other stripes, spots or other patterns on the rest of the abdomen. Although, in some cases the last segment can be completely dark brown. Compared to male *D. suzukii* adults, female flies neither have sex combs on their front feet nor do they have a black spot on their wings (Vlach, 2010).

1.3 Life cycle

Female *D. suzukii* lay approximately one to three eggs per oviposition site and 7-16 eggs per day (Calabria et al., 2012). This means that they can lay up to 600 eggs in a lifetime (Cini et al., 2012) if they are able to oviposit up to 51 days (Lin et al., 2014). Nevertheless *D. suzukii* females lay on average around 380 eggs (Calabria et al., 2012). These eggs are laid in ripening fruits, so the larvae can easily feed themselves.

Eggs are milky-white and glossy (Walsh et al., 2011) with two breathing tubes that are visible on the surface of infected fruit (*Figure 5, A. and B.)*. The eggs are approximately 0,6 mm long (Walsh et al., 2011).

Depending on the temperature eggs hatches between 2 and 72 hours (Cini et al., 2012). The larval development occurs inside the fruit, feeding upon the host fruit. The larvae are milky-white with black mouthparts (Walsh et al., 2011). When they emerge they are approximately 0,6 mm in length and after three larval instars (Lin et al., 2014), within a period of 3 to 13 days they become a pupae (Cini et al., 2012). The feeding of the larvae creates soft, sunken and brown areas on the fruit. So the fruit cannot be consumed anymore (*Figure 5, D. and E.*)(Walton et al., 2010).

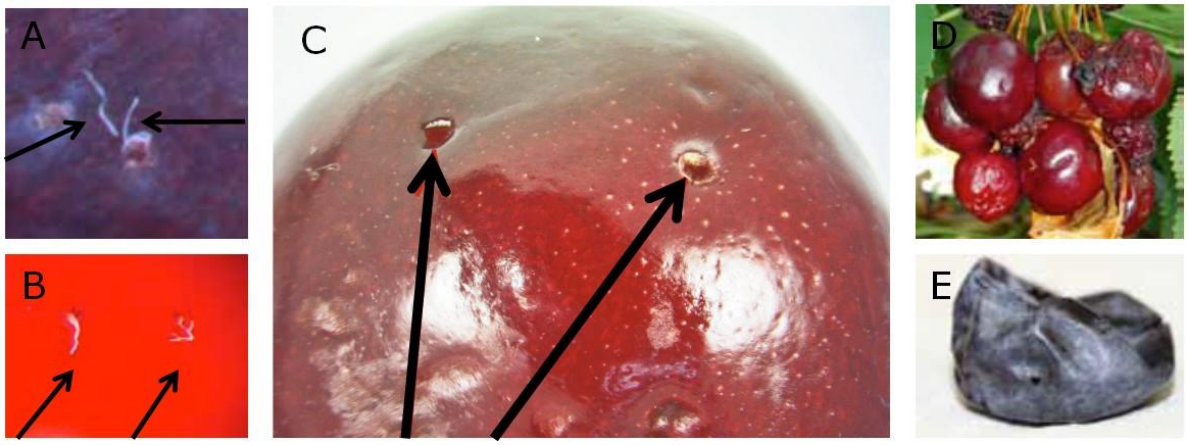

Figure 5: Fruit damage caused by D. suzukii. ***A.*** *Egg with two visible breathing tubes in grape (30x magnification) (Walton et al., 2010).* ***B.*** *Two D. suzukii eggs with visible breathing tubes in cherry (30x magnification). On the right oviposition site there are two berating tubes visible whereas the left oviposition site only one breathing tube is visible (Walton et al., 2010).* ***C.*** *Cherry damage by D. suzukii. Two puncture wounds are visible as oviposition site (Dreves, Walton, & Fisher, 2009).* ***D.*** *Cherry damage with secondary infections more than five days after egg laying (Walton et al., 2010).* ***E.*** *Collapsed blueberry more than five days after egg laying (Walton et al., 2010)*

The pupae vary from brown to yellowish. Pupation occurs for 3 to 15 days, depending on the temperature and can occur inside or outside the host fruit. However outside pupation happens less frequently (Cini et al., 2012).

Depending on the temperature adults emerge after a minimum of 8 days after oviposition (Cini et al., 2012). This can vary between 8 and 33 days (*Figure 6*) (Walsh et al., 2011). According to Cini et al. the adults need 2 days to mature before they are able to lay eggs (Cini et al., 2012); however, according to Kinjo et al. the pre-oviposition period is suggested to be three days (Kinjo, Kunimi, & Nakai, 2014). Because of this short life cycle and extreme fecundity, *D. suzukii* could produce up to 15 generations a year under

optimal climatic conditions (Walsh et al., 2011). Under these optimal conditions the adults have a maximum life expectancy of 53 days according to Cini et al. and Lin et al. (Cini et al., 2012; Lin et al., 2014; Walsh et al., 2011). This is in contradiction with the results found by Emiljanowicz et al. of a mean life expectancy of 86 days with a maximum of 154 days (Emiljanowicz, Ryan, Langille, & Newman, 2014).

The life cycle becomes longer in autumn and before winter, due to temperature decreases (Tochen et al., 2014). *D. suzukii* overwinters as an adult in reproductive diapause to conserve energy (Dalton et al., 2011). This reproductive diapause seems to be affected by winter climatic conditions, the nutritional status and food availability (Zerulla, Schmidt, Streitberger, Zebitz, & Zelger, 2015). Winter forms are recognisable by their darker colour (Goffin & Beliën, 2015) and can live up to 150 days (Goffin & Beliën, 2015). Even though there is knowledge about the existence of winter forms, there is very little known about the feeding sources and shelter during the winter period.

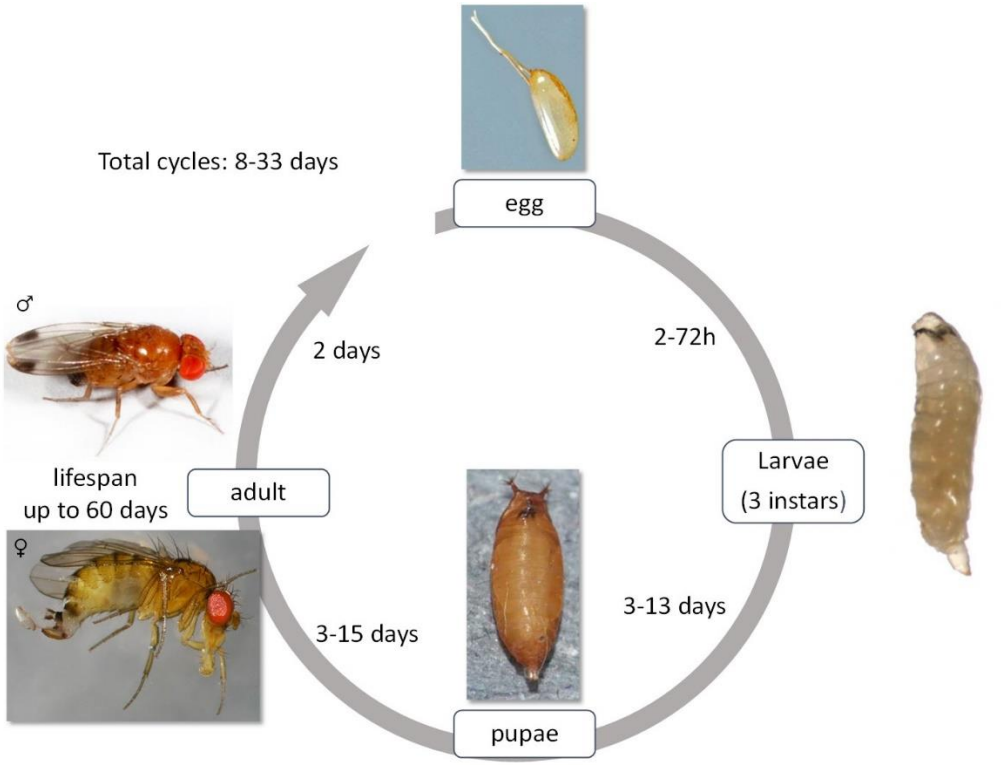

Figure 6: Life cycle of D. suzukii (Cini et al., 2012; Emiljanowicz et al., 2014; Kinjo et al., 2014; Walsh et al., 2011)

1.4 Evolution and origin

Before *D. suzukii* became known in the western world little knowledge about its evolution and origin was available. Certainly in comparison to *D. melanogaster* which is probably been one of the most studied insects all over the world for more than 100 years (Markow & O'Grady, 2006). The research on *D. melanogaster* reached its apogee in 2000 with the publication of the complete genomic sequence (Myers et al., 2000). This was a major breakthrough in evolutionary studies because of an invaluable combination of fast reproduction and a short generation time. In addition, *Drosophila* has ecologically interesting characteristics such as a particular fast adaptability to different environments and is therefore able to occupy a wide variety of niches (Markow & O'Grady, 2006). Therefore *D. melanogaster* was able to expand all over the world starting from Africa approximately 15 000 years ago (David & Capy, 1988). Due to climatic adaptations, in order to survive in different environments, *D. melanogaster* changed genetically resulting in several different genotypes.

Early observations of *D. suzukii* were made by Kanzawa (Cini et al., 2012) in the early nineteen hundreds in Japan and indicate that *D. suzukii* has originated in Southeast Asia. Only very recently it started to spread out all across the whole world. Recent studies by Ometto et al. have genetically confirmed, by comparing different genomic datasets, that *D. suzukii* has indeed its origin in Asia and more specifically around the Himalaya mountains (Ometto et al., 2013). With a relaxed clock analysis, used in the research done by Ometto, it is possible to determine changes in the genetic information of organisms and link these changes to a geological timeline (Drummond, Ho, Phillips, & Rambaut, 2006); especially to ordain the time that two taxa diverged (Thorne, Kishino, & Painter, 1998). Relaxed clock analysis of the *Drosophila* species showed that *D. suzukii* diversified from the other *Drosophilidae*. It particularly differentiated from *D. biarmipes* 7.3 million years ago when there was a monsoon intensification in the region and the Himalayan / Tibetan plateau had an increase in uplift (*Figure 7*). Therefore it can be concluded that *D. suzukii* has adapted to more temperate mountain environments than *D. biarmipes* (Ometto et al., 2013). Monitoring *D. suzukii* on different altitudes in the European Alps have confirmed the hypothesis that *D. suzukii* developed during the uplift op the Himalayan / Tibetan plateau. During the monitoring there were more *D. suzukii* flies found on altitudes above 600 meters above sea level than below (Calabria et al., 2012; Ometto et al., 2013). This is in contradiction with the available food sources in Europe and North America. The majority of the host plants and commercial fruits can be found on much lower altitudes than 600 meters above sea level. Which food source *D. suzukii* used at the place of its origin and how it developed despite a lower presence of food is still unknown.

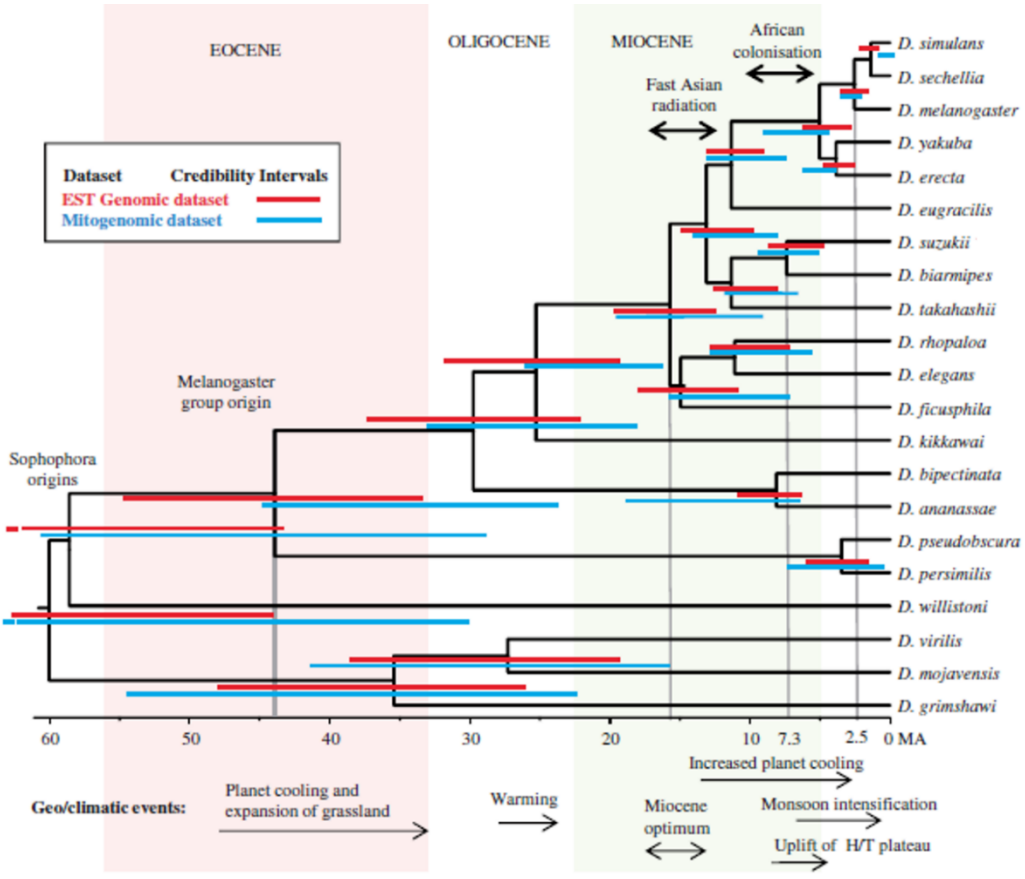

Figure 7: Molecular time tree and paleoclimate with major geoclimatic events. To build the taxonomic tree, two genetic dataset, expressed sequence tags (EST) and mitogenomic datasets, were compared with each other. The (Ometto et al., 2013)

In only a couple of years *D. suzukii* managed to spread from Southeast Asia across Northern America and Europe and to occupy different environments (Cini et al., 2012).

This recent spread across the whole world can be used in evolutionary studies to see how *D. suzukii* genetically adapts to different environments and can give an answer how *D. suzukii* is able to survive in all those different environments. Opposite to other *Drosophilidae*, *D. suzukii* lays eggs in ripening fruits. To achieve this, the females needed morphological adaptations. This resulted in an enlarged serrated ovipositor which is able to puncture the skin of ripening fruit (Walsh et al., 2011). In Europe and Northern America they filled up an empty niche without competitors. Apart from the physical adaptations *D. suzukii* has also physiological and neurological adaptations, so they can find and feed on unripe food sources (Keesey, Knaden, & Hansson, 2015). *D. suzukii* and its close relative *D. biarmipes* and *D. subpulchrella* are less attracted to fermented food resources compared to *D. melanogaster* and more sensitive to volatiles that are associated with the process of ripening fruit (Keesey et al., 2015). *D. suzukii* uses this sensitivity to ripening fruit to find suitable oviposition sites for the development of the larvae (Lee et al., 2011). Nevertheless *D. suzukii* and *D. biarmipes* are also attracted by fermented fruit (Keesey et al., 2015) as a feeding place for adults (Bellamy, Sisterson, & Walse, 2013; Lee et al., 2011).

1.5 Integrated pest management (IPM)

With the arrival of a new pest other management strategies have to be found in order to deal with potentially massive crop losses in soft fruits (Goodhue, Bolda, Farnsworth, Williams, & Zalom, 2011). The *D. suzukii* pest creates a lot of challenges for our current pest management strategies such as IPM, waste management, biological and chemical control, preventing new introduction and infestation, olfactory interferences and others (Cini et al., 2012; Walsh et al., 2011).

1.5.1 Monitoring

Monitoring is essential to assess the damage from a potential pest organism such as *D. suzukii* (Cini et al., 2012). A monitoring setup can be split up in to two components: the trap and the bait or lure.

Traps come in all sorts of colours and shapes. When *D. suzukii* first arrived in Europe and North America traps used to monitor or catch other *Drosophilidae* (Cini et al., 2012) were used. These traps were mainly clear plastic bottles or cups with small or big holes around the top (Beers, Van Steenwyk, Shearer, Coates, & Grant, 2011; Lee et al., 2012) and are currently used in the Belgian national monitoring (Van Delm, Melis, Stoffels, & Baets, 2015). However these kind of traps are not ideally suited for *D. suzukii*, which is mainly attracted to ripeing fruits. Experiments indicated that yellow-coloured and red-coloured traps caught significantly more *D. suzukii* flies than clear white and black traps (Lee et al., 2013). Although the number of trapped *D. suzukii* may be influenced by crop type and stage of crop colouring, it is not yet clear if the colour of the trap needs to match the crop colour or that the trap colour must be different so that there is a visible contrast for the flies (Lee et al., 2013).

Another feature of the trap design is the placement of the holes. There are different options available such as top, side and bottom holes. The top holes are considered less useful than the side holes because they need an additional rain tent in order to prevent the bait solution from diluting (Lee et al., 2012). Side holes are more often used in the design of monitoring traps (Vaccari, Caruso, Nouhaud, & Maistrello, 2015). However a dome trap with a bottom hole seems to have a better chance at catching *D. suzukii* (Landolt, Adams, & Rogg, 2012).

Also the diameter of the holes has an influence on the number of *D. suzukii* caught by the traps. The bigger the hole, the more attracting volatiles are released from the trap

(Cha et al., 2014), but it also enlarges the entry area for other insects. Non-target insects that are trapped, i.e. the bycatch, is preferably very low to reduce determination work after checking the traps.

The right lure is key to attract *D. suzukii.* At the moment there are a lot of different lures used but none of them seems to be specific enough for *D. suzukii*. The most commonly used baits are apple cider (Lee et al., 2013) or fermented food materials. This is in contradiction with the egg laying pattern of *D. suzukii* because they prefer to lay their eggs in ripening fruit in opposition to other *Drosophila* flies that are attracted by fermented and rotten fruit (Cha et al., 2014; Cini et al., 2012). These baits are not able to detect *D. suzukii* in early infection stages and in full season the host plants are more attractive than the traps (Cha et al., 2014).

A more attractive bait than apple cider vinegar is a mixture of wine and vinegar. The mixtures used by Landolt et al. was a 40:60 mixture of the diluted vinegar and the wine (red grape wine) with a 2% acetic acid and 7.2% ethanol content (Landolt et al., 2012). This bait captured larger numbers of *D. suzukii* in field tests in Oregon (US). Simultaneously Landolt et al. (2012) also compared also the mixture of wine and vinegar to a combination of ethanol and acetic acid in order to have a consistent medium that is easy to reproduce. They found that the combination of wine and vinegar is more effective than the combination of ethanol and acetic acid (Landolt et al., 2012). On top the effect of the ethanol and acetic acid seems to amplify each other. Other studies confirmed the attractiveness of wine and vinegar mixtures (Cha, Adams, Rogg, & Landolt, 2012). Further research revealed a five compound mixture of ethanol, acetic acid, acetoin, ethyl lactate and methionol to have almost the same attractiveness as the wine and vinegar mixture (Cha et al., 2014). However, these five compounds worked the best in the research performed by Cha et al. They concluded upon a four compound mixture of ethanol, acetic acid, acetoin and methionol, which can be found in Merlot wine (Cha et al., 2012). This four compound lure is used in the national monitoring of the UK as part of the SF 145 project led by EMR. The lure for national monitoring consists of a water mixture combined with 7.2% ethanol, 1.6% acetic acid and 1% boric acid (to inhibit bacterial growth). To lower the surface tension a 0.01% odourless detergent has been added. Methionol and acetoin are impregnated in cotton plugs and are placed in vials for a controlled release rate of the volatiles (Buss, 2015). This four component lure is a step in the right direction to a high performing attractant with a minimal amount of bycatch. In order to achieve such a lure further research is needs to be performed. Current research is focused on discovering extra volatiles in the field (Keesey et al., 2015), although other options such as pheromones or microbial volatiles can also have potential.

If the right attractant has been found it is only a small step to mass trapping and attract and kill strategies as a control measurement. Mass trapping is already used on a small scale in Switzerland by placing traps around and in the field to keep *D. suzukii* from infecting any fruit (Baroffio et al., 2015) and in combination with chemical control in Italy (Profaizer, Grassi, Zadra, & Maistri, 2015)

1.5.2 Sanitary measurements

In order to give *D. suzukii* no extra chance to lay eggs in fruits it is important to take sanitation measures. Some sanitary measurements are disposal and destroying of waste fruit contaminated with *D. suzukii* and removal of unmarketable fruits in the crop (Buss & Fountain, 2015; Cini et al., 2012; Walsh et al., 2011). Waste fruit cannot be composted because it is still a breeding source for *D. suzukii* (Keesey et al., 2015).

Currently a few possibilities such as solarisation, airtight storing (EMR), burying and fermentation in bioreactor have been examined. At the moment most of the fruit waste of the Hoogstraten area (Belgium) has been collected and used in a bioreactor to minimise possible breeding sources (personal communication, Stoffels K., Proefcentrum Hoogstraten).

1.5.3 Biological management strategies

When *D. suzukii* invades new areas around the world and fills empty niches, the species encounters different environmental conditions compared to its origin in Southeast Asia. Therefore, they were subjected to a strong selective pressure to survive in those new conditions. A key element in the problematic invasion is the absence of natural enemies of *D. suzukii,* also known as the Enemy Release Hypothesis (ERH) (Chabert, Allemand, Poyet, Eslin, & Gibert, 2012). This hypothesis states that natural enemies are important regulators of a population and that prey switching of specialized enemies rarely happens (Chabert et al., 2012). The hypothesis also assumes that parasitoids are more effective against native species than invasive species (Keane & Crawley, 2002), because exotic species can also fill an empty niche in such a way that the available resources are relatively higher than coexisting native species. Therefore they have more chance to survive when they can fill in an empty niche (Blumenthal, 2006).

Certain European parasitoid wasps already have a natural population on the European continent and can affect *D. suzukii. Pachycrepoideus vindemmiae*, Rondani 1875 (Hymenoptera: Pteromalidae) (Marchiori & Barbaresco, 2007) is a promising generalist that lays eggs in the pupae of many tephritid fruit flies and several *Drosophila* species (Wang & Messing, 2004), including *D. suzukii* at the same rate as *D. melanogaster* (57%) *(Chabert et al., 2012)*. Another pupae generalist is *Trichopria drosophilae*, Perkins (Hymenoptera: Diapriidae) (Rossi Stacconi et al., 2013). Both species were able to reduce the population of *D. suzukii* in laboratory tests (Chabert et al., 2012; Rossi Stacconi et al., 2013). Larval parasitoids such as *Asobara tabida,* Förster 1862 (Hymenoptera: Braconidae), *Leptopilina heterotoma,* Thomson 1862 (Hymenoptera: Eucoilidae) and *Leptopilinia boulardi*, Barbotin, Carton & Keiner-Pillault 1979 (Hymenoptera: Eucoilidae) were not as effective as the pupal parasitoids. Because *D. suzukii* has a higher production of constitutive blood cells (hemocyte) compared to *D. melanogaster.* These hemocytes act as an immune response to the eggs that are laid in *D. suzukii* larvae (Kacsoh & Schlenke, 2012). Specialized parasitoids can also shift to another prey, but this often requires a genetic change. The difficulty of prey shifting is illustrated with two different populations of *A. tabida*. The population in Japan is adapted to parasitize *D. suzukii* (Mitsui, Van Achterberg, Nordlander, & Kimura, 2007), whereas the population in France is not yet adapted to parasitize on *D. suzukii (Chabert et al., 2012)*.

A real potential to control *D. suzukii* biologically exists but at the moment tests have only been performed in controlled environments where the conditions for the wasps are ideally and there is no competition. The outcome of these tested species cannot be extrapolated yet to field tests to indicate the effectiveness of these parasitoids outside in a natural environment. In order to obtain more biological control organisms further research is needed.

1.5.4 Chemical management strategies

Because of a zero tolerance towards damaged fruits, growers have changed their pest management programs (Cuthbertson, Collins, Blackburn, Audsley, & Bell, 2014). With the aid of monitoring traps, growers are currently spraying proactively with insecticides that have a broad-spectrum working mechanism. Their goal is to stay ahead of *D. suzukii* and to protect their crops by applying particular insecticides five to eight times or even more often (Van Timmeren & Isaacs, 2013).

In Belgium there three different active ingredients are allowed to manage *D. suzukii*, i.e. spinosad, dimethoate and λ-cyhalothrin (FOD Volksgezondheid, 2016). All three active ingredients are approved for the production of cherries but only spinosad and λ-cyhalothrin are registered in the strawberry production (FOD Volksgezondheid, 2016).

Extensive trials by different groups have confirmed that spinosyns, pyrethroids and organophosphates have a controlling effect on the number of *D. suzukii*. These insecticides provide a decrease of *D. suzukii* by applying a residual layer for 5 to 14 days (Beers et al., 2011; Bruck et al., 2011; Van Timmeren & Isaacs, 2013). This layer of insecticides on the fruits protect the fruit from penetration by *D. suzukii*. In addition Van Timmeren & Isaacs shows that malathion, spinetoram and spinosad all cause a high adult mortality rate. However, even with this high adult mortality, *D. suzukii* still managed to lay eggs in the treated fruits to deliver adult offspring. Of course a lower number of flies was detected in comparison to non-treated fruits (Van Timmeren & Isaacs, 2013). Malathion is not an ideally suited as insecticide because it is sensitive to ultraviolet light causing break down in a minimum of 3 days (Awad, Vinson, & Brazzel, 1967). Therefore malathion proved ineffective as control measure for *D. suzukii (Profaizer et al., 2015)* on the longer term. In addition malathion has been removed from the list of approved insecticides, 91/414/EEG: annex I and is currently not allowed anymore as insecticide (Kyprianou, 2007).

It is also important to notice that some active ingredients such as methomyl and acetamiprid cause a high mortality for the larvae but show a low adult mortality (Van Timmeren & Isaacs, 2013). Therefore it is not easy to decide which insecticide to use for a sufficient adult mortality rate and at the same time minimizing the number of sprayings.

Neonicotinoids shows less mortality of *D. suzukii* adults than other active ingredients with a contact activity (Bruck et al., 2011). They have only a short contact period with the adults. However they could potentially have a long-term effect, due to their systemic working mechanism (Wise et al., 2006).

A factor that mainly influences the period of the effective residue layer of contact insecticides is rainfall. Most contact insecticides are negatively influenced by rainfall because the protecting layer gets easily diluted (Van Timmeren & Isaacs, 2013).

Another challenge related to chemical management are the maximum residue levels (MRL) of the insecticides that are allowed (Bruck et al., 2011). An MRL is the maximum amount of active ingredient that is tolerated to be found on harvested crops. These levels are set by law and are specifically for each country and crop (Haviland & Beers, 2012). MRL values determine final application time of an insecticide that is allowed before harvest and enforces a waiting time (Aktar, Sengupta, Alam, & Chowdhury, 2010). As such, a gap of no protection between the last application and harvest is inevitable (Bruck et al., 2011). In addition ripening fruits are also the most vulnerable for *D. suzukii* (Keesey et al., 2015). For the insecticides allowed in Belgium the current MRL values are 0,5 ppm for λ-cyhalothrin, 0,3 ppm for spinosad and 0,02 ppm for dimethoate. The MRL values for the other insecticides described in Bruck et al. and Van Timmeren & Isaacs are 0,5 ppm for acetamiprid, 0,2 ppm for spinetoram, 0,02 ppm for methomyl and for malathion 0,02 ppm (Homologa, 2015). These numbers are the MRL values for Europe. In other countries outside Europe the levels will generally be higher (Haviland & Beers, 2012). As such, export to other countries outside the European Union is not a problem unless those countries have not established an MRL value for that particular insecticide. Import of fruits into the EU can be problematic due to the lower European MRL values (Haviland & Beers, 2012).

A third problem with chemical management of *D. suzukii* is the resistance against insecticides. *D. suzukii* has a high fecundity with 7 to 15 generations a year (Cini et al., 2012). This allows *D. suzukii* to generate rapid adaptations towards insecticides (Crow, 1957; Oakeshott, Horne, Sutherland, & Russel, 2003). To what extent *D. suzukii* is resistant to insecticides has not been determined yet.

2 OBJECTIVE 1: COMMERCIAL TRAP COMPARISON

2.1 Introduction

The monitoring of *D. suzukii* is key to control this insect, certainly as part of an IPM strategy. It assists growers to know whether *D. suzukii* is present in their crops and when they need to apply control measures. In addition, monitoring can provide more information about feeding patterns, behaviour throughout the year and population dynamics. Therefore it is important to use the best traps available in order to obtain the most information.

The first trap designs and volatiles were based on traps developed for *D. melanogaster* and other *Drosophila* flies. They were clear plastic cups with various sizes of holes and apple cider vinegar as an attractant (Dalton et al., 2011). These straps are still used by the Belgian national monitoring (Van Delm et al., 2015) as part of an ADLO project (Departement Landbouw en Visserij: Afdeling Duurzame Landbouwontwikkeling) and IWT project (project number 135079). However, apple cider vinegar attracts a lot of other insects as well as other *Drosophila* flies. In 2012 Cha et al. found that a combination of wine and vinegar had more potential to attract more *D. suzukii* flies and less bycatch (Cha et al., 2012). Cha et al. (2014) tried to isolate the actual active volatiles of the wine and vinegar combination and decided upon a four component attractant that consists of ethanol, acetic acid, methionol and acetoin (Cha et al., 2014). This attractant gave far better results than the apple cider vinegar but is still not selective enough for *D. suzukii* to use it as a attract and kill setup or masstrapping device. More recent research done by Keesey et al. shifted the search to new volatiles by examining whole strawberry plants. They found that the leafs of strawberry also emits volatiles, such as β-cyclocitral, that have potential to attract *D. suzukii* (Keesey et al., 2015). In order to find more selective volatiles more research is needed. Possibly the attractants are related to different crop stages (Revadi et al., 2015) or to different crops (Cini et al., 2012).

A plethora of different attractants is currently available, making the decision process for growers to select optimal commercially available traps for *D. suzukii* a challenge. Therefore a field comparison was performed with ten different trap, lure and drowning solution combinations in order to give growers advice about the most optimal trap for monitoring *D. suzukii*. Information from this field trial can also be very valuable to develop attract and kill or masstraping setups as control measures (Hammack, 2003). The development of these two control measures as well as the monitoring traps is still ongoing due to the nonspecific attractants. At the moment ongoing research is trying to determine which volatiles are responsible for attracting female *D. suzukii* flies to ripening soft and stone fruit (Cini et al., 2012; Keesey et al., 2015; Revadi et al., 2015).

2.2 Materials and methods

2.2.1 Traps

Four designs of traps were selected to accommodate the lures and baits. The first trap, i.e. New Droso Trap (Biobest NV., Ilse Velden 18, B-2260 Westerlo, BE) (the new variant of their old Droso Trap) has a round bottom and three groups of seven holes in the side. These holes have a bar in the middle that reduces the entry opening intended to reduce the bycatch. The colour is red and the trap can hold up to 300 ml of drowning solution which is indicated by a horizontal line on the inside of the trap. According to the technical sheet of Biobest they recommend, to put two to four traps per hectare for monitoring purposes. If the goal is a perimeter trapping as a preventive method Biobest recommends of 80 to 100 traps per hectare. These New Droso Traps cost £6.00 (€7.50) per trap (*Figure 8, A.*).

The New Pherocon SWD trap (Trécé Inc, 7569 Highway 28 West Adair, Oklahoma 74330, US) consist of a clear plastic cup with two holes in the side covered with a red mesh to minimise bycatch. Compared to the older version of the trap the mesh is now red instead of white. Trécé recommends to place one trap per ten acres (4.05 ha) in cherry, peaches and plums orchards. In blueberries and strawberries they recommend to use six traps per 4.05 ha. Each trap costs $5.80 (€5.00). (*Figure 8, B.*)

The Scentry SWD trap (Scentry Biologicals Inc., 610 Central Avenue Billings, Montana 59102, US) is made out of a clear plastic cup, covered with a red strip, with three black entry points which consist out of eight smaller holes. There was no information available about the placement of the traps. The trap costs $8.30 (€7.30). (*Figure 8, C.*)

The fourth selected trap is coded in order to comply to the confidentially agreement with EMR and the manufacturer of the trap. The trap will further be referred to as EMR Coded.

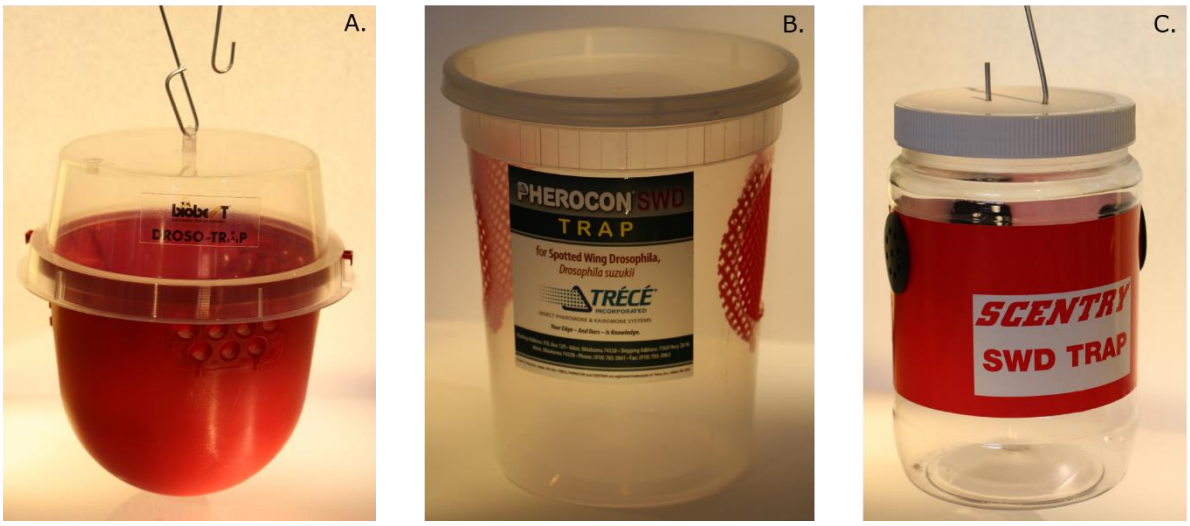

Figure 8: Three of the four different traps used in this experiment. ***A.*** *New Droso Trap (Biobest NV.)* ***B.*** *Pherocon SWD Trap (Trécé Inc.)* ***C.*** *Scentry SWD Trap (Scentry Biologicals Inc.)*

2.2.2 Lures and drowning solutions

Although lures and drowning solutions can both attract *D. suzukii,* they are not essentially the same. The main goal of a lure is to attract *D. suzukii* were as the main goal of a drowning solution, also called bait, is to capture the insects that fly into the traps.

In this trial eight lures and three drowning solutions were selected (*Table 1*). The first selected lure was the Scentry lure (Scentry Biologicals Inc., 610 Central Avenue Billings, Montana 59102, US). The lure consists of a clear gel package that was suspended from the top of the trap. The lure was used in combination with a water and detergent drowning solution (400 ml) and a Scentry SWD trap. The Scentry lure costs $6.60 (€5.80) for one lure and has a field life between four and six weeks according to the manufacturer.

Table 1 Different combinations of traps lures and drowning solutions for D. suzukii that were used in the field trial. The main trap used is the New Droso Trap of Biobest. Some lures (i.e. Pherocon SWD lure and NRI Dry Lure) are twice used in combination with different drowning solutions. The detergent used in all combinations with water & detergent is a soap without perfume to decrease the surface tension of the water.

Treatment number	Trap	Lure	Drowning solution
1	Scentry SWD trap	Scentry lure	water & detergent
2	New Pherocon SWD trap	Pherocon SWD lure	apple cider vinegar
3	New Droso Trap	Koppert	Koppert
4	New Droso Trap	Pherocon SWD lure	apple cider vinegar
5	New Droso Trap	Russell IPM solid bait	water & detergent
6	New Droso Trap	NRI Dry Lure	water & detergent
7	New Droso Trap	NRI Dry Lure	diatomaceous earth
8	New Droso Trap	Dros' Attract	Dros' Attract
9	New Droso Trap	Super Gasser	Super Gasser
10	EMR Coded	EMR Coded	water & detergent

The Pherocon SWD lure (Trécé Inc, 7569 Highway 28 West Adair, Oklahoma 74330, US) is the high specificity lure of Trécé with three components. The lure, supplied as a strip, has a plastic cover that had to be removed before placement. According to the manufacturer the Pherocon SWD lure lasts six to eight weeks and can be used in combination with or without a natural bait. To improve the attractiveness of the lure for *D. suzukii* a combination with an apple cider vinegar was preferred (Biona Organic Cider Vinegar with the mother unpasteurised, unfiltered and oak matured, Biona, Cliftonroad 34a, Kinston KT2 6PH, GB). The Pherocon SWD lure was placed in two different traps, i.e. the New Pherocon SWD trap and the New Droso Trap. The holes in the lid of the New Pherocon Trap were not big enough to support the Pherocon lure strip and had to be enlarged. In the New Droso Trap a hole was made in the lid in order to hang the lure strip from the top. The Pherocon SWD lure costs $3.65 (€3.20) per lure.

The Russell IPM solid bait (Russell IPM Ltd., Unit 45 First Avenue, Deeside, Flintshire, CH5 2NU, GB) is a black sachet that had to be double folded in order to fit in the New Droso Trap preventing contact with the drowning solution. Similar to the previous lure the New Droso Trap had to be modified in order to accommodate the Russell IPM solid bait in the trap.

The NRI Dry Lure (National Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, GB) is an improved version of the Cha and Landolt (Cha et al., 2014) four component lure developed by the National Resources Institute, University of Greenwich (NRI). The four components are methionol, acetoin, ethanol and acetic acid and are already been used in the national monitoring of *D. suzukii* in the UK. Methionol and acetoin are impregnated in cotton buds and placed in vials in the trap. The two other components are dissolved in water with a concentration of 7.2% ethanol and 1.6% acetic acid and make up the drowning solution which is further complemented with 1% boric acid and 0.01% detergent. These last two components do not add any attractiveness to the trap but the detergent reduces the surface tension of the drowning solution and the boric acid works as an anti-microbial agent. NRI improved this trap by putting all four components in cotton buds, placed in sachets. These sachets are developed by NRI with a controlled release rate of the components. Because there is no liquid needed to accommodate all four components the lure received the name of dry lure by NRI. The NRI Dry Lure was placed in a New Droso Trap and suspended from the top of the lid in a similar way as the previous lures. Two drowning solutions were selected

to use with the NRI Dry Lure and New Droso Trap. The first drowning solution is just a water and detergent solution whereas the second drowning solution is diatomaceous earth (Agralan Ant & Flea powder puffer, Agralan Ltd., The Old Brichyard, Ashton Keynes, Swindon, Wilts, SN66QR, GB), which works as an insecticide to catch the insects. This combinations does not contain any liquid. Because the NRI Dry lure is in development, no price is yet available for this lure.

The next three lures also serves as a drowning solution i.e. Dros'Attract ® New Formula, Super Gasser and Fruit Fly Attractant. Dros' Attract ® New Formula (Biobest NV., Ilse Velden 18, B-2260 Westerlo, BE) is the lure that Biobest recommends their traps to be used with and costs £22 (€28) per five litres Dros' Attract ® New Formula. The Dros' Attract ® New Formula is based on fermented wine and is a red solution. According to the technical sheet of Dros' Attract ® New Formula a sugar cube was added to each trap and the solution was replaced after two weeks. The Super Gasser solution (Ten litres attractant for *Drosophila suzukii*, Super Gasser, Riga, Schützenhausstrasse 7, Ellikon an der Thur 8548, CH) is according to Riga derived from precision monitoring traps but is not yet available on the market at this moment. The last lure is made by Koppert (Fruit Fly Attractant, Koppert B.V., Veilingweg 14, 2651 BE Berkel en Rodenrijs, NL). It is a clear, yellow solution. According to Koppert there are ten to 20 traps per hectare needed for monitoring purposes and between 75 and 100 per hectare to catch *D. suzukii* as a preventive measure, or even 200 traps when damaged fruit is detected. The Fruit Fly Attractant is already on the market but the price of a five litre container is unknown.

2.2.3 Assessments

The assessments of the *D. suzukii* trap catches were done over a period of three weeks from 28th of July until 19th of August 2015.

The traps were checked weekly for insect catches at the same day of the week. Therefore each week the drowning solution was collected each week from the traps and poured over a filter in order to collect all caught insects. Afterwards the drowning solutions were poured back in the traps. Due to the recommendations of the technical sheet only Dros' Attract was replaced after two weeks. After collecting the filtered trap catches they were counted with a microscope in order to divide them into four categories: male and female *D. suzukii* flies, other drosophila flies and insects bigger than five millimetres. The last two categories are further on referred to as bycatch.

2.2.4 Experimental layout

This experiment to test different trap combinations was conducted in a cherry orchard at East Malling Research (EMR) (New Road, East Malling, Kent, ME19 6BJ, United Kingdom). The cherry orchard is labelled as Rookery Field (RF 181) by EMR. In Rookery Field two cherry varieties (Penny and Sweetheart) are combined into one orchard. Each other row of the orchard contains only the variety Penny whereas the other rows consist out of alternating the Penny and Sweetheart varieties. The distance between the trees, inside a row, measures two meters and each row is placed four meters apart. Rookery field has a North-northeast by South-southwest orientation of the rows with a slight slope towards the South of the orchard. The South and West side of the orchard are protected by a hedgerow to create a micro climate. In the North of the orchard there are some houses with private gardens. At the East of the orchard the EMR gene bank of different cherry varieties, used in breeding, is located (*Figure 9*). The gene bank receives a minimal amount of chemical control during the year and the cherries are never harvested. The private gardens and gene bank could be possible sources of *D. suzukii*. In order to increase the natural population without developing any resistance towards insecticides, Rookery Field was last sprayed with λ-cyhalothrin at the beginning of May.

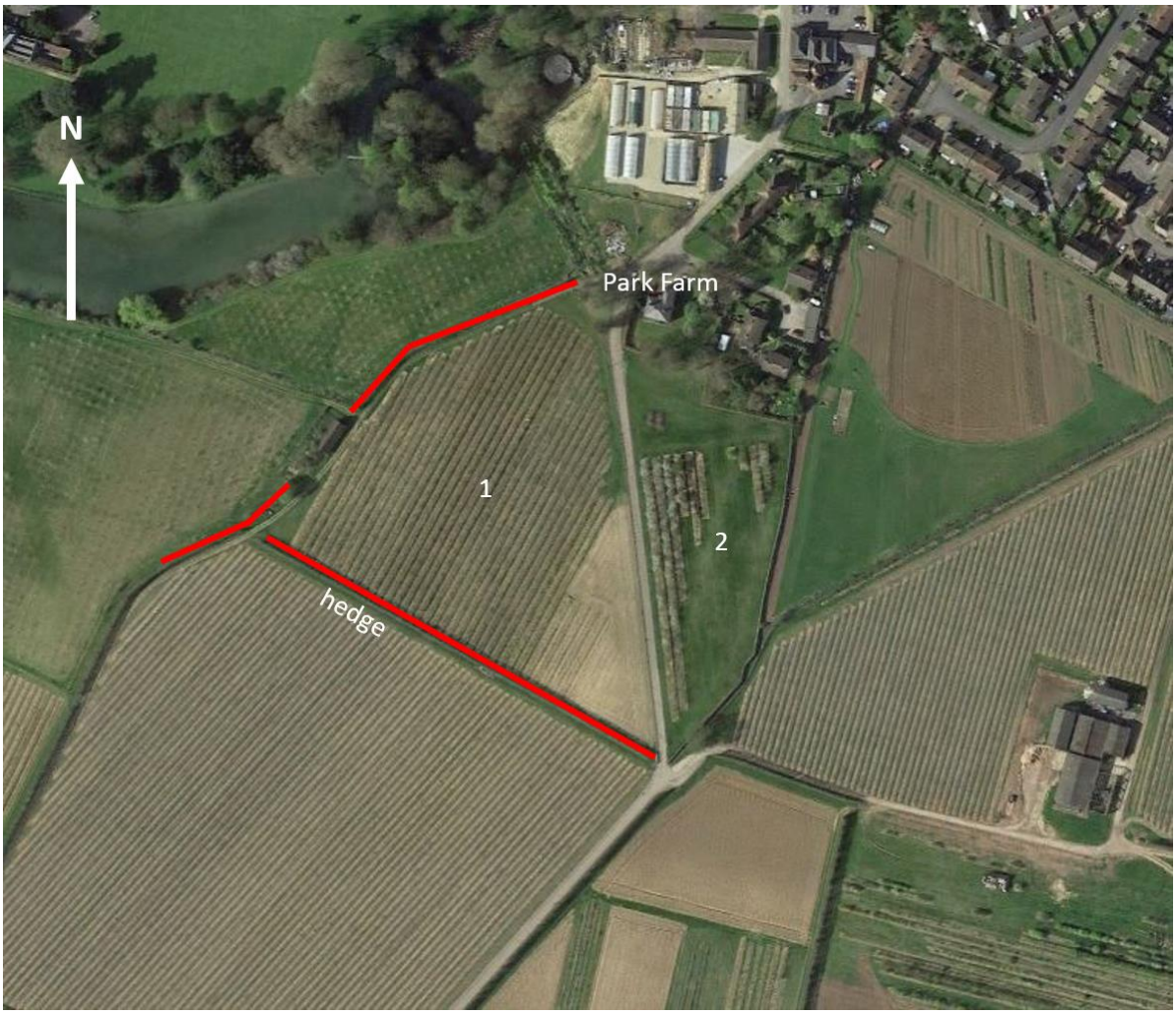

Figure 9: Satellite image of Rookery Field (Google Earth, picture taken on 20/04/2015, 51°17'34.06" N and 0°26'46.33" E). The red lines represent the hedges adjoining Rookery Field. Number one on the map is Rookery Field and number two is the cherry gene bank used for breeding new varieties.

As shown in *Figure 10* the area around East Malling Research and the Rookery Field orchard has a freely draining, slightly acid but base rich loamy soil. In the North of East Malling there are limestone rock formations which are part of the same rock formation (the dark brown and yellow colour) as the white cliffs of Dover. Between East Malling and the limestone rock formation there is a small depression. This area is connected to the river Medway and has a higher water table than the EMR orchards.

The traps were laid out in a block system where each block contained all ten, completely randomised, trap combinations. There were six blocks which meant that all ten trap combinations were six times replicated throughout the orchard.

The traps were placed at least one tree away from the edge of the orchard. Between each trap, placed in a single row, there were five trees placed. Therefore the traps were in the row 12 meters apart. Side to side there was a guard row placed in between the rows were the traps were positioned. The traps were alternating placed in such a way that each trap has at least a circle of ten meters were no other traps were placed (*Appendix, Objective 1: Commercial Trap Comparison, Figure 28*). This was done to minimize the influence of the different traps. All traps were suspended from the cherry trees in the orchard on an approximate height of one meter above the ground.

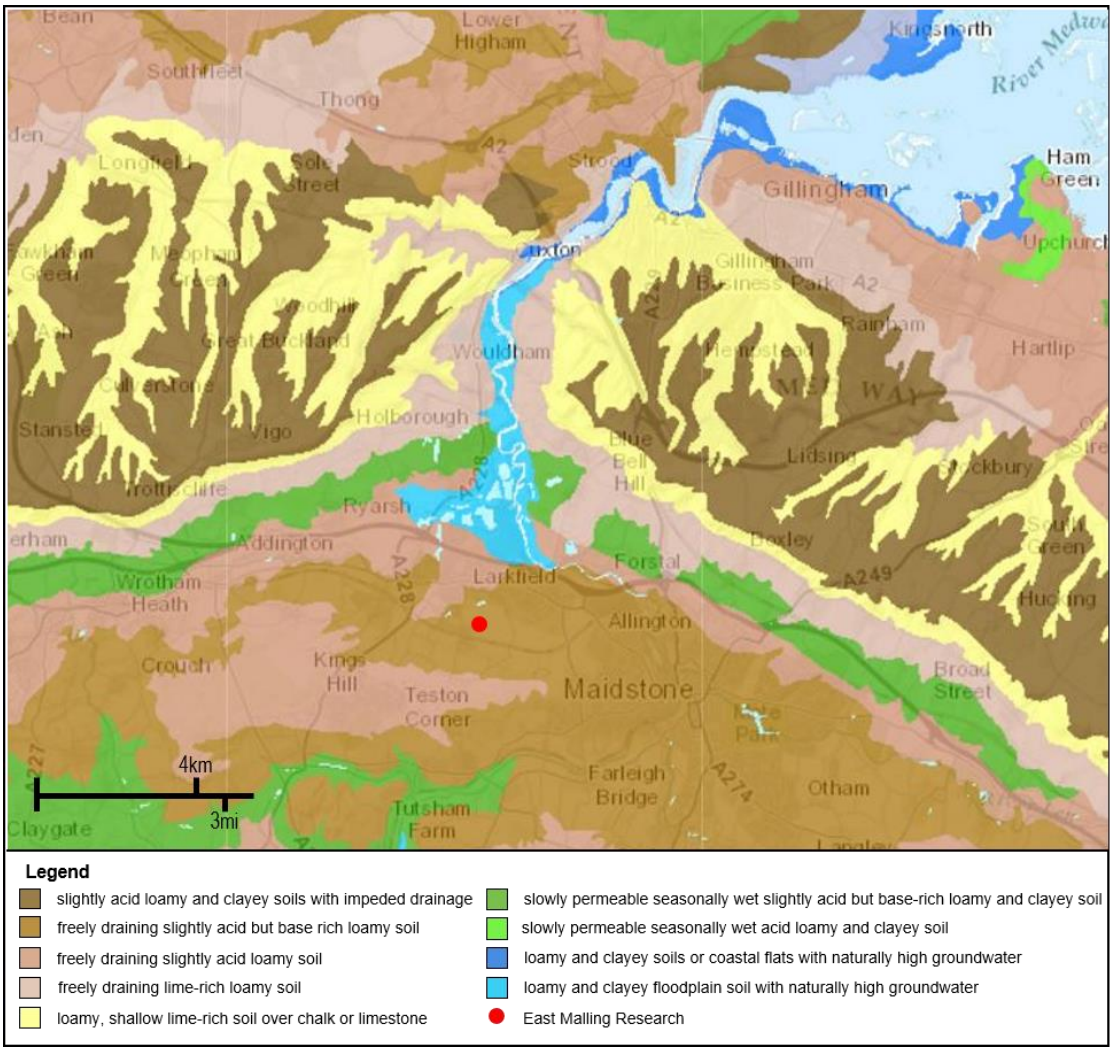

Figure 10: The upper 30 centimetre soil around East Malling Research (Cranfield University & Department for Environment Food & Rural Affairs (DEFRA))

The choice of the right traps depends of different variables. In order to guide growers in their trap combination choice the trap combinations were given a score to scale their ease of handling and installation. There was also a price comparison done between the different trap combinations. The last factor that was scored is the amount of *D. suzukii* flies caught compared to the amount of bycatch, which is preferably low in order to make determination of the traps more easy.

2.2.5 Statistics

The results of the total *D. suzukii* catches and the ratio with the bycatches were not normally distributed. In order to transform the data a Box-Cox power transformation was tried. Because the Box-Cox power transformation gave a lambda value of one, the date could not been transformed to get it normally distributed. Therefore a general linear hypotheses and multiple comparisons for parametric models combined with a Poisson distribution and a Tukey test were used to analyse the data. All statiscs were done using R version 3.2.1 "World-Famous Astronaut" (R Core Team, 2015) and package "multcomp" (Hothorn, Bretz, & Westfall, 2008).

2.3 Results

In order to give advice to growers, four different factors of the traps were taken into account. The ease of handling and installing the traps, the price, the total amount of *D. suzukii* flies caught and the ratio of the bycatch compared to the *D. suzukii* catches.

2.3.1 Ease of handling and installing of the traps

The first factor, the ease of handling and installing the traps, was scored with a number between zero and 15, with 15 the best score. The overall score was calculated by taking the sum of three categories, each scored with a number between zero and five, with five the best score. The first category scored the initial placing of the traps in the orchard with ropes or hooks. The second category gave a score to the ease of use in order to refill the traps and to replace the drowning solutions. This was done each week to check the traps for insect catches. The last category scored the modifications that had to be made before the traps could be deployed in the field, with a score of five representing no changes. (*Table 2*)

Table 2: Overview of handling scores for each trap. The total score, between zero and 15, was made by taking the sum of three categories, placement, refilling and modifications of the traps. Each category received a score between zero and five with five the highest score.

	Combination name	Placing	Refilling	Modifications	**Total**
1	Scentry	5	5	5	**15**
2	Pherocon	3	1	3	**7**
3	DT + Koppert	5	3	2	**10**
4	DT + Pherocon	5	3	2	**10**
5	DT + Russell	5	3	1	**9**
6	DT + Dry lure + water	5	3	2	**10**
7	DT + Dry lure + Diatomaceous	5	0	2	**7**
8	DT + Dros'Attract	5	3	2	**10**
9	DT + Super Gasser	5	3	2	**10**
10	EMR Coded	4	5	4	**13**

The initial placing of the traps went well for most of the traps. Only the Pherocon trap had to be attached with a rope to the branches of the cherry trees, therefore it received a three as score. All the other traps had hooks that could easily be placed over a branch without any problem.

The Pherocon trap, with the reservoir, was attached with a rope to the tree. Therefore it was difficult to replace the drowning solution. All the other traps were only attached to the tree with the lid and the reservoir could be screwed down. This made it easy to check the traps and replace the drowning solutions. However the New Droso Trap has a screw down reservoir, the bottom of this trap is rounded and could not be placed on the ground in order to replace the drowning solution. In addition to the round bottom leeks around the holes made pouring out the liquid more difficult. Therefore the New Droso Trap received a score of three for ease of refilling. The worst trap to refill was the Droso trap in combination with the diatomaceous earth (combination seven). Because the diatomaceous earth is a fine powder that can have an irritating effect in case of inhalation, skin contact or eye contact, gloves and safety glasses were required to replace the diatomaceous earth. The diatomaceous earth also clothed when there was any moisture in the Droso Trap, which made it hard to determine which insects were caught in the trap. Another disadvantage of the diatomaceous earth is related to its mode of action as an insecticide. The diatomaceous earth absorbs lipids from the exoskeleton of insects (Athanassiou et al., 2005; Korunic, 1998) drying them completely out and making identification of the female *D. suzukii* flies difficult.

Almost all traps needed some modifications in order to receive their lure. Because each trap is designed with a certain lure and drowning solution in mind the score only considered the work that had to be done as intended by the manufacturer. For example the New Droso Trap of Biobest is designed to be used with Dros' Attract and has therefore no holes or hooks in the lid to accommodate any hanging, solid lures. Consequently the extra holes that were needed in this experiment to place the solid lures in the Droso Trap were not considered in the score. On the other hand, the New Droso Traps came in boxes without the inserts of the three times seven holes. These inserts needed to be placed into the New Droso Traps, which made it a labour intensive work. The inserts were also very cumbersome to get into the slots. Therefore the New Droso Trap did not score well in the category modification. The combination with the Russell IPM solid bait lure was even worse because the sachet of the lure was too big to fit in the New Droso Trap. In order to fit the lure inside the trap, the lure needed to be folded up and taped together to keep the lure dry. The Pherocon trap had slots in the top to hang the Pherocon lure, but the slots were not big enough to accommodate the Pherocon lure and needed to be enlarged. The handle had to be attached to the Pherocon trap, which made the trap not ideal to deploy it quickly.

The Scentry trap (trap combination one) scored the best across all categories due to the easy screw one lid and integrated hook to hang the Scentry lure at the top.

2.3.2 Price comparison

D. suzukii is a relative new pest organism in Western Europe which means that extra costs are needed in order to control this organism. Due to the current economic situation the cost price of new measurements could be a limiting factor for growers to choose a trap and lure combination. All available prices are listed in below in *Table 3.*

Table 3: Comparison of the total costs per combination. All prices are estimations and may vary due to import costs, different retailers or bulk purchases. If the trap is not yet on the market, it has been marked with "NA". The products that are available with unknown prices are marked with "??". The drowning solutions that act also as a lure are only counted as a lure.

	Combination name	Trap (£)	Lure (£)	Drowning solution (£)	**Total (£)**	**Total (€)**
1	Scentry	5.77	4.60	0.00	10.36	13.20
2	Pherocon	4.00	2.55	0.76	7.34	9.35
3	DT + Koppert	6.00	??	/	??	??
4	DT + Pherocon	6.00	2.55	0.76	9.31	11.80
5	DT + Russell	6.00	??	0.00	??	??
6	DT + Dry lure + water	6.00	NA	0.00	NA	NA
7	DT + Dry lure + Diatomaceous	6.00	NA	1.70	NA	NA
8	DT + Dros'Attract	6.00	1.32	/	7.32	9.30
9	DT + Super Gasser	6.00	NA	/	NA	NA
10	EMR Coded	NA	NA	NA	NA	NA

The prices in *Table 3* are only estimations and may vary due to different retailers, bulk purchases and import costs of United States based lures. The prices of the Fruit Fly attractant by Koppert and the Russell IPM solid bait lures were not available, although they are for sale, and are marked with "??". The prices that are marked with "NA" were the prices that were not available because the lure or trap was not yet available on the market. To express total costs in euro there was an estimation made with the help of the exchange course of 1.2686 euros to one pound on 30 March 2016.

The total costs for each trap were based on the initial cost of the trap, the lure and the drowning solution, taking possible replacement into account. The total costs do not include the life time of the drowning solution or lure because the manufactures do not

give a fixed replacement time. Therefore this cost depends upon the time interval of replacing the drowning solution, chosen by the grower.

2.3.3 Total amount of *D. suzukii* and bycatch ratio

To compare the trap catches, the total number of *D. suzukii* flies caught and the ratio of *D. suzukii* and bycatch are the most import parameters to consider. During the weekly assessments the traps were checked for male and female *D. suzukii* flies, other drosophila species and insects bigger than five millimetres.

In the charts below the mean amount of caught *D. suzukii* flies is visualized for the different combinations of traps and lures. The values were statistically compared by a Tukey test with $P<0.05$.

The overall trap catches of adult *D. suzukii* flies are shown in *Figure 11.* Values represent means of the three assessments during the three weeks of the experiment. Trap combination nine shows the highest trap catches of *D. suzukii* followed by trap combination three. On the other hand, four trap combinations showed less attractiveness to *D. suzukii*. These trap combinations are combination two, combination four, combination seven and especially combination ten with very low trap catches. All other trap combinations did not show any significant differences with the best and least attractive trap combinations.

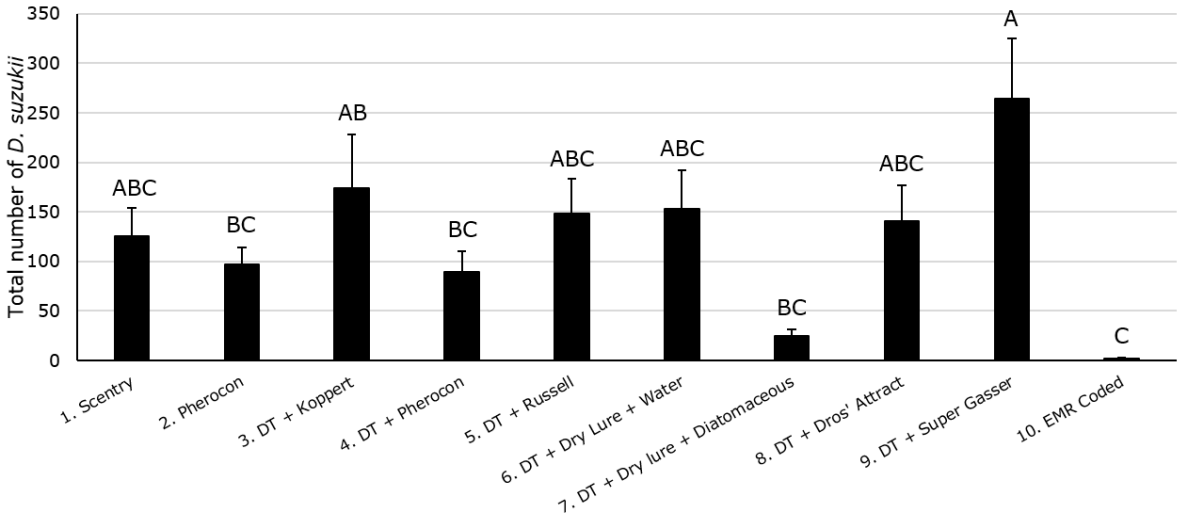

Figure 11: The mean of the total number of D. suzukii flies caught by each trap combination across all three weeks. The different combinations are divided in different groups if they are significantly different from each other (P<0.05). Different groups are represented by the different letters.

The amount of bycatch also determinates the efficacy of the trap for *D. suzukii*. A minimal bycatch is preferred in order to make the monitoring for *D. suzukii* easier and reduces the entrapment of beneficial insects. In *Figure 12* the overall bycatch compared to the *D. suzukii* catches for the entire experiment is shown. The ratio is calculated by dividing the total number of *D. suzukii* flies caught in the traps with the bycatch, which consist out of the sum of the other drosophila species and the insects bigger than five millimetres. This means that a high ratio represents a low bycatch compared to the caught *D. suzukii* flies.

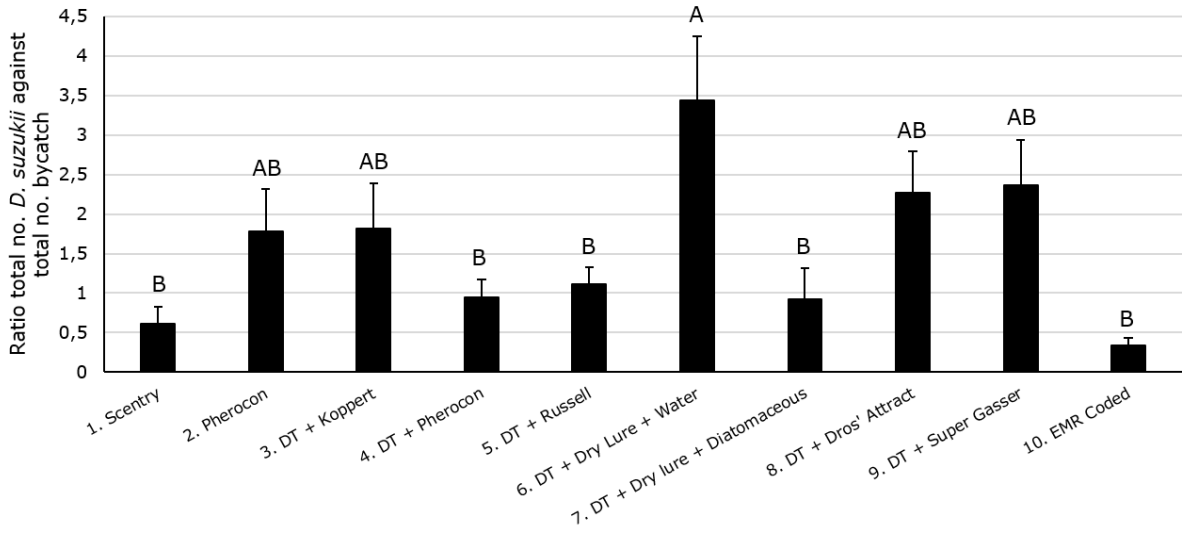

Figure 12: The mean ratio of total number of D. suzukii flies divided by the total number of bycatch caught by each trap combination across all three weeks. A higher ratio means less bycatch compared to the D. suzukii catch. The different combinations are divided in different groups if they are significantly different from each other (P<0.05). Different groups are represented by the different letters.

The combination with the Droso Trap, the NRI Dry Lure and the water as drowning solution (trap combination six) had the best overall ratio (*Figure 12*). The Scentry combination, the Droso Trap with the Pherocon lure, The Droso Trap with the Russell IPM solid bait, the Droso Trap combined with the NRI dry lure and diatomaceous earth and the EMR coded had the lowest ratio which means a relative high bycatch compared to the *D. suzukii* catches. These trap combinations are less selective to *D. suzukii* than the other trap combinations.

Figure 11 and *Figure 12* show the overall ratio and total trap catches for the whole period of the experiment. In order to compare each week separately, each trap combination was statistically compared in the same way as described above for the three weeks combined (*Appendix, Objective 1: Commercial Trap Comparison*). The comparison between the three weeks separately made it possible to distinguish any difference of the attractants throughout the experiment.

Figure 29, *Figure 30* and *Figure 31* (*Appendix, Objective 1: Commercial Trap Comparison*) show the total number of *D. suzukii* caught for each week. The same trend as previous described in *Figure 11* can be seen in these figures. Trap combination nine, the Droso Trap combined with Super Gasser, has the highest number of *D. suzukii caught* of all trap combinations and combination 10, EMR Coded, showed the lowest numbers. In week one there was a distinct variation between the traps but by week three the same result as in *Figure 11* could be seen for the different trap combinations.

Figure 32, *Figure 33* and *Figure 34* (*Appendix, Objective 1: Commercial Trap Comparison*) show the ratio of bycatch for each week. In week one (*Figure 32*) of the experiment trap combination six (Droso Trap and NRI Dry lure combined with water and detergent) showed the highest ratio of all trap combinations. All other trap combinations were not significantly different from each other. By week two (*Figure 33*) the difference between the different trap combinations became less obvious. Although trap combination six remained the combination with the highest ratio together with trap combination nine (Droso Trap and Super Gasser), they were not significantly different from the combinations two, three, five and eight. This trend remained the same until week three of the experiment. The trap combinations one (Scentry), seven (Droso Trap, NRI Dry lure with diatomaceous earth) and ten (EMR Coded) remained the traps with the lowest ratio in week two and three which is also shown in the overview (*Figure 12*).

2.4 Discussion

The main goal of this trial was to advise growers which traps would be the best to use for *D. suzukii* monitoring. In order to answer this question four different factors were taken into account: the ratio of bycatch, the total number of *D. suzukii* caught, the price and the ease of handling and placement of the traps.

Because the cherries were not harvested at Rookery Field, the traps were subjected to competition of the cherries that were still hanging from the trees. Therefore it could be a valuable trial to see how the different traps perform in field conditions.

A first analysis of all the results across the three weeks showed that the combination of the Droso Trap and Super Gasser together with the Droso Trap and the Koppert solution gave the best results in catching the most *D. suzukii* flies across all three weeks. However the Droso Trap in combination with the NRI Dry lure and water gave the best results with the lowest bycatch. The two traps with the least *D. suzukii* caught and the highest bycatch compared to their *D. suzukii* catch were the Droso Trap with the NRI Dry lure and the diatomaceous earth as a drowning solution and the EMR Coded trap.

The NRI Dry lure was used in two setups with the same trap, the Droso Trap, but with a different drowning solution. The setup in combination with water and detergent performed well both for total *D. suzukii* counts as well as for the ratio against the bycatch. Whereas the combination with the diatomaceous earth had a very low total number of caught *D. suzukii* flies and a high bycatch compared to the caught *D. suzukii* flies. This bycatch was mainly due to the high number of earwigs that were caught in the trap, very little flying insects were found in the diatomaceous earth. The flies were probably not immediately caught by the diatomaceous earth and could escape out of the trap. On top of the poor *D. suzukii* catch, the diatomaceous earth was difficult to refill and made it difficult to determine the catches.

The Pherocon lure was placed in two different trap setups, i.e. New Droso trap and Pherocon trap. Due to the modifications that had to be made to the Pherocon trap it took a lot of time to setup and replace the liquid. Although it was easy to see through the clear cup if there were any insects caught in the trap. However this advantage did not outweigh the time loss to set the trap up. Both traps had around the same *D. suzukii* caught however the Pherocon trap had a little bit less bycatch than the New Droso trap. This could be explained by slightly larger entry holes of the New Droso trap.

The setup with the Scentry trap was the most expensive setup. It performed average on the *D. suzukii* catch but it did have a high number of bycatch. However the trap was easy to use and setup.

Throughout the three weeks the setup with Super Gasser had immediately a positive effect on the total number of *D. suzukii* caught. This effect was not observed with the other trap setups. They needed a week longer to attract more *D. suzukii* flies. It could be that the plastic strips or sachets with the lures prevent the volatiles from dispersing as fast as Super Gasser (Ashley, 1985). However the slower release of volatiles could increase the lifetime of the lure. The exact life time of the lures compared to the manufacturer recommendations has to be further tested.

According to this trial the setup with Super Gasser and the NRI Dry lure, both with the New Droso Trap performed the best for the total amount of *D. suzukii* caught as well as the reduced number of bycatch. However, both attractants are not yet commercially available therefore the Koppert solution, or Dros' Attract, both with the New Droso trap can be recommended to growers awaiting the release of the other two attractants. The two trap combinations that performed the least across all characteristics were the EMR Coded trap and the NRI Dry lure combined with diatomaceous earth.

3 OBJECTIVE 2: INSECTICIDE EFFICACY

3.1 Introduction

The most effective control measure against *D. suzukii* is currently chemical control. In order to further improve this control strategy and to reduce the chance of resistance development it is key to establish the efficacy of existing and new insecticides. This becomes even more important due to changing governmental rules regarding insecticide use. The main trend in Europe is to diminish the use of insecticides. A way to reduce the number of applications is to use insecticides that have a controlling effect on *D. suzukii*.

The insecticides which are currently approved on cherry in the UK are acetamiprid, cyantraniliprole, λ-cyhalothrin, pyrethrins, spinosad and thiacloprid as shown in *Table 4*. Only acetamiprid and pyrethrins are fully approved. The other insecticides are only temporarily approved with an extension of authorisation for minor use (EAMU) (Agriculture and Horticulture Development Board, December 2015). In Belgium there three different active ingredients are allowed to manage *D. suzukii*, i.e. spinosad, dimethoate and λ-cyhalothrin (FOD Volksgezondheid, 2016).

Table 4: Approved insecticides in the UK with activity against D. suzukii in cherry. The EAMU approvals (Extension of Authorisation for Minor Use) are limited extended approvals for the use of insecticides against a particular pest. (Agriculture and Horticulture Development Board, December 2015)

	Active ingredient	Approval	Max no. applications	Max. rate	Harvest interval (days)
Outdoor	acetamiprid	full	1	0.375 kg/ha	14
	cyantraniliprole	emergency EAMU	2	0.9 l per 1,000 l/ha	7
	λ-cyhalothrin	EAMU	2	90 ml/ha	7
	pyrethrins	full	no limit	0.02 l per 5l	1
	spinosad	emergency EAMU	3	0.25 l/ha	5
	thiacloprid	EAMU	2	0.313 l/ha	14
Protected	acetamiprid	full	1	0.375 kg/ha	14
	cyantraniliprole	emergency EAMU	2	0.9 l per 1,000 l/ha	7
	pyrethrins	full	no limit	0.02 l per 5l	not stated

3.2 Materials and methods

3.2.1 Experimental layout

The testing of insecticide efficacy was conducted on a cherry orchard at EMR (New Road, East Malling, Kent, ME19 6BJ). This cherry orchard, called Rookery Field is the same orchard as described in 2. Objective 1: Commercial trap comparison. In this cherry orchard two cherry varieties (Penny and Sweetheart) are combined. Each other row of the orchard contains only the variety Penny whereas the other rows consist out of alternating the Penny and Sweetheart varieties. Only in the rows where Penny is the single variety, the insecticides were applied.

Adjoining Rookery field in the North there are some private gardens which could be a possible source of *D. suzukii*. On the east side of the orchard there is also a possible source of *D. suzukii* where the cherry gene bank of EMR is located. This gene bank only receives spray in order to preserve the trees but not to preserve any harvest (*Figure 13*).

In order to increase the natural population of *D. suzukii* without developing any resistance towards any insecticides, Rookery Field was last sprayed with λ-cyhalothrin and did not received any other treatments.

The insecticide efficacy study was laid out in the orchard in a block system where each block was replicated six times. Each block contained eight randomised treatments. All treatments were applied on one tree and were separated from each other with a guard tree in between. Blocks were arranged end to end in a row, therefore two rows were needed to accommodate for all replicates. The two rows were covered with polytunnels (ethylene-vinyl acetate) and separated by a guard row in order to minimize the influence of neighbouring plots.

These tunnels were set up at the first of July, nine days before the start of the experiment, and served as rain cover (*Figure 13*). The polytunnels were open on the sides, front and back, allowing *D. suzukii* free entry into the crop. This way the insecticides were applied without any risk of washing of their residues and reducing the efficacy of the insecticides (Gautam et al., 2016).

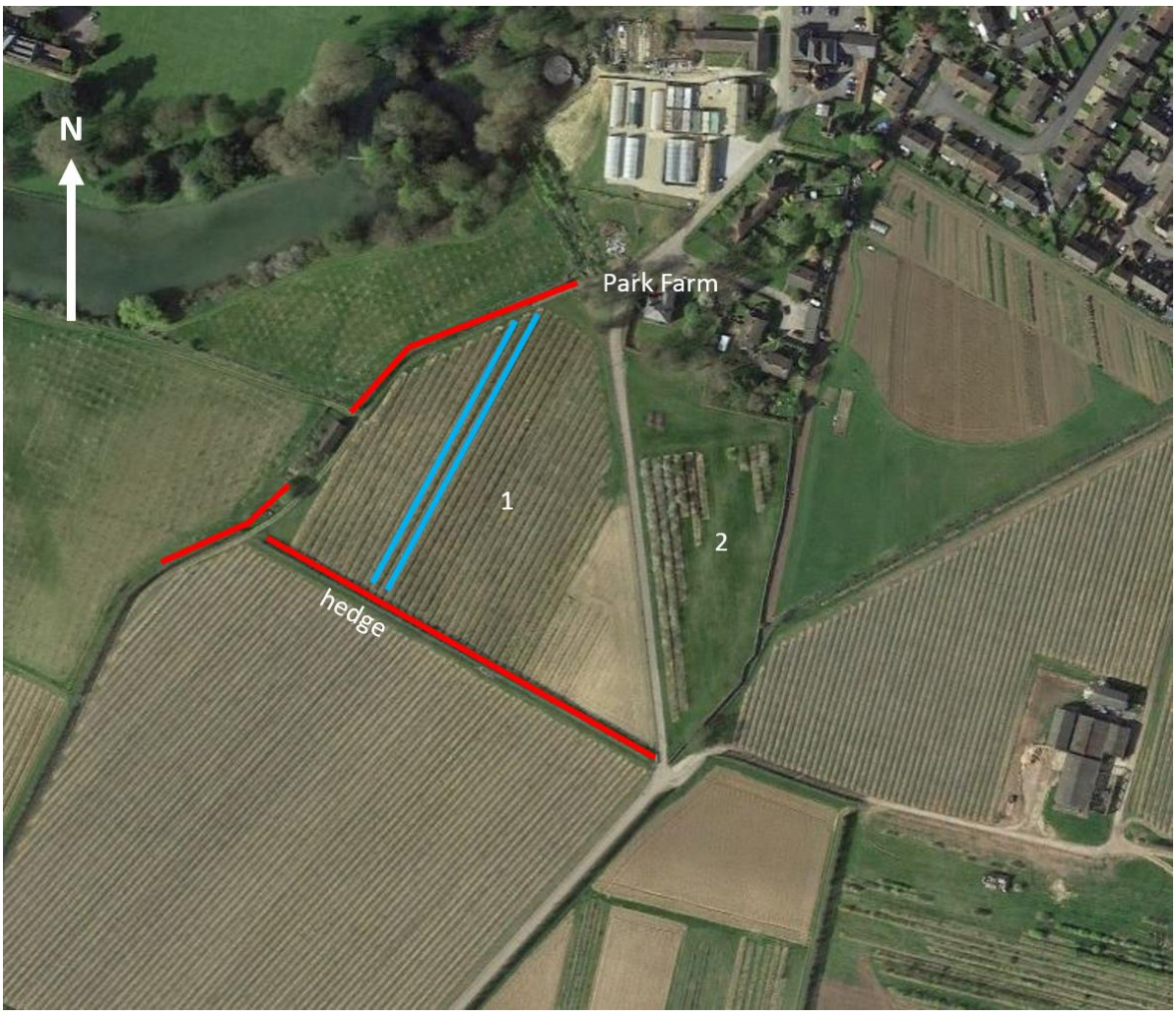

Figure 13: Satellite image of Rookery Field (Google Earth, picture taken on 20/04/2015, 51°17'34.06" N and 0°26'46.33" E). The red lines represent the hedges adjoining Rookery Field. The two blue lines are the two rows of the variety Penny who were covered with polytunnels for the insecticide efficacy trial. The North side of Rookery field is further on called as the Park Farm side and the South side of the orchard is the hedge side. Number one on the satellite image is Rookery Field and number two is a cherry gene bank used for breeding new varieties. This gene bank only received insecticide treatments to conserve the trees but did not receive any pesticide applications to conserve a cherry harvest. The fruits of this gene bank were not harvested.

The population of *D. suzukii* was monitored in neighbouring orchards with a four component lure based on the Cha and Landolt bait (Cha et al., 2014) and a modified Droso Trap (Biobest, NV., Ilse Velden 18, B-2260 Westerlo, BE). This setup is also used in the national monitoring of the UK.

3.2.2 Assessments

In order to assess the efficacy of the used insecticides the EPPO guideline for the efficacy evaluation of insecticides (EPPO, 2013a) was used as a reference for the assessments. The fruits were picked on 0 DAT (Days After Treatment), 1 DAT, 4 DAT, 7 DAT and 14 DAT with 0 DAT the start date of the experiment at the ninth of July. Each assessment day, 20 fruits were picked for each plot and put in modified clear polystyrene presentation boxes (228x121x86mm Transpack, Total Packaging product code TPCC6107). The middle of the lids of the boxes was cut out and replaced by a mesh. This mesh allowed air to circulate in the boxes but prevented *D. suzukii* from escaping (*Figure 14)*.

After each sampling date the lids of the boxes with collected fruit were sealed and put in a controlled temperature room at 21°C (Tochen et al., 2014). Over a period of three weeks after the sampling date the boxes were checked on adult emergence of *D. suzukii* twice a week. The emerged adult flies were removed with the help of an electric pooter. This was necessary to prevent *D. suzukii* from laying a second generation of eggs. Because of the high temperature and infection by *D. suzukii* the cherries began to liquefy. To prevent the adult flies from drowning and to aid the adult count, blue tissue paper was added.

Figure 14: Clear polystyrene presentation boxes (228x121x86mm) with modified lid. The middle section of the lids was removed and resealed with a fine mesh to allow for ventilation. The fine mesh made sure no larvae or adult D. suzukii flies could escape.

3.2.3 Insecticides

In this insecticide efficacy trial seven insecticides were used as shown in *Table 5*. These insecticides were applied once during the morning of 0 DAT of the experiment by a Birchmeier B 245 sprayer with micron restrictor nozzles. Each plot was individually sprayed with a target volume rate of 1000 L/ha and a maximum product concentration as stated in *Table 5*. The insecticides were applied on the ninth of July 2015 between 06h50 and 10h00 on a sunny morning. The start and end temperatures were 16 and 19°C (dry bulb temperatures). The wet bulb temperatures at the start and end of the insecticide application were respectively 11 and 17°C. During the insecticide application a wind speed of 0 km/h was registered.

Some of the insecticides used in this trial were not approved yet by legislation at the time of the experiment. Therefore all harvested fruits were destroyed.

Table 5: Insecticides used in the insecticide efficacy trial. The lime treatment, treatment five, is a combination of four active ingredients. In the last column the target spray (%) is calculated by dividing the volume sprayed by the theoretical volume required. The classification of the modes of action for each active ingredient are in accordance with the IRAC Mode of Action Classification Scheme, Version 8.0 (Insecticide Resistance Action Committee (IRAC), December 2015).

	Active ingredient	Product name	Company	IRAC code	AI concentration	Dose rate product / ha	Target spray (%)
1	acetamiprid	Gazelle	Certis	4A	200 g/kg	375 g	91
2	cyantraniliprole	Exirel	DuPont	28	100 g/l	900 mL	95
3	deltamethrin	Decis	Bayer	3A	25 g/l	200 mL	100
4	λ-cyhalothrin	Hallmark	Syngenta	3A	100 g/l	90 mL	98
5	lime	Ds-Lime	Plantosys	NA	≥ 96%	2 000g	96
	micro copper	Cuprum	Plantosys	NA	10 g/l	1000 mL	
	micro manganese	ManZincum	Plantosys	NA	NA	250 mL	
	micro zinc	ManZincum	Plantosys	NA	NA	250 mL	
6	pyrethrin	Spruzit	Certis	3A	4.59 g/l	12 L	93
7	spinosad	Tracer	Dow AgroSciences	5	480 g/l	250 mL	91
8	untreated	untreated	/	/	/	/	/

3.2.4 Weather

Throughout the experiment the temperature and relative humidity were measured by a SEN-R combi sensor (Adcon telemetry, Austria). This sensor is part of a weather station on the EMR site (Agrii intelligence, MetQuest weather stations, GB) and gives measurements with 15 minute intervals. As shown in *Figure 15* the air temperature averages around 18°C. The relative humidity fluctuates mainly between 60 and 90%.

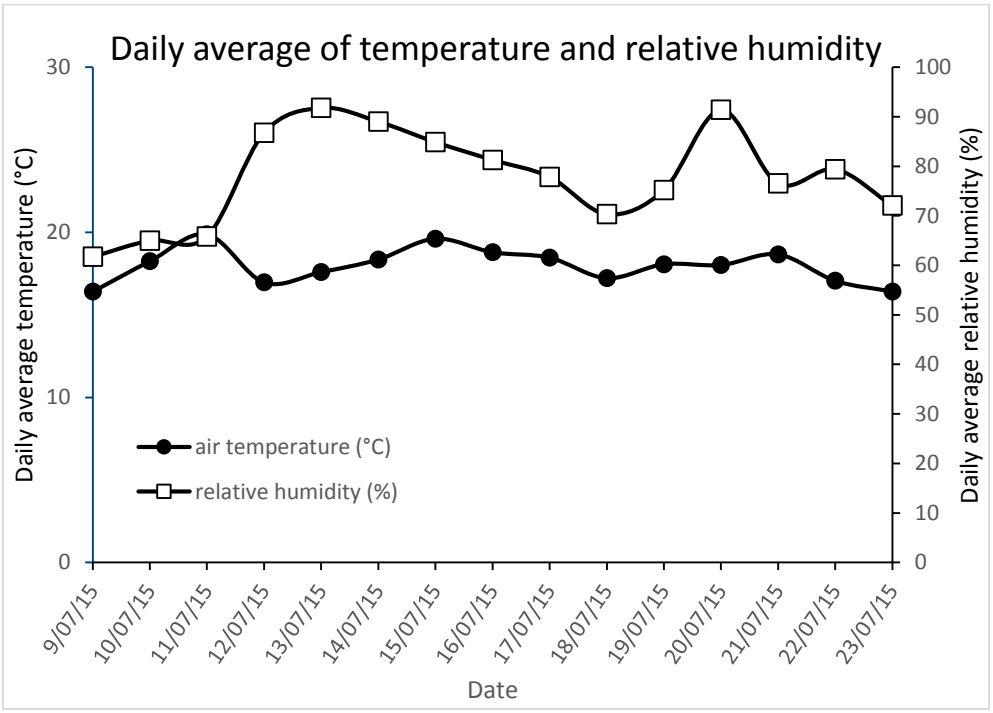

Figure 15: Daily average air temperature and relative humidity during the experiment. The averages were calculated based on 15 minute interval measurements of a 24 hour day period.

3.2.5 Statistics

The results of each assessment day were statistically analysed using R version 3.2.1 "World-Famous Astronaut" (R Core Team, 2015) and package "multcomp" (Hothorn et al., 2008). Because of the high number of zero's in the *D. suzukii* counts the results were analysed with a general linear hypotheses and multiple comparisons for parametric models combined with a Poisson distribution and a logarithmic offset of the amount of collected cherries. To compare the different treatments for each assessment day a Dunnett or Tukey test was used, depending on the comparison against the untreated treatment or as for 14 DAT, against each treatment separately.

3.3 Results

At 0 DAT and 1 DAT no significant differences were detected between the treatments nor in comparison to the untreated (*Figure 16* and *Figure 17*). At 4 DAT only acetamiprid and spinosad were significantly different to the untreated ($P<0.01$) (*Figure 18*). By 7 DAT acetamiprid, cyantraniliprole, deltamethrin, lime and spinosad were significantly different from the untreated ($P<0.001$) (*Figure 19*). At 14 DAT a difference between all treatments was observed and cyantraniliprole and λ-cyhalothrin showed the best possibilities to control *D. suzukii* at this point (*Figure 20*).

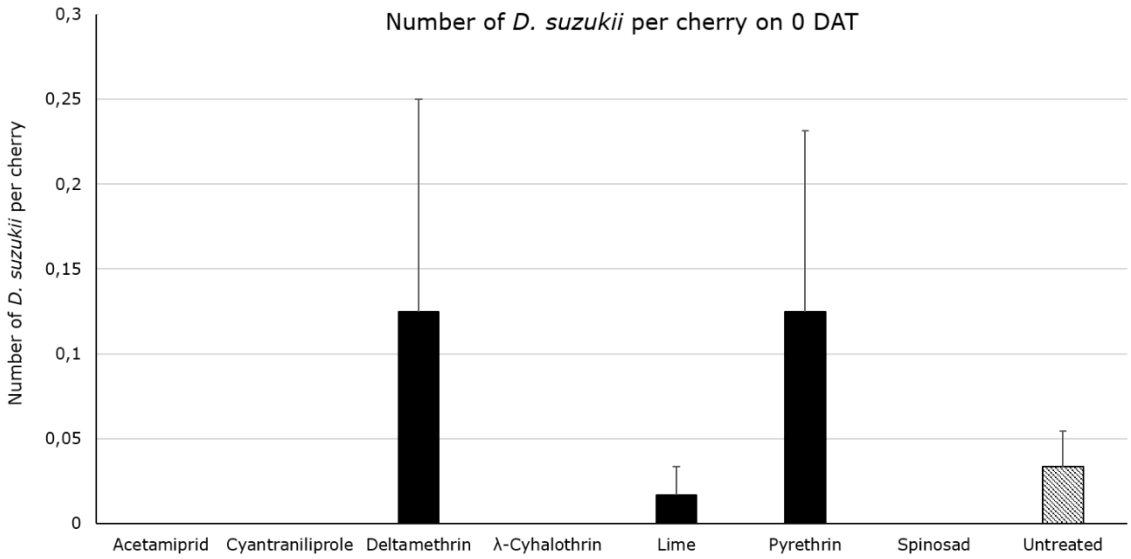

Figure 16: Number of D. suzukii adults per cherry for each treatment on 0 DAT. No significant differences were observed between the different treatments and untreated (Dunnett test).

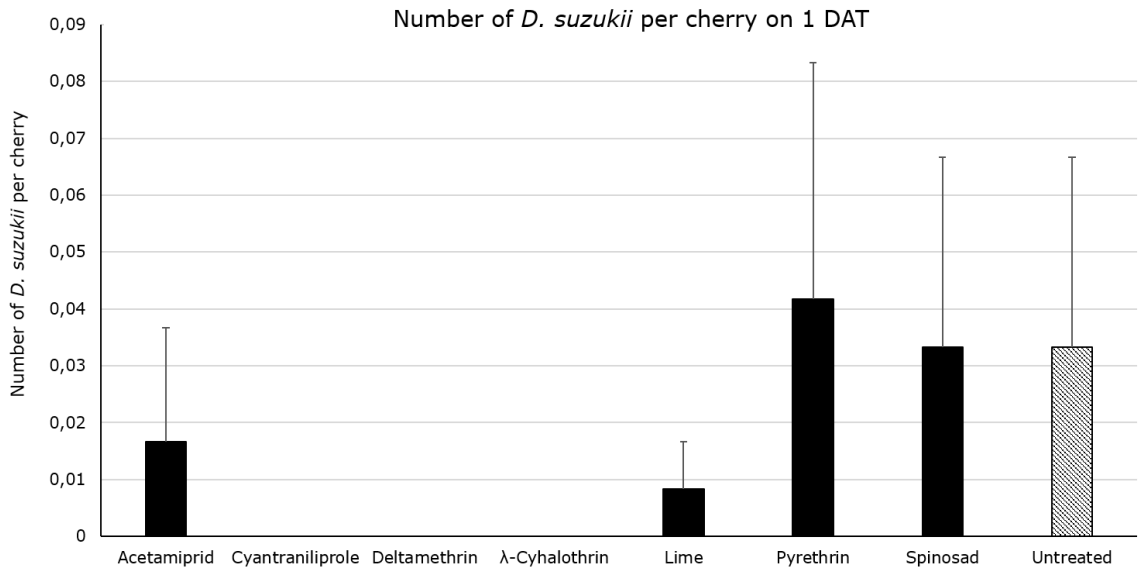

Figure 17: Number of D. suzukii adults per cherry for each treatment on 1 DAT. No significant differences were observed between the different treatments and untreated (Dunnett test).

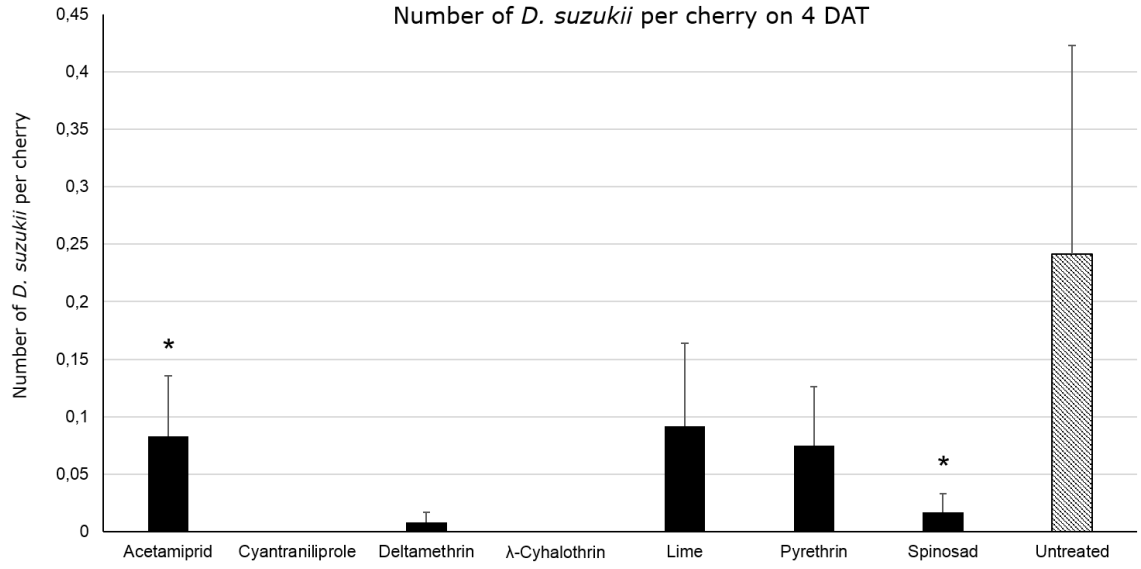

Figure 18: Number of D. suzukii adults per cherry for each treatment on 4 DAT. Acetamiprid and Spinosad were significantly different to untreated (P<0.01) (Dunnett test).

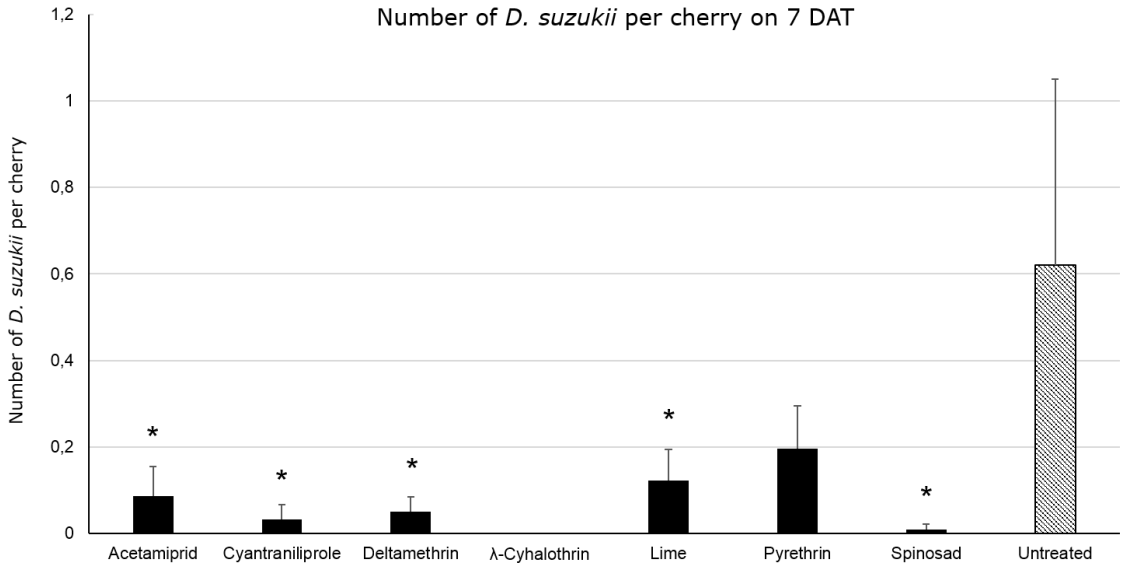

Figure 19: Number of D. suzukii adults per cherry for each treatment on 7 DAT. λ-Cyhalothrin and pyrethrin were not significantly different to untreated. All other treatsments were significantly different (P<0.001) (Dunnett test).

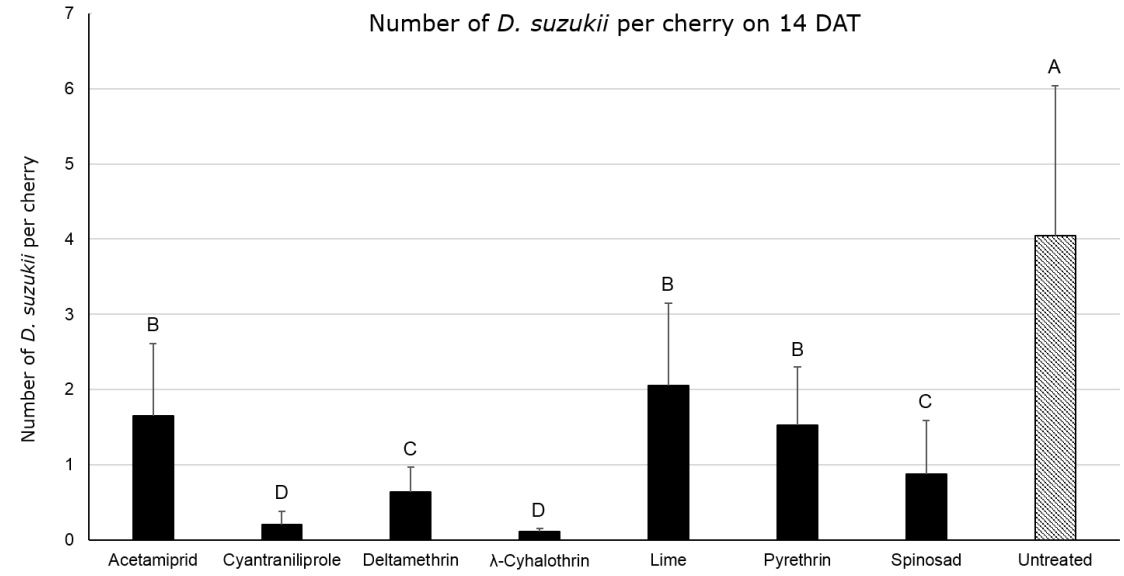

Figure 20: Number of D. suzukii adults per cherry on 14 DAT. A significant difference between the different treatments was observed. The letters show the different groups of significant difference with a Tukey test as post hoc test. Each letter represents a group of treatments which are significantly different to the other groups but not within their own group. At 14 DAT all treatments were significantly different to untreated.

Figure 21 shows the mean normalised results. It represents the relative infection compared to untreated and was done by dividing each assessment of each treatment by the mean of untreated. The normalisation had as goal to filter the influence of the variations of the infection pressure out of the results. Therefore no influence of the gradual increase in the number of *D. suzukii* caught in monitoring traps throughout the two weeks of the experiment was taken into account.

With the exception of two out layers of deltamethrin and pyrethrin at 0 DAT all treatments had an immediate effect on *D. suzukii*. The different treatments can be compared across the different assessment days. Cyantraniliprole and λ-cyhalothrin have a fast and long working period. Acetamiprid deltamehrin and spinosad also have an effect on *D. suzukii*. But they effectiveness decreases by 14 DAT. The lime treatment has an effect on *D. suzukii* infestation but gives inconclusive results in this trial. Between 0 DAT and 1 DAT pyrethrin has an effect but this effect stagnates by 4 DAT.

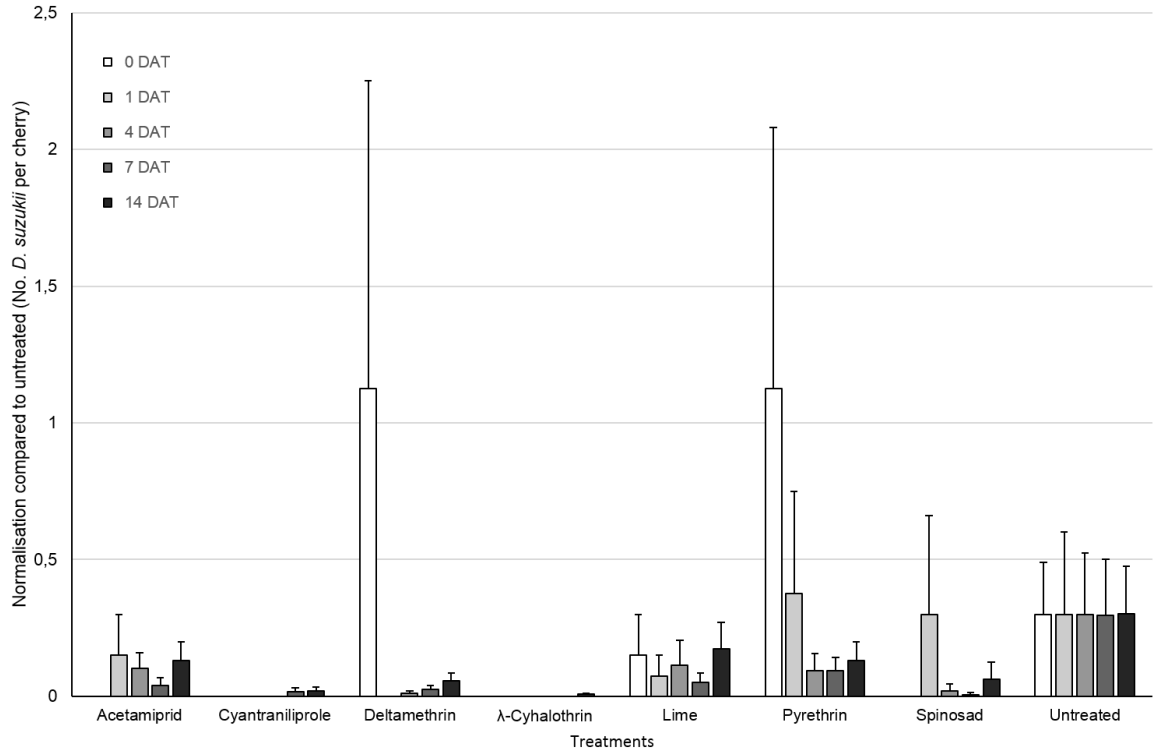

Figure 21: Normalised results compared to untreated of the insecticide efficacy trial. At 0 DAT there were two out layers on the deltamethrin and pyrethrin treatments.

3.4 Discussion

In this field study seven insecticides, with different modes of action were used, to establish their efficacy against *D. suzukii* on cherry. Because of the development of *D. suzukii* as a pest organism in the western world a lot of attention is currently paid to practical and environmental solutions in order to manage this drosophila species. *Figure 21* gives an indication of the efficacy of potential insecticides that can be used against *D. suzukii*.

Some of the insecticides used in this trial showed high numbers of emerged *D. suzukii* on 0 DAT and 1 DAT. These high numbers result from eggs laid previously to the insecticide application. This is the case for the pyrethin and spinosad treatments. Therefore it can be concluded from the results that acetamiprid, cyantraniliprole, deltamethrin, λ-cyhalothrin and lime do have an instant effect on killing *D. suzukii* larvae or pupae in contradiction to pyrethrin and spinosad who have a slower effect. Pyrethrin and spinosad do not seem to kill larvae or pupae but prevent *D. suzukii* from laying new eggs. Therefore it is probably better to use spinosad as a preventive measure.

In contradiction with earlier laboratory studies pyrethrin does not show promising results in this trial (Cuthbertson et al., 2014). Although it has an effect by 14 DAT of this study (*Figure 21*), the number of *D. suzukii* emerged from the collected cherries was too high to use the cherries for consumption. The susceptibility of natural pyrethrins to rapid degradation under sunny conditions might underpin this inferior result (Crosby, 1995) as high temperatures (Atkinson, Blackman, & Faber, 2004) were present during the experiment. As a result to these weather conditions the data also suggest that the pyrethrins were not effective by 1 DAT. Deltamethrin and λ-cyhalothrin are part of the same group as pyrethrin which are known for their rapid photo degradation (Shukla, Omkar, & Kulshrestha, 1998). Although deltametrhin and λ-cyhalothrin show good potential to control *D. suzukii* in this trial in contradiction with pyrethrin. Under the right conditions pyrethrin can be used to prevent *D. suzukii* from laying eggs especially to bridge the waiting periods of the harvest intervals of other insecticides. With a harvest interval of one day it can still be used just before harvest. In a complete spraying program, that can be integrated in a IPM strategy, pyrethrins are not the best choice due to the short working period and high frequency of applications, which is most likely to enhance resistance. Deltamethrin and λ-cyhalothrin, active ingredients of the same group, do appear to have a better result in this trial and are preferred above pyrethrin due to their fewer applications.

Acetamiprid, which is part of the group of neonicotinoids, appears to have an effect but not in such a way that acetamiprid can control *D. suzukii.* The results show that the effect wears out after 7 DAT. Acetamiprid is already used against *Rhagoletis cerasi* L. (Diptera: Tephritidae) in Europe (Daniel & Grunder, 2012). Acetamiprid can be used in combination with a *R. cerasi* IPM strategy. However the insecticides that are effective against *D. suzukii* such as λ-cyhalothrin, cyantraniliprole, deltamethrin and spinosad (Yee & Alston, 2006) are also effective for *R. cerasi*, which decreases the need of an extra spray with the less effective insecticide acetamiprid.

The lime treatment in combination with micro particles of copper, zinc and manganese did not show definite results. Lime showed an effect compared to the untreated control but this was not consistent for all the different assessment days. In addidition, lime left a white residue on the cherries, which is not acceptable in order to market the cherry yield. The experiments done by Baroffio (Baroffio et al., 2015) in Switzerland also showed some effect of lime treatment in comparison to untreated strawberries.

Spinosad showed good control potential after 4 DAT until 7 DAT. This is consistent with previous research results (Baets et al., Unpublished Data).

Due to the specific mode of action of cyantraniliprole as a ryanodine receptor modulator (IRAC number 28, diamides) (Insecticide Resistance Action Committee (IRAC), December 2015)and the effectiveness against *D. suzukii* shown in this trial, cyantraniliprole is an ideal insecticide to use in an insecticide resistance management strategy.

To conlude this trial, cyantraniliprole and λ-cyhalothrin showed an extensive (until 14 DAT) and good control of *D. suzukii,* both preventive and curative. Deltamethrin and spinosad showed also an adequate control of *D. suzukii*, but it seems that both insecticides wear out after 7 DAT. Deltamethrin also showed a curative and preventive control. However Spinosad seems to have a slower effect to control *D. suzukii*. Due to their different modes of action these four insecticides can be safely combined in a spraying schedule with a minimal risk of developing resistance. According to this trial also acetamiprid, lime and pyrethrin did show an effect to control *D. suzukii* however. However, their effect was not adequate enough to protect the fruit yield.

This study is only a good first exploration of the possible insecticides to manage *D. suzukii*. Further research in field trials has to be conducted to establish a baseline to advise growers on spray programs.

3.5 Acknowledgements

For this experiment in particular I want to thank all summer casuals of the entomology lab and Gloria Endredi who helped with their technical support of the research. I also like to thank Phil Brain for his insight and guiding into the statistics of this research. This study was supported through funding by the Agriculture and Horticulture Development Board (AHDB) under guiding of Hariet Duncalf.

4 OBJECTIVE 3: THEORETICAL POPULATION MODEL

4.1 Introduction

In order to get a better understanding of *D. suzukii* as an invasive pest an understanding of the population dynamics throughout the season of this insect is important. Currently it is unclear how *D. suzukii* populations behave throughout the year and at which time of the season the adults are utilising different crops, such as cherries, strawberries and raspberries. A population model would help to understand and predict *D. suzukii* population dynamics. This model could be used to predict the first egg laying of female *D. suzukii* and identify different intervals with a high egg laying pressure. These intervals would be useful to enable growers to plan the application of insecticide spray programmes. Furthermore a population model could be used for a better understanding of the life cycle of *D. suzukii* and to develop a more accurate prediction tool for a more sustainable integrated pest management.

4.2 Materials and methods

The population model uses an algorithm to calculate the population size. These calculations were done in R (R Core Team, 2015) combined with R Studio (RStudio Inc, Version 3.2.1, 18-06-2015) as an integrated development environment. A mathematical package 'stringi' (Gagolewski and Tartanus, 2015) and statistical package 'hydroGOF' (Zambrano-Bigiarini, 2014), were used to execute the algorithm.

4.2.1 Weather data

Before an algorithm can be calculated it needs input values. One of the key parameters that influences the outcome the *D. suzukii* population growth is the weather. The algorithm uses weather data as a basis for almost all calculations. These weather data were provided by Agrii (Agrii intelligence, MetQuest Weather Stations). All imported weather data were collected from different weather stations and were checked for errors made by the electronics of the weather stations. The files were also checked for consistent measurements during 15 minute intervals. In preparation for further calculations each file with weather data was provided with cumulative degree days (DD) based on the temperature. The calculation with cumulative DD was used to eliminate temperature variances during the different seasons and across multiple years and places. It is a tool used to compare different climatological circumstances. For this data the DD were calculated with 10°C and 30°C as a lower and upper limit, above and below which *D. suzukii* reproduction will cease (*Equation 1*). The range between 10 and 30°C corresponds with the normal development of *D. suzukii* (Kinjo and Kunimi, 2014). Each degree above the lower limit was multiplied by the interval time between two measurements. If the temperature rose above the upper limit of 30°C or dropped below the lower limit of 10°C no DD were added. The cumulative DD is the sum of the previous measuring points and the DD calculated at a given time. This method of calculating cumulative DD was more accurate than using a single or double sine method with the average daily temperatures, more frequently used in population models.

Equation 1: Calculation of DD. With T_x= air temperature on a given $Time_x$, T_{lower}=10°C, T_{upper}=30°C. Because of the 15 minute intervals a conversion factor was needed to convert the DD in minutes to a DD in days by multiplying by 1440.

$$DD\ (\text{days}) = [(T_x - T_{lower}) * (Time_x - Time_{x-1})] * 1440$$
$$if\ T_{lower} \leq T_x \leq\ T_{upper}\ else\ DD(days) = 0$$

The weather data consisted of 15 minute intervals and included air temperature, relative humidity, rain and solar radiation. Currently the model only uses the air temperature to model the population dynamics. Under the assumption that the temperature has the major influence on the development of this invasive insect, this parameter is assumed to have the greatest impact in the model. In a later phase relative humidity and rain could be implemented as these are known to affect activity of the adults (Tochen et al., 2015). Potentially rain and dry conditions may decrease activity and prevent mating and thereby effecting the next generation.

4.2.2 Estimation of first egg laying

To obtain growth of a population eggs need to be laid and larvae to develop through all of the stages. Therefore, the time of the first egg laying is a critical time point for the algorithm. The estimation of the first egg laying in spring was found by comparing the calculated cumulative DD with the ovary development. The time points of general ovary development were selected as points of the first egg laying.

The ovary development was monitored by EMR, at the two sites used in the habitat monitoring by dissecting five female *D. suzukii* from each site each week and assessing their ovaries (funded by AHDB). The habitats were monitored with modified drosotraps (Biobest) with Cha-Landolt bait. Each ovary received a number based on the stage of development (developed by Beverley Gerdeman USA, *Table 6, Figure 22*).

Table 6: stage of ovarian development determination key (developed by Beverley Gerdeman, Washinton State University, USA)

Number	***Stage of development***
1	No distinguishable ovaries when opened
2	Ovaries are distinguishable when abdomen opened but no eggs within
3	Ovaries distinguishable full of eggs without filaments when opened
4	Mature eggs with filaments
5	Ovaries with few mature eggs, many wrinkled, may look slightly yellow

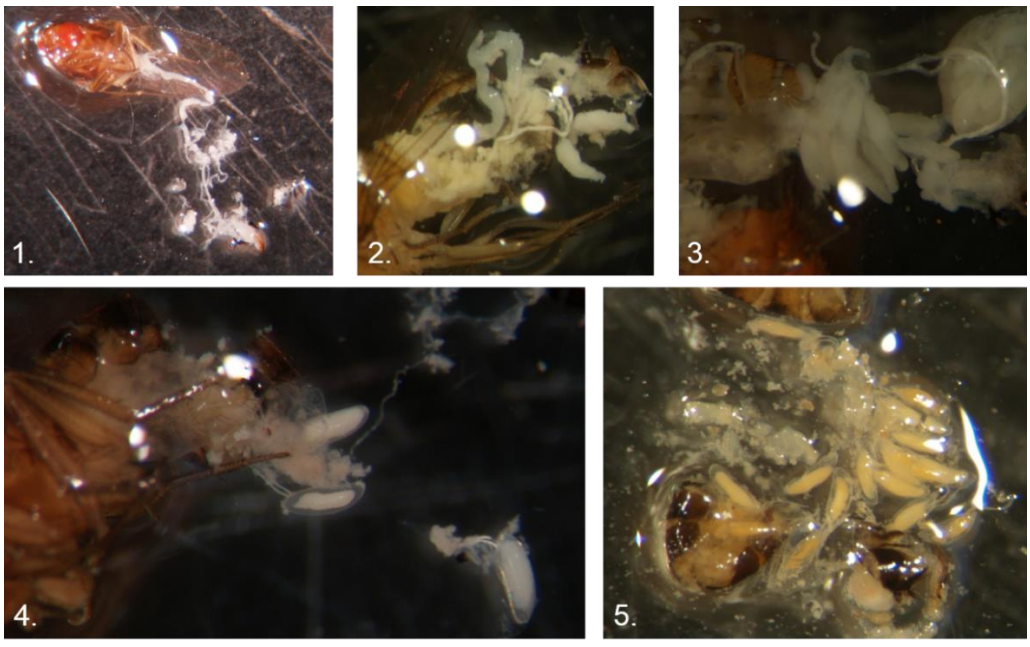

Figure 22: The five different ovary stages as described in Table 6 (photographs by Bethan Shaw)

4.2.3 Temperature-dependent mortality rate

To calculate the mortality rate of female *D. suzukii* flies *Equation 2* was used (Asplen et al., 2015). If the temperature fell outside the [0°C, 31°C] interval a mortality rate of 0.5 was chosen. This was made under the assumption that outside this temperature interval the adult population will decrease with 50% a day. Recalculated to a 15 minute time period means a mortality rate of 0.0052083.

Equation 2: Temperature-dependent mortality rate of adult D. suzukii flies (Asplen et al., 2015). Temperature is expressed in °C.

$$M(T) = 0.00035\,(T-15)^2 + 0.01$$

4.2.4 Temperature- and age dependent fecundity

The number of eggs laid by female *D. suzukii* is influenced by temperature as well as the age (in days) given by *Equation 3*. This equation is based on laboratory results done by Ryan et al. (2016) and is combined with the age specific egg laying observed by Asplen et al. (2015) at a temperature of 21°C (*Equation 4*). For other temperatures there is no age specific fecundity available yet. To combine this two formulas the influence of the age was estimated by normalising the age specific fecundity. To increase the temperature dependence of the fecundity a factor four was added (*Equation 3*). If the temperature fell in the range of [5°C, 30°C] (Ryan, Emiljanowicz, Wilkinson, Kornya, & Newman, 2016) *Equation 3* is used, otherwise the fecundity is set to 0.

The constants in *Equation 3* are α = 659.06, γ = 88.53, λ = 52.32, δ = 6.06 and τ = 22.87 (Ryan et al., 2016). With an optimal temperature of 22.87°C when $\frac{\delta y}{\delta T} = 0$.

Equation 3: Temperature- and age dependent fecundity of female D. suzukii adults. This function is constrained over an interval [T_{min}, T_{max}] with T_{min} = 5°C and T_{max} = 30°C (Ryan et al., 2016; Saryazdi & Cheriet, 2007). The factor $A \in [0,1]$ represents the influence of the age of the female adults on the fecundity by normalising Equation 4 (Asplen et al., 2015). The age factor is multiplied by four to increase the temperature dependence of the fecundity.

$$Y = \begin{cases} \alpha\left[\frac{\gamma+1}{\pi\lambda^{2\gamma+2}}\left(\lambda^2 - ([T-\tau]^2+\delta^2)\right)^{\gamma}\right] 4A & if\ T_{min} < T < T_{max} \\ 0 \end{cases}$$

Equation 4: Age specific egg laying of female at 21°C with the age in days (Asplen et al., 2015).

$$A(age) = \frac{0.585\ age}{1.0475^{age}}$$

The assumption was made to start the female fecundity estimation after a three day pre-oviposition period (Kinjo et al., 2014) with a maximum female age of 86 days (Emiljanowicz et al., 2014).

4.2.5 Temperature-dependent development egg-to-adult rate

By fitting a Brière function to the laboratory results observed by Ryan et al. (2016) the estimation of the temperature-dependent development egg-to-adult rate is given by *Equation 5*. If the temperature fell not in the range of [8.1°C, 31.4°C] (Ryan et al., 2016) the development rate is set to 0. According to this formula the optimal development temperature is 28.2°C when $\frac{\delta D(T)}{\delta T} = 0$

Equation 5: Brière function to estimate temperature-dependent development egg-to-adult rate (Brière, Pracros, Le Roux, & Pierrre, 1999). (a = 0.00020 and m = 2.51). The temperature thresholds are T_l = 8.1 °C and T_u = 31.4°C (Ryan et al., 2016).

$$D(T) = aT(T - T_l)(T_u - T)^{\frac{1}{m}}$$

4.2.6 Population calculations

The population model requires different inputs, shown in *Figure 23* (R-code can be found in *Appendix: Objective 3: Theoretical population model, p.66*). In the pre-processing process the weather data which consist of 15 minute intervals (Agrii intelligence, MetQuest weather stations, GB) were manually checked on missing values. With *Equation 1* the air temperature was recalculated cumulative DD. At the same time the estimation of the first egg laying by dissection of the ovaries was calculated. Both inputs were used as base for the population calculations.

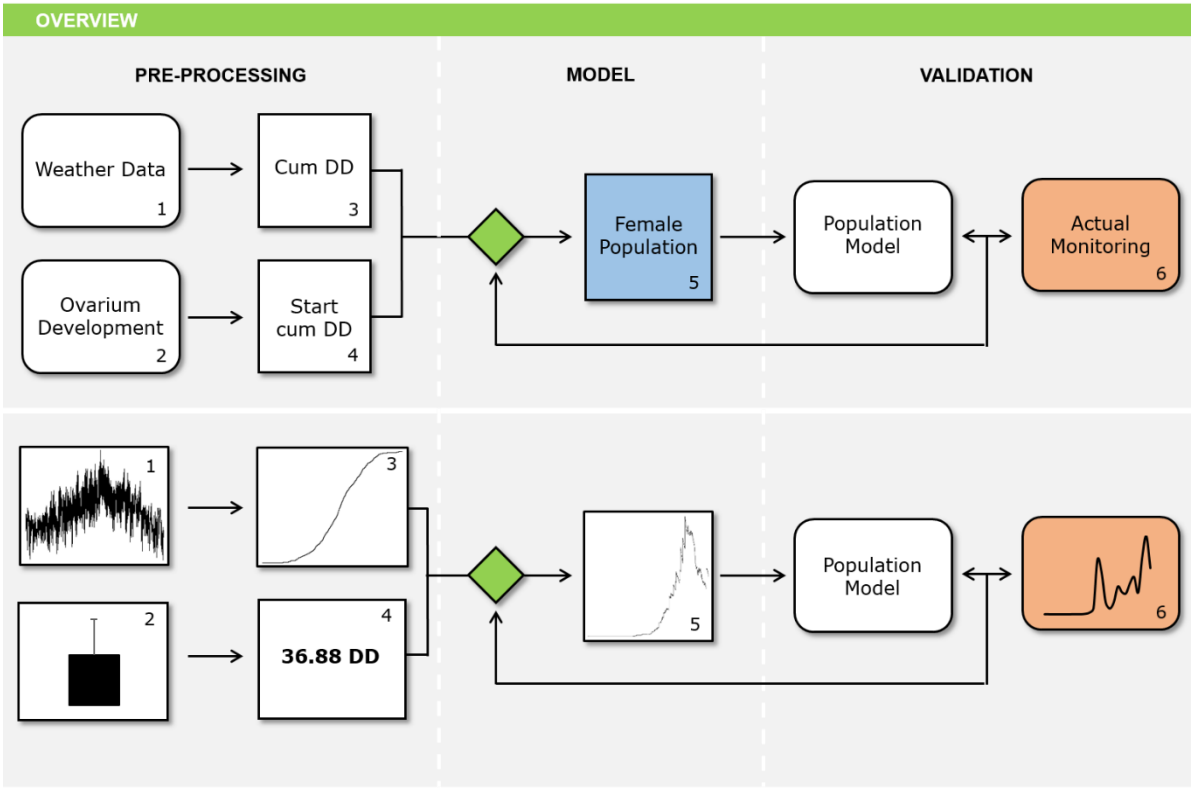

Figure 23: Overview of the population model. The left hand side shows the input needed to start the calculations. The middle section is the main part of the model and is further discussed in Figure 24. The blue box is the output of the theoretic population model. The right side represents the validation of the model with the monitoring data of the UK national monitoring. The bottom part of the figure represent visually the top part.

The actual model calculations are represented by the calculation diagram in *Figure 24*. If the start cum DD were not reached the starting population decreased according to *Equation 2.* When the start cum DD was reached the algorithm divided the population calculations in to two groups, i.e. every 15 minutes and every day. The calculations that had to be made every 15 minutes were the egg development and mortality rate of adult *D. suzukii* flies. In order to increase the age of each female *D. suzukii* adult with one day the right side of the calculation diagram showed the calculations needed at the beginning of every day. In theory this should happen every 96 times the 15 minute interval calculations were calculated (each day contains 96 hour quarters).

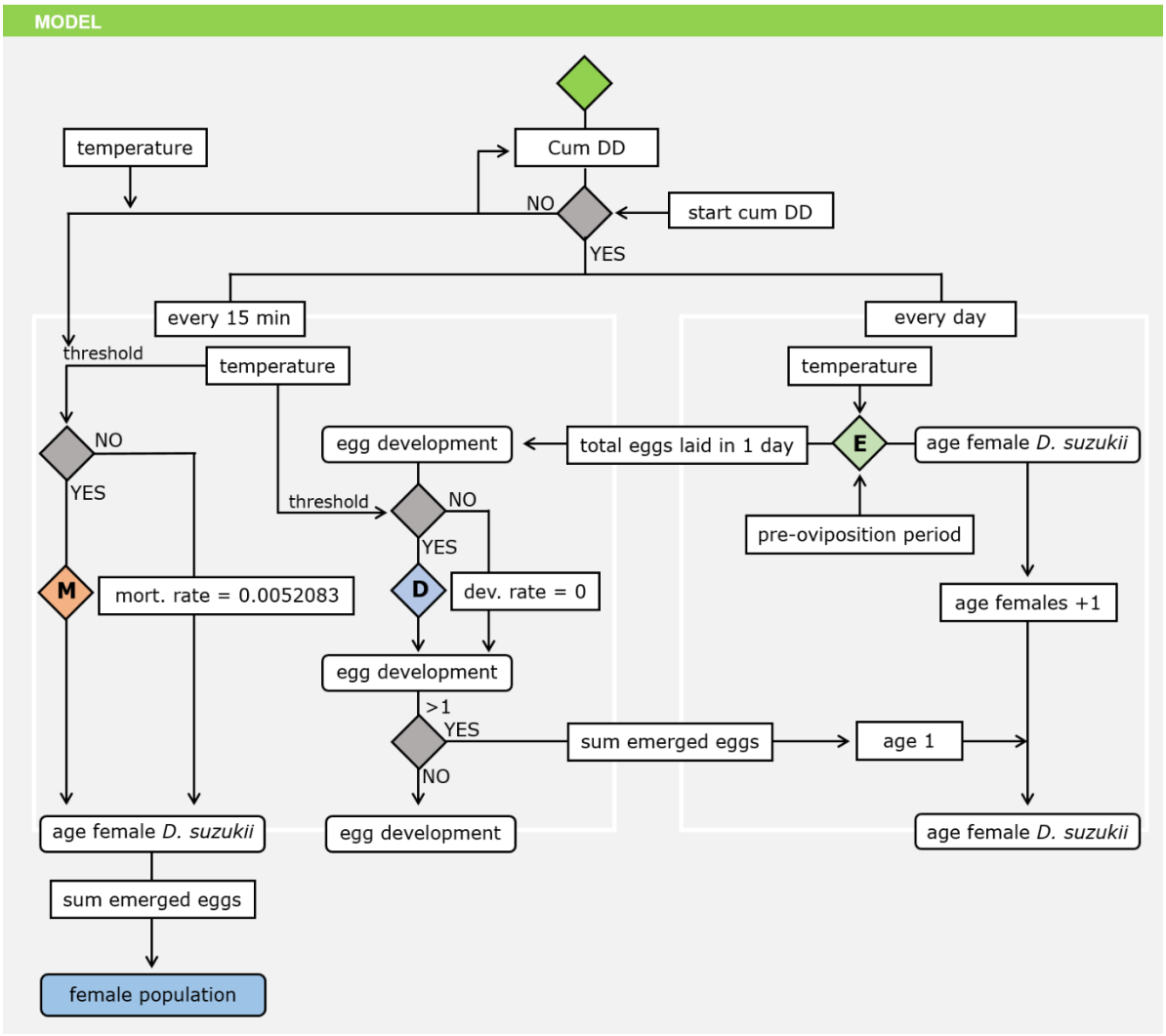

Figure 24: Calculation diagram to predict the female D. suzukii population. The actual population calculations are split up in two groups. The first set of calculations are placed in the left, white box. These set of calculations are done every 15 minutes. The other group of calculations has to be done each day and are placed in the right, white box. The orange square represents the mortality rate and is calculated by (Equation 2) (Asplen et al., 2015). The blue square represents the development rate of the egg-to-adult phase (Equation 5) (Ryan et al., 2016). The fecundity or the amount of eggs each female lays during the day is represented by de green square and is based on the combination of age and temperature (Equation 3) (Asplen et al., 2015; Ryan et al., 2016).

To simplify the model a couple of assumptions were made. All eggs laid by *D. suzukii* females were assumed to emerge. Currently no information is available about how many eggs survive the three larval stages and pupation before becoming an adult. Furthermore, the pre-oviposition period was set on three days (Kinjo et al., 2014). A third assumption was made, based on field data collected over three years in the UK at EMR, about the proportion of male / female adults. The proportion was assumed to be 50/50. Therefore 50% of the eggs will become females.

4.2.7 Validation

The population output generated by the calculation algorithm is validated by seven different farms of the national monitoring which are divide in to three groups, i.e. South-East (SE), East (E) and West Midlands (W ML) (*Figure 25*). The theoretic model as well as the national monitoring data is normalised in order to compare the population dynamics. The absolute values of the model does not represent any actual population that could be present in a certain area. The model output depends on the start population of the model and is arbitrarily chosen. The national monitoring does represent the actual population but only the trap catches only represent a fraction of the actual number of adults. The normalised numbers are compared by their correlation coefficient.

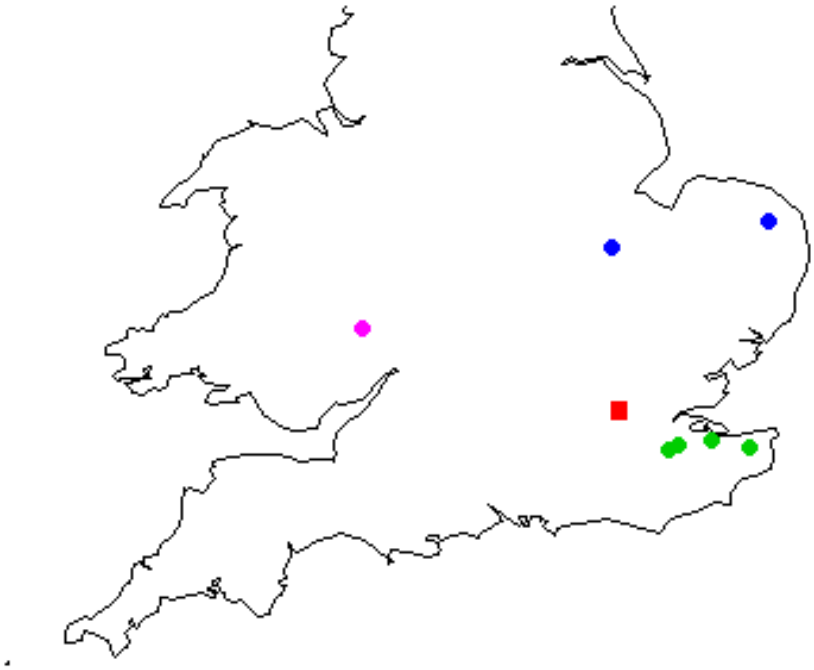

Figure 25:The seven different farms of the national monitoring. The farms are divided in three groups, i.e. South-East, East and West Midlands. The South-East farms are represented by green dots, the East farms by blue dots and the West Midland farm by a purple dot. London is represented by the red square.

4.3 Results

Table 7 shows the calculated estimation of the possible first and second egg laying in cumulative degree days (DD). These results are based on the ovarian development dissection done by EMR. The estimation for the second spike of egg laying has a high variance, making it difficult to pinpoint the exact cumulative DD around which female *D. suzukii* lay their first eggs. Furthermore, the lower threshold of the 95% significance interval was also too early compared to the assessments done by the dissections of the ovaries. In order to estimate an accurate first egg laying further research is needed. The earliest record of developed ovaries was found at 25 cumulative DD.

Table 7: Estimation of first and second egg laying in cumulative degree days DD. The model used the 95% significance interval of the first egg laying to establish the time range of the first egg laying.

DD (day)	Mean	S.E.	68% significance		95% significance	
			lower threshold	upper threshold	lower threshold	upper threshold
1st egg laying	36,88	13,12	23,76	50,01	10,64	63,13
2nd egg laying	88,82	42,42	46,40	131,25	3,98	173,67

For two years the cum DD were calculated for seven farms who are part of the national monitoring in the UK. The start times of the population model are established by the intersection of the first egg estimations (*Table 7*) and the cum DD of the different farms (*Figure 26*).

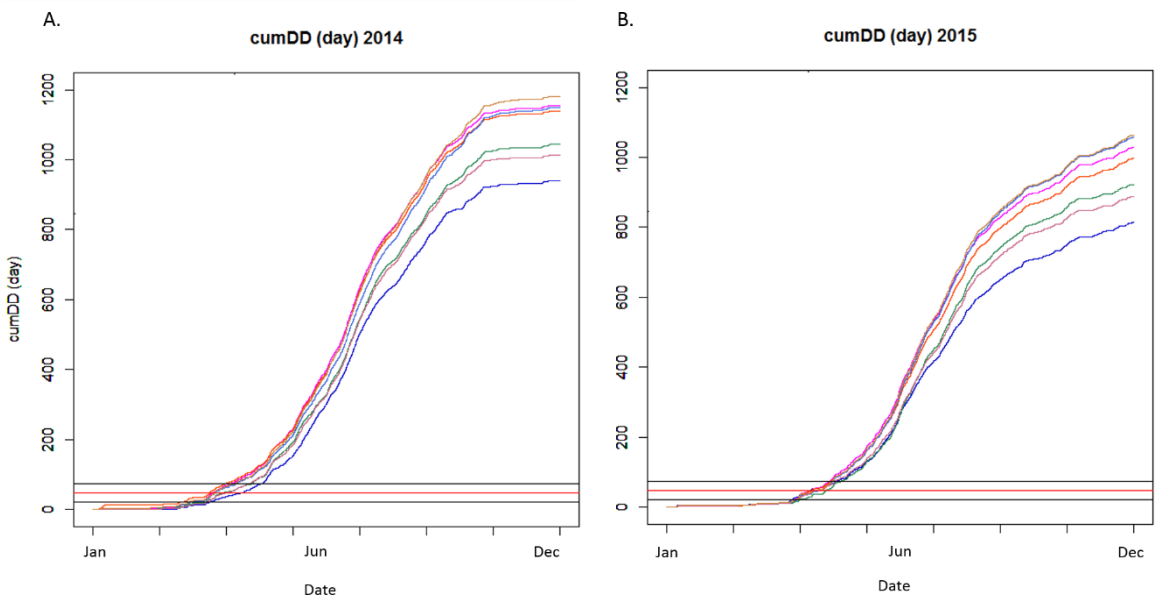

Figure 26: The intersection of the first D. suzukii egg laying estimation with the cum DD (days). The two black horizontal lines are the upper and lower thresholds of the 95% significance interval (Table 7), the red horizontal line is the mean (Table 7). Graph A. shows the calculated cum DD of 2014 for seven farms. Graph B. shows also the calculated cum DD of 2015 the same seven farms. Those farms are part of the national monitoring programme of D. suzukii in the UK. The start time of the model is established by the intersection between the first egg laying and the cum DD.

4.3.1 Population estimation

For each year and each farm the theoretic population was calculated with the population model. These calculations were done for four start times, i.e. mean first egg laying, lower threshold of first egg laying, upper threshold of first egg laying and at one cum DD (*Table 7*). An example for one year, one farm and mean first egg laying can be found in *Figure 27*.

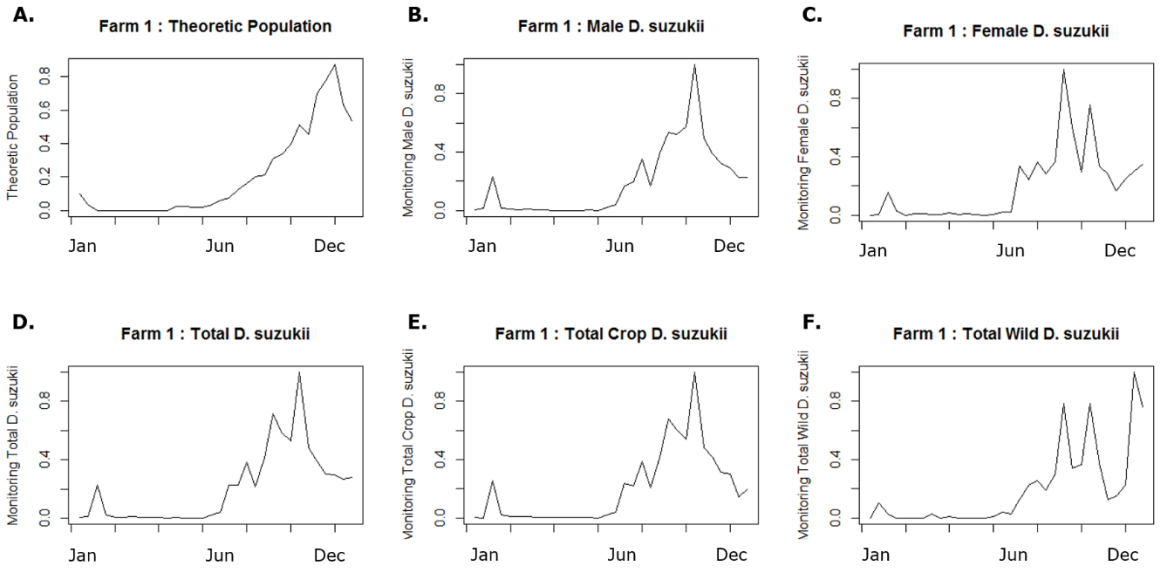

Figure 27: Example of normalised validation output for farm 1. A. Theoretic population. B. Male D. suzukii counts of the national monitoring. C. Female D. suzukii counts of the national monitoring. D. Total number of D. suzukii adults on one farm. E. Total number of D. suzukii numbers collected from traps placed in the crop. F. Total number of D. suzukii collected from traps placed on the edge of the crop or in hedges or woods.

4.3.2 Validation

By establishing the correlation coefficient between the normalised, theoretic population output and the different national monitoring categories, i.e. male, female, total, total crop and total wild (*Table 8)*. The correlation coefficient has a value between zero and one, with the best fit when the correlation coefficient is one. All correlation coefficients higher than 0.5 do have a positive correlation. This means that the model does not give an accurate prediction for the region East. Overall the population estimations for all start times are almost the same. For the region SE gives the model the best prediction for the total crop trap catches of the national monitoring with the mean start value as best result (R = 0.89). The total crop trap catches gives the best results for the region East but the correlation coefficient is too low (R < 0.50) to be taken into account. The correlation coefficient for the region West Midlands gives equally accurate predictions for the male and total trap catches (R > 0.74) although for the calculations with start value 1 DD the wild trap catches seems to be the most accurate.

Table 8: Results of population model validation with the correlation coefficient. The results are the mean of both years. The results are divided in the three different regions, i.e. South-East (SE), East (E) and West Midlands (W ML) and give separate reading for each start value of the model.

		1 DD	lower	mean	upper
SE	male	0.798684	0.795067	0.810722	0.813744
	female	0.773838	0.770535	0.778117	0.782261
	total	0.792826	0.778576	0.791088	0.795031
	crop	0.844117	0.891173	0.890564	0.881582
	wild	0.767513	0.761561	0.757497	0.750035
	Mean	0.795396	0.799383	0.805598	0.804531
E	male	0.3753	0.34309	0.354141	0.346231
	female	0.225467	0.161945	0.164663	0.141643
	total	0.390814	0.342598	0.35045	0.341088
	crop	0.394987	0.282237	0.292645	0.27869
	wild	0.445116	0.450903	0.450229	0.436922
	Mean	0.366337	0.316155	0.322425	0.308915
W ML	male	0.774148	0.761005	0.758259	0.786215
	female	0.712647	0.569013	0.590504	0.641829
	total	0.771217	0.747269	0.753884	0.786873
	crop	0.711109	0.668934	0.684274	0.721987
	wild	0.822647	0.667805	0.671026	0.703534
	Mean	0.758354	0.682805	0.69159	0.728087

4.4 Discussion

The validation results give a first indication of the accuracy of the population model. This means that a prediction can be made about the population dynamics of *D. suzukii*. The results show that the interval of the first egg estimation is valuable as a starting point for the population growth of *D. suzukii*. T

However the population does only fit for certain regions of the UK. Which means that an extra factor is needed to account for the placement of a farm. The most accurate estimations were given by the model in the South East region of the UK. To incorporate this variations into the model the coordinates of the different farms can be taken into account. The validation data can also be improved through the improvement of the monitoring data. In order to get accurate readings it is best to monitor from day to day certain areas. This is however a lot of work that cannot be done for each of the seven farms. Another improvement can be made by improving the traps and lures to be more selective and to catch more *D. suzukii* adults. The trap catches are also succeptible to variations in rain, temperature and relative humidity who influences the amount of *D. suzukii* flies caught.

During the year the moment of rapid, exponential growth can also be pinpointed, giving important information to the farmers. Because if a grower can intervene at the beginning of an exponential growth the further development of a large population can be decreased. Furthermore the model is an important step towards a clear life cycle follow-up of *D. suzukii*, providing a useful tool for research.

In a next step the harvest dates of the different crops can also be taken into account together with the influence of the relative humidity and rain on the *D. suzukii* population.

In this model there were no factors such as parasitism and predation taken into account due to the lack of information. Further research is also needed to establish more accurate estimations of the mortality rate and development rate of *D. suzukii*.

To conclude, the population model in its current form delivers useful information and is a good starting point for the estimation of population dynamics. This gives valuable information to farmers to start their pest management of *D. suzukii* at the right time. Together with the above mentioned improvements this model an become even more relevant and result in huge advantages for the horticultural fruit sector.

4.5 Acknowledgements

Many thanks to Agrii Intelligence for providing the weather data used in this model and to EMR and the AHDB for providing the national monitoring data.

GENERAL CONCLUSION

The research done in this master thesis all contributed to an improved IPM strategy which can be used to control *D. suzukii* in soft and stone fruit. The commercial trap trial gives valuable information to growers which trap to choose for monitoring purposes with the price, ease of handling and installing and the actual performance to attract and catch *D. suzukii* adults with a minimal bycatch. A good trap and lure combinations can determine when to apply the insecticide treatments to minimize yield losses. The insecticide efficacy determined that cyantraniliprole and λ-cyhalothrin showed the best control against *D. suzukii* with the longest working period in cherry. A combination of both the best trap combination and efficient use of insecticides do contribute to the improvement of an IPM strategy.

A more theoretical approach of *D. suzukii* as a population was explained by the theoretic population model of *D. suzukii*. This model, validated by UK's national monitoring, gives growers and scientists a better insight in the population dynamics and weather factors that influence the *D. suzukii* population during the year. This model still needs further improvement but can in the near future be used to advice growers when to apply their IPM control measures.

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APPENDIX

Objective 1: Commercial Trap Comparison

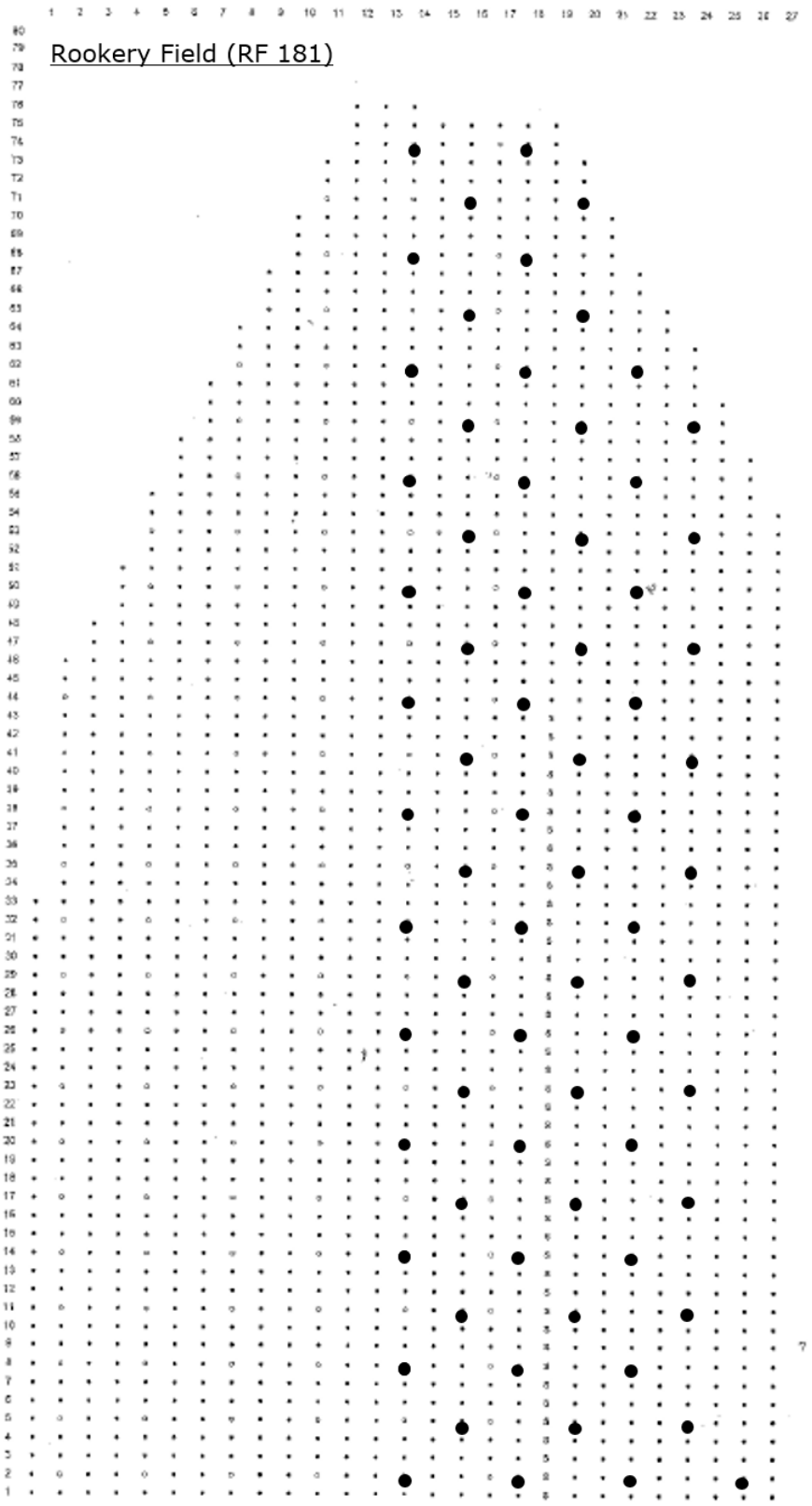

Figure 28: Orchard layout of Rookery Field (RF 181) at EMR. The black dots represent the placement of the traps.

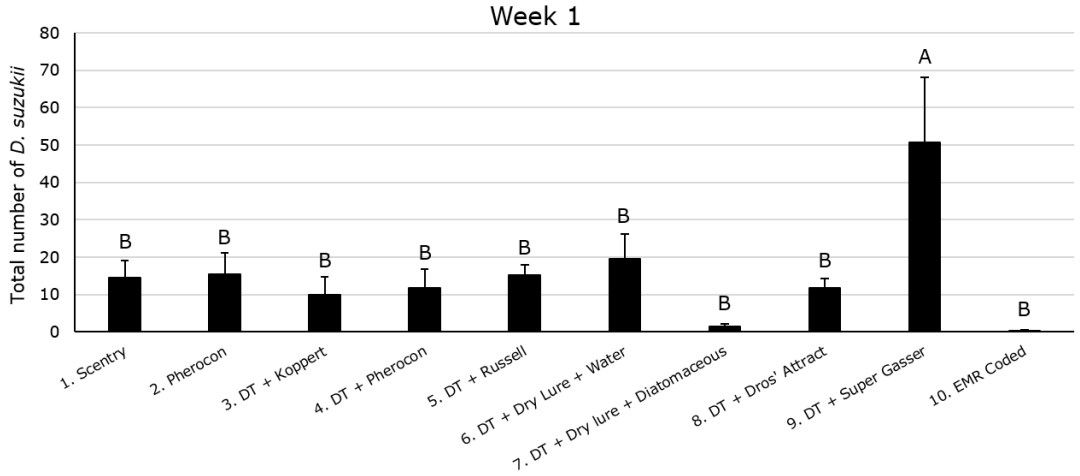

Figure 29: The mean of the total number of D. suzukii flies caught by each trap combination in week one of the experiment.

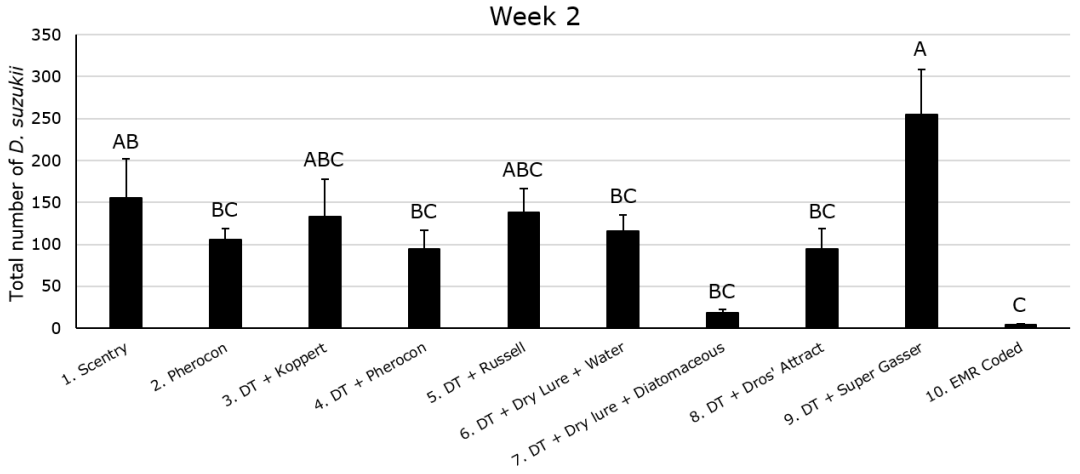

Figure 30: The mean of the total number of D. suzukii flies caught by each trap combination in week two of the experiment.

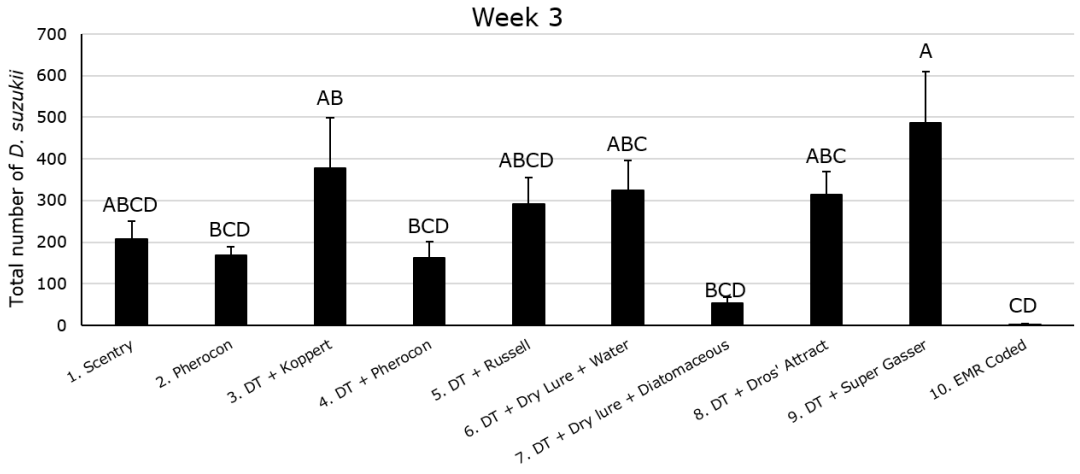

Figure 31: The mean of the total number of D. suzukii flies caught by each trap combination in week three of the experiment.

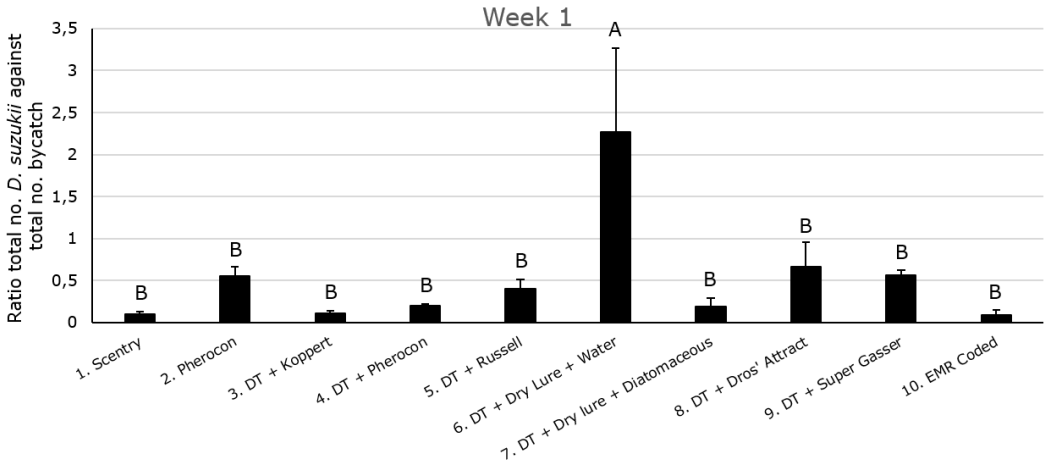

Figure 32: The mean ratio of the total number of D. suzukii flies divided by the total number of bycatch caught by each trap combination for week one. The higher the ratio the less bycatch there was compared to the D. suzukii catch.

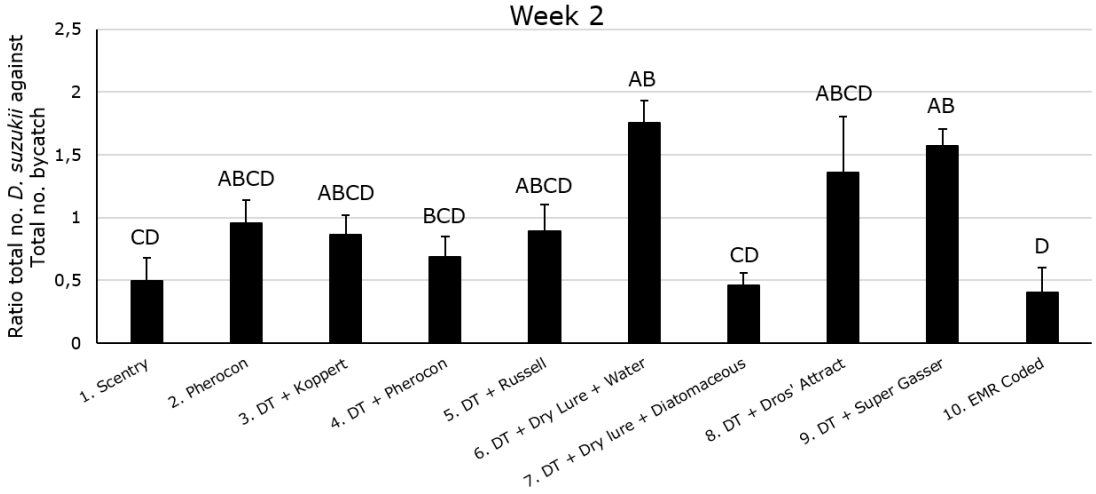

Figure 33: The mean ratio of the total number of D. suzukii flies divided by the total number of bycatch caught by each trap combination for week two. The higher the ratio the less bycatch there was compared to the D. suzukii catch.

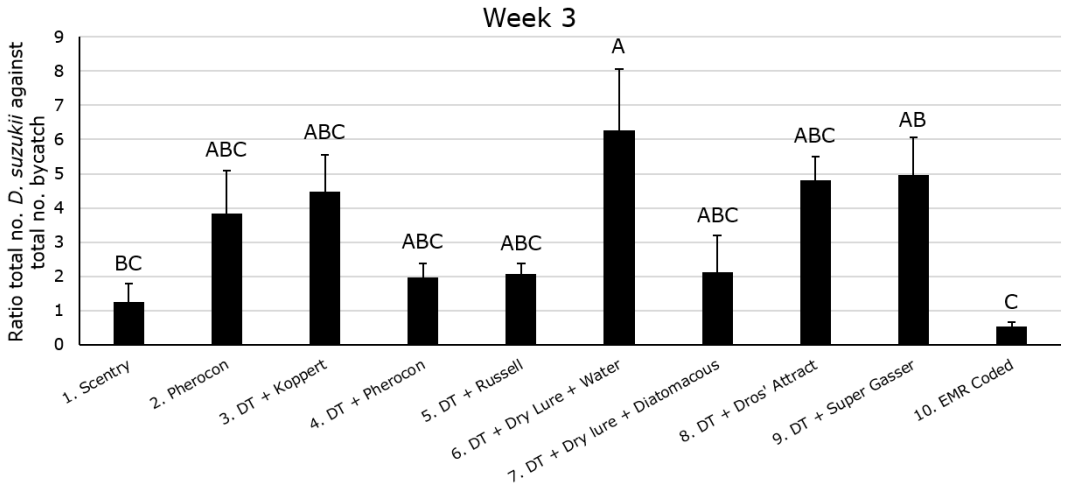

Figure 34: The mean ratio of the total number of D. suzukii flies divided by the total number of bycatch caught by each trap combination for week three. The higher the ratio the less bycatch there was compared to the D. suzukii catch.

Objective 3: Theoretical population model

```
################################################################################
################### Drosophila suzukii Population Model ########################
################################################################################
# Dieter Baets, 2016

# Packages
library("stringi", lib.loc="~/R/win-library/3.2")
library("hydroGOF", lib.loc="~/R/win-library/3.2")

# Working Directory
setwd("E:/PopulationModel_DrosophilaSuzukii")

# variables
# fixed
  # estimation of first egglaying (Bethan Shaw, EMR, Ovary development)
firstEgg.mean <- 68983        # cumDD (min)
firstEgg.lower <- 31192       # cumDD (min)
firstEgg.upper <- 106773      # cumDD (min)

  # temperature dependent fecundity (Ryan et al., 2016)
cAlpha <- 659.06
cGamma <- 88.53
cLambda <- 52.32
cDelta <- 6.06
cTau <- 22.87

cTmin <- 5                    #(°C)
cTmax <- 30                   #(°C)

  # maximum fecundity dependent age (Asplen et al., 2015)
cMaxFecundityEgg <- 4.63649558808703

  # temperature dependent Development Rate (Egg to Adult) (Saryazdi and Cheriet,
2007; Ryan et Al., 2016)
cA <- 0.00015
cM <- 4.39

cTlower <- 8.1                #(°C)
cTupper <- 30.9               #(°C)

  # cumulative Degree Day sum (cumDD) upper and lower thresholds
threshold.lower <- 10         #(°C)
threshold.upper <- 30         #(°C)

  # maximum age of female Drosophila suzukii (Emiljanowicz et al., 2014)
ageMax <- 86

  #pre-oviposition period of female Drosophila suzukii (Kinjo et al., 2014,
supplementary material)
preOvipositionPeriod <- 3     #(days)

  # start female population
cStartPopulation <- 8000
cMean <- 30
cSD <- 20

  # start date population model (min)
```

```
setStartCalculation <- 60
setStartCalculation_2 <- setStartCalculation / 2

# initiating variables
calculationDataList <- list()
outputPopulation <- list()


# Agrii Weather Data and calculation of cumDD
################################################################################
# the following layout for the weather data is mandatory
# date (number)
# time
# air temperature
# relative humidity
# rain
# pyranometer
# leaf wetness
# soil temperature (15cm)
# soil temperature (45cm)

# list with the files to import
listFiles <-list.files(paste(getwd(),"R_Input","AgriiWeather","DataFrame_csv", sep
= "/"))

# loop through all the weather files in the "DataFrame_csv" folder
for(j in 1:length(listFiles)){
  no.file<-j

  # importing file into temporary file
  metData <- read.csv(file =
paste(getwd(),"R_Input","AgriiWeather","DataFrame_csv",listFiles[no.file], sep =
"/"), sep = ";",dec = ".",header = T)

  # tempory file to store weather data (date, time, airTemp, RH, rain)
  DD<- data.frame(date = metData$Date, time = metData$Time, airTemp = metData[,3],
RH = metData[,4], rain = metData[,5])
  DD$cumDD_Min <- NA
  DD$cumDD_Day <- NA

  # calculating DD
  for(i in 1:length(metData[,3])){
    if(i==1){
      if(metData[i,3]>threshold.lower){
        DD$cumDD_Min[i]<-(15*(metData[i,3]-threshold.lower))
      }else{DD$cumDD_Min[i]<-0}
    }else if (is.na(metData[i,3])){
      DD$cumDD_Min[i]<-DD$cumDD_Min[(i-1)]
    }else if(metData[i,3]>threshold.lower && metData[i,3]<threshold.upper){
      DD$cumDD_Min[i]<-DD$cumDD_Min[(i-1)]+(15*(metData[i,3]-threshold.lower))
    }else if(metData[i,3]>threshold.upper){
      #whitout vertical cutoff
      DD$cumDD_Min[i]<-DD$cumDD_Min[(i-1)]+(15*(threshold.upper-threshold.lower))
    }else{
      DD$cumDD_Min[i]<-DD$cumDD_Min[(i-1)]
    }
  DD$cumDD_Day[i] <- DD$cumDD_Min[i] / 1440
  }
```

```
  #save file in list of dataframes
  calculationDataList[[no.file]] <- DD

  #clear variables
  metData <- NA
  DD <- NA
}

#clear workspace
remove(DD,i,j,metData,no.file)

# cumDD plots per farm
for (i in 1:length(listFiles)){
  no.file <- i
  DD <- calculationDataList[[no.file]]

  nameFile <- paste("DD_", substr(listFiles[no.file],start = 0,stop =
(stri_length(listFiles[no.file])-4)), ".jpg", sep = "")
  outputPath <- paste(getwd(),"R_Plot","DDSum",nameFile,sep = "/")
  jpeg(file = outputPath,width = 600,height = 600,units = "px")
  plot(DD$cumDD_Day, col="blue",type = "l",axes = T,ylab = "cumDD (day)",xlab =
"Date (rownumber)",xlim = c(0,35060),ylim = c(0,1500))
  title(paste(substr(listFiles[no.file],start = 0,stop =
(stri_length(listFiles[no.file])-4)),": cum DD (day)",sep = ""))
  dev.off()
}

#clear workspace
remove(DD,i,no.file)

# output file to output folder
for (i in 1:length(listFiles)){
  no.file <- i
  DD <- calculationDataList[[no.file]]

  nameFile <- paste("DD_", substr(listFiles[no.file],start = 0,stop =
(stri_length(listFiles[no.file])-4)), ".csv", sep = "")
  outputPath <- paste(getwd(),"R_output","DDSum",nameFile,sep = "/")
  write.table(DD,file = outputPath, sep=";",row.names = F)
}

#clear workspace
remove(nameFile,outputPath,no.file,i,DD)


# Combined cumDD plot for 2014 and 2015 for all farms
################################################################################
#sort filenames
name.2014 <- NA
name.2015 <- NA
a <- 1
b <- 1
for(i in 1:length(listFiles)){
  if(i<8){
        name.2014[a] <- substr(listFiles[i],start = 0,stop =
(stri_length(listFiles[i])-4))
    a <- a+1
  }else{
```

```
        name.2015[b] <- substr(listFiles[i],start = 0,stop =
(stri_length(listFiles[i])-4))
    b <- b+1
  }
}

# combined cumDD plots
colPlot <-
c("mediumblue","magenta","orangered","royalblue","seagreen","palevioletred3","peru
","purple","sienna")
dev.new()
j <- 1
for (i in 1:length(listFiles)){
  if(i<8){
    plot(calculationDataList[[i]]$cumDD_Day, col=colPlot[j],type = "l",axes =
T,ylab = "cumDD (day)",xlab = "Date (rownumber)",xlim = c(0,35060),ylim =
c(0,1200))
    par(new = T)
    j <- j + 1
  }else if(i==8){
    legend("topleft", "(x,y)",legend = name.2014)
    abline(h=firstEgg.lower/1440, col = "black")
    abline(h=firstEgg.mean/1440,col = "red")
    abline(h=firstEgg.upper/1440, col = "black")
    title("cumDD (day) 2014")
    par(new = F)

    j <- 1
    dev.new()
    plot(calculationDataList[[i]]$cumDD_Day, col=colPlot[j],type = "l",axes =
T,ylab = "cumDD (day)",xlab = "Date (rownumber)",xlim = c(0,35060),ylim =
c(0,1200))
    par(new = T)
    j <- j + 1
  }else{
  plot(calculationDataList[[i]]$cumDD_Day, col=colPlot[j],type = "l",axes = T,ylab
= "cumDD (day)",xlab = "Date (rownumber)",xlim = c(0,35060),ylim = c(0,1200))
  par(new = T)
  j <- j + 1
  }
}
legend("topleft", "(x,y)",legend = name.2015,col = colPlot[1:9])
abline(h=firstEgg.lower/1440, col = "black")
abline(h=firstEgg.mean/1440,col = "red")
abline(h=firstEgg.upper/1440, col = "black")
title("cumDD (day) 2015")
par(new = F)

#clear workspace
remove(a,b,i,j)
```

```
# Female Population modelling
################################################################################

# assumptions:
# All the eggs laid by Drosophila suzukii females emerge
# Pre-oviposition period of 3 days (Kinjo et al., 2014)
# Male / Female => 50 / 50
# The number of eggs laid by female D. suzukii flies depends of the age of the
females and the temperature

# Loading Data Frames
listFiles <-list.files(paste(getwd(),"R_Output","DDSum", sep = "/"))
for(no.file in 1:length(listFiles)){
  calculationDataList[[no.file]] <- read.csv(file =
paste(getwd(),"R_Output","DDSum",listFiles[no.file], sep = "/"), sep = ";",dec =
".",header = T)
}

# importing development and mortality rate (min)
DevAndMortRate <- read.csv(file =
paste(getwd(),"R_Input","FormulaLitrature","DevelopmentMortalityRateMIN.csv", sep
= "/"), sep = ";",dec = ".",header = T)

# for each file the same calculation
for (no.file in 1:length(listFiles)){
weatherInput <- calculationDataList[[no.file]]  #tempory file
yearlength <- length(weatherInput$date)
weatherInput$femalePopulation <- NA

#initiating variables
ageCategory <- data.frame(age = NA, noFemales = NA)
for (i in 1:ageMax){
  ageCategory[i,1] <- i
  ageCategory[i,2] <- (dnorm(ageCategory$age[i],mean = cMean,sd = cSD, log =
FALSE))*cStartPopulation
}

eggDevelopment <- data.frame(noEggs = NA, development = NA,keep = NA)
for(i in 1:3){
  eggDevelopment[1,i] <- NA
}

# temporary variables
temp.mortality <- NA
temp.development <- NA
temperature <- NA
temp.population <- NA
temp.eggs <- NA
sumEmergedEggs <- 0
sumEggs <- NA
daycounter <-NA
newFile <- FALSE
startCalculate <- FALSE
changeDay <- FALSE

newFile <- TRUE
startCalculate <- FALSE

# sum start population 1st of January
```

```
startPopulation <- 0
for (i in 1:length(ageCategory$age)){
  startPopulation <- startPopulation + ageCategory[i,2]
}
weatherInput$femalePopulation[1] <- startPopulation

# For each row in each file
for (year.rows in 1:yearlength){
  # each row <- save temperature
  temperature <- weatherInput$airTemp[year.rows]

  # check if there is a new day (exception for day 1)
  if(year.rows == 1){
    daycounter <- weatherInput$date[year.rows]
  }else{}

  if(weatherInput$date[year.rows] != daycounter){
    daycounter <- weatherInput$date[year.rows]
    changeDay <- TRUE
  }else{
    changeDay <- FALSE
  }

  #check if the start cumDD is reached
   if ((newFile == TRUE) &&
(weatherInput$cumDD_Min[year.rows]>setStartCalculation)){
    newFile <- FALSE
    startCalculate <- TRUE
   }else{}


  if (startCalculate == TRUE){
    if(changeDay==TRUE){
      changeDay <- FALSE
      #ageclass +1
      temp.population <- ageCategory$noFemales
      for (i in 1:length(ageCategory$age)){
        if (i==1){
          ageCategory$noFemales[i] <- 0
        }else{
          ageCategory$noFemales[i] <- temp.population[i-1]
        }
      }

      # Female fecundity for each day
      sumEggs <- 0
      for (a in preOvipositionPeriod:length(ageCategory$age)){
        # for each age category the fecundity per female (number of eggs per day
per female)
        percentageAge <-
((0.585*ageCategory$age[a])/(1.0475^ageCategory$age[a]))/cMaxFecundityEgg
        if((temperature>cTmin) && (temperature<cTmax)){
          temp.eggs <-
(cAlpha*(((cGamma+1)/(pi*cLambda^(2*cGamma+2)))*((cLambda^(2)-((temperature-
cTau)^(2)+cDelta^(2))))^(cGamma)))*percentageAge *4
        }else{temp.eggs <- 0}

        temp.eggs <- (ageCategory$noFemales[a]*temp.eggs)
        sumEggs <- sumEggs + temp.eggs
```

```
      }
      temp.row <- c(sumEggs,0,TRUE)
      eggDevelopment <- rbind(eggDevelopment,temp.row)

      #sum of emerged eggs to age class 0
      ageCategory$noFemales[1] <- sumEmergedEggs*0.5 #50/50 males/females
(assumption)
      sumEmergedEggs <- 0

    }else{}

    #calculations every 15 min
    # lookup mortality rate
    if(temperature >= 0 | temperature <= 31){
      for (i in 1:length(DevAndMortRate$temp)){
        if (temperature == DevAndMortRate$temp[i]){
          temp.mortality <- DevAndMortRate$MortalityRate[i]
        }else{
          paste("Error:","temperature not found in table range",sep = " ")
        }
      }
    }else{
      temp.mortality <- 0.005208 #mortality rate of 0.5/day recalculated to 15min
    }

    # calculate egg development
    if((temperature>cTlower) && (temperature<cTupper)){
      temp.development <- (cA*temperature*(temperature-cTlower)*((cTupper-
temperature)^(1/cM)))/(1440/15)
    }else{temp.development <- 0}


    # population decrease for each age category
    temp.sumFemales <- 0
    for (i in 1:length(ageCategory$age)){
      ageCategory$noFemales[i] <- ageCategory$noFemales[i]-
(ageCategory$noFemales[i]*(temp.mortality*15))
      temp.sumFemales <- temp.sumFemales + ageCategory$noFemales[i]
    }
    weatherInput$femalePopulation[year.rows] <- temp.sumFemales

    # development rate for each egg (15min) sum eggs gather untill the and of the
day
    for (i in 1:length(eggDevelopment$noEggs)){
      if (eggDevelopment$noEggs[i]==0 | is.na(eggDevelopment$noEggs[i])){
        #eggDevelopment$keep[i]<- FALSE
      }else{
        eggDevelopment$development[i] <-
(eggDevelopment$development[i]+(temp.development))
        if (eggDevelopment$development[i]>1){
          eggDevelopment$keep[i] <- FALSE
          sumEmergedEggs <- sumEmergedEggs+eggDevelopment$noEggs[i]
        }else{
          eggDevelopment$keep[i] <- TRUE
        }
      }
    }
    # remove the emerged eggs
    eggDevelopment <- eggDevelopment[eggDevelopment$keep == TRUE,]
```

```
  }else{
    if(year.rows!=1){
      if ((weatherInput$cumDD_Min[year.rows]>setStartCalculation_2)){
        temp.sumFemales <- 0
        if(temperature >= 0 | temperature <= 31){
          for (i in 1:length(DevAndMortRate$temp)){
            if (temperature == DevAndMortRate$temp[i]){
              temp.mortality <- DevAndMortRate$MortalityRate[i]
            }else{
              paste("Error:","temperature not found in table range",sep = " ")
            }
          }
        }else{
          temp.mortality <- 0.005208 #mortality rate of 0.5/day recalculated to
15min
        }
        for (i in 1:length(ageCategory$age)){
          ageCategory$noFemales[i] <- ageCategory$noFemales[i]-
(ageCategory$noFemales[i]*(temp.mortality*15))
          temp.sumFemales <- temp.sumFemales + ageCategory$noFemales[i]
        }
        weatherInput$femalePopulation[year.rows] <- temp.sumFemales
      } else {
        temp.population <- 0
        for (i in 1:length(ageCategory$age)){
          temp.population <- temp.population + ageCategory[i,2]
        }
        weatherInput$femalePopulation[year.rows] <- temp.population
        }
      }else{}
  }
}
outputPopulation[[no.file]]  <- weatherInput
}

# ouput to .csv files
populationYear <-2014
i<-1
for (no.file in 1:length(outputPopulation)){
  outputDataFrame <- outputPopulation[[no.file]]
  if(no.file == 8){
    i <- 1
    populationYear <- 2015
  }else{}
  nameFile <- paste(populationYear,"_","Population", "Farm", i, ".csv", sep = "")
  outputPath <- paste(getwd(),"R_output","PopulationData",nameFile,sep = "/")
  write.table(outputDataFrame,file = outputPath, sep=";",row.names = F)
  i <- i+1
}
```

```
# Validation Population Model
################################################################################
# loading theoretical population files (2015 = last seven files of the map)
listFiles <-list.files(paste(getwd(),"R_Output","PopulationData", sep = "/"))
theoreticalPopulation <- list()
for(no.file in 8:length(listFiles)){
  theoreticalPopulation[[no.file-7]] <- read.csv(file =
paste(getwd(),"R_Output","PopulationData",listFiles[no.file], sep = "/"), sep =
";",dec = ".",header = T)
}
# loading monitoring files (2015)
listFiles <-list.files(paste(getwd(),"R_Input","NationalMonitoring_2015", sep =
"/"))
monitoringPopulation <- list()
for(no.file in 1:length(listFiles)){
  monitoringPopulation[[no.file]] <- read.csv(file =
paste(getwd(),"R_Input","NationalMonitoring_2015",listFiles[no.file], sep = "/"),
sep = ";",dec = ".",header = T)
}

# normalisation
# theoretical population
norm.theoreticalPopulation <- list()
for (no.file in 1:length(theoreticalPopulation)){
temp.theoPopulation <- theoreticalPopulation[[no.file]]
temp.max <-0
for (i in 1:length(temp.theoPopulation$date)){
  if (temp.max < temp.theoPopulation$femalePopulation[i]){
    temp.max <- temp.theoPopulation$femalePopulation[i]
  } else {}
}

temp.theoPopulation$normal <- NA
for (i in 1: length(temp.theoPopulation$date)){
  temp.theoPopulation$normal[i] <-
temp.theoPopulation$femalePopulation[i]/temp.max
}
norm.theoreticalPopulation[[no.file]] <- temp.theoPopulation
}
# monitoring population
norm.monitoringPopulation <- list()
for (no.file in 1:length(monitoringPopulation)){
  temp.moniPopulation <- monitoringPopulation[[no.file]]
  x <- !is.na(temp.moniPopulation$total)
  removeNA <- NA
  r <- 1
  # remove NA lines
  for (i in 1:length(x)){
    if(!x[i]){
      removeNA[r] <- i
      r <- r + 1
    }
  }
  temp.moniPopulation <- temp.moniPopulation[-removeNA,]

  temp.moniPopulation$normalMale <- NA
  temp.moniPopulation$normalFemale <- NA
  temp.moniPopulation$normalTotal <- NA
  if(no.file == 2 || no.file == 3){}else{
```

```
  temp.moniPopulation$normalCrop <- NA
  temp.moniPopulation$normalWild <- NA
  }
  temp.maxMale <-0
  temp.maxFemale <- 0
  temp.maxTotal <- 0
  temp.maxCrop <- 0
  temp.maxWild <- 0
  # male
  for (j in 1:length(temp.moniPopulation$date)){
    if (temp.maxMale < temp.moniPopulation$male[j]){
      temp.maxMale <- temp.moniPopulation$male[j]
      } else {}
  }
  # female
  for (j in 1:length(temp.moniPopulation$date)){
    if (temp.maxFemale < temp.moniPopulation$female[j]){
      temp.maxFemale <- temp.moniPopulation$female[j]
    } else {}
  }
  # total
  for (j in 1:length(temp.moniPopulation$date)){
    if (temp.maxTotal < temp.moniPopulation$total[j]){
      temp.maxTotal <- temp.moniPopulation$total[j]
    } else {}
  }
  if(no.file == 2 || no.file == 3){}else{
  # crop
  for (j in 1:length(temp.moniPopulation$date)){
    if (temp.maxCrop < temp.moniPopulation$crop[j]){
      temp.maxCrop <- temp.moniPopulation$crop[j]
    } else {}
  }
  # wild
  for (j in 1:length(temp.moniPopulation$date)){
    if (temp.maxWild < temp.moniPopulation$wild[j]){
      temp.maxWild <- temp.moniPopulation$wild[j]
    } else {}
  }
  }

  # normalisation
  for (i in 1: length(temp.moniPopulation$date)){
    temp.moniPopulation$normalMale[i] <- temp.moniPopulation$male[i]/temp.maxMale
    temp.moniPopulation$normalFemale[i] <-
temp.moniPopulation$female[i]/temp.maxFemale
    temp.moniPopulation$normalTotal[i] <-
temp.moniPopulation$total[i]/temp.maxTotal
    if(no.file == 2 || no.file == 3){}else{
    temp.moniPopulation$normalCrop[i] <- temp.moniPopulation$crop[i]/temp.maxCrop
    temp.moniPopulation$normalWild[i] <- temp.moniPopulation$wild[i]/temp.maxWild
    }
  }

  norm.monitoringPopulation[[no.file]] <- temp.moniPopulation
  }

# select dates in theoretical population that match the monitoring population
validation <- list()
```

```
for(no.file in 1: length(norm.theoreticalPopulation)){
 temp.theoretic <- norm.theoreticalPopulation[[no.file]]
 temp.monitoring <- norm.monitoringPopulation[[no.file]]
 temp.dataFrame <- data.frame(date = NA,airTemp = NA, RH = NA, cumDD_Day = NA,
femalePopulation = NA, theoreticNormal = NA,
                              actualMale = NA, actualFemale = NA, actualTotal =
NA, actualCrop = NA, actualWild = NA, normalMale = NA,
                              normalFemale = NA, normalTotal = NA, normalCrop =
NA, normalWild = NA)
 temp.date <- NA
 temp.airTemp <- NA
 temp.RH<- NA
 temp.cumDD_Day<- NA
 temp.femalePopulation<- NA
 temp.theoreticNormal<- NA
 temp.normalFemale<- NA
 temp.normalMale<- NA
 temp.normalTotal<- NA
 temp.normalCrop<- NA
 temp.normalWild<- NA
 temp.actualMale<- NA
 temp.actualFemale<- NA
 temp.actualTotal<- NA
 temp.actualCrop<- NA
 temp.actualWild<- NA

 i <- 1
 for (rowNumber in 1: length(temp.theoretic$date)){
   if((temp.monitoring$date[i] == temp.theoretic$date[rowNumber]) &&
(i<=length(temp.monitoring$date))){
     temp.date <- temp.monitoring$date[i]
     temp.airTemp <- temp.theoretic$airTemp[rowNumber]
     temp.RH <- temp.theoretic$RH[rowNumber]
     temp.cumDD_Day <- temp.theoretic$cumDD_Day[rowNumber]
     temp.femalePopulation <- temp.theoretic$femalePopulation[rowNumber]
     temp.theoreticNormal <- temp.theoretic$normal[rowNumber]

     temp.actualMale <- temp.monitoring$male[i]
     temp.actualFemale <- temp.monitoring$female[i]
     temp.actualTotal <- temp.monitoring$total[i]
     temp.actualCrop <- temp.monitoring$crop[i]
     temp.actualWild <- temp.monitoring$wild[i]
     temp.normalMale <- temp.monitoring$normalMale[i]
     temp.normalFemale <- temp.monitoring$normalFemale[i]
     temp.normalTotal <- temp.monitoring$normalTotal[i]
     if (no.file == 2 || no.file == 3){} else{
     temp.normalCrop <- temp.monitoring$normalCrop[i]
     temp.normalWild <- temp.monitoring$normalWild[i]
     }
     newRow <-
c(temp.date,temp.airTemp,temp.RH,temp.cumDD_Day,temp.femalePopulation,

temp.theoreticNormal,temp.actualMale,temp.actualFemale,temp.actualTotal,

temp.actualCrop,temp.actualWild,temp.normalMale,temp.normalFemale,
                 temp.normalTotal,temp.normalCrop,temp.normalWild)
     temp.dataFrame <- rbind(temp.dataFrame,newRow)
     i <- i +1
   }else{}
```

```
 }
  temp.dataFrame <- temp.dataFrame[-1,]
  validation[[no.file]] <- temp.dataFrame
}

# statistical comparison
output.validation <- list()

for (no.file in 1:length(validation[])){
  output.dataframe <- data.frame(male = NA, female = NA, total = NA, crop = NA,
wild = NA)

  output.dataframe[1,1] <-
cor(validation[[no.file]]$theoreticNormal,validation[[no.file]]$normalMale, use =
'complete.obs',method = "spearman")
  output.dataframe[1,2] <-
cor(validation[[no.file]]$theoreticNormal,validation[[no.file]]$normalFemale, use
= 'complete.obs',method = "spearman")
  output.dataframe[1,3] <-
cor(validation[[no.file]]$theoreticNormal,validation[[no.file]]$normalTotal, use =
'complete.obs',method = "spearman")
  if (no.file == 2 || no.file == 3){} else{
  output.dataframe[1,4] <-
cor(validation[[no.file]]$theoreticNormal,validation[[no.file]]$normalCrop, use =
'complete.obs',method = "spearman")
  output.dataframe[1,5] <-
cor(validation[[no.file]]$theoreticNormal,validation[[no.file]]$normalWild, use =
'complete.obs',method = "spearman")
  }
  output.dataframe[2,1] <-
NSE(validation[[no.file]]$theoreticNormal,validation[[no.file]]$normalMale)
  output.dataframe[2,2] <-
NSE(validation[[no.file]]$theoreticNormal,validation[[no.file]]$normalFemale)
  output.dataframe[2,3] <-
NSE(validation[[no.file]]$theoreticNormal,validation[[no.file]]$normalTotal)
  if (no.file == 2 || no.file == 3){} else{
  output.dataframe[2,4] <-
NSE(validation[[no.file]]$theoreticNormal,validation[[no.file]]$normalCrop)
  output.dataframe[2,5] <-
NSE(validation[[no.file]]$theoreticNormal,validation[[no.file]]$normalWild)
  }

  row.names(output.dataframe) <- c("correlation","NSE")

  output.validation[[no.file]] <- output.dataframe
}
# output validation
populationYear <- 2015
for (no.file in 1:length(output.validation)){
  outputDataFrame <- output.validation[[no.file]]
  nameFile <- paste(populationYear,"_","Validation", "Farm", no.file, ".csv", sep
= "")
  outputPath <- paste(getwd(),"R_output","Validation",nameFile,sep = "/")
  write.table(outputDataFrame,file = outputPath, sep=";",row.names = T)
}
```

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