Euphresco Network for phytosanitary research coordination and funding

Final Report

For more information and guidance on completion and submission of the report contact the Euphresco Call Secretariat (bgiovani@euphresco.net).

Project Title (Acronym)
IPM Strategies against *Drosophila suzukii* (IPMDROS)

Project Duration:

Start date:	01/07/14
End date:	31/12/16

1. Research Consortium Partners

Coordinator – Partner 1			
Organisation	National Institute for Agricultural and Food Research and Technology - Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)		
Name of Contact (incl. Title)	Dr. Ismael Sánchez Ramos (Project Coordinator) Ms Anabel de la Peña (Topic Coordinator)	**Gender**:	M F
Job Title	Research Scientist Head of Service of relations with EU		
Postal Address	Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Carretera de La Coruña, Km 7.5, 28040 Madrid, Spain		
E-mail	ismael@inia.es anaisabel.delapena@inia.es		
Phone	00 34 913473998 00 34 913478776		

Partner 2			
Organisation	Austrian Agency for Health and Food Safety (AGES) Institute for Sustainable Plant Production		
Name of Contact (incl. Title)	Mag. Dr. Christa Lethmayer DI Alois Egartner Univ. Doz. DI Dr. Sylvia Blümel	**Gender**:	F M F
Job Title	Research scientist, local coordinator Research scientist Head of department		
Postal Address	Spargelfeldstrasse 191, A-1220 Vienna, Austria		
E-mail	christa.lethmayer@ages.at alois.egartner@ages.at sylvia.bluemel@ages.at		
Phone	0043/50555-33311 0043/50555-33316 0043/50555-33300		

Partner 3			
Organisation	Research Council for Agriculture and Economics – Research Centre for Agrobiology and Pedology (CREA-ABP).		
Name of Contact (incl. Title)	Dr. Sauro Simoni Dr. Elisabetta Gargani	**Gender**:	M F
Job Title	Research scientist Research scientist		
Postal Address	via di Lanciola 12/a 50125 Florence (Italy)		
E-mail	sauro.simoni@crea.gov.it elisabetta.gargani@crea.gov.it		
Phone	+39 055 2492229 +39 055 2492241		

Partner 4			
Organisation	Institute for agricultural and Fisheries Research (ILVO)		
Name of Contact (incl. Title)	Hans Casteels Madelena de Ro	**Gender**:	M F
Job Title	Research scientist and head of the Entomology lab PhD Student		
Postal Address	Institute for agricultural and Fisheries Research (ILVO), Plant Science Unit, Crop Protection, Burgemeester Van Gansberghelaan 96, B-9820 Merelbeke, Belgium		
E-mail	hans.casteels@ilvo.vlaanderen.be madelena.dero@ilvo.vlaanderen.be		
Phone	(32)-9-272 24 56 (32)-9-272 24 65		

Partner 5			
Organisation	General Directorate of Agricultural Research (GDAR)- Plant Protection Central Research Institute (PPCRI)		
Name of Contact (incl. Title)	Dr. Vildan Bozkurt (Local coordinator) Dr. Ayse Ozdem Mr. Erdogan Ayan	**Gender**:	F F M
Job Title	Research Scientist Research Scientist and Vise Manager of the Institute Research Scientist		
Postal Address	Plant Protection Central Research Institute. Gayret Mahallesi, Fatih Sultan Mehmet Bulvari No: 66 Yenimahalle Ankara / Turkey		
E-mail	vildan.bozkurt@tarim.gov.tr ayse.ozdem@tarim.gov.tr erdogan.ayan@tarim.gov.tr		
Phone	00-90-312 344 59 94 /1123 00-90-312 344 74 30 00-90-312 344 59 94/1124		

2. Executive Summary

Project Summary

The spotted-wing drosophila (SWD), *Drosophila suzukii*, is a polyphagous invasive pest species native to Asia that is able to attack a wide variety of small-fruit crops. This fly is able to lay eggs in fresh ripening fruits due to the serrated ovipositor of the female. The insertion of the ovipositor produces physical damage to the host fruits, and the feeding larvae cause soft and rot fruits. Resulting damage can be up to 80% crop loss. In this context, the development of IPM programs for the control of *D. suzukii* is of great importance to reduce the huge economic impact that this fly can potentially exert on European agriculture.

The main objectives of the project were: to improve the basic knowledge about the biology of the fly, including the effect of temperature on development, reproduction and population increase and the overwintering behaviour; to develop effective trapping systems for population reduction; to evaluate alternative methods for control, such as insect growth regulators and entomopathogenic microorganisms; and to perform monitoring surveys for early detection and to develop quarantine measures and effective surveys of goods in global trade among countries.

Effect of temperature on biological parameters of *D. suzukii*

Laboratory assays were performed to quantify the effect of constant temperatures on the development and reproduction of the fly using artificial diet as food source. Different mathematical models were fitted to the results obtained. The range of temperatures to complete the preimaginal period of this fly is between 8.7°C and 30.9°C. The highest survival is observed around 20°C, but the development period is the shortest at 28°C and in general, it takes less than two weeks above 22°C, indicating that multiple generations can exist after winter if suitable food is available. This fly has a high reproductive potential, and the highest fecundity was registered at 22°C. However, the greatest population increase was around 25°C. At this temperature, a population of SWD doubles its numbers in only three days. This explains the huge potential of this fly to produce damage in susceptible crops. Longevity can be prolonged for more than 100 days at 16°C, and this is surely higher at lower temperatures. This result explains the overwintering capacity of this fly and the recolonization of susceptible crops after winter break.

Cold tolerance and overwintering biology of *D. suzukii*

Cold tolerance and overwintering potential of SWD is crucial to anticipate population build-up and dynamics in the early season. Different experiments revealed that winter morph adults were more resilient to direct and indirect chilling injury than summer morphs. In winter conditions, oviposition by winter morph females was ceased for approximately six weeks. Females of any age exposed to more favourable conditions could resume egg-laying within one week. These results could indicate the occurrence of a short and shallow reproductive diapause in winter morph females of SWD.

The influence of temperature on SWD populations was also evaluated under field conditions. Positive correlation between increasing field temperatures and abundance of SWD adults was found in coastal and inland areas of central Italy in winter and early spring. The SWD abundances recorded were better fit by temperature data interpolated by the Allen method than by the average daily temperature. The Allen interpolation of temperature data seems to give stable forecasting to be considered in strategies to SWD control.

Evaluation of alternative control strategies

Different insect growth regulators (lufenuron, chitin-synthesis inhibitor; pyriproxyfen, juvenile hormone mimic; cyromazine, moulting disruptor; and azadirachtin, botanical compound which inhibits reproduction and has sterilising effects) were tested against SWD adults to establish their effect on fertility and fecundity. The products were administered by ingestion mixed with the food. The emergence of the offspring of treated flies decreased drastically for cyromazine, lufenuron and pyriproxyfen, but a continuous exposure to the insecticides was necessary to achieve this effect. Further experiments suggested a vertical transmission on the compounds assayed from the female to their offspring so that, although the eggs were able to hatch, the larvae could not continue the development. The practical use of these compounds under real conditions seems not feasible because is not likely that flies feed always from the same food sources.

In addition, the efficacy of organic labelled products for controlling the pest was evaluated. Promising results were obtained with *Metarhizium anisopliae*, an entomopathogenic fungus that caused a mortality of more than 80% on SWD adults and around 60% on SWD preimaginal stages in direct toxicity trials.

An important contribution for the control of SWD would be to find efficient traps for monitoring and mass-trapping. Enhanced self-made traps and commercially available traps were compared according their catch efficiency for SWD and the position of the traps in the orchard. A significantly higher number of SWD were collected in the self-made traps and in traps positioned at a height of 1.5 m. In addition, significantly more females were caught in the traps. Therefore, the self-developed traps are an effective, suitable alternative to commercially available traps.

Surveys and protocols for early detection of *D. suzukii* in non-invaded countries

The status of SWD in Turkey, which is considered a non-invaded country, tried to be clarified. A trapping system baited with apple vinegar was used to determine the presence of the pest in the major Turkish fruit grown areas. Training activities were organized at national level and survey and plant protection technical instructions were prepared including description, biology, hosts, pathways and damages of the pest. A leaflet was printed and distributed to technical staffs and producers to increase public awareness. SWD has not been detected during the activities of the project but a recent paper from colleagues from Turkish universities showed that the fly is already present in the East of the country.

3. Report

INTRODUCTION

The spotted-wing drosophila (SWD), *Drosophila suzukii*, is a polyphagous invasive pest species native to Asia that is able to attack a wide variety of small-fruit crops like cherries, strawberries, blackberries, blueberries, raspberries, grapes or plums (Walsh *et al*., 2011). This species is currently undergoing a rapid expansion in North America and Europe. Contrary to most of drosophilid flies that oviposit on overripe fruits, *D. suzukii* is able to lay eggs in fresh ripening fruits due to the serrated ovipositor of the female (Walsh *et al*., 2011). The insertion of the ovipositor causes physical damage to the host fruits, and the larvae that develop from the eggs cause the fruit to become soft and rot rapidly. Secondary infections by pathogens via the oviposition wound can increase the damage. Furthermore, a high variety of wild fruits can also host the spotted-wing drosophila, thus acting as reservoirs from which the fly can infest cultivated fruits (Cini *et al*. 2012). Damage produced by this pest species can be up to 80% crop loss (Walsh *et al*., 2011).

Since the first European record of the fly in Spain and Italy in 2008 (Raspi *et al*., 2011; Calabria *et al*., 2012), the spotted-wing drosophila rapidly spread through most of the Mediterranean countries and continued its colonization toward north and east (Cini *et al*., 2012) until the actual distribution almost in all countries of Europe (Cini *et al*., 2014; Asplen *et al*., 2015). In fact, it has been pointed out that the lack of reports from many areas is probably due to a lack of monitoring rather than to an actual absence of *D. suzukii* (Cini *et al*., 2012), since the ecological requirements of this fly make regions with various climatic conditions (from subtropical to continental) suitable for colonization (Walsh *et al*., 2011).

The control of this pest supposes a challenge for the production of many stone and soft fruits in Europe. The current methodologies employed for the control of *D. suzukii* are mainly based in the use of insecticides with the objective of protecting the fruits from the moment when they are nearly ripe until the harvest. Several insecticide classes have proven to be effective against this fly, like spinosyns, organophosphates, pyrethroids and neonicotinoids (Walsh *et al*., 2011). However, the range of insecticides currently available for the control of *D. suzukii* is very limited, since highly efficient broad spectrum products are being progressively restricted due to associated problems such as the accumulation of residuals, the development of resistance or negative effects on pollinators and other beneficial species (Cini *et al*., 2012).

In this context, the development of IPM programs for the control of *D. suzukii* is of great importance to reduce the huge economic impact that this fly can potentially exert on European agriculture. The IPMDROS project included research activities with the aim of generating practical tools to be used in the IPM strategies for this pest.

The General Objectives of the project were:

-To improve of basic knowledge about the biology of the fly, including overwintering behaviour and effect of temperature on development, reproduction and population increase.
-To develop effective trapping systems for population reduction.
-To evaluate alternative methods for control, such as insect growth regulators and entomopathogenic microorganisms.
-To organise surveys for early detection and development of quarantine measures and effective surveys of goods in global trade among countries.

All the objectives have been successfully achieved.

The project was organised in 5 Work Packages detailed below

Work Packages (WP)	
No. of WP	**Title**
1	Project management and co-ordination Lead : INIA
2	Effect of temperature on biological parameters of *D. suzukii* Lead: INIA
3	Cold tolerance and overwintering biology of *D. suzukii* Lead: CREA-ABP ILVO. Cold tolerance and overwintering strategies of *D. suzukii* CREA-ABP. Seasonal field trapping and laboratory evaluation aimed at definition of intervals for switch on/off overwintering *D. suzukii* times
4	Evaluation of alternative control strategies. Lead: AGES CREA-ABP. Evaluation of entomopathogenic microorganisms. AGES. Enhancing of trapping systems against *D. suzukii*. INIA. Effect of insect growth regulators on the fecundity and fertility of *D. suzukii*.
5	Surveys and protocols for early detection of *D. suzukii* in non-invaded countries Lead: GDAR

WP1: PROJECT MANAGEMENT AND CO-ORDINATION

The objectives of WP1 were:

- To coordinate the research activities of the consortium.
- To act as contact point for partners regarding project issues.
- To organize scientific work meetings about the progress of the research components committed.
- To coordinate dissemination activities of the results obtained in the project.

Three consortium meetings were held throughout the project, in which members of all work packages participated. Progress and plans for experiments were discussed and cooperative work was coordinated:

- Kick-off meeting, 30 October 2014, INIA, Madrid, Spain.

Each research team made a brief presentation of the main activities performed in their institutions. In addition, the background of the teams and their main research lines were presented. Then, each research team made a brief presentation of the activities committed in the framework of the IPMDROS project. Background, rationale of the proposed research, methodologies and expected results were presented. A discussion time followed each presentation.

- Second meeting, 3 December 2015, CREA-ABP, Florence, Italy.

Each research team made a presentation of the results and advances obtained during the first year of the project. A discussion time followed each presentation and new ideas and suggestions were shared to improve different problematic aspects originated during the development of the research activities proposed in the project by some partners.

- Final meeting, 15 December 2016, AGES, Vienna, Austria.

The results obtained from the activities developed during the project were presented. The results were discussed and a time schedule was agreed for the elaboration of the final report.

To ensure successful progress of the project, the project leader was in regular e-mail contact with the other WP leaders and members of the project.

WP2: EFFECT OF TEMPERATURE ON BIOLOGICAL PARAMETERS OF *D. suzukii* (INIA)

The objectives of WP2 were:

- To establish the effect of temperature on survival and development of immature stages of *D. suzukii*.
- To study the effect of temperature on adult survival and reproduction.
- To calculate the life table parameters of the fly at different constant temperatures.
- To obtain non-linear models for the relationship between temperature and developmental rate and population growth.

Methods

Assays were carried out in rearing units consisting of plastic tubes (15 cm high × 5.5 cm diameter) placed vertically in the upper half of a 5.8 cm diameter Petri dish with the upper opening covered by a piece of translucent nylon cloth held in place by a rubber band, which provided ventilation for the rearing unit (Figure 1). A round box (4 cm diameter x 2 cm height) filled with artificial diet (1.5 cm height) was introduced within each rearing unit. The diet served as food source and as oviposition substrate.

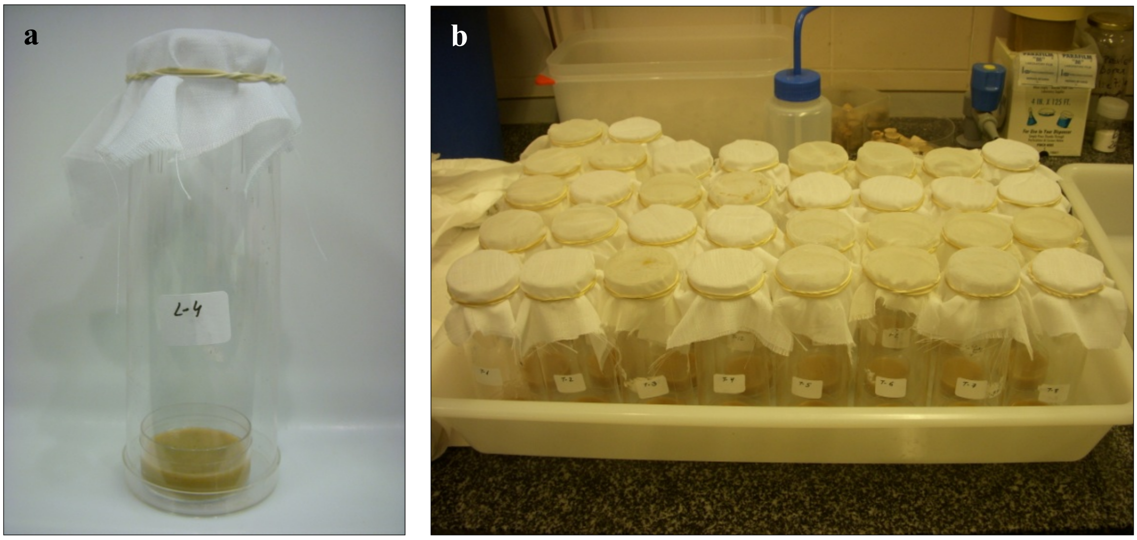

Figure 1. Rearing unit with artificial diet at the bottom (a). Plastic tray with several rearing units (b).

To determine the effect of temperature on the survival and development of the immature stages of *D. suzukii*, eggs < 2 h were used. Eleven temperatures were tested (10, 13, 16, 19, 22, 25, 27, 28, 29, 30 and 31°C). In all cases, assays were performed at 80% relative humidity (RH) and 16:08 h light:dark photoperiod.

To obtain the eggs, 10-day old adults from acclimatized cultures were introduced in six rearing cages with new artificial diet. Eggs laid in these cages were collected individually by scooping a small amount of diet around the egg with a stainless laboratory spatula and transferring the mass to round boxes containing artificial diet. Each round box containing a single egg was introduced without cover in a rearing unit.

Development was checked daily until the adult stage was reached or the immature stage died. The preimaginal survival, duration of the egg-larva and pupa stages, total duration from egg to adult and sex ratio was established.

Mortality data were fitted to the function $y = e^{a+bT+cT^2}$. The linear degree-day model and the non-linear Lactin model (Lactin *et al*., 1995) were used to fit the developmental rate data (the inverse of the developmental durations) obtained at each temperature.

For the reproduction assays, pairs of < 24 h old adult flies were used. To obtain these adults, all the adults already present in an acclimatized culture were removed, so that only pupae remained on the diet. The next day, all the adults present in the cage were < 24 h old.

The same rearing units shown in Figure 1 were used. The number of eggs laid was counted daily, and the survival of adults registered. A stock of males was maintained in additional rearing units to substitute those that died before their corresponding female during the experiment. The percentage of fertile mating, preoviposition, oviposition and postoviposition periods, the total fecundity per female, the daily fecundity per female and the male and female longevity were established. Six constant temperatures were evaluated: 16, 19, 22, 25, 27 and 29°C.

A Maxima function (Richter & Söndgerath, 1990) was used to fit the mean daily fecundity per female. The equation is

$$f(t) = \alpha t e^{(-\tau t)}$$

where *f(t)* represents the mean daily fecundity per female, α and τ are parameters, and t is time.

Survival of males and females were fitted by a Weibull function (Pinder III *et al*., 1978), whose general form is

$$S(t) = e^{[-(t/b)^{\beta}]}$$

where *S(t)* represents the probability of surviving to a given age, *b* is the parameter that describes the scale, β is the parameter that describes the shape of the curve and *t* is time.

The preimaginal development, immature and adult survival and reproduction data were used to calculate the life table parameters of *D. suzukii*. The estimated parameters were the intrinsic rate of natural increase (r_m), that is, the difference between the daily birth rate and the daily death rate; the finite rate of natural increase (λ), that is, the number of times that a population multiplies in a unit of time; the net reproductive rate (Ro), that is, the rate of multiplication in one generation; the mean generation time (T), that is, the time between the birth of a generation and the mean moment of birth of all its progeny; and the population doubling time (PDT), that is, the period of time required for a population to double in size. The Briere 2 model (Briere *et al*., 1999) was used to fit the r_m values obtained at each temperature.

In all cases, model fitting was done using Tablecurve 2D software (SYSTAT, 2002).

Results

Results obtained regarding immature development and mortality are shown in Tables 1 and 2.

Table 1. Preimaginal mortality (%) of the different developmental stages of *D. suzukii* at eleven constant temperaturas.

Temperature (°C)	Egg + Larva	Pupa	Total
10	53.3	29.6	67.1
13	30.4	1.4	31.4
16	15.5	2.8	17.8
19	4.6	0.8	5.4
22	11.3	2.9	13.9
25	13.7	0.0	13.7
27	37.6	5.1	40.8
28	48.2	7.7	52.2
29	52.2	10.4	57.2
30	64.6	32.9	76.2
31	76.6	100	100

Table 2. Duration (mean ± SE) of the different developmental stages of *D. suzukii* at eleven constant temperatures.

Temperature (°C)	Initial N	Stage				
		Egg + Larva	Pupa	Egg to Adult	Egg to Adult (male)	Egg to Adult (Female)
10	152	36.4 ± 0.4 (71)	30.0 ± 0.2 (50)	65.6 ± 0.5 (50)	65.8 ± 0.9 (21)	65.6 ± 0.7 (29)
13	102	19.0 ± 0.1 (71)	17.0 ± 0.1 (70)	36.0 ± 0.2 (70)	36.1 ± 0.2 (31)	35.9 ± 0.3 (39)
16	129	12.9 ± 0.1 (107)	11.0 ± 0.1 (104)	23.9 ± 0.1 (104)	23.8 ± 0.1 (53)	24.1 ± 0.1 (51)
19	130	9.1 ± 0.1 (124)	7.9 ± 0.1 (123)	16.9 ± 0.1 (123)	16.9 ± 0.1 (66)	17.0 ± 0.1 (57)
22	115	6.8 ± 0.1 (102)	5.7 ± 0.1 (99)	12.5 ± 0.1 (99)	12.4 ± 0.1 (52)	12.7 ± 0.1 (47)
25	102	5.7 ± 0.1 (88)	4.6 ± 0.1 (88)	10.3 ± 0.1 (88)	10.3 ± 0.1 (40)	10.4 ± 0.1 (48)
27	125	5.5 ± 0.1 (78)	4.1 ± 0.1 (74)	9.6 ± 0.1 (74)	9.5 ± 0.1 (37)	9.6 ± 0.1 (37)
28	226	5.6 ± 0.1 (117)	4.0 ± 0.1 (108)	9.5 ± 0.1 (108)	9.5 ± 0.1 (46)	9.6 ± 0.1 (62)
29	201	5.6 ± 0.1 (96)	4.2 ± 0.1 (86)	9.8 ± 0.1 (86)	9.8 ± 0.1 (40)	9.7 ± 0.1 (46)
30	429	5.7 ± 0.1 (152)	4.3 ± 0.1 (102)	9.9 ± 0.1 (102)	9.8 ± 0.1 (51)	9.9 ± 0.1 (51)
31	175	6.6 ± 0.2 (41)	-	-	-	-

Initial N: initial number of eggs established at each temperature. The sample size for each developmental stage is indicated within brackets under each value.

The mortality of the immature stages followed a typical U-shaped pattern, with high mortality values at temperatures near the developmental thresholds and low mortality values in the optimum range of temperatures. Figure 2 shows the fitting obtained to the function $y = e^{a+bT+cT^2}$. In all cases, the coefficients of determination were higher than 0.94. This model predicted lower and upper thresholds for survival of immature stages around 8-9ºC and 31ºC, respectively (Table 3).

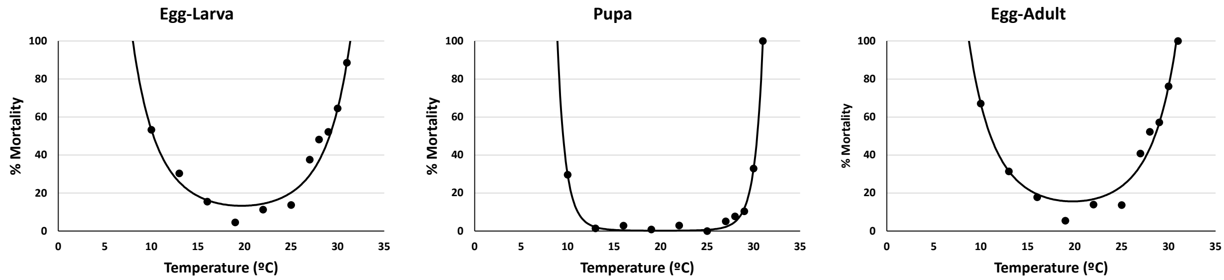

Figure 2. Percentage of mortality for the egg-larva, pupa and for the total developmental period from egg to adult of *D. suzukii* obtained at different temperatures and model fitting according to the equation $y = e^{a+bT+cT^2}$.

Developmental time decreased as temperature increased from 10 to 28°C (Table 2). Above this temperature, a small increase in developmental time was observed. The degree-day linear model was fitted to the range of temperatures 10-27°C (range for which the response of the developmental rate to temperature is linear) and the thermal constants and the lower developmental thresholds were estimated to be 104.3, 78.2 and 182.5 DD and 7.4, 8.2 and 7.8°C, respectively, for the egg-larva, pupa and egg-adult periods (Table 3). These thresholds are quite similar to those obtained by the non-linear Lactin model (Table 3). The Lactin model allowed also to estimate the optimum temperature for development (temperature at which the developmental period is shortest) around 28.5°C and the upper developmental threshold around 32-34°C. The Lactin models obtained for the egg-larva, pupa and egg-adult periods are shown in Figure 3.

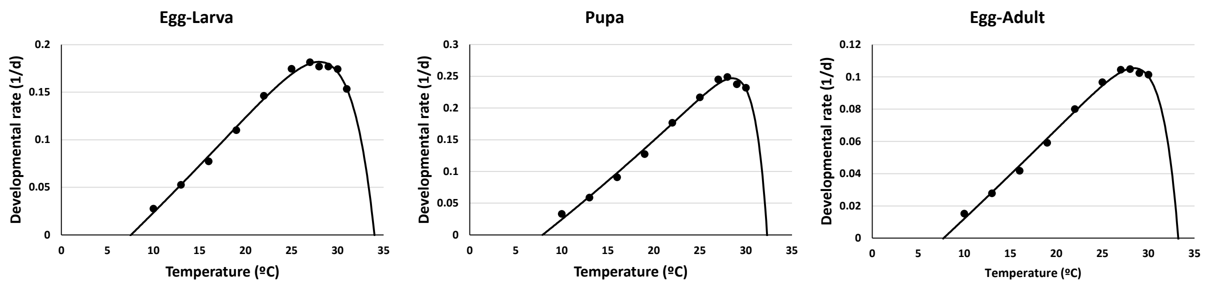

Figure 3. Lactin model for the rate of development (1/day) of *D. suzukii* egg-larva, pupa and egg-adult as a function of temperature (ºC).

Table 3. Lower and upper thresholds, optimum temperature for development and thermal constant for the immature stages of *D. suzukii*.

	Egg + Larva				Pupa				Egg to Adult			
Model	**LT**	**UT**	**OT**	**K**	**LT**	**UT**	**OT**	**K**	**LT**	**UT**	**OT**	**K**
Mortality	8.0	31.4	-	-	8.9	31.0	-	-	8.7	30.9	-	-
Linear	7.4	-	-	104.3	8.2	-	-	78.2	7.8	-	-	182.5
Lactin	7.5	34.0	28.0	-	7.9	32.4	28.5	-	7.7	33.3	28.4	-

LT: Lower developmental threshold (ºC); UT: Upper developmental threshold (ºC); OD: Optimum temperature for development (ºC); K: Thermal constant (ºDD)

Results regarding adult survival and reproduction are shown in Table 4. No significant effect of temperature was observed on the percentage of fertile matings (χ^2: 3.722, d.f.: 5, p = 0.5901). However, general linear models indicated a significant quadratic effect of temperature on the other parameters studied ($p < 0.005$). The equations of the fitted models are shown in Table 5.

Table 4. Reproductive parameters and longevity (mean ± SE) of *D. suzukii* at six constant temperatures.

Temp. (°C)	N	Fertile Matings (%)	Preoviposition Period (days)	Oviposition Period (days)	Postoviposition Period (days)	Fecundity (eggs)	Daily fecundity (eggs)	Male Longevity (days)	Female Longevity (days)
16	45	100.0	8.6 ± 0.5 (45)	57.0 ± 3.2 (45)	1.4 ± 0.2 (45)	228.4 ± 18.5 (45)	4.2 ± 0.3 (45)	83.0 ± 3.6 (45)	67.1 ± 3.2 (45)
19	45	95.6	5.8 ± 0.3 (43)	45.0 ± 2.5 (43)	1.0 ± 0.2 (43)	296.5 ± 23.7 (43)	6.7 ± 0.5 (43)	61.0 ± 3.5 (43)	51.8 ± 2.4 (43)
22	40	100.0	3.2 ± 0.2 (40)	29.5 ± 1.5 (40)	0.5 ± 0.1 (40)	341.3 ± 25.8 (40)	11.6 ± 0.7 (40)	40.9 ± 2.3 (39)	33.1 ± 1.5 (40)
25	44	97.7	3.1 ± 0.2 (43)	28.2 ± 1.6 (43)	0.7 ± 0.2 (43)	243.1 ± 17.7 (43)	8.9 ± 0.5 (43)	34.4 ± 1.7 (43)	31.9 ± 1.7 (43)
27	46	97.8	2.9 ± 0.1 (45)	26.1 ± 1.0 (44)	1.2 ± 0.3 (44)	208.5 ± 13.8 (44)	8.4 ± 0.6 (44)	27.6 ± 1.5 (44)	30.2 ± 1.2 (44)
29	49	95.9	3.5 ± 0.3 (47)	18.9 ± 1.1 (45)	1.4 ± 0.3 (45)	102.5 ± 12.3 (45)	5.1 ± 0.5 (45)	22.7 ± 1.0 (45)	23.7 ± 1.0 (45)

N: initial number of pairs established at each temperature. The sample size used to calculate the reproductive and longevity parameters is indicated within brackets after each value.

Table 5. General linear models for the temperature response of reproductive and longevity parameters of *D. suzukii*.

Parameter	N	Model
Preoviposition Period	263	$y = 44.2663 – 3.2341T + 0,0631T^2$
Oviposition Period	260	$y = 173,343 – 9,78272T + 0,1563T^2$
Postoviposition Period	260	$y = 10,4444 – 0,8702T + 0,0193T^2$
Fecundity	260	$y = –1254,05 + 149,059T – 3,5276T^2$
Daily fecundity	260	$y = –606,122 + 61,7337T – 1,3456T^2$
Male Longevity	259	$y = 283,3 – 16,9661T + 0,2765T^2$
Female Longevity	260	$y = 229,012 - 13,9804T + 0,2407T^2$

N is the total sample size used to calculate the reproductive and longevity parameters at all temperatures. The model with the highest significant term of temperature ($p \leq 0.05$) was selected. All models were highly significant ($p < 0.005$).

The graphical representation of the Maxima and Weibull models obtained are shown in Figures 4, 5 and 6.

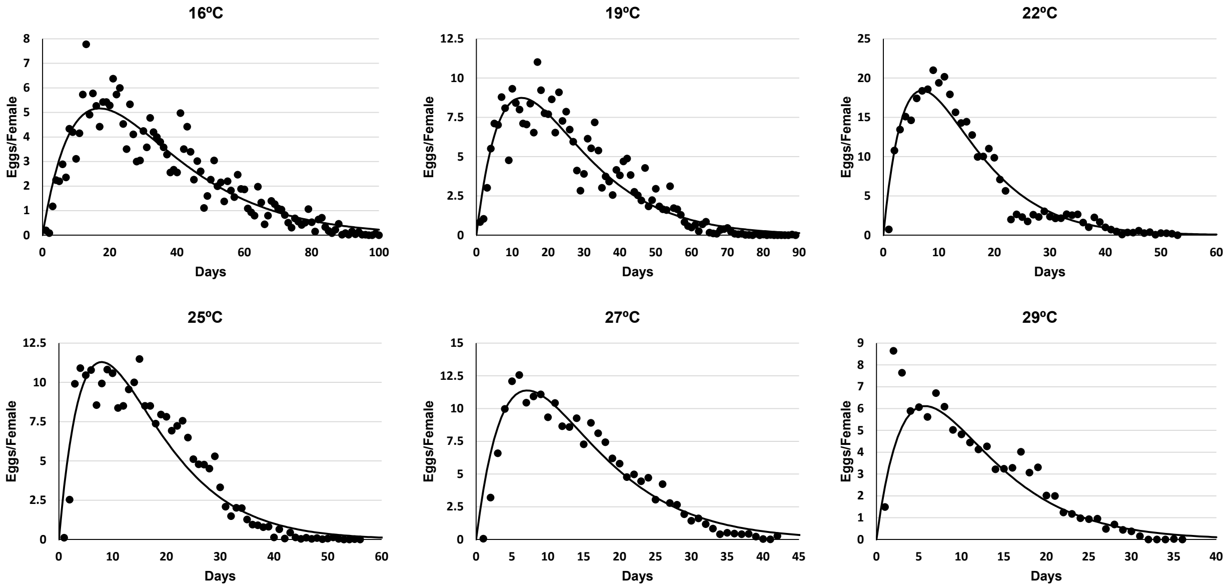

Figure 4. Maxima models for the mean daily fecundity per female of *D. suzukii* at six constant temperatures.

The Maxima function fitted well the population mean daily fecundity data for all temperatures, with R^2 values greater than 0.83. Once oviposition began, a rapid increase in egg production was observed until the maximum daily egg production was reached. The oviposition peaks predicted by the Maxima function were at 17, 13, 7, 8, 7 and 6 days after the preoviposition period for 16, 19, 22, 25, 27 and 29 °C, respectively. The number of eggs per female predicted at these peaks were 5.2, 8.7, 18.4, 11.3, 11.4 and 6.1 eggs. After that, a progressive reduction in daily egg production was observed until oviposition stopped, which extended between 40 and 100 days, depending on temperature.

The R^2 values obtained by the Weibull distribution for survival were greater than 0.99 for both sexes at all temperatures. A differential pattern for males and females was obtained in the range of temperatures between 16 and 22°C (with greater longevity for males compared to females), whereas no difference was obtained at temperatures ranging between 25 and 29°C. Differences observed at low temperatures could be due to the greater physiological cost of egg production in females, while at higher temperatures this effect might be counteracted by the smaller size of males that produces higher dehydration because of their greater surface/volume ratio.

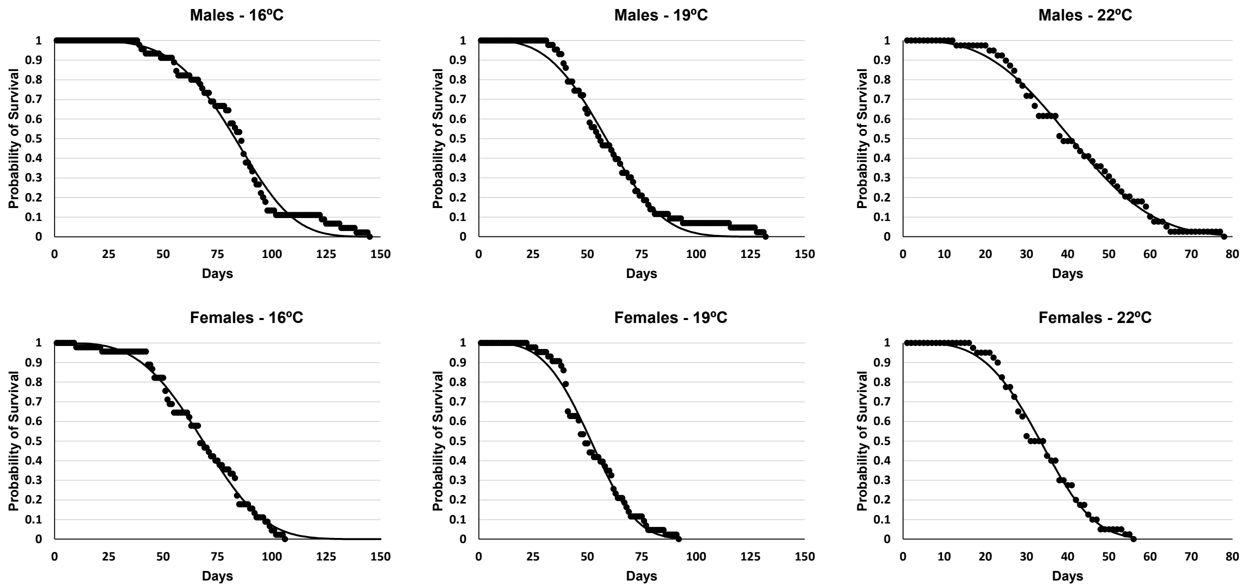

Figure 5. Weibull models for the male and female survivorship of *D. suzukii* at 16, 19 and 22°C.

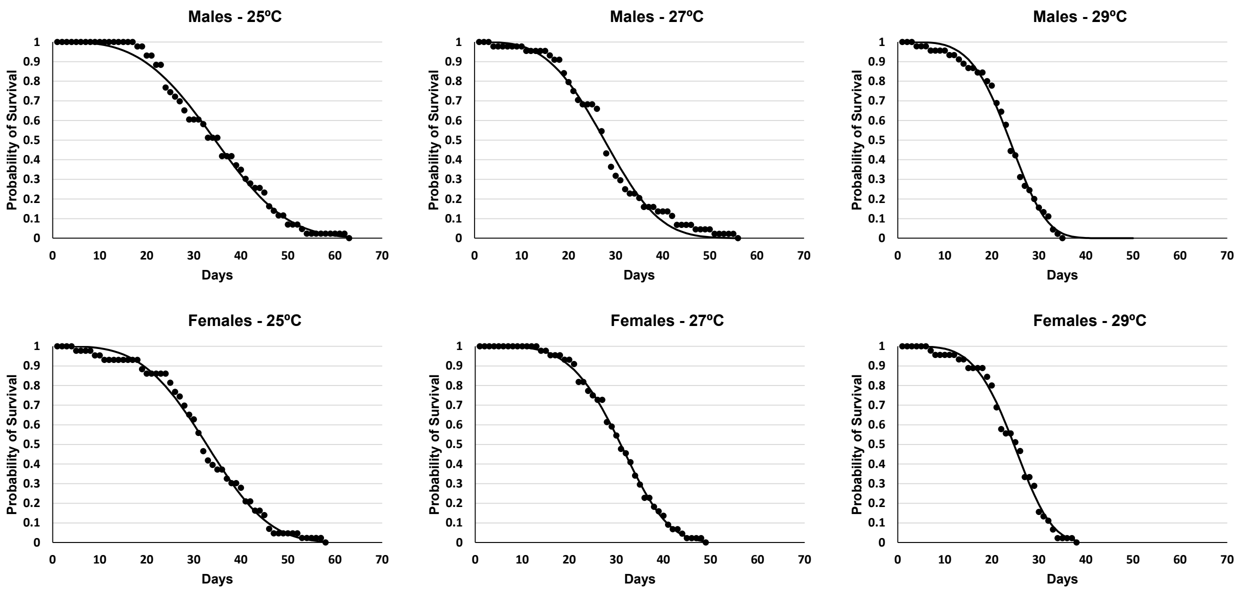

Figure 6. Weibull models for the male and female survivorship of *D. suzukii* at 25, 27 and 29ºC.

Results regarding the effect of temperature on life table parameters are shown in Table 6. The population doubling time obtained at the studied temperatures is lower than 8 days and slightly higher than 3 days at the optimum temperature (25°C), what shows the great potential for population increase of *D. suzukii*. This explains the condition of pest of this insect and its demographic explosions when the climatic conditions are favorable, as well as the damage produced in susceptible crops.

The Briere 2 model was fitted to the relationship between the intrinsic rate of increase and temperature (Figure 7). The model predicted the lower and upper thresholds for population increase at 12.0 and 31.6°C, respectively. Therefore, below 12.0°C and above 31.6°C, the populations of the fly cannot thrive.

Table 6. Life table parameters of *D. suzukii* at six constant temperatures.

Temperature (°C)	$r_m \pm$ SE	(95% CI) [1]	λ	R_0	T (days)	PDT (days)
16	0.09140 ± 0.00210	(0.08719 – 0.09561)	1.09571	93.87240	49.6930	7.6
19	0.14180 ± 0.00327	(0.13526 – 0.14834)	1.15235	133.99564	34.5402	4.9
22	0.21604 ± 0.00335	(0.20928 – 0.22281)	1.24116	146.90812	23.0967	3.2
25	0.22414 ± 0.00483	(0.21448 – 0.23381)	1.25125	102.44202	20.6536	3.1
27	0.20955 ± 0.00542	(0.19871 – 0.22039)	1.23312	60.33138	19.5650	3.3
29	0.16576 ± 0.00797	(0.14981 – 0.18171)	1.18029	21.93262	18.6292	4.2

r_m: intrinsic rate of natural increase; [1] 95% Confidence interval for r_m; λ: finite rate of natural increase; Ro: net reproductive rate; T: mean generation time; TDP: population doubling time.

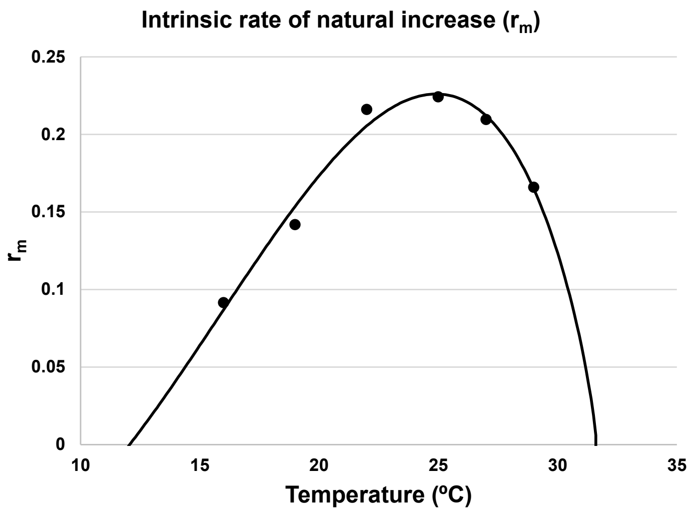

Figure 7. Briere 2 model for the intrinsic rate of natural increase of *D. suzukii* with regard to temperature.

Discussion

The results obtained reasonably agree with other studies about immature development of *D. suzukii*, although slightly shorter development periods and higher survival have been registered. This can be due to the different food employed, to the acclimatization period or to the high relative humidity employed in the assays, because it has been reported the high susceptibility of this fly to low environmental moisture. According to the mortality model obtained, the range of temperatures to complete the preimaginal period of this fly is between 8.7°C and 30.9°C. On the contrary, the Lactin model predicts the lower and upper thermal thresholds at 7.7ºC and 33.3°C, respectively. When fitting models to developmental rate data, model predictions should not be extended to the temperature range in which the species cannot survive (Okuyama, 2014). Therefore, the predictions given by the mortality model seems more accurate.

The highest survival is observed around 20°C, but the development period is shortest at 28°C and in general, it takes less than two weeks above 22°C, indicating that multiple generations can exist after winter through the seasons if suitable food is available.

This fly has a high reproductive potential, being able to lay a mean number of more than 340 eggs per female at 22°C during its life, with almost 12 eggs per day per female. Moreover, fecundity is highest in the first part of the oviposition period, so at that temperature the mean number of eggs laid per female can reach around 20 eggs. However, the greatest population increase was around 25°C. This is because the intrinsic rate of increase is a result of egg production and the speed of development and survival from egg to adult, and the combination of these parameters maximizes population increase at 25°C. At this temperature, a population of *D. suzukii* doubles its numbers in only three days. This explains the huge potential of this fly to produce damage in susceptible crops.

The threshold for population increase have been estimated at 12°C and 31.6°C. This upper threshold is not consistent with the estimated upper developmental threshold (30.9°C), since it is not possible to have population increase at a temperature higher than that resulting in 100% mortality of the immature stages. Therefore, new reproduction experiments might be necessary to obtain more accurate estimations.

Some females are able to live and lay eggs for more than 100 days at 16°C. This can be prolonged even more at lower temperatures what explains the overwintering capacity of this fly and the recolonization of susceptible crops after winter break.

The different models obtained could be used in integrated pest management programs of this pest to predict risk situations and possible new colonization areas. However, refinement of the models obtained, especially the model for the intrinsic rate of population increase *vs.* temperature, seems recommendable by studying the reproduction of the fly at a lower and a higher temperatures than those here assayed.

WP3: COLD TOLERANCE AND OVERWINTERING BIOLOGY OF *D. suzukii* (ILVO, CREA)

The objectives of WP3 were:

- To study the cold tolerance and overwintering strategies of *D. suzukii* (ILVO):
  - Study of the influence of cold temperatures on the biology of *D. suzukii*, i.e. map the cold hardiness of the species.
  - Assessment of the overwintering potential of *D. suzukii* for the European member states.
  - Identification of possible overwintering strategies exploited by *D. suzukii.*
- Seasonal field trapping and laboratory evaluation aimed at definition of intervals for switch on/off overwintering *D. suzukii* times (CREA-ABP).

Cold tolerance and overwintering strategies of *D. suzukii*

The specific objectives were to perform a characterisation of cold hardiness for various European populations of *D. suzukii* and to assess the role of diapause and cold adaptability as overwintering strategies in the establishment of *D. suzukii* in Europe

1. Characterisation of cold hardiness for various European populations of *D. suzukii* (ILVO)

Methods

Laboratory culture

A laboratory culture of *D. suzukii* was established with insects obtained from a field collection in Strijtem, Belgium (50°50'33.5"N, 4°06'41.4"E) in August 2015.

Summer morphs were reared in 50 ml tubes on an artificial corn meal diet at 25°C, 16:8h (L:D) and 65 ± 10% RH. Winter morphs were acquired by transferring the eggs of summer morph females to 10°C, 8:16h (L:D) and 65 ± 10% RH; at these conditions they were allowed to further develop.

Experiments

In this study, the cold hardiness of both summer and winter morphs of a Belgian *D. suzukii* population was examined by means of three common indices: (1) supercooling point (SCP), (2) the lower lethal temperature (LLTemp) and (3) the lower lethal time (LLTime).

Supercooling point

The SCP experiments were performed on pupae in the pharate adult stage P10 - P12 (Ashburner *et al*., 2005) and on 3-day-old males and females of both summer and winter morphs. A discrimination was made between unmated and mated females of the summer morphs. All treatment groups consisted of approximately 30 individuals. Each Individual was brought into contact with a type-T 30-gauge copper-constantan thermocouple that was put through the opening of a 1mL pipet tip and connected to a data logger (TC-08 Thermocouple Data Logger, Pico Technology, Eaton Socon, UK). Another 1mL pipet tip was mounted onto the first one to prevent the insect from escaping. This setup was placed in a Haake G50 alcohol bath (Thermo Fisher Scientific, Waltham, MA, USA), using glass tubes. Starting from 25°C, the insects' surrounding temperature (-0.5°C/min) were gradually lowered until the freezing temperature of the haemolymph was reached. The lowest temperature that was measured before the release of latent heat was determined as the SCP.

Lower lethal temperature

The LLTemp was investigated for 3-day-old males and females of winter and summer morphs. Using the same method as for the SCP, individuals were cooled down from 25°C at a rate of 0.5°C/min to a range of sub-zero temperatures and maintained at each of those temperatures for one minute (Table 7). Hereafter, the temperature was gradually increased (0.5°C/min) to 25°C and individuals were transferred to an incubator (25°C, 16:8h (L:D), 65 ± 10% RH) in separate 2 mL microcentrifuge tubes containing 0.5 mL of artificial corn meal diet. Survival of *D. suzukii* adults was assessed after 24h.

Table 7. Temperatures used to determine the LLTemp50 of summer and winter morph males and females of *D. suzukii.*

Morph	Sex	n	Temperatures (ºC)
Summer	Males	130	-8, -9, -10, -11, -11.5, -12.5, -13, -13.5
	Females	190	-5.5, -6, -7, -8, -9, -10, -11, -11.5, -12.5, -13, -13.5
Winter	Males	101	-13.5, -14, -14.5, -15, -16
	Females	99	-13.5, -14, -15, -16

Lower lethal time

LLTime experiments were performed on summer and winter morph adults kept at -5°C, 0.8°C and 6.5°C. During these tests, no light source was used and relative humidity was not controlled. Prior to the experiments, 3- to 6-day-old summer and winter flies were collected from the laboratory cultures at 25 and 10°C, respectively, and cooled with stepwise increments (30 min at 15°C and/or 30 min at 5°C) to the aspired temperature. At certain periods, subsamples of approximately 3 x 10 adults were taken and transferred stepwise to 25°C. After 24h, the number of surviving adults was recorded. At 6.5°C, adults were provided with fresh artificial diet every 6-8 weeks.

Statistical analysis

Data analysis was carried out using R version 1.0.44 (R Core Team, 2015). A significant α-value of 0.05 was used for all analyses.

The effect of mating status (virgin or non-virgin female) on the SCP of summer females was determined with an unpaired t-test (normally distributed data). Analysis of the SCPs of the treatment groups sex (male or female), morph (summer or winter) and life stage (pupa or adult) were performed using a non-parametric Kruskal-Wallis H test. Post-hoc tests for pairwise comparisons between the different treatment groups were done by a Dunn test.

LLtemps and LLtimes were analysed using generalized linear models with a logit-link function and binomial error distribution to test for significant differences between morph (summer, winter) and sex (male, female). LLTemp50 (temperature at which 50% of the population dies after exposure for 1 min) and LLTime50 values (time after which 50% mortality occurs in a population at a certain temperature) were calculated by means of logistic regression analysis.

Results

Supercooling point

Table 8 and Figure 8 show the SCPs of the different treatment groups, ranging from -20 to -16°C. Mating status did not significantly affect the SCP of summer morph females. The sex of *D. suzukii* adults did not seem to influence the SCP on a significant level either, and this for both summer and winter morphs. Further, no statistical differences could be found between the supercooling points of pupae reared under summer conditions and those reared under winter conditions. Summer morph adults had a significantly lower/colder SCP (-20.09°C) than winter morph adults (-16.79°C).

Table 8. Supercooling points of pupae, males and females of summer and winter morphs of *D. suzukii*. For summer morphs, a distinction was made between mated and unmated females. Values having a different letter are significantly different based on the Kruskal-Wallis test ($p \leq 0.05$).

	Summer morphs				Winter morphs		
	Pupae	Males	Females		Pupae	Males	Females, mated
			Mated	Unmated			
SCP (ºC)	-16.02 ± 0.34 b	-20.65 ± 0.31 a	-19.54 ± 0.38 a	-20.24 ± 0.42 a	-15.89 ± 0.27 b	-17.38 ± 0.24 b	-16.40 ± 0.51 b

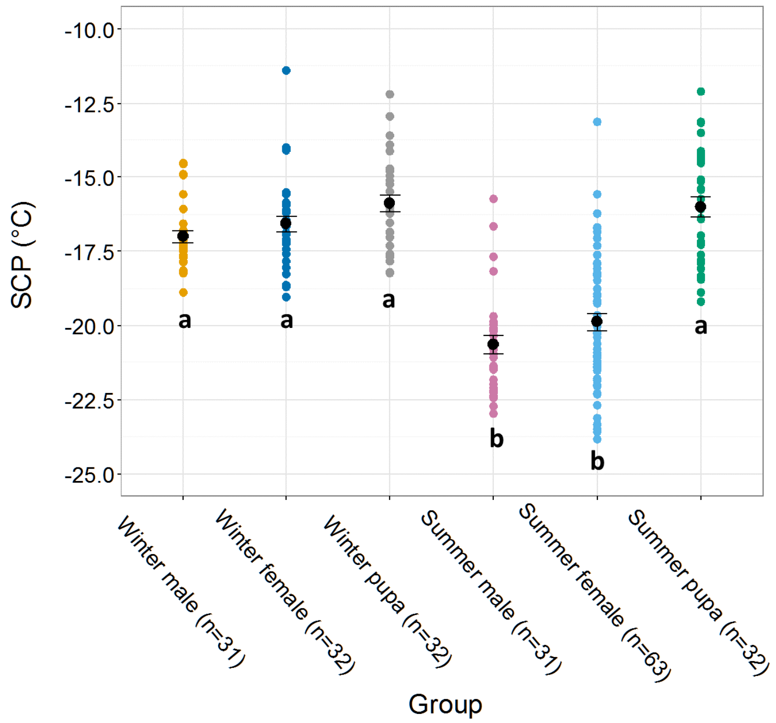

Figure 8. Supercooling points of pupae, males and mated females of summer and winter morphs of *D. suzukii*. Sample sizes (n) are listed for each group. Error bars represent standard errors of the means. Values having a different letter are significantly different based on the Kruskal-Wallis test ($p \leq 0.05$).

Lower lethal temperature

The regression curves of the survival of male and female summer and winter morph adults at different temperatures are illustrated in Figure 9. The corresponding LLTemp50-values and the response of these treatment groups to low temperatures are shown in Table 9.

A significant interaction was found between temperature and sex and between temperature and morph type. The LLTemps of summer morph adults were significantly higher/warmer than those of winter morph adults, meaning winter morphs had a considerably higher direct accumulated chill injury tolerance than summer morphs.

Compared to the LLTemp50 of summer morph females of *D. suzukii*, summer morph males had a distinctly higher/warmer LLTemp50 value (-7.93 °C versus -10.42°C, respectively). This difference could not be found in winter morph males and females. However, the chill injury response of all four groups to the low temperatures differed significantly.

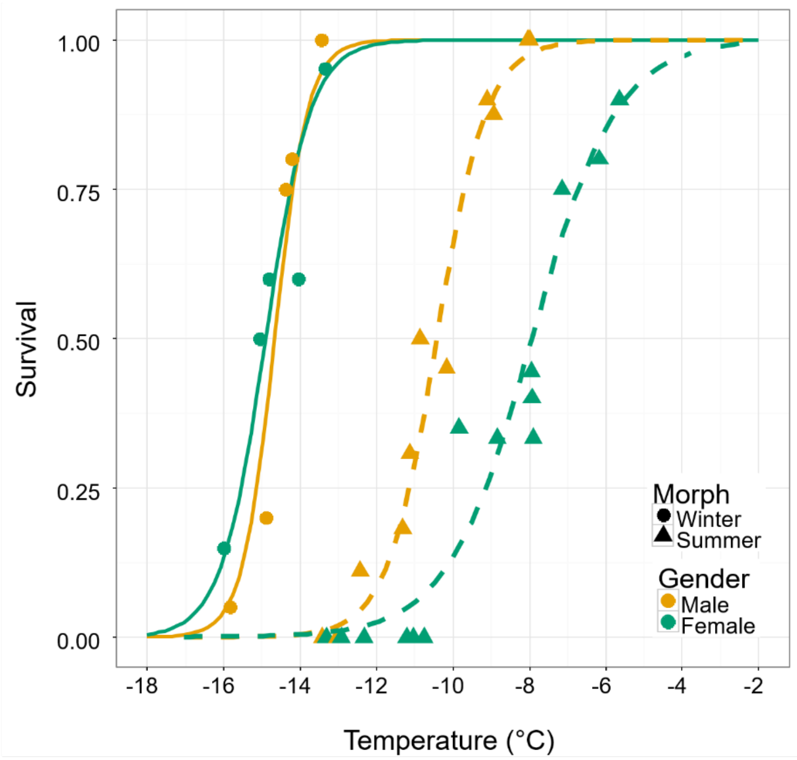

Figure 9. LLTemp-values of male and female summer and winter morphs of *D. suzukii*. The models determined by a logistic regression for winter females (solid green line), winter males (solid orange line), summer females (dashed green line) and summer males (dashed orange line) are illustrated in the figure.

Table 9. Mean SCP, LLTemp50 and LLTime50 of different life stages, sex and morphs of *D. suzukii.* Values having a different letter within a column are significantly different (p ≤ 0.05).

Morph	**Gender**	**SCP (ºC)**	**LL Temp 50 (ºC)**	**LL Temp response**	**LL Time 50 (h)**		**LL Time response**	
					0.5ºC	**-5ºC**	**0.5ºC**	**-5ºC**
Summer	Pupa	-16.02 b						
	Male	-20.65 a	-10.42	a				
	Female	-19.88 a	-7.93	b				
	Adult (♂+♀)				17.5	4.71	a	a
Winter	Pupa	-15.89 b						
	Male	-17.38 b	-14.66	c				
	Female	-16.40 b	-14.90	d				
	Adult (♂+♀)				163	37.7	b	b

Lower lethal time

At 6.5°C, both summer and winter morph adults had a long life span, which led to the inability to complete this experiment within the time frame of the project. The LLTime50s at this temperature could thus not be determined yet. The survival of summer and winter morph adults at 6.5°C in function of time (up to 296 days) is presented in Figure 10. The dotted green and orange lines represent the exponential trend lines of survival of the summer and winter morph adults, respectively. Both morphs were able to survive for more than 300 days under these conditions, but winter morphs clearly had a higher survival rate than summer morphs. After 140 days, 80% of the winter morph adults were still alive, whereas - according to the trend line - only 20% of the summer morphs survived.

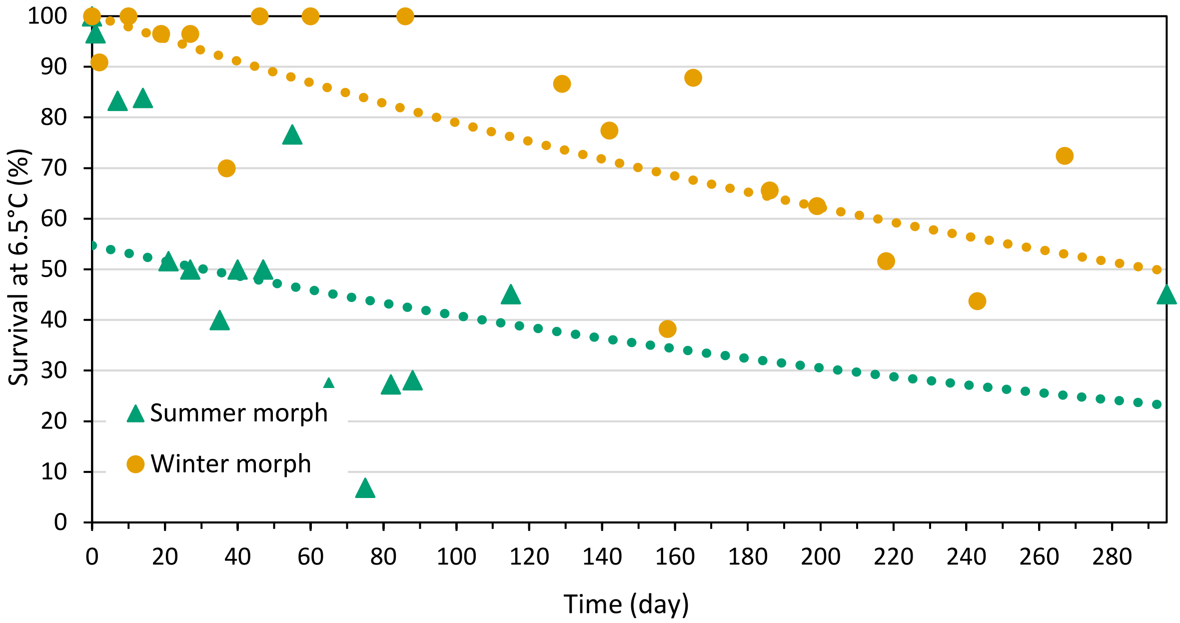

Figure 10. Survival response of summer and winter morph adults of *D. suzukii* towards continuous exposure to 6.5°C, illustrated by means of the exponential trend lines (dotted green line: summer morph adults, dotted orange line: winter morph adults).

Figure 11 displays the regression curves of the survival of summer and winter morph adults of spotted wing drosophila at 0.5 and -5°C. A significant interaction between exposure time, morph type and temperature was found. At the same periods, higher mortality occurred in summer morph individuals than in winter morph adults at both temperatures. The logistic regression models had significantly different slopes, indicating that summer and winter morphs had a different indirect chill injury response (Table 9).

At -5°C, the predicted LLTime50-values were 4.71h for summer morph adults and 37.7h for winter morph adults. Summer morphs exposed to -0.5°C had an LLTime50 of 17.5h, whereas winter morphs had a much higher LLTime50-value of 163h.

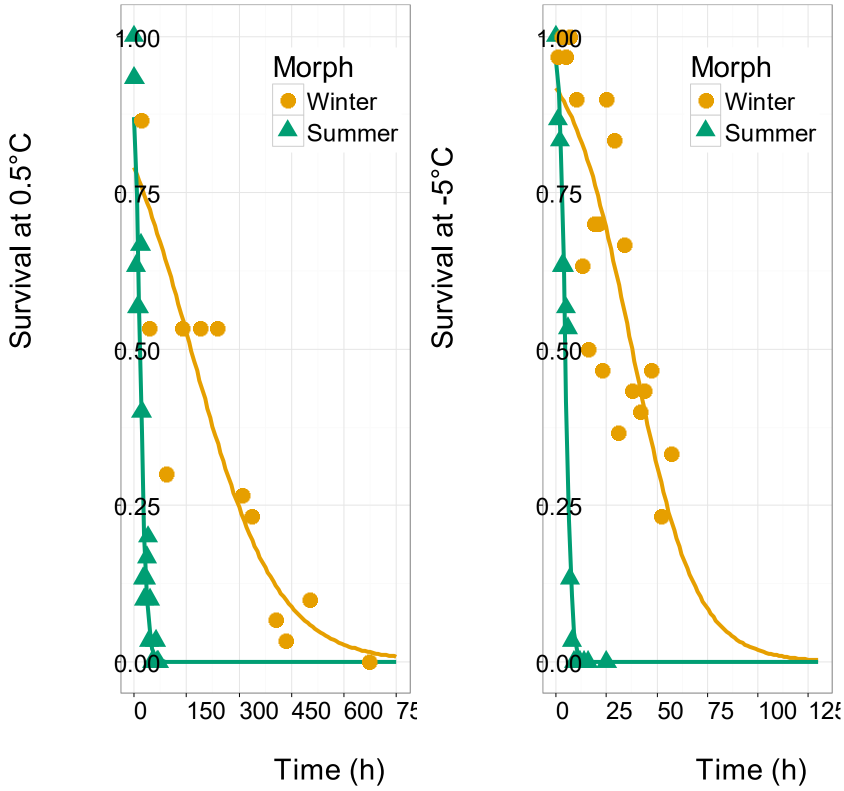

Figure 11. LLTime values of summer and winter morphs of *D. suzukii* at 0.5°C (left graph) and -5°C (right graph). The models determined by a logistic regression for winter and summer morphs are illustrated in both figures by a solid green and orange line, respectively.

Discussion

This study provides an insight in the cold tolerance of *D. suzukii* populations in Northwestern Europe.

The LLTemp-experiments revealed that mortality of the fruit fly occurs at sub-zero temperatures due to chill injury factors rather than freezing of the haemolymph. This might indicate that a portion of the winter morphs is chill tolerant, whereas summer morphs appear to be chill intolerant.

Although the SCP of winter morph adults was higher/warmer than that of summer morphs of the Asian fruit fly, winter morphs showed higher LLTime50s and lower/colder LLTemp50s than summer morph adults. Thus, it appears that winter morphs increased their SCP, i.e. their SCP occurred at “warmer” sub-zero temperatures, to strengthen their chill injury tolerance. This would enable them to survive long term exposure at mild temperatures, but makes them more sensitive to acute and extreme temperature drops. The exact reason for this phenomenon and the underlying mechanism is still unknown and should be further studied.

The average temperature in Belgium between December and February is 3.6°C (KMI, 2016). Based on the results of the LLTime- and LLTemp-experiments, it is very likely that spotted wing drosophila can overwinter in Belgium and in other countries with a similar temperate maritime climate and mild winters (most of Northwest Europe). However, sheltered environments are probably needed during the colder days and a drop back in the population during winter is to be expected. Year-round monitoring programmes on *D. suzukii* populations in Belgium confirm this (pcfruit, personal communication).

2. Assessment on the role of diapause and cold adaptability as overwintering strategies in the establishment of *D. suzukii* in Europe (ILVO)

Methods

Reproductive diapause

One of the believed overwintering strategies exploited by *D. suzukii* is reproductive diapause. To explore this hypothesis, couples of winter morph adults were exposed to winter conditions (10°C, 8:16h (L:D), 65-85% RH). At set intervals (week 0, 1, 2, 3, 4, 6 and 9), 10 couples were transferred to summer conditions (25°C, 16:8h (L:D), 65-85% RH). Their oviposition was monitored frequently to determine the preoviposition period of winter morph females after exposure to a cold period. Dissections were executed frequently to assess the ovarian development of the winter morph females at 10°C.

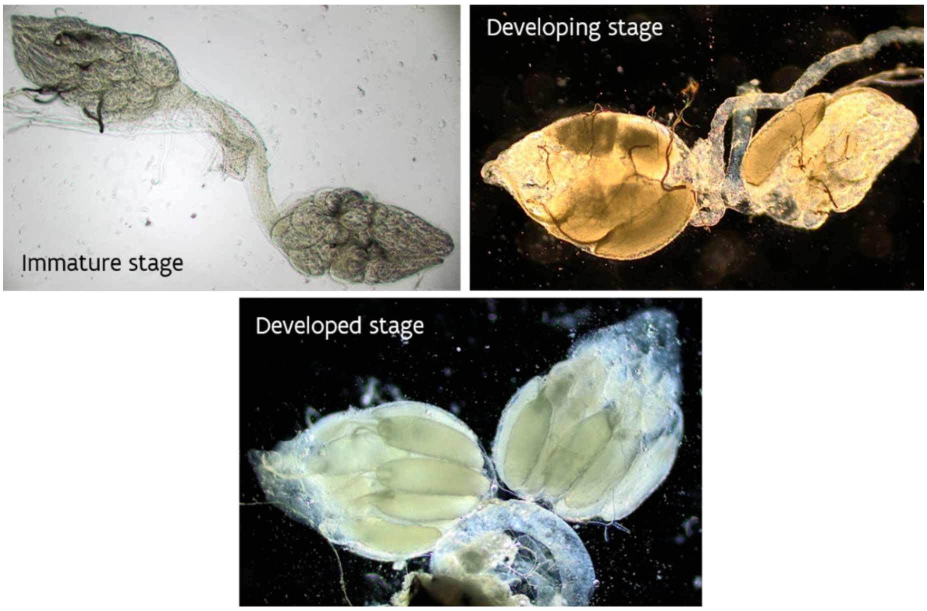

Figure 12. Classification of the developmental stages of the ovaries of *D. suzukii*: immature stage (= immature ovaries without yolk accumulation), developing stage (= developing ovaries with incomplete vitellogenesis) and developed stage (= developed ovaries filled with mature eggs) (@ De Ro M.).

The ovaries were classified into three developmental stages (Figure 12). In the first stage (immature stage), no yolk accumulation had occurred yet. This corresponds with the ovaries of newly hatched summer females or females believed to be in reproductive diapause. In ovaries in the developing stage (the second stage), yolk accumulation had started (= vitellogenesis) and a few eggs might be fully formed. In the third and last stage, the ovaries were fully developed and thus filled with mature eggs.

Semi-field overwintering

To get an indication of the ability of *D. suzukii* to overwinter in the Belgian climate, a small experiment was set up at the end of October 2015 (October 27th) under semi-field conditions and was continued until mid-April 2016. Summer and winter morphs were brought into small bug dorms (30x30x30 cm) and placed at three different locations, including location-adapted diets, each representing a different habitat. On each location, temperature and relative humidity were measured continuously.

A first series of winter and summer morph adults were put in vials filled with a standard artificial cornmeal diet at a sheltered location (south-faced staircase), where temperatures could go up to 10°C higher compared to the other locations (Figure 13).

At the start of the experiment, 319 summer morph and 162 winter morph adults (4 to 10 days old) were taken from their respective cultures at 25°C and 10°C (see Materials and methods of WP3.1), acclimated for 1 day at 10°C and transferred to two outside cages ('summer' and 'winter' cage) on the first location. On December 24th, another 140 summer adults and 163 winter morph adults were added to the cages.

Adults were transferred two times a week to new vials, in order to get an idea of the survival rate of the adults, as well as to assess the potential oviposition and larval and pupal development.

Figure 13. The three locations and their food sources: sheltered habitat (under staircase; artificial diet) (top left), (abandoned) fruit orchard (shrubs; fruits and artificial diet) (right) and edge of deciduous forest (trees and shrubs; wild fruits and artificial diet) (bottom left).

On the second location, a summer and a winter cage were placed under shrubs and small trees. The *D. suzukii* adults were put in a bug dorm filled with leaf litter, small branches, a strawberry plant and fruit to simulate the environment of an (abandoned) fruit orchard (Figure 13). New fruit or semi-rotten fruit was provided on a regular basis.

The third location was situated at the edge of a small deciduous forest. Leaf litter, small branches, moss, tree bark, rotten wood, some flowers and wild berries, being replaced if available, were added to the cages (Figure 13).

At the second and third location, initially 250 summer morph and 150 winter morph adults (4-10 days old) were put in the cages, after being acclimated during 1 day at 10°C. On December 21st and December 24th another 200 summer and winter adults were added to these cages.

To ensure that adults at the last two locations did not die of starvation, small petri dishes with artificial diet, yeast drops and sugar water were provided and replenished on a regular basis. This made it possible to monitor oviposition and immature development, assuming that the artificial diet was preferred above the natural sources present in the bug dorm cages.

Results

Reproductive diapause

Figure 14 shows the ovarian development of newly hatched to 9-week-old *D. suzukii* winter morph females at 10°C. Developing and developed ovaries could be found from week 3 onwards and in general, the proportion of females with developed ovaries increased over time. The length and width of the ovaries also increased in function of time and this growth was most obvious during the first four weeks (Figure 15).

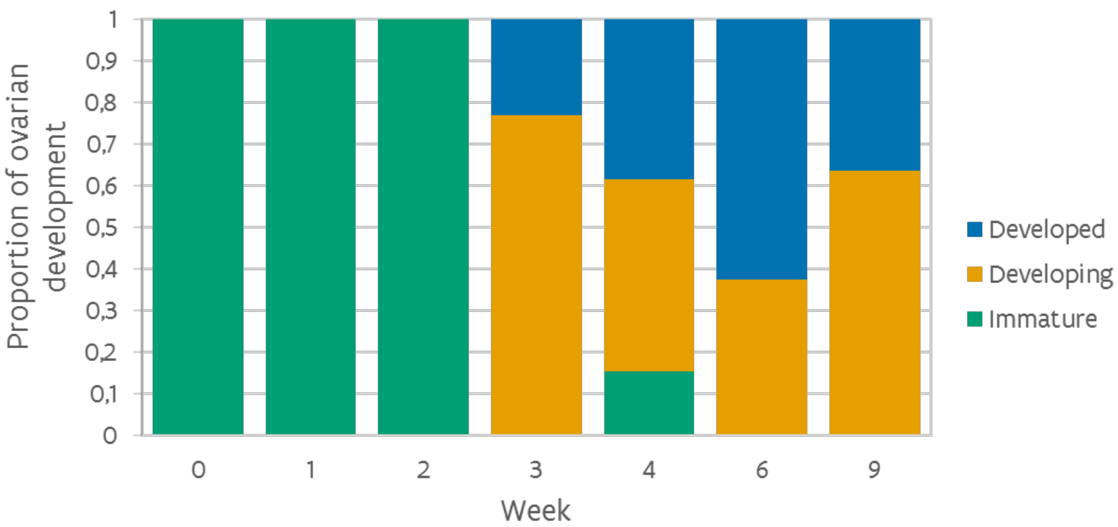

Figure 14. Proportion of development of the ovaries of winter morph females of *D. suzukii* at 10°C in function of time.

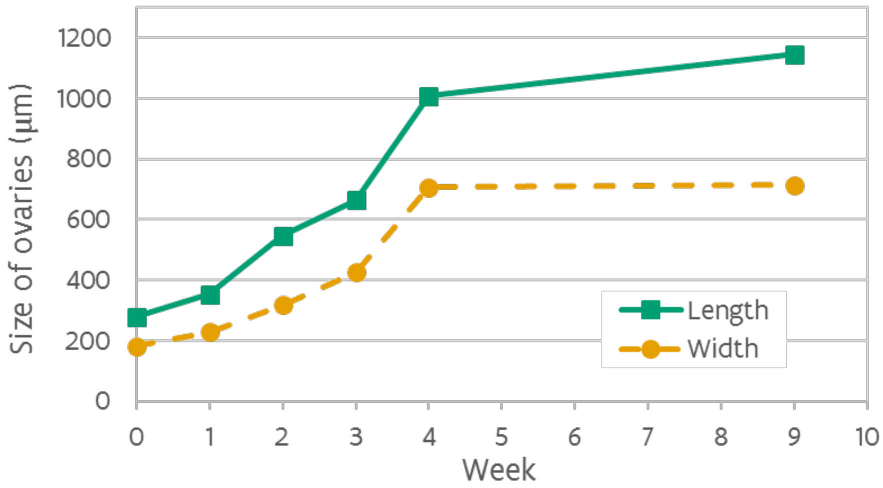

Figure 15. Length and width of the ovaries of newly-hatched-to-9-week-old winter morph females of *D. suzukii* at 10°C.

About 60% of the couples that were transferred to 25°C after being exposed to 10°C for four or more weeks started ovipositing within 7 days. The remaining fraction of the *D. suzukii* couples never laid a single egg. Females younger than four weeks, i.e. winter females that were transferred from 10 to 25°C before week 4, were able to oviposit as well, but their preoviposition period was more variable and ranged from 3 to 20 days.

In addition, winter morph females that stayed at 10°C for 6 weeks (or more), were observed to start laying eggs that hatched spontaneously.

Semi-field overwintering

An overview of the average daily temperatures at the three different locations and in open air during the winter of 2015-2016 are shown in Figure 16.

In general, the average temperature per day was the lowest in open air and the highest at the sheltered location. There, sub-zero temperatures were merely recorded during 6 days, while on sunny days temperatures could rise to as much as 36°C. The average temperature curves in the fruit orchard and at the edge of the deciduous forest were very similar, although actual temperatures below zero were observed more often at the forest rim than in the orchard.

Figure 17 displays the total amount of adult winter and summer flies of *D. suzukii* being counted at different time points in the sheltered habitat, in combination with the mean daily temperature curve at this location.

Between November 25th and December 15th, a downward trend was observed in both the summer and winter morph population. This was most likely due to the growth of fungi on the artificial diet, as a result of incorrect preparation of the medium. Once new medium was made, the decline disappeared.

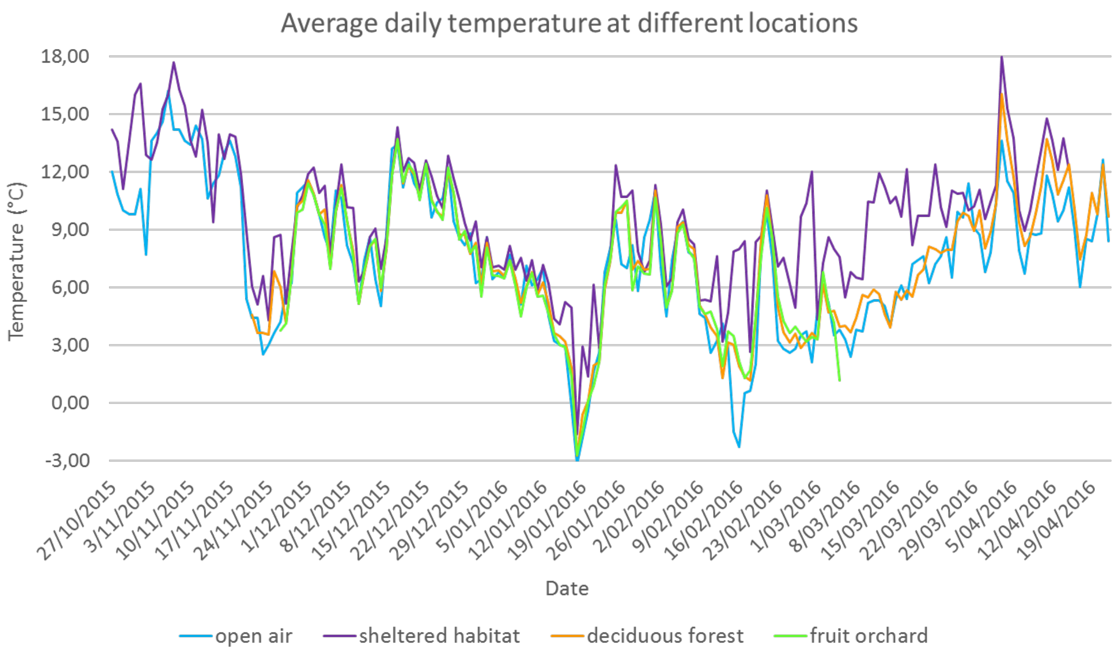

Figure 16. Average daily temperature curves in open air (blue line), in the sheltered habitat (purple line), at the edge of the deciduous forest (orange line) and in the fruit orchard (green line).

Approximately two months after the start of the experiment, new winter morph adults started to emerge in both winter and summer cages, though the reproduction rate slope of the summer morph females appeared higher compared to that of winter morphs.

From February 8th onwards, both summer and winter population sizes decreased drastically. Presumptively, this was caused by a less frequent replacement of the artificial diet, which led to dessication of the medium.

At the end of the experiment, 250 *D. suzukii* adults were counted in the summer cage and 230 adults in the winter cage. Compared to the total amount of adult flies that were added to the cages, winter morphs could increase their population size with 110%. In the summer cage, a population of 126% was calculated. On February 8th, i.e. before the medium started to dessicate, the summer and winter population had grown by 214% and 169%, respectively.

In the simulated fruit orchard, fruit flies were observed in the cages throughout the winter, but as temperatures dropped, their number decreased significantly as well. After completion of the experiment, many invertebrates (e.g., barklice, woodlice, springtails, etc.) could be found (Table 10). In the summer cage a small amount of winter morph flies of spotted wing drosophila were counted, indicating that the adults were able to reproduce to some extent during this study. However, the net summer and winter population survival rates were only 16% and 14%, respectively.

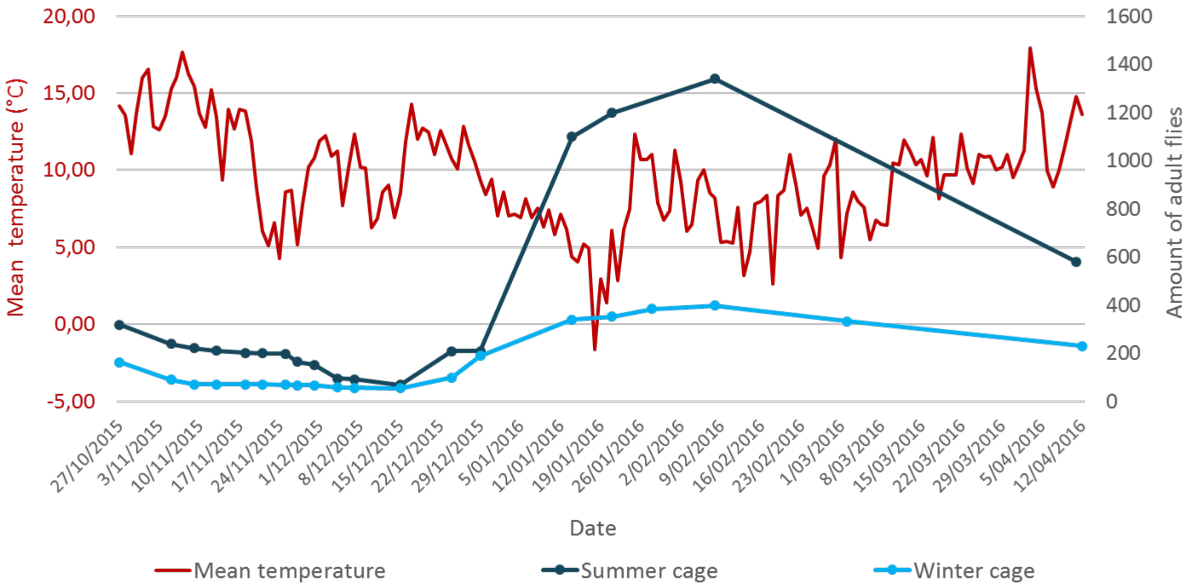

Figure 17. Average daily temperature and total amount of summer morph (solid dark blue line) and winter morph adults (solid light blue line) of *D. suzukii* in the sheltered habitat in function of time.

Table 10. The total amount of fruit flies and other invertebrates counted in the winter and summer cage at the end of the experiment in the fruit orchard.

	Summer adults *D. suzukii*		**Winter adults *D. suzukii***		**Survival *D. suzukii* (%)**	**Other fruit flies (*D. subobscura*,...)**		**Other invertebrates**
	Males	**Females**	**Males**	**Females**		**Males**	**Females**	
Summer cage	17	61	9	20	16.46	0	0	barklice (Psocoptera), rove beetles (Staphylinidae), Latridiidae, woodlice, centipedes, aphids, Heteroptera, spring tails (Collembola)
Winter cage	0	0	23	54	14.00	58	46	barklice, rove beetles, Latridiidae, woodlice, centipedes, aphids, Heteroptera, spiders, Opomyzidae

At the third location, fruit flies (assumingly *D. suzukii*) could be observed until the end of January. No adults of spotted wing drosophila were found during the final counting at the end of the experiment. In fact, besides a small number of woodlice, no invertebrates were detected at all. Most likely, this was a result of a lack of moisture and other water sources. The artificial diet in the petri dishes and the sugar water on the cotton balls dessicated very quickly at this location.

Discussion

At winter conditions, oviposition by winter morph females ceased for approximately 6 weeks. Winter morph females of any age that were exposed to more favourable conditions resumed egg-laying within one week. These results may indicate the occurrence of a short and shallow reproductive diapause in winter morph females of *D. suzukii*. However, this could also be attributed to a slower development of genitalia at lower temperatures and thus needs further research.

In Belgium, the winter of 2015-2016 was unusually mild, which could explain the observed continuation of the development of immature stages of *D. suzukii* during the semi-field experiments. This species was able to survive the winter months in both the sheltered habitat and the fruit orchard. However, the presence of a suitable food and water source is crucial.

Seasonal field trapping and laboratory evaluation aimed at definition of intervals for switch on/off overwintering *D. suzukii* times (CREA)

The evolutionary capacity of *D. suzukii* lends support to the possibility that different populations present in Europe, particularly in Italy, may not have exactly the same life history characteristics of flies previously studied in other locations. This wide range in adaptability could imply different overwintering strategies at different latitudes and altitudes.

Methods

An assessment of the effect of different climatic conditions and of different combinations of temperature and photoperiod on *D. suzukii* overwintering was performed by means of monitoring stations.

From winter 2013-2014 until spring 2015, survey in different climatic regions (coastal and internal areas of Tuscany, Central Italy, Figure 18) was carried out with traps baited with food Droskii-drink mix. In each survey site, temperature and relative humidity were daily registered. The collected adults were examined at the laboratories of CREA - ABP.

Figure 18. Location of sampling areas in Central Italy.

-Farm Case Basse (★), in the southwest area of Montalcino (Siena province): var Sangiovese Grosso (Brunello wine); over 23 hectares, it is in a perfect situation to the hilly land of Eocene origin; altitude: 320 meters; exposure: South – West; Climate dry, sunny.

-Farm Le Mortelle (★), Castiglione della Pescaia (Grosseto province): 270 overall hectares, 160 of which planted to vineyards. 15 hectares of organically cultivated fruit orchards, (peaches, plums, apricots, pears, and blueberries) along with two artificial lakes, the larger of which covers a surface of six hectares (15 acres). The property is surrounded by low hills covered by olive groves and woods. The 160 hectares of vineyards are planted principally to Cabernet Sauvignon and Sangiovese.

The temperature data were considered both according to the usual synthesis of the average daily temperature and by the method of Allen with the interpolation of the data of the daily thermal extremes. The acquired data were used to calculate the cumulative amount of degree-days for the progress of insect development.

In addition, during winter 2015-2016 two entomological cages were set up in a sheltered environment (a courtyard close to a CREA laboratory), introducing inside them, 15 couples of *D. suzukii* (from the Lab rearing - 25°C, 16:8h (L:D) and 65±10% RH), a layer of leaves and weeds and a pot containing *D. suzukii* artificial diet. Temperature and Relative Humidity were registered in continuous with data logger.

In the laboratory, a climatic chamber was set up at 10°C, 08:16h (L:D) and 65 ± 10% RH. *Drosophila suzukii* eggs obtained from the laboratory rearing adults were inserted in the chamber and control were carried out every two days.

Results

Figures 19 and 20 show the regression lines obtained for the catches of *D. suzukii* in two Italian regions with regard to the cumulative degree-days calculated according to the methods of average daily temperature and to the Allen interpolation.

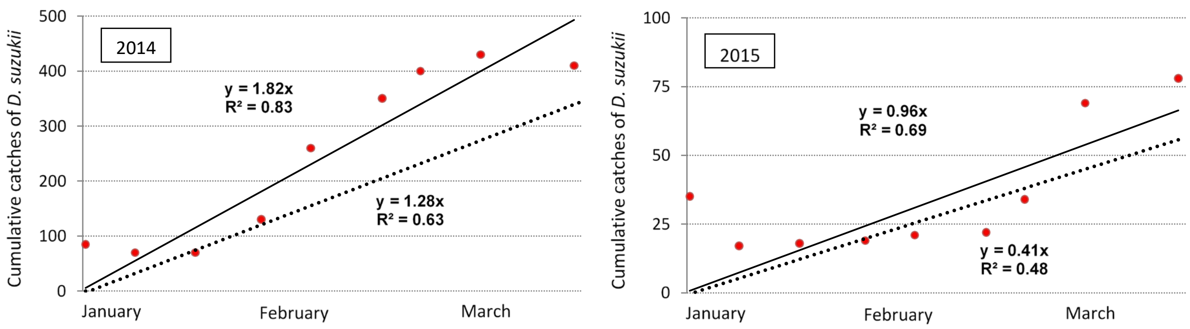

Figure 19. Regressions based on cumulative catches (red) recorded in Montalcino farm in 2014 and in 2015 in accordance to the methods of average daily temperature (dotted line) and of Allen interpolation (solid line).

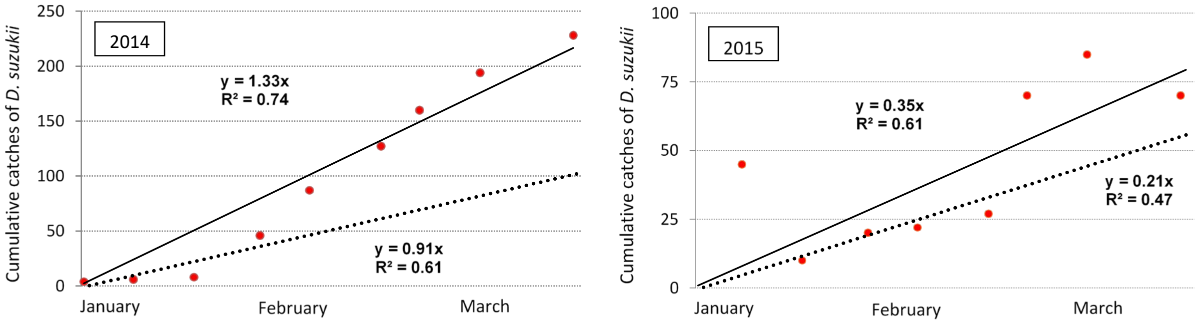

Figure 20. Regressions based on cumulative catches (red) recorded in Castiglione della Pescaia farm in 2014 and in 2015 in accordance to the methods of average daily temperature (dotted line) and of Allen interpolation (solid line).

As the fits registered, especially considering short periods, eg a few weeks, characterized by high thermal excursions, at different latitudes and conditions, the accumulation of degree-days through the Allen interpolation can be useful in forecasting processes to be adopted in the implementation of control strategies of the insect.

In the semi-field condition, new adults were obtained in the cage left in the courtyard after 68 days from the introduction of the *D. suzukii* adults. The temperatures registered with the data logger in this period are shown in Table 11.

Table 11. Average, highest and lowest temperatures (ºC) registered within the entomological cages in the semi-field experiment.

Time period	Average T °C	Highest T °C	Lowest T °C
2015/12/29 to 2016/01/02	5.6	12.3	-0.7
2016/01/02 to 2016/01/07	7.8	17.5	3.3
2016/01/07 to 2016/01/13	3.5	19.2	-4.4
2016/01/13 to 2016/01/21	6.6	22.3	-2.4
2016/01/21 to 2016/01/28	12.2	18.2	4.0
2016/01/28 to 2016/02/04	10.1	14.9	5.2
2016/02/04 to 2016/02/12	10.1	14.9	5.2
2016/02/12 to 2016/02/18	11.2	17.9	3.2
2016/02/18 to 2016/02/25	10.0	16.2	2.4

In the climatic chamber maintained at 10°C, we obtained new adults after 35 days from the introduction of the diet with *D. suzukii* eggs. These new adults were equally active but the colour of the body was darker than the "normal" one. The female did not lay eggs during the period of the observation and also in the dissected female we could not find mature eggs, with the exception of two of them.

Discussion

The field and semi-field observations allowed to better understand *D. suzukii* performance in relation to temperature. The findings is a valuable tool in building provisional models to set control strategy methods. In particular, the consideration of different interpolation, in more limited time scale, may be better fitting the dynamics and spreading of *D. suzukii*, in consideration both of its intrinsic biological potential and of the environmental differences registered in large country areas.

WP4: EVALUATION OF ALTERNATIVE CONTROL STRATEGIES (AGES, CREA, INIA)

The objectives of WP4 were:

- To enhance the efficacy of trapping systems against *D. suzukii* (AGES).
- To evaluate the use of entomopathogenic microorganisms (CREA-ABP).
- To investigate the efficacy of insect growth regulators for the control of the *D. suzukii* populations (INIA).

Enhancement of trapping systems against *D. suzukii* (AGES)

Methods

Trial site

The trial site was located in the south-eastern part of Austria (Nitschaberg; Styria) in a small-structured landscape with fruit orchards, vineyards, meadows, woodland and small towns. Here a commercial raspberry orchard (cultivar Himbotop, 8 years, about 1.5 ha) with known *D. suzukii* infestation (since 2011) was chosen for the study; about half of the orchard was surrounded by wood, the other part by meadows and buildings.

Material

In this trial two trap types (Figure 21) were compared in two different positions in the raspberry orchard.

The “Swiss cup trap®” (RIGA AG) was a transparent small plastic cup with 9 openings (about 3 mm Ø) in the lid and a separate white roof above as rain cover. The traps were filled with 100 ml of the original lure of the manufacturer (unkown composition). The self-made trap type (“green bottle trap”) was a commercially available green-coloured 1.5 l water bottle with 48 openings (about 3 mm Ø) evenly distributed in three rows in the upper fifth of the bottle. The trapping liquid was 300 ml of the Italian “Droskidrink”, a mixture of 750 ml apple cider vinegar (5%), 250 ml red wine and 20 g of unrefined sugarcane for 1 litre (Grassi *et al*., 2015).

Both trap types were tested in two different positions in the raspberry rows: at 40 to 50 cm height (lower position) and 130 to 150 cm height (upper position); all traps were placed in shady positions (Figure 22).

Figure 21. “Swiss cup trap®” (left) (© J. Altenburger) and the self-made trap type “green bottle trap” (right) (© C. Lethmayer)

Figure 22. Position of the traps in the raspberry orchard (Nitschaberg, Austria) (© C. Lethmayer)

The trial set up consisted of four variants (2 trap types and 2 trap positions) with 11 replicates for each variant resulting in a total of 44 traps. They were randomly distributed in 11 rows with four traps per row (based on Von Lochow & Schuster, 1961), having a distance of 6 m to each other (Figure 23). The traps were installed on the 20th of August 2014 (week 34) and removed on the 1st of October 2014 (week 40) due to the clearing of the raspberry plants. With the weekly change of the trapping liquid (except the first change after a two week period in week 36) data of five catching periods were achieved.

A1	D2	B3	C4	A5	A6	B7	D8	C9	C10	A11
B1	C2	A3	D4	B5	C6	A7	B8	D9	A10	B11
C1	B2	D3	A4	C5	D6	D7	C8	A9	B10	C11
D1	A2	C3	B4	D5	B6	C7	A8	B9	D10	D11

Figure 23. Test design for the trial (11 rows with 4 traps): A = “green bottle trap” below, B = “green bottle trap” above, C = “Swiss cup trap®” below, D = “Swiss cup trap®” above.

The numbers of caught spotted wing drosophila individuals were statistically analysed with a mixed Poisson-Model with a log-link in order to test if there are significant differences for the mean number of flies in the traps regarding the trap type ("green bottle trap", "Swiss cup trap"), position of the trap (above, below) and the sex (male, female) of caught flies. The different time points of the trapping liquid change were incorporated as random effect, in order to take time dependencies of different catching periods into account. In all analyses the level of significance was α = 0.05. Data analyses were conducted using the statistical software R Version 3.1.3 (R Core Team, 2015) and R package lme4 (Bates *et al*., 2014).

Results

In Figure 24 the number of caught *D. suzukii* for each trial variant and catching period in the raspberry orchard is shown. During the first catching weeks from mid August to mid September 2014 the number of caught flies in all traps and variants ranged between 9 and 120 flies per trap in average which changed obviously from week 38 (mid of September) onwards to an increase of fly catches (minimum of 57 flies and maximum of 279 flies per trap in average). The number of caught spotted wing drosophila was higher in "green bottle traps" (2.72 times more) than in the "Swiss cup traps®".
In both trap types the number of caught *D. suzukii* was higher in traps with position in 1.5 m height compared to traps in lower position (in average 12% less individuals than in traps with higher position).

In addition, the number of caught *D. suzukii* for each trial variant and catching period was differentiated between caught males and females of *D. suzukii* (Figure 25). The achieved results showed that in both trap types the number of caught females was higher than the number of caught males, independently from the trap type.

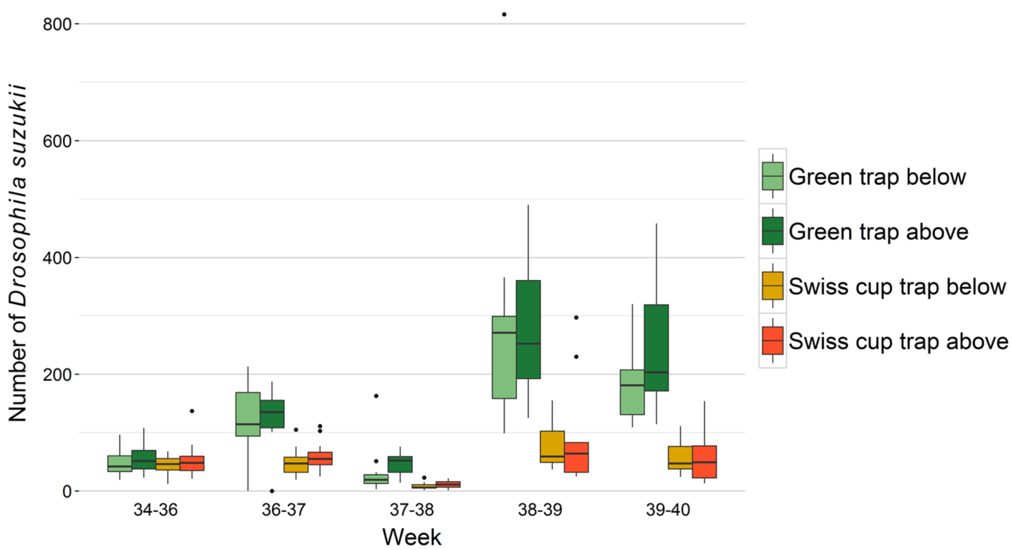

Figure 24. Boxplots for the number of caught *Drosophila suzukii* for each trial variant and catching period (from mid August until October 2014) in the raspberry orchard (Nitschaberg, Austria).

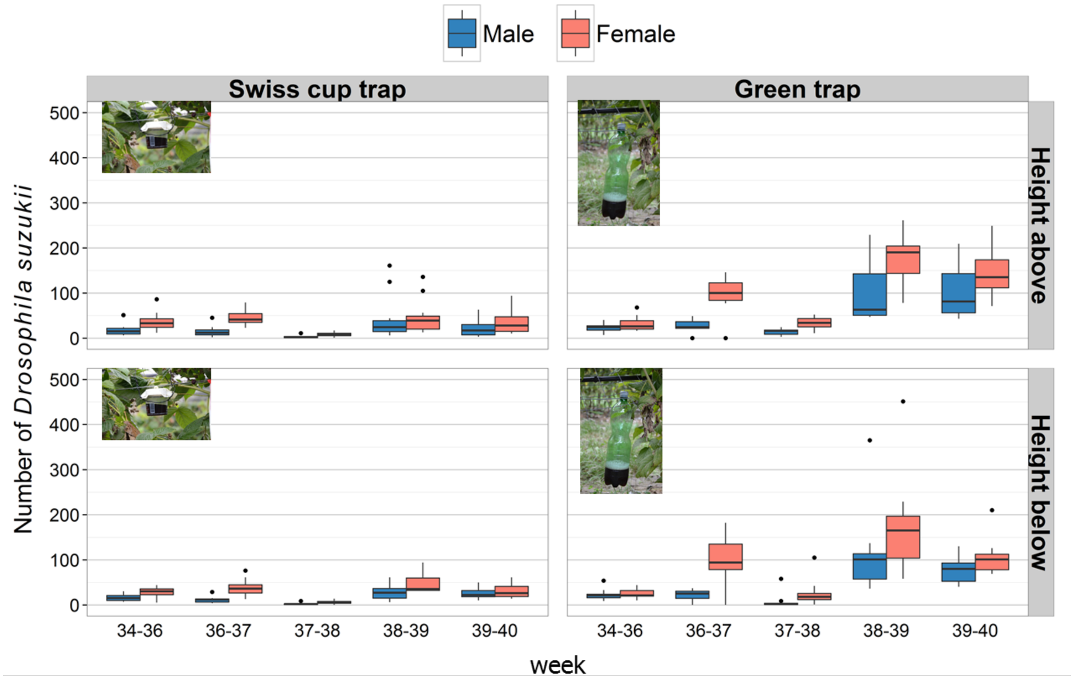

Figure 25. Boxplots for the number of caught *Drosophila suzukii* for each trial variant and catching period (from mid August until October 2014), differentiated between males and females, in the raspberry orchard (Nitschaberg, Austria).

Statistical analysis revealed significant differences for the number of caught flies concerning the type of trap, height of trap and the ratio of spotted wing drosophila males and females in the traps ($p \leq 0.05$).

Discussion

Several studies about the use and development of traps show that there are many possibilities to catch a high number of spotted wing drosophila if certain criteria are considered – for example a high number of entry points, an attractive lure and the trap's position on cool and shady places. The influence of other decisive factors like the trap's shape and colour are still not sufficiently known.

In general, a direct comparison of traps is difficult because in most cases, the traps are different concerning size and volume of trapping liquid, so standardised reference parameters would be needful. Thus, new developed trap types should be compared with well working and accepted traps - as it is done in the present study with the widely approved "Swiss cup trap®" from RIGA AG. The results showed satisfying trapping efficiency of the self-made traps having higher numbers of caught *D. suzukii* with these new traps. Although the self-developed traps caught significantly more *D. suzukii* it has to be mentioned that the size and the trapping liquid volume of the "Swiss cup trap®" is smaller than that of the self-made trap. This could explain the reason for the lower number of catches to some extent. Nevertheless, this comparison demonstrates that the self-developed trap type is an effective trap for catching spotted wing drosophila.

In addition, it is indicated that traps in the height of the fruits - like here for raspberries in a 1.5 m position – seem to be more effective than in lower positions, which was achieved with both trap types.

Interesting is the fact that both trap types caught more *D*. *suzukii*-females than males.

Evaluation of the use of entomopathogenic microorganisms (CREA)

Introduction

Several chemical products available within the EU have shown good potential as control agents against *D. suzukii*. However, organic fruit growers still have limited options as few products appropriate for organic use have been found to be effective against *D. suzukii*. Equally, the use of chemicals can be very disruptive to natural enemies; therefore, there is the need to screen for potential biological control agents for *D. suzukii*.

Methods

Laboratory bioassays were performed to investigate effectiveness of entomopathogenic products using formulated insecticide products registered in Italy. All the biological agents investigated are commercially available and were tested in different lab trials. The products were Naturalis (*Beauveria bassiana*), MET52 *Metarhizium anisopliae* and NoFly *Paecilomyces fumosoroseus*. In previous laboratory trials (Gargani *et al*., 2013, 2014) Naturalis (*Beauveria bassiana* strain ATTC74040, g 7.16, Equal to 2.3 x 107 viable spores/ml) have shown a certain control action of the fly. *Metarhizium anisopliae* is a fungus that grows naturally in soils throughout the world and causes disease in various insects. Even if it lacks a rapid efficacy against insect population, it may represent an important alternative control measure. In fact, it attacks the host through the cuticle and no insect resistance towards this fungus has been reported. Finally, *Paecilomyces fomosoroseus* is a well-known entomopathogenic fungus with a worldwide distribution in temperate and tropical zones and a relatively wide host range, which makes it interesting agent for the development of biocontrol methods.

Source of Insects

Drosophila suzukii used in the experiments came from bug-dorm insect cages maintained at CREA ABP laboratories (T 25°C, 65% R.H. and 16:8 hr L:D). The insects were reared on an artificial diet.

Laboratory trials

First trial: direct toxicity test of MET52 on *D. suzukii* adults

Petri dishes containing 6 grams of commercial product Met52, granular formulation based on sterile rice, containing the fungus *M. anisopliae* (*Metarhizium anisopliae* Strain F52, it contains 2g 9 x 10^8 (CFU)/gram) or 6 grams of untreated rice (control) and a cotton ball with water and honey, were set up (Figure 26). Twenty-four couples of *D. suzukii* were left moving for two hours inside Petri dishes and then transferred into others, containing Saboraud substrate.

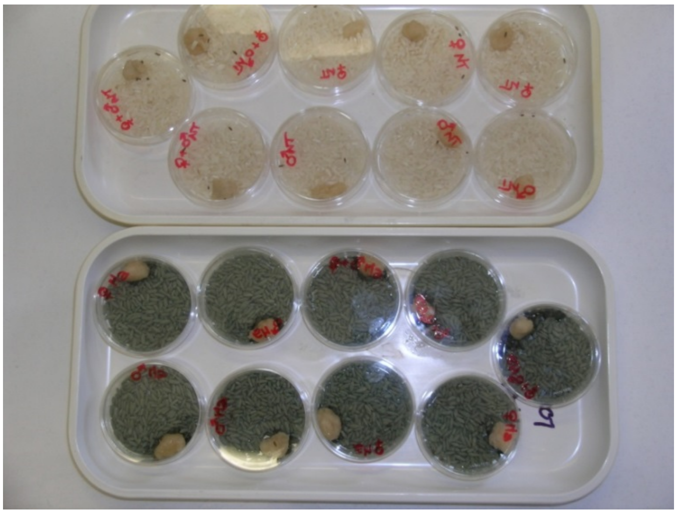

Figure 26. Control and treated Petri dishes for the evaluation of direct toxicity of *M. anisopliae* on *D. suzukii* adults.

Controls were carried out 2, 24 and 72 hours after the treatment, to verify adult mortality.

To verify the cause of *D. suzukii* adult mortality, the dead individuals were put singly into Petri dishes on filter paper saturated daily with water to achieve 100% RH to promote conidial growth. The dishes were maintained at room temperature (20-25°C). Then, fungal propagules, grown on the cadavers, were identified.

Second trial: direct toxicity test MET52 on *D. suzukii* adults, crossing treated and non-treated individuals

Twelve couples of *D. suzukii* were left for two hours walking inside treated Petri dishes and 12 couples were left on untreated rice. Then the individuals, in couples crossed as describe in Table 12, were transferred onto other Petri dishes containing Saboraud substrate. Control were made after 6 days.

Table 12. Crosses performed to test the transmission of *M. anisopliae* from treated to untreated adults.

Thesis 1	♀ MET52 + ♂ MET52
Thesis 2	♀ MET52 + ♂ NT
Thesis 3	♀ NT + ♂ MET52
Thesis 4	♀ NT + ♂ NT

<u>Third trial: direct toxicity test MET52 on *D. suzukii* preimaginal stages</u>

Blueberries were infested for 72 hours within a bug-dorm culture cage containing approximately 60 adult of *D. suzukii*. Following this infestation period, the blueberries were cleared of adult flies and 40 berries, randomly chosen, were dipped in 5g MET52/250ml water suspension (in Italy there is not a commercial conidial suspension, thus the granular product was chopped and then diluted in water to create a suspension), while a water treatment acted as a control. After dipping, berries were returned to a controlled environment cabinet and incubated for a further 10 days at 25°C, within ventilated plastic pots. Following this, the number of flies emerged were counted and the efficacy of the treatment was calculated using the Abbott formula.

<u>Fourth trial: direct toxicity of Naturalis and NoFly on *D. suzukii* preimaginal stages</u>

In this experiment, the product Naturalis (*Beauveria bassiana* strain ATTC74040, g 7.16, Equal to 2.3 x 107 viable spores/ml) was compared with another product based on the fungus *Paecilomyces fomosoroseus,* the commercial product NoFly (18% *Paecilomyces fumosoroseus* ceppo FE 9901 (2 x 109 CFU / g). The addition of sucrose in two of the thesis was done following the indications reported in Cowles *et al*. (2015). Blueberries (n.400) were infested for 72 hours within a bug-dorm culture cage containing approximately 150 adult *D. suzukii*. After this period, the blueberries were cleared of *D. suzukii* adult and 60 berries, randomly chosen, were dipped in 250ml of solution/emulsion for 1 min for each thesis, as described in Table 13.

Table 13. Products employed to evaluate direct toxicity and side effect against *D. suzukii* preimaginal stages.

Product	**Dose**
Naturalis	0.3 ml/200 ml H_2O
NoFly	0.8 g/200 ml H_2O
Naturalis+No Fly	0.3 ml + 0.8 g/200 ml H_2O
Naturalis+sucrose	0.3 ml/200 ml H_2O + sucrose 0.6 g
NoFly+sucrose	0.8 g/200 ml H_2O + sucrose 0.6 g
Control	200 ml water

<u>Fifth trial: side toxicity test</u>

Fifty blueberries not infested for each tested product were dipped in 200 ml of solution/emulsion (Table 13), and then dried for 2 hours; for each thesis/product, 10 blueberries were placed into 5 Petri dishes and then they were put in different five cages with 200 *D. suzukii* adults for 48 hours.

The coefficient of toxicity, (E) was calculated according to Overmeer (1988):

$$E = 100\% - (100\% - M) \times R$$

where M is the percentage of mortality calculated according to Abbott; R is the ratio between the average number of eggs and larvae counted for each product and the average number of eggs and larvae produced by females in the untreated thesis.

Results

<u>First trial - direct toxicity test: MET52 on *D. suzukii* adults</u>

Increased percentages of adult mortality were obtained through time until the greatest value was obtained at 72 h (Table 14). The fungal propagules grown on the cadavers were identified as *M. anisopliae* (Figure 27).

Table 14. Direct effect of *M. anisopliae* on *D. suzukii* adults at different time intervals.

	Mortality % 2 h	**Mortality % 24 h**	**Mortality % 72 h**
MET52	0	50%	83.3%

Figure 27. Identification of fungal propagules of *M. anisopliae* on cadavers of *D. suzukii* adults.

<u>Second trial - direct toxicity test MET52: *D. suzukii* adults, crossing treated and non-treated individuals</u>

Insects that came in contact with infected insects also become infected (Table 15).

Table 15. Percentages of mortality and infection of *D. suzukii* in crosses between *M. anisopliae* treated and untreated adults.

Thesis	Mortality after 6 days	Infection
1): ♀ MET52 + ♂ MET52	100 %	*100% M.anisopliae*
2): ♀ MET52 + ♂ NT	100 %	*100% M.anisopliae*
3): ♀ NT + ♂ MET52	100 %	*100% M.anisopliae*
4): ♀ NT + ♂ NT	100 %	*No infection*

Despite the complete mortality of the *D. suzukii* adults, MET52 would not seem efficient in controlling *D. suzukii* numbers, as the next generation of larvae were already coming through in the feeding media one week after application, without significantly differences among treated and untreated couples. The mortality of the check adults after 6 days may be due to the absence of water and feed.

<u>Third trial - direct toxicity test: MET52 on *D. suzukii* preimaginal stages</u>
Result obtained is shown in Table 16. This result is similar to that one obtained treating infested blueberries with Naturalis a commercial product based on *Beauveria bassiana* (Gargani et al., 2013, 2014).

Table 16. Mean number of adults emerged from eggs treated with *M. anisopliae* and corrected percentage of emergence.

Product	Number of adults (Mean ± SD)	Abbott index %
MET52	1.75 ± 0.5	58.01
Control (water)	6.5 ± 2.08	

<u>Fourth trial - direct toxicity on *D. suzukii* preimaginal stages</u>
Table 17 shows the number of adults emerged and the percentages of emergence obtained when infested blueberries were treated with Naturalis and NoFly and with their mixture. The evidence of this trials highlighted that the thesis with the mixture of the two commercial products Naturalis and No Fly gave promising results such as that one adding sucrose to Naturalis (the addition of sucrose improves the performance oft he entomopathogenic fungi by faavouring their development in the substrate).

Table 17. Mean number of adults emerged from eggs treated with different products and/or their mixtures and corrected percentages of emergence.

Product	Number of adults (Mean ± SD)*	Abbott index (%)
Naturalis	6.75 ± 4.11 b	42.55
NoFly	5.5 ± 2.8 bc	53.19
Naturalis+No Fly	2.75 ± 1.26 c	76.60
Naturalis+sucrose	2.75 ± 0.96 c	76.60
NoFly+sucrose	4.75 ± 3.59 bc	59.57
Control	11.74 ± 2.50 a	

* Different letters indicate significant differences, Tukey b test, $p \leq 0.05$.

<u>Fifth trial: side toxicity test</u>

Concerning the evidence of the side effect toxicity, Naturalis+sucrose, compared with the others products, showed a quite good efficacy, reducing the *D. suzukii* oviposition on treated fruits and confirming a coefficient of toxicity, E, over 50% (Table 18).

Table 18. Percentage of efficacy of different products employed to test their side toxicity against *D. suzukii* preimaginal stages.

Product	Efficacy % (E)
Naturalis	33.38
NoFly	46.75
Naturalis+sucrose	51.38
NoFly+sucrose	42.73
Naturalis+NoFly	35.13

Discussion

Drosophila suzukii presents a real challenge to a large variety of fruit crops. Containment and/or eradication of this pest will prove difficult. None of the products tested provided complete mortality, in fact, the biological agents (entomopathogenic fungi) can cause reductions in population numbers of *D. suzukii* but are unlikely to control/eradicate populations. However, they will be more easily incorporated into existing invertebrate control programmes compared to conventional pesticides.

Effect of insect growth regulators (IGRs) on the fertility and fecundity of *D. suzukii* (INIA)

Methods

The effect of lufenuron (chitin-synthesis inhibitor), pyriproxyfen (juvenile hormone mimic), cyromazine (moulting disruptor) and azadirachtin (botanical compound which inhibits reproduction and has sterilising effects) on fertility and fecundity of *D. suzukii* was evaluated. These products were mixed with food attractants simulating bait applications frequently used for the control of other Diptera (Sánchez-Ramos *et al*., 2013).

Assays were carried out in the same type of rearing units described in WP2.

Eight to twelve replicates were used per product. Experiments were performed at 20°C, 70% HR and a 16:08 h light:dark photoperiod.

Three experiments with different periods of exposure to the insect growth regulators were performed. In the first assay, adults < 2h old were fed for 1-2 days with a mixture of sucrose and hydrolysed proteins containing each product in a final dose equivalent to the maximum field recommended concentration (MFRC). In the second assay, adults were fed with the treated diet for 3-4 days. In the last assay, adults were fed continuously with the treated diet for 15 days. In this case, the products were administered again at the MFRC by treating the surface of the artificial diet employed as oviposition substrate.

Pairs of treated adults were established and the percentage of fertile mating, the preoviposition period, the total fecundity for 10-20 days, the daily fecundity and the percentage of adults emerged from the total number of eggs laid were evaluated. The number of eggs laid was counted daily and the diet containing the eggs was replaced by new fresh diet. The old diet with the eggs was transferred to plastic cages where the development from egg to adult occurred.

Choice experiments were performed to establish the possible repellent effects of those products that showed effect in the previous assays (cyromazine, lufenuron and pyriproxyfen) and to determine if these effects were due to interference on the larvae development or to alterations of the reproduction because of the adult feeding on treated diet.

The same rearing units employed in the previous assays were used and a pair < 24 h old was introduced inside (Figure 28a). Each product was administered at the MFRC by treating the surface of the artificial diet (Figure 28b). Pairs were exposed for 6 days to the treated diet (Figure 28c). After that, pairs were transferred to plastic cages (8.5 x 8.5 x 6.5 cm) covered with a ventilated lid, containing two round boxes, as described above, filled with artificial diet (1.5 cm height) (Figure 28d). One of the boxes was treated with the product to be evaluated and the other was treated with the same quantity of distilled water acting as a control. A double-control with both diets treated with distilled water was also established for comparative purposes. Pairs were maintained for two days in these cages and, after that, the number of eggs laid in each substrate was counted. Then, the boxes containing the eggs were transferred individually to disposable plastic boxes to check the immature development and the adult emergence (Figure 28e). Experiments were performed at 20°C, 70% HR and a 16:08 h light:dark photoperiod. Eleven replicates per product were used.

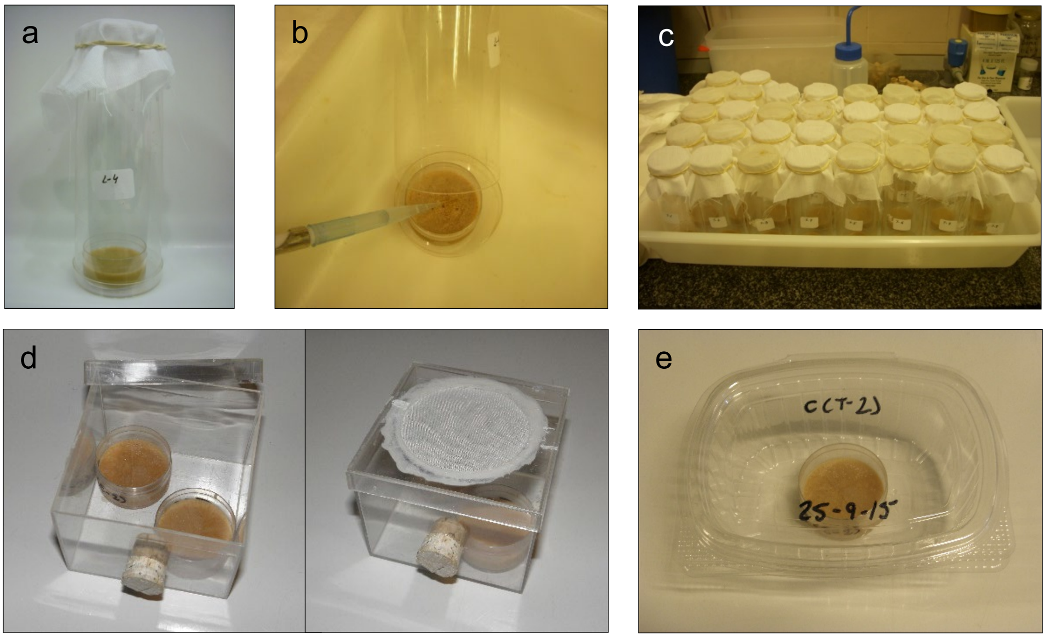

Figure 28. Experimental procedure employed in choice assays for *D. suzukii* against different IGRs. Rearing unit with the diet on the bottom (a). Application of the IGR on the diet (b). Rearing units containing a pair of *D. suzukii* and the treated diet (c). Plastic cages containing a treated and an untreated diet for the choice assay (d). Disposable plastic boxes to check adult emergence (e).

The number of eggs laid in each substrate was used to calculate the oviposition deterrence index according to the following equation:

$$DI = 100 \times \frac{C - T}{C + T}$$

Where *DI* is the oviposition deterrence index, *C* is the number of eggs laid in the control diet and *T* is the number of eggs laid in the treated diet. The *DI* was also established for the double-control replicates for comparative purposes, since its value should not be statistically different from zero.

Results

No significant differences were observed when the adults fed for 1-2 days with treated diet were compared to the control (Figure 29). Similarly, none of the products assayed affected the fertility, fecundity or emergence of the treated flies when they were fed for 3-4 days, although in the case of lufenuron, a slight reduction compared to the control was observed, but this effect was not statistically significant (Figure 3). Finally, when the adults were fed continuously on treated diet, fertility and fecundity were not affected (although again a slight reduction was observed with lufenuron compared to the control), but a drastic reduction in the emergence of adults was observed (Figure 3). Thus, no adult emerged from the eggs laid by females treated with lufenuron and cyromazine, and only 1% of the eggs reached the adult stage for those females treated with pyriproxyfen, compared with the 81% obtained for the control. Azadirachtin had no significant effect, because 72% of the eggs laid reach the adult stage. Regarding the percentage of pupation, it was 0% for lufenuron and cyromazine and 49% for pyriproxyfen, compared with 82% and 76% obtained for the control and azadirachtin, respectively.

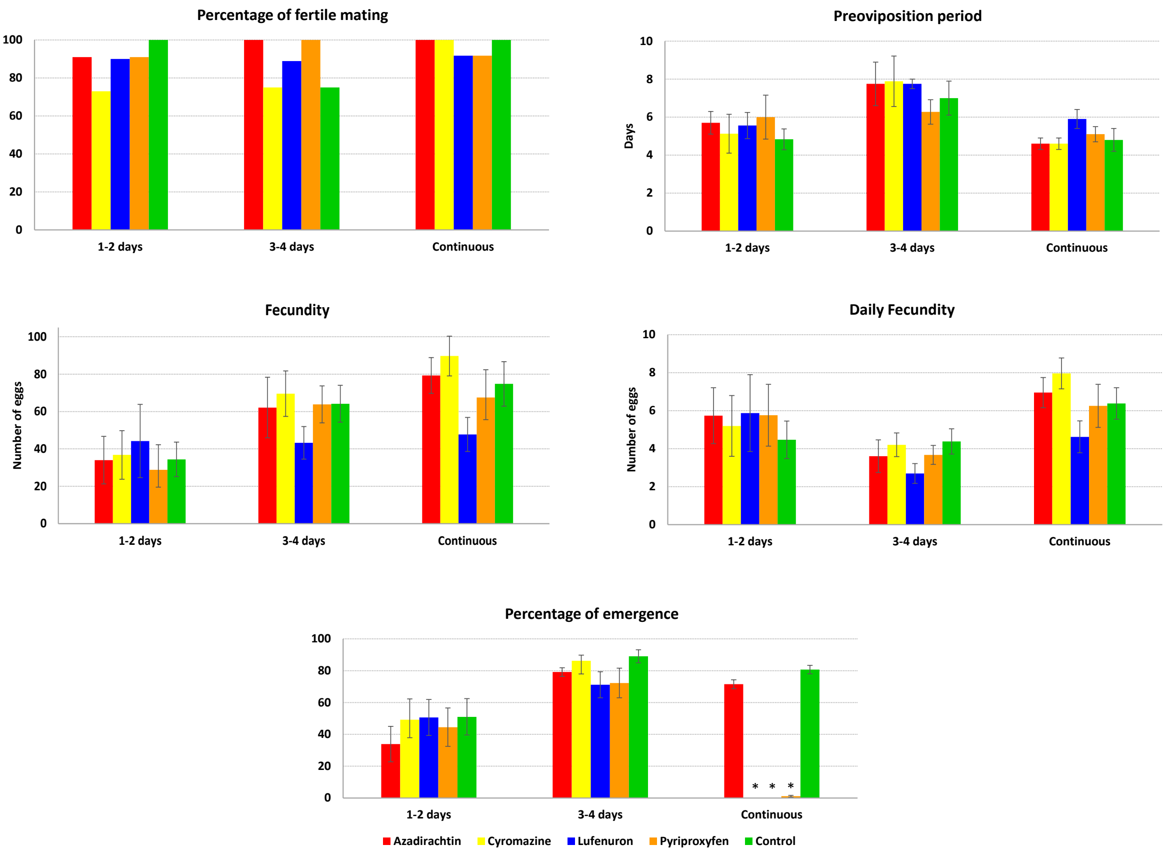

Figure 29. Preoviposition period (days), percentage of fertile mating, fecundity (total number of eggs laid per pair), daily fecundity (number of eggs laid per pair and day) and percentage of adult emergence from offspring of *D. suzukii* pairs fed for 1-2 days, 3-4 days or continuously with diet treated with azadirachtin, cyromazine, lufenuron and pyriproxyfen at the maximum field recommended concentration and with untreated diet (control). * indicates significant differences compared to control ($p \leq 0.05$; Kruskall-Wallis test followed by Dunn test).

Figure 30 shows the results obtained regarding the oviposition deterrence index. None of the products showed repellent effect, since no significant differences were found between the DI calculated for each product compared with the control. Only a significant difference was observed between pyriproxyfen and lufenuron, although this can be considered an artifact of the statistical analysis due to the reduced sample size.

When the adult emergence was checked in the treated and untreated diets, no emergence was obtained for cyromazine and lufenuron in the treated diets but, surprisingly, the same occurred in their corresponding control diets, whereas for pyriproxyfen, no emergence was observed in the treated diets and less than 18% was registered in their controls (Table 19). For the double-control diets, percentages of emergence ranged 50-80%.

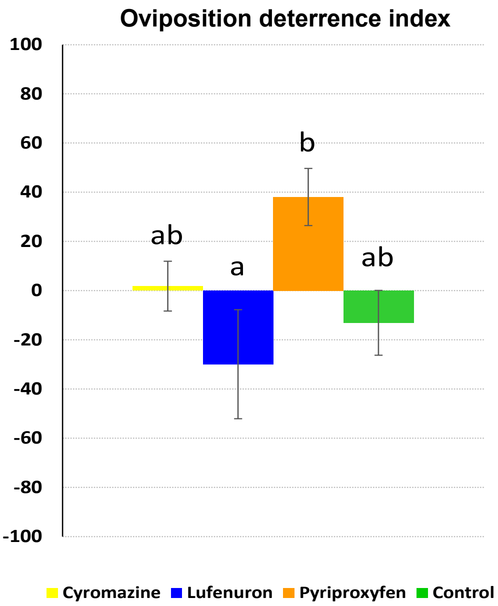

Figure 30. Oviposition deterrence indices obtained for *D. suzukii* in choice assays.

Table 19. Percentage emergence of *D. suzukii* adults coming from eggs laid in treated diets and in their corresponding control diets, and in the double control.

Treatment	Emergence (%)*
Cyromazine	0.0 ± 0.0 a
Control (Cyromazine)	0.4 ± 0.4 a
Lufenuron	0.0 ± 0.0 a
Control (Lufenuron)	0.0 ± 0.0 a
Pyriproxyfen	0.0 ± 0.0 a
Control (Pyriproxyfen)	17.4 ± 4.6 b
Control	50.0 ± 9,0 c
Control'	79.4 ± 9,7 c

*Different letters indicate significant differences.

Discussion

The products assayed have shown a limited efficacy for the control of *D. suzukii*. Thus, azadirachtin had no effect on the reproduction of the fly whereas cyromazine, lufenuron and pyriproxyfen prevented adult emergence of the offspring only when the flies fed continuously on treated diet. Emerged larvae were seen for all the treatments during the daily observations, so it was hypothesized initially that the effect found could be due to direct feeding by the larvae on the treated diet instead of the alteration of the reproductive processes of the adults. This would limit the possible use of these products to control this pest by means of bait treatments directed against adults. However, the results observed in the choice assays suggest a vertical transmission on the compounds assayed from the female to their offspring so that, although the eggs are able to hatch, the larvae cannot continue the development. This is so because if the observed effects were due to the feeding of the larvae on the treated diet, similar percentages of adult emergence should have been obtained in the control diets corresponding to each treatment and in the double control diets. This might open the possibility for the use of these products applied in chemosterilant bait stations for the control of the fly, although the need for a continuous exposure is a limiting fact, because is not likely that flies feed in the field always from the same food sources.

WP5: SURVEYS AND PROTOCOLS FOR EARLY DETECTION OF *D. suzukii* IN NON-INVADED COUNTRIES (GDAR)

The objectives of WP5 were:

- To conduct surveys for early detection of the pest.
- To prepare technical instructions for the control of the pest.
- To prepare a critical review of management practices that could be applied in case of detection of the pest.
- To propose IPM approaches and future research objectives.

WP5 aims to determine the status of *D. suzukii* in Turkey, so far known as non-invaded country. Objectives of WP5 study were to perform monitoring activities and organise training sessions in some provinces of Turkey. Surveys have been conducted to determine the status of *D. suzukii* in the country and eventually implement control measures against this pest in infested production areas. Survey studies were conducted in fruit orchards including fruit species such as cherry, peach, plum, fig, strawberry, grape, persimmon and kiwi fruit. Baited with apple vinegar as attractant, a trapping method was used to determine the presence of the pest in the surveyed areas. Traps were placed in major fruit growing areas in cities, towns, villages and packing houses to detect *D. suzukii* in economically important hosts. Training activities were organized at national level and technical instructions were prepared including the description of the pest and information on its biology, hosts, pathways and damages it may induce. Leaflets were printed and distributed to technical staffs and producers to increase public awareness.

Although it was planned to conduct life table studies in the laboratory in case of presence of *D. suzukii* in Turkey, these studies were not performed because *D. suzukii* was not detected during the surveys.

Methods used and Results obtained

At the begining of the study, a survey protocol was prepared. Then training activities were organized two times a year for technical staffs who work in extension services in provinces. Training was given on how to conduct surveys, monitoring and identification of the pest and on the damages it produces on plant hosts. More than 5000 leaflets were printed and distributed to farmers and technicians. A poster presentation was prepared and presented in a horticultural congress in Çanakkale (Bozkurt, 2015). Survey studies were organized and a trapping method was implemented by using apple vinegar as attractant to determine absence or presence of the pest in major fruit growing areas (Figure 31). Traps were hung on trees in different fruit orchards and in packing houses. Traps consisted of plastic bottles with 8-10 holes (3 mm Ø) baited with apple vinegar. Traps were checked at weekly intervals between April and November in both years.

Figure 31. Transparent bottle traps used in survey studies (© V. Bozkurt)

Collected samples were preserved in boxes containing 70% ethanol (Figure 32). Samples were collected every week and identified using laboratory binocular microscope. During the study, 6000 insect samples were collected from different fruit growing areas and were inspected in the laboratory.

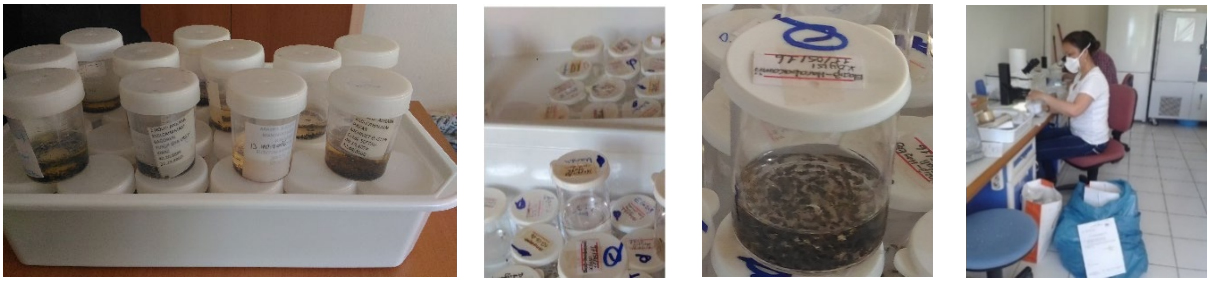

Figure 32. Collected samples preserved in plastic cups with 70% ethanol.

Discussion

Survey studies have been conducted between 2015 and 2016 in Afyon, Aydın, Bursa, Balıkesir, Çanakkale, Denizli, Isparta, Izmir, Konya, Manisa provinces. Although other Drosophilidae species such as *Drosophila melonogaster*, *D. simulans* and *Zaprionus* spp. were present, *Drosophila suzukii* was not identified during the survey in the Eupresco study. However, *D. suzukii* was found in Turkey in Erzurum province (in east part of Turkey) during the course of another study (Orhan *et al*., 2016). Erzurum province was not surveyed during the Euphresco study. The pest was found on stawberry fruits in the campus of the Atatürk University by university researchers. Eradication measures were established and applied to eradicate the pest in Erzurum.It is not known how the infestation occurred but it is thought that it could have been caused by the transportation of infested fruits from the neighbouring country Iran, which is a country infested with *D. suzukii* (Parchami *et al*., 2015).

MAIN CONCLUSIONS

-In laboratory experiments, *D. suzukii* is able to complete the preimaginal development in the range of temperatures between 10 and 30°C. Models estimate the developmental thresholds around 9°C and 31°C. The optimal temperature for immature survival is around 20°C, but the fastest development occurs at 28°C. In general, immature development takes less than two weeks above 22°C, indicating that multiple generations can exist after winter through the seasons if suitable food is available.

-The short developmental period and the high reproductive potential result in high capacity for population increase, which explains the huge potential of this fly to produce damage in susceptible crops. The optimal temperature for population increase has been estimated at 25°C. A great longevity has been registered at low temperatures, explaining the overwintering capacity of this fly and the recolonization of susceptible crops after winter break. New studies of reproduction might be necessary for refinement of the model obtained for the intrinsic rate of natural increase.

-Different models have been obtained describing the effect of temperature on the biological parameters studied, that could be used in integrated pest management programs of this pest to predict risk situations and possible new colonization areas.

-*Drosophila suzukii* most likely overwinters as winter morph adults, which seek refuge in sheltered locations during winter. Further research should focus on the cold hardiness of more northern and southern located populations in Europe, to have a better understanding of the cold tolerance and the adaptability of this species to sub-zero temperatures.

-*Drosophila suzukii* adults are likely capable of overwintering mild winters in temperate maritime climate zones, although sheltered places with suitable food sources are probably needed during the colder days and a drop back in the population during winter is to be expected.

-A short and shallow reproductive diapause could occur in winter morph females, but this hypothesis should be further explored.

-There is a positive correlation between increasing field temperatures and abundance of *D. suzukii* adults in winter and early spring. The *D. suzukii* abundances recorded are better fit by temperature data interpolated by the Allen method. This method seems to give stable forecasting to be considered in strategies to *D. suzukii* control.

-The self-developed traps represent a suitable and potential alternative to commercial available traps to catch *D. suzukii*, especially for private persons/gardeners.

-However, in future, research and further development of traps (including attractants) and their way of use are still important aims for improving monitoring activities and control of *D. suzukii*.

-Of the biological control agents tested, the entomopathogenic fungus *Metarhizium anisopliae*, caused more than 80% mortality on *D*. *suzukii* adults and around 60% on *D*. *suzukii* preimaginal stages in direct toxicity trials.

-However, none of the biological control products tested resulted in complete mortality and, although they can reduce population numbers of *D. suzukii*, they are unlikely to control/eradicate populations. In any case, they could be more easily incorporated into existing control programmes compared to conventional pesticides.

-Cyromazine, lufenuron and pyriproxyfen prevent adult emergence of the offspring of flies that feed continuously on treated diet. This is due to a vertical transmission of these compounds from the female to the offspring: the eggs laid are able to hatch but the larvae cannot continue the development. However, the possible use of these products applied in chemosterilant bait stations for the control of *D. suzukii* seems not feasible because is not likely that flies feed always from the same food sources. In any case, field trials might be performed to check this fact.

-This work is the first study conducted about *D. suzukii* in Turkey. Results of survey studies and information about importance of *D. suzukii* have been given to Ministry of Food, Agriculture and Livestock.

-In another study, *D. suzukii* was found on strawberry plants in a limited area of the Erzurum province. Since surveys had been focused mainly on the west part of Turkey, detailed surveys were started in east part of country especially Erzurum and vicinity of that region. As a result, strawberry fruits were found infested with *D. suzukii* in a restricted area of Erzurum province. All infested fruits and plants were eradicated. It can be said that *D. suzukii* was eradicated in Erzurum province in Turkey. Although eradication process was completed by Ministry of Food, Agriculture and Livestock, official survey and identification studies will be continued in fruit growing areas in whole region of Turkey.

-To avoid damages of *D. suzukii*, monitoring and identifying of the pest is very important and reviewing the effectiveness of control measures has to be undertaken in major fruit growing areas in Turkey. IPM strategies has to be applied in order to effectively control *D. suzukii* in infested production areas to reduce population and damages.

PUBLICATIONS AND DISSEMINATION ACTIVITIES

INIA

Published papers:

González-Núñez, M. & Sánchez-Ramos, I. (2015). Desarrollo de estrategias para el manejo sostenible de *Drosophila suzukii*. Phytoma, 269: 29-31.

Conference presentations:

González-Núñez, M. & Sánchez-Ramos, I. Desarrollo de estrategias para el manejo sostenible de *Drosophila suzukii.* Ecología y control de Drosophila suzukii tras cinco años en nuestros cultivos. Encuentro Internacional PHYTOMA-España sobre *Drosophila suzukii*, Valencia (Spain), 5-6.05.2015.

Guzmán, V., González-Núñez, M., Fernández, C.E., Pascual, S. & Sánchez-Ramos, I. Estudios de laboratorio para el desarrollo de estrategias de manejo integrado de *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). IX Congreso Nacional de Entomología Aplicada. XV Jornadas Científicas de la Sociedad Española de Entomología Aplicada, Valencia (Spain), 19-23.10.2015.

AGES

Published papers:

Lethmayer, C. & Egartner, A. (2017). Enhancement of *Drosophila suzukii* trapping. – IOBC-WPRS Bulletin, Vol. 123, 143-149.

Conference presentations:

Lethmayer, C. & Egartner, A.: *Drosophila suzukii* (Kirschessigfliege) in Österreich – Status Monitoring und Bekämpfung. 55. Österreichische Pflanzenschutztage, Seggau (Austria), 26.11.2014.

Lethmayer, C. & Egartner, A.: *Drosophila suzukii* (Kirschessigfliege) – Statusbericht Österreich. 3. Treffen der AG Kirschessigfliege, Freiburg (Germany), 09.12.2014.

Lethmayer, C.: Situationsbericht *Drosophila suzukii* 2014 in Österreich und Nachbarländern. Rebschutzgebietsleitertagung, Vienna (Austria), 29.01.2015.

Lethmayer, C.: Kirschessigfliege – Biologie und aktueller Stand in Österreich; Steinobstfachtag 2015, Silberberg (Austria), 12.02.2015.

Lethmayer, C.: Kirschessigfliege (*Drosophila suzukii*) – Biologie und Status in Österreich. Weinbau Pflanzenschutzabend 2015, Baden (Austria), 19.02.2015.

Lethmayer, C.: Aktuelle Situation der Kirschessigfliege (*Drosophila suzukii*) in Österreich. Besprechung SWD-Strategie Weinbau 2015, Klosterneuburg (Austria), 13.04.2015.

Lethmayer, C. & Egartner, A.: Einsatz von Fallen gegen die Kirschessigfliege (*Drosophila suzukii*). Besprechung SWD-Strategie Weinbau 2015, Klosterneuburg (Austria), 13.04.2015.

Lethmayer, C. & Egartner, A.: *Drosophila suzukii* (Kirschessigfliege) - Fallenvergleich und Status Monitoring Österreich 2015. Österreichische Pflanzenschutztage, Rust (Austria), 24.11.2015.

Lethmayer, C. & Egartner, A.: Neue Erkenntnisse über den Einsatz von Fallen zum Vergleich von *Drosophila suzukii* (Kirschessigfliege). 4. Treffen der AG Kirschessigfliege, Erfurt (Germany), 09.12.2015.

Lethmayer, C.: Erfahrungen mit der Kirschessigfliege in Österreich. Symposium zur Kirschessigfliege, Friedrichshafen (Germany), 20.02.2016.

Lethmayer, C., Egartner, A. & Strauß, G.: Kirschessigfliege *(Drosophila suzukii)* - Monitoring 2015. Rebschutzgebietsleitertagung 2016, Vienna (Austria), 25.02.2016.

Lethmayer, C., Egartner, A., Oberhuber, M., Strauß, G. & Blümel, S.: Aktuelle Projekte über die Kirschessigfliege (*Drosophila suzukii*). Klosterneuburger Forschungsdialog, Klosterneuburg (Austria), 21.06.2016.

Lethmayer, C., Egartner, A.: Enhancement of trapping systems against *Drosophila suzukii*. 9th International Conference on Integrated Fruit Production (IOBC), Thessaloniki (Greece), 06.09.2016.

Lethmayer, C.: Aktuelles zur Kirschessigfliege (*Drosophila suzukii*) 2016. 57. Österreichische Pflanzenschutztage, Wels (Austria), 29.11.2016.

Technology transfer:

Lethmayer, C., Egartner, A. & Blümel, S.: Infoveranstaltung Kirschessigfliege (*Drosophila suzukii*) - Organization of an information-meeting about *D. suzukii* for national phytosanitary advisors and practitioners, Vienna-AGES (Austria), 25th January 2015.

Lethmayer, C., Egartner, A. & Blümel, S.: Infoveranstaltung Kirschessigfliege (*Drosophila suzukii*) - Organization of an information-meeting about *D. suzukii* for national phytosanitary advisors and practitioners, Vienna-AGES (Austria), 17th February 2016.

Lethmayer, C. & Egartner, A.: input on the AGES-webpage about *D. suzukii*: https://www.ages.at/themen/schaderreger/kirschessigfliege/

Technical publications:

Lethmayer, C. & Egartner, A. (2015). Explosionsartige Vermehrung der Kirschessigfliegen. - Besseres Obst 1, 6-7.

Lethmayer, C. & Egartner, A. (2016). Kirschessigfliege: Welche Falle eignet sich am besten? - Besseres Obst 2, 14-16.

CREA

Published papers:

Gargani E., Bagnoli B., Simoni S. (2015). *Drosophila suzukii* in Tuscany (Italy), from cherry crops to vineyards and beyond. CIHEAM, Watch Letter n.33

Conference presentations:

Landi S., E. Gargani, F. Paoli, S. Simoni, P.F. Roversi (2014). Sviluppo embrionale in *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). Riassunti XXIV Congresso Nazionale di Entomologia, Orosei 9-14 giugno, 2014: 43-44.

Simoni S., D. Goggioli, S. Guidi, F. Tarchi, E. Gargani (2016). Valutazione di parametri di interpolazione in report di temperatura ambientale per la conoscenza della dinamica stagionale di *Drosophila suzukii*. – Atti XXV Congresso Nazionale Italiano di Entomologia (a cura di M. Faccoli, L. Mazzon e E. Petrucco-Toffolo) – Padova 20-24 giugno 2016: 133.

ILVO

Published papers:

De Ro, M., Devos, T., Berkvens, N., Casteels, H., Goffin, J., Beliën, T. & De Clercq, P. (2017). Overwintering capacity of *Drosophila suzukii* (Diptera: Drosophilidae) in Belgium. – IOBC-WPRS Bulletin, Vol. 123, 178-179.

Conference presentations:

De Ro, M., Devos, T., Berkvens, N., Casteels, H., Goffin, J., Beliën, T. & De Clercq, P.: Cold hardiness of *Drosophila suzukii* (Diptera: Drosophilidae) in Belgium. 68th International Symposium on Crop Protection, Gent (Belgium), 17.05.2016.

De Ro, M., Devos, T., Berkvens, N., Casteels, H., Goffin, J., Beliën, T. & De Clercq, P.: Overwintering capacity of *Drosophila suzukii* (Diptera: Drosophilidae) in Belgium. 9th International Conference on Integrated Fruit Production (IOBC), Thessaloniki (Greece), 06.09.2016.

GDAR

Conference presentations:

Bozkurt, V. (2015). Önemli Karantina Zararlısı *Drosophila suzukii* (Matsumura 1931) (Diptera: Drosophilidae) Uzerine Inceleme. VII. Ulusal Bahçe Bitkileri Kongresi. Bildiri Özetleri Kitabı. 25-29 Ağustos 2015. Çanakkale-Turkiye. (Poster).

Technical publications:

Anonymous (2012). Survey Instruction of *Drosophila suzukii* (Diptera: Drosophilidae). Republic of Turkey, Ministry of Food Agriculture and Livestock. General Directorate of Food and Control. Plant Health and Quarantine Directorate. Ankara, Turkey.

Bozkurt, V. (2015). Leaflet about description, hosts and damages of *Drosophila suzukii* (Diptera: Drosophilidae). Republic of Turkey, Ministry of Food Agriculture and Livestock. General Directorate of Food and Control. Plant Health and Quarantine Directorate. Ankara, Turkey.

Anonymous (2016). Plant Protection Technical Instruction of *Drosophila suzukii* (Diptera: Drosophilidae). Republic of Turkey, General Directorate of Agricultural Research and Policy (GDAR). Plant Health Research Directorate. Ankara, Turkey.

Bozkurt, V., Ozdem, A. (2016). Control and Eradication Technical Instruction of *Drosophila suzukii* (Diptera: Drosophilidae). General Directorate of Agricultural Research and Policy (GDAR). Plant Health Research Directorate. Ankara, Turkey.

Training activities:

Bozkurt, V., Ozdem, A., Ayan, A. -Training about conducting survey, damages and description of *Drosophila suzukii* (Diptera: Drosophilidae) for technical staff and National Plant Protection Organisation's staff. Ankara-GDAR (Turkey), 26th February 2015.

Bozkurt, V., Ozdem, A., Ayan, A.- Provincial training about conducting survey, damages and description of *Drosophila suzukii* (Diptera: Drosophilidae). Ankara-GDAR (Turkey), 10th March 2016.

Bozkurt, V., Ozdem, A., Ayan, A.- Regional training about conducting survey of *Drosophila suzukii* (Diptera: Drosophilidae). Ankara-GDAR (Turkey), 31th March 2016.

Bozkurt, V., - National training about description, hosts, survey, damages and control measures of *Drosophila suzukii* (Diptera: Drosophilidae). Antalya-GDAR (Turkey),16th April 2016.

ACKNOWLEDGEMENTS

This research was supported and funded by:

- National Institute for Agricultural and Food Research and Technology (INIA, ES).

- Austrian Agency for Health and Food Safety (AGES, AT).

- Research Council for Agriculture and Economics – Research Centre for Agrobiology and Pedology (CREA-ABP, IT).

- Institute for Agricultural, Fisheries and Food Research (ILVO, BE), funded by Flanders Innovation & Entrepreneurship (VLAIO, BE) (Grant Nr. IWT/LATR/135079).

- General Directorate of Agricultural Research and Policy (GDAR, TR).

REFERENCES

1. Ashburner, M., Golic, K.G. & Hawley, R.S. (1961). *Drosophila*: a laboratory handbook, 2nd edn. Cold Spring Harbor Laboratory Press, New York, USA, pp. 1409.
2. Bates, D., Maechler M., Bolker B. & Walker S. (2014). lme4: Linear mixed-effects models using Eigen and S4. R package version 1: 1-7.
3. Bozkurt, V., (2015). Önemli Karantina Zararlısı *Drosophila suzukii* (Matsumura 1931) (Diptera: Drosophilidae) Uzerine Inceleme. VII. Ulusal Bahçe Bitkileri Kongresi Bildiri Özetleri Kitabı. 25-29 Ağustos 2015. Çanakkale, Turkiye (Poster).
4. Briere, J.-F., Pracros, P., Le Roux, A.-Y. & Pierre, J.-S. (1999). A novel rate model of temperature-dependent development for Arthropods. Environ. Entomol. 28: 22-29.
5. Cowles, R.S., Rodríguez-Saona, C., Holdcraft, R., Loeb, G.M., Elsensohn, J.E. & Hesler, S.P. (2015). Sucrose Improves Insecticide Activity Against *Drosophila suzukii* (Diptera: Drosophilidae). J. Econ. Entomol. 108(2): 640–653.
6. EPPO (2017). https://www.eppo.int/QUARANTINE/listA2.htm
7. Gargani, E., Tarchi, F., Frosinini, R., Mazza, G. & Simoni, S. (2013). Notes on *Drosophila suzukii* Matsumura (Diptera: Drosophilidae): Field survey in Tuscany and laboratory evaluation of organic products. Redia XCVI: 85-90.
8. Gargani E., Tarchi F., Frosinini R., Mazza G., Lazzeri L., Matteo R., Simoni S., 2014 - Evaluation of some organic products for Spotted Wing Drosophila control. - Book of Abstract VIII Workshop on Integrated Soft Fruit Production, Vigalzano di Pergine Valsugana (TN), 26-28 May 2014: 84-85.
9. Grassi, A., Anfora, G., Maistri, S., Gottardello, A., Maddalena, G., De Cristofaro, A., Savini, G. & Ioriatti, C. (2015). Development and efficacy of Droskidrink, a food bait for trapping *Drosophila suzukii*. IOBC-WPRS Bulletin Vol. 109, 197-204.
10. KMI (2017). http://www.meteo.be (read on 10-01-2017)
11. Lactin, D.J., Holliday, N.J., Johnson, D.L. & Craigen R. (1995). Improved rate model of temperature-dependent development by arthropods. Environ. Entomol. 24: 68-75.
12. Okuyama, T. (2014). On the estimation of temperature-dependent development rate. Appl. Entomol. Zool. 49: 499-503.
13. Orhan, A., Aslantaş, R., Önder, B. Ş. & Tozlu, G. (2016). First record of the invasive vinegar fly *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) from eastern Turkey.» Turk J. Zool. 40: 290-293.
14. Overmeer, W.P.J. (1988) Laboratory method for testing side-effects of pesticides on the predaceous mites *Typhlodromalus pyri* and *Amblyseius potentillae* (Acari: Phytoseiidae). IOBC/WPRS Bulletin. 11: 65-69.

15. Parchami-Araghi, M., Gilasian, E. & Keyhanian, AA. (2015). Spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Dip.: Drosophilidae) an invasive fruit pest new to the Middle East and Iran. *Drosophila Information Service* no. 98, 59-60.

16. Pinder III, J.E., Wiener, J.G. & Smith, M.H. (1978). The Weibull distribution: a new method of sumarizing survivorship data. Ecology 59: 175-179.

17. R Core Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

18. Richter, O. & Söndgerath, D. (1990). Parameter estimation in ecology: The link between data and models, VCH Verlagsgesellschaft, Weinheim, Germany, pp. 218.

19. Sánchez-Ramos, I., Fernández, C.E., González-Núñez, M. & Pascual, S. (2013). Laboratory tests of insect growth regulators as bait sprays for the control of the olive fruit fly, *Bactrocera oleae*(Diptera: Tephritidae). Pest Manag Sci. 69: 520–526.

20. SYSTAT. (2002). TableCurve 2D 5.01 for Windows User's manual. SYSTAT Software Inc, Richmond, CA, USA, 672 pp.

21. Von Lochow, J. & Schuster, W. (1961). Anlage und Auswertung von Feldversuchen: Anleitungen und Beispiele für die Praxis der Versuchsarbeit. Dlg-Verlag, Frankfurt, Germany, pp. 130.