EUPHRESCO Final Report (NC)
for Non-Competitive research projects

Please send the final report to all your project partners, to the NC topic coordinators and to the EUPHRESCO Secretariat (euphresco@fera.gsi.gov.uk).

Project Title and Acronym
Damage potential of *Drosophila suzukii* and development of risk management and control measures (DROSKII)

Project Duration:

Start date:	01/06/12
End date:	30/05/14

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2. Executive Summary

Project Summary

Title
Damage potential of *Drosophila suzukii* and development of risk management and control measures (DROSKII)

Introduction
Drosophila suzukii, the Spotted Wing Drosophila (SWD), is a vinegar fly native to Asia, which recently has been introduced to Europe (Calabria *et al.*, 2012). *Drosophila suzukii* infests thin-skinned fruits and poses a significant risk to soft fruits, stone fruits as well as grapes (Cini *et al*., 2012). Although this pest is present in Europe since only a few years, severe damages were observed on sweet cherries, strawberries, raspberries, black and blueberries. New findings in EU countries account for infestation on some grape varieties. The economic damage potential of *D. suzukii* is therefore enormous due to its high biotic potential. Experiences with insecticide applications show that efficient crop protection is difficult to attain. Furthermore, the range of registered products against *D. suzukii* is quite limited (Boselli *et al*., 2012). Thus, the elaborated pest risk assessment of the EPPO (European and Mediterranean Plant Protection Organization) concluded that *D. suzukii* is an important pest of soft fruits, stone fruits and grapes in most parts of the EPPO region.

Main objectives
To characterize/identify:

- the potential dispersal of *D. suzukii* in Europe by large scale monitoring;
- the most behaviourally-active volatiles emitted by fruits host of *D. suzukii* in order to develop more selective and powerful attractant lures;
- the applicability and efficacy of environmentally sound methods such as physical barriers, mass-trapping and mating disruption;
- the occurrence and level of incidence of natural enemies, in particular parasitoids, and their even management to control.

Methods
Monitoring - activities, assessment, refinement and forecasting, at local-regional-transnational scale.
Susceptibility testing of different fruit varieties
Field survey in different susceptible agro ecosystem in order to verify occurrence and incidence of *D. suzukii* on different hosts. Extraction and identification of volatile compounds emitted by its main host fruits.
Survey on the infestation of grapes and grape varieties in Europe
Monitoring of infestation on grapes and screening the susceptibility of grape vars.
Actions for containment and control of D. suzukii by environmentally-friendly measures.
Assessment of appropriate approved chemicals for fruit against adults and/or larvae. Screening and assessment of new control measures and management approaches. Field survey to verify occurrence and incidence of natural enemies on SWD.

Results and Conclusion
WP2: *D. suzukii* is very rapidly spreading in Europe. In 2012-2013, SWD was

recorded over the winter as well as in spring. The peak of population was in late October/early November. In 2013-2014, the mild winter allowed the *D. suzukii* population to build up very early in the spring.

Different traps and lures were tested: the ideal trap is put on a shady place and has a high number of small openings (2 to 3 mm), which are rain-proofed; yeast baits or e.g. the mixture of apple cider vinegar and wine as trapping lures delivered the best results but development is required to improve the efficiency of traps - e.g. the number and position of traps per site and/or the timing of control measures. Mass-trapping is implemented on a broad scale in berry production and producers were satisfied with the achieved protection.

WP3: Different volatiles from host plants influence the olfactory behaviour of *D. suzukii*. Mated females were attracted to rubber septa loaded with synthetic isoamyl acetate. The release rate of isoamyl acetate from attractive rubber septa was comparable to that by fresh fruits. The almost complete putative full repertoire of genes encoding odorant receptors normally activated by isoamyl acetate in *D. melanogaster* are present in *D. suzukii*. The results indicate that *D. suzukii* uses olfactory cues to select oviposition sites.

Preliminary lab trials using Y-olfactometer to evaluate the response of *D. suzukii* to the yeast *Hanseniaspora uvarum,* frequently present on healthy fruits, were carried out. The comparison among three yeast strains artificially reared, highlighted that only one of them is attractive in comparison to the control represented by the growing media.

WP4: An online survey on the threat that *D. suzukii* poses to European table and wine grape production was developed and emailed to phytosanitary services, industry partners, policy makers and scientists all over Europe in the winter 2013 and 2014. It results that *D.suzukii* is present in most European vineyards, but it did not cause any major damages on table or wine grapes in 2012 as well as 2013. The obtained responses cover more than 10% of the viticultural area of Europe. It was confirmed that grapes can be damaged by SWD even if they are not very suitable for larval development and the buildup of large populations. The interaction of *D. suzukii* with native vinegar flies and fungal pathogens is partly understood. There are no clear evidences that SWD presence favors the development of other drosophilids or sour rot even if such interactions cannot definitely be excluded.

WP5: The application of sanitation measures and mass-trapping proves to be very effective against *D. suzukii*.

Spinosad, chlorantraniliprole and the experimental product, TA2674, showed excellent potential as control agents for blueberries with mortalities of 100%, 93% and 98%, respectively. Entomopathogenic agents (fungi: *Lecanicillium muscarium* as Mycotal as well as *Beauveria bassiana* as Naturalis and Botanigard; nematodes: *Steinernema carpocapsae*, *S. feltiae*, *S. kraussei*) were tested and appeared to reduce *D. suzukii* population development. However, they are probably unable to prevent outbreaks. *Pachycrepoideus vindemiae* (Hym.: Pteromalidae), a generalistic parasitoid was collected from both *D. suzukii* and *D. melanogaster* pupae in traps deployed in different sites in Northern Italy (Trento province). The record of *P. vindemiae* represents the first identification of a *D. suzukii* parasitoid in Europe.

WP1 - Project Management and Coordination

Leader: Sauro Simoni (CRA-ABP, Italy)

WP1 activity have ensured running and accomplishment of the Project activities by:

- Definition of WP leaders
- Coordination of information and data exchange among WPs, prevalently based on e-mails.

The Project activities and the partners' interactions have been defined within the Kick-off Meeting, held in Pergine Valsugana (Trento, Italy) on 2012, September, the 7th. The Project progress has been verified with an intermediate Meeting, held in Sion (Switzerland) on 2013, November, the 26th-27th. The final event of the Project – with some conclusions and results presented – was during the IOBC Meeting, IOBC Working Group "Integrated Plant Protection in Fruit Crops" Subgroup "Soft Fruits" held in Vigalzano (Trento, Italy) on 2014, May, the 26-28th.

No major problems were encountered, so that all the project objectives were achieved and the deliverables made available according to the contract.

WP2: Monitoring – activities, assessment, refinement and forecasting

Lead: AT-AGES (Christa Lethmayer, Alois Egartner, Sylvia Blümel)

Partners involved: CH-FOAG (Catherine Baroffio), JKI (Peter Baufeld)

Objectives:

- quick and efficient detection of SWD (with traps)
- practicability and cost effectiveness of traps for application/disposal/use
- successful use of traps for SWD control and ability to regulate the suitable lures to attract one or both of the sexes to the trap
- assessment of trap types depending on the use, the development and/or evaluation of appropriate matrices for the release of the lure compound(s)
- knowledge on the biology and ecology preferences of SWD under European conditions and information about the geographical distribution of SWD in Europe
- forecasting of populations density, expected damages and economic consequences depending on habitats, availability of suitable hosts over the season and climatic conditions

Methods and Results

1 Monitoring activities for information of SWD-distribution

1.1.1. Results of monitoring activities for information of SWD-distribution in Switzerland 2012-2014 (CH-FOAG)

1.1.2 Material and Methods

The monitoring was conducted in all Swiss cantons all over the country. Between 2012 and 2014, the network consisted of about 200-300 traps distributed each year according to the importance of fruit growing areas (2 to 15 traps/canton). The traps consisted of a polystyrene box of 1300 ml (Agroscope trap) containing 200 ml of attractive liquid. The liquid was composed of a mixture of equal parts of apple cider vinegar and water, a small amount of red wine (about 5%) in order to increase the mixture's attractiveness, as well as a drop of detergent to reduce the surface tension of the liquid. Between May and July 2012, the monitoring traps were placed in cherry and strawberry crops, followed by raspberry, blackberry, blueberry crops and in autumn they were finally set-up in vineyards. The traps were checked on a weekly basis for identification of captured vinegar flies and other insect species.

1.1.2 Results

In 2012, more than 60’000 individuals were captured. The recordings of the population development including crops affected, progression and location of spread were published on the open access website www.drosophilasuzukii.agroscope.ch. The first discovery was reported in a cherry orchard of the canton of Ticino (southern part) in the month of May 2012. *Drosophila suzukii* was registered from July onwards over the whole of Switzerland. The insect was captured from the plain to the subalpine zone of the Jura and the Alps. The number of individuals captured increased over the season as a result of the buildup of pest populations over the summer . The peak of insect activity was thus recorded between mid-September and late October as in neighboring countries. Overall, the number of *D. suzukii* catches was highest in late fruit crops such as raspberries, blackberries and blueberries and in the surrounding of vineyards (Fig. II-1, II-2). Monitoring traps placed in hedges, including elderberry and *Viburnum* sp. also captured large numbers of *D. suzukii*. In these natural areas, monitoring was continued over the winter 2012-13 and 2013-2014. Catches fell sharply in the end of November 2012 stopping altogether in January (snow being present). Individual captures were once again recorded in February 2013. But with mild winter 2013-2014 continuous catches and a high potential reservoir of insects were present for the beginning of the season 2014 (Fig. II-3).

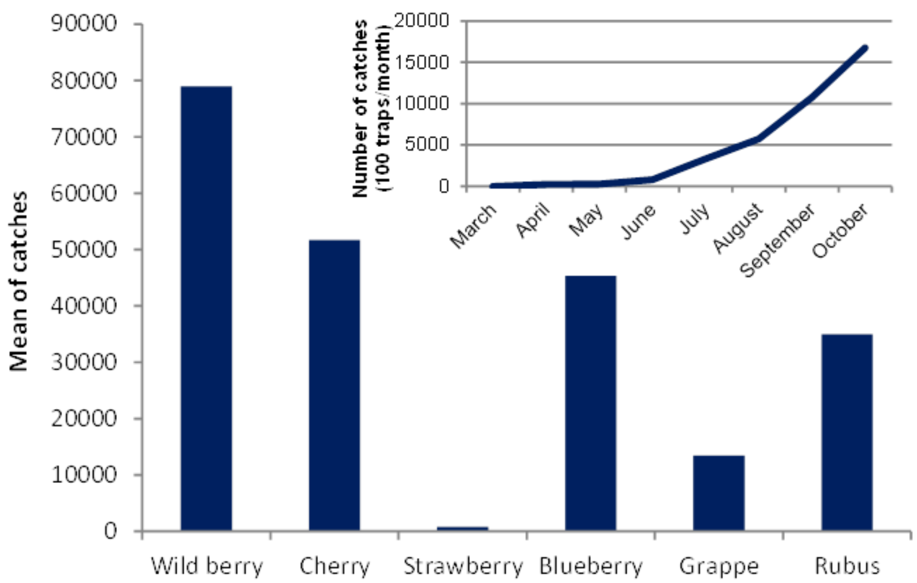

Fig. II-1: Captures of *D. suzukii* on 200 traps per crop and per month. Log. Scale

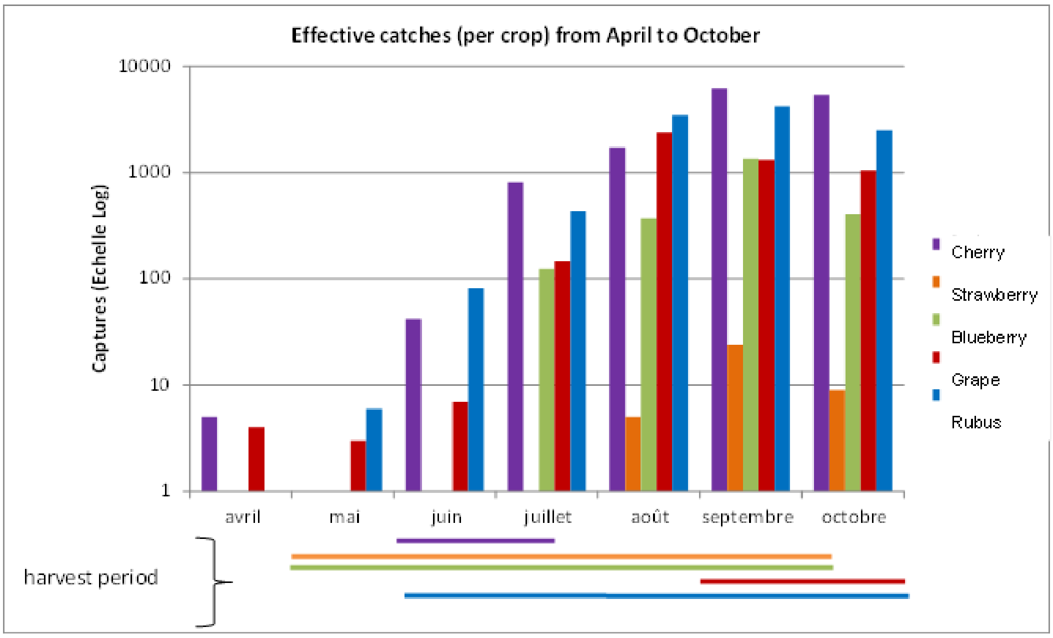

Fig. II-2: SWD catches in 2013 on cherry, strawberry, blueberry, grapes and *Rubus* in Switzerland

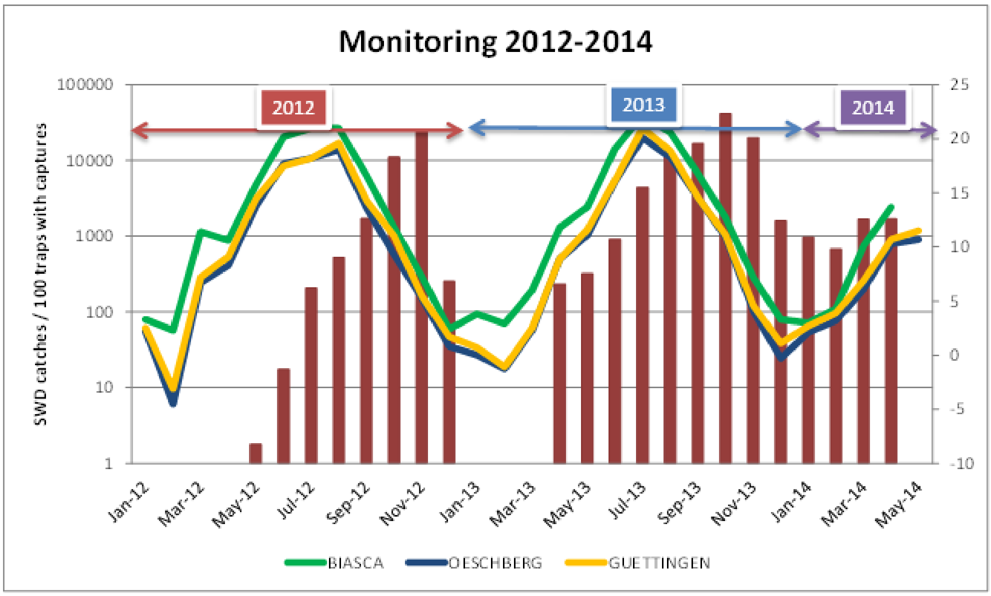

Fig. II-3: SWD catches between 2012 and 2014 with the mean temperatures of 3 different places in Switzerland

1.2. Results of monitoring activities for D. suzukii *in Austria 2012-2013 (AGES)*

In Austria a nation-wide monitoring with traps was carried out in 2012 (133 sites) and 2013 (22 sites) in orchards (pome, stone and berry fruits), private gardens, vineyards and the natural surroundings of orchards (forest). Results of 2012 and 2013 revealed that *D. suzukii* is distributed over all Southern and Western regions of Austria (mostly

between 1 and 30 individuals per trap per week), partially with higher abundance (up to more than 100 individuals per trap per week in 2013). The area with confirmed SWD trap catches expanded to the North-Eastern regions in 2013. However, only single individuals were caught in these regions, mainly in late October 2013. In total more than 40 locations with SWD occurrence were confirmed in Austria.
Regarding the phenology and distribution of SWD it can be summarized that in the Southern and Western regions first single individuals of SWD occurred in July and August, with a slight increase in September and an obvious peak at the end of October. In contrast, only single individuals occurred at the North-Eastern regions later in the year, for the first time in October and November.
While in 2011 infestation of fruits from elderberries and strawberries was assessed, no visible damage of fruits was reported from producers for 2012 and 2013.
At the moment the risk of infestation seems to be present only for late ripening fruits as raspberries, elderberries and blackberries in Austria.

2. SWD Control

2.1. Development of efficient traps for monitoring and controlling SWD (CH-FOAG)

2.1.1. Material and Methods

Selectivity of monitoring traps and lure comparison

The three commercial traps “Droso-Trap”, “McPhail” and “Sentomol” were compared to the Agroscope trap (Table 1).

Table II-1: Description of the 4 traps used in the trial in 2012

			
Droso-Trap Vol.: 1300 ml 3 lateral openings Ø 1.2cm	**McPhail** Vol.: 2600 ml 1 basal opening Ø 4.5cm	**Sentomol** Vol.: 1000 ml 12 lateral openings Ø 1cm	**Agroscope** Vol.: 1300 ml 16 lateral openings Ø 3mm

The trial first took place in three cherry orchards in June 2012 and in July the traps were placed in two raspberry crops. The four trap models were placed in a line in a distance of 2 meters between traps. All traps were filled with 250 ml attractive liquid composed of 50% water, 40% apple vinegar, 10% red wine and a drop of liquid detergent. The traps were checked each week and captured insects were subsequently identified. Captured insects were assigned in the following categories: a) *D. suzukii*, b) *Drosophila* sp., c) Muscidae d) Aniposodidae, e) Scatopsidae, f) Lepidoptera, g) Hymenoptera and h) others.
A lure comparison trial was set in August and September 2013 on *Rubus* crop (Fig. II-4).

Lure Comparison:

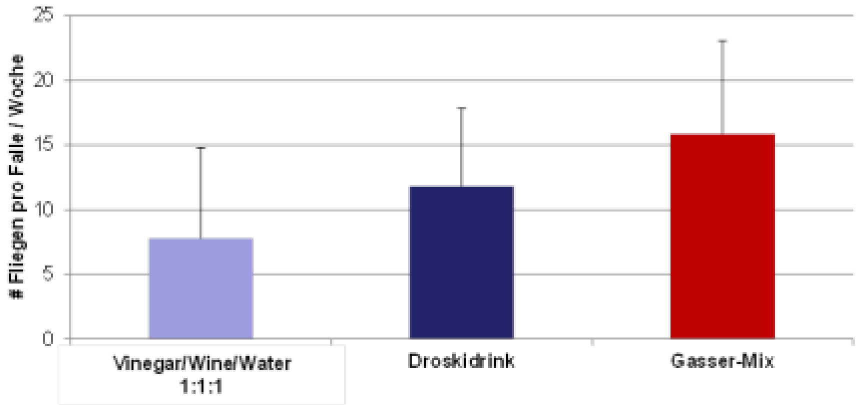

Fig. II-4: Crop: *Rubus* sp. Period: 29.8.-12.9.2013. Trap: ACW-trap 1.8 dl, transparent, Layout: randomized block design

2.1.2. Results

- *Selectivity of monitoring traps*

Unfortunately the spread of *D. suzukii* began in this region of Valais after the trapping period and therefore not a single individual was captured in the four trap designs tested from June to July 2012. In the traps set-up in the cherry orchards, 46% of the insects captured were native vinegar flies or other small Dipterans and 14% Scatopsidae. In traps located in raspberry, native vinegar flies and small Dipterans made up 40% of the catch and Muscidae 30%. Only the Agroscope trap with openings of 3 mm showed a high selectivity for vinegar and other small flies. The openings of the commercialized trap models were of at least 1 cm and the "Droso-Trap", "McPhail" and "Sentomol" captured consequently a large number of Lepidopterans, and the latter two also many Hymenopterans. These results show that the first primary criterion for a selective monitoring trap is the diameter of the openings, which should not exceed 3 mm. Following this observation, the selectivity of the "Droso-Trap" was enhanced by adding a fine mesh tulle over the openings. The ideal trap has an attractive lure and has a high number of small openings.

2.2. Evaluation of different trap types for catching SWD (AGES)

2.2.1. Material and Methods

Investigation sites

The trap comparison was carried out at two locations with *D. suzukii* infestation confirmed in 2011: one raspberry orchard (site 1) in the South-Eastern part of Austria (Nitschaberg; Styria) and one private garden (site 2) (with different fruits and vegetables) in the southern part of Austria (Dölsach; Eastern Tyrol). At site 1 the trial place was changed at the end of September due to the clearing of raspberry plants and traps were transferred to a part of the orchard close to the original trial place (about 50 m distance).

Material
Two commercial available traps – modified DROSO-trap® (type 2012) from Belgium (‘Biobest Belgium N.V.’) and the Swiss cup trap (RIGA AG) – were compared with a self-developed trap, called ‘green trap’ (Fig. II-5).

Fig. II-5: modified DROSO-trap (left), green trap (middle) and Swiss cup trap (right)

At each investigation site, the 3 trap types were used in 5 replicates (15 traps per site, 30 traps in total). They were randomly distributed in 3 rows with a distance of 6 m to each other and a distance to the margin of the rows of about 3 m (Fig. II-6). The trap content of the traps was changed regularly in fortnight intervals from July 2nd (week 27) to November 20th of 2013 (week 47), representing data of 10 consecutive catching periods each with two week duration.

CH 5	PET 5	DROSO 5
DROSO 4	CH 4	PET 4
PET 3	CH 3	DROSO 3
CH 2	DROSO 2	PET 2
DROSO 1	PET 1	CH 1

Fig. II-6. Trial plan for each investigation site (3 rows with 5 traps; distance of traps: 6 m, distance of traps to margin: ca. 3 m) - DROSO = DROSO-trap, PET = green trap, CH = Swiss cup trap, numbers are the replicates of traps.

The mean *D. suzukii* catches per trap, trap type and trial site were statistically analyzed for those trial periods in which increased numbers of adults were caught and for which potential significant differences in the mean trap catches between the trap types were visible in the box-plot graphs.
Mean SWD catches per trap, trap type and trial site were statistically analyzed per trial period with non-parametric tests for independent samples (Kruskal-Wallis test, SPSS for Windows) and one-way ANOVA (Bonferroni-Test) if homogeneity of variances was not significantly different (Levene-Test).

2.2.2. Results
Site 1 (raspberries‘ crop)
Until October only individual SWD flies were caught with the traps in the raspberry orchard, followed by an obvious increase during the following weeks resulting in a peak of SWD catches at the end of October/beginning of November. Here the average number of flies in a trap within one period (2 weeks) was between 20 and 50 flies (Fig. II-7).
The mean SWD catches per trap were significantly different in the trial periods 6, 7, 8, 9 and 10 ($\alpha < 0.05$). The DROSO-traps showed significantly lower mean trap

catches in all five above mentioned trial periods, whereas mean catches in green traps and Swiss cup traps were significantly different only in period 6.

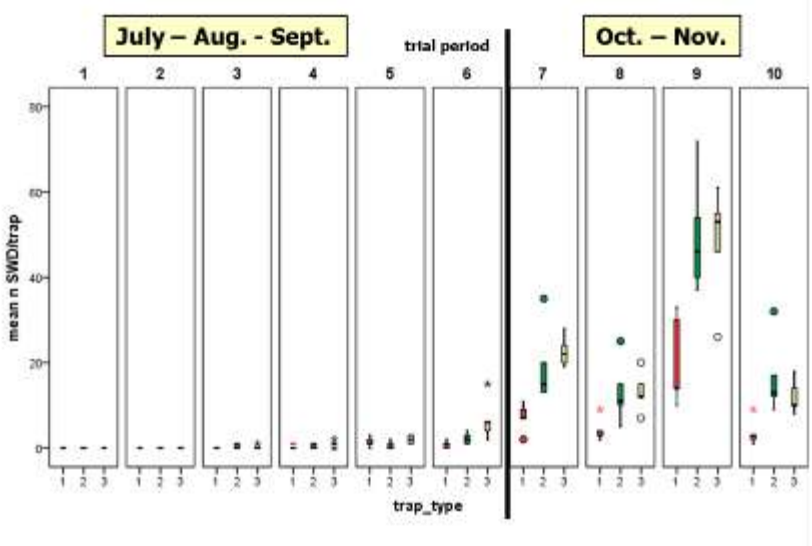

Fig. II-7: Mean number of caught SWD with traps (1 = DROSO-trap, 2 = green trap, 3 = Swiss cup trap) from July to November 2013 respectively trial period 1 to 10 (1 = week 27+29, 2 = week 29+31, …, 10 = week 45+47) on site 1 (raspberries, Styria).

Site 2 (private garden)

The number of caught flies was very low at this site with a slight peak in October comparable to site 1 (Fig. II-8). The average number of caught drosophila flies per trap within one period was one individual fly up to a maximum of about 15 individual flies. Three traps (one of each trap type) caught conspicuous high numbers of flies compared to the other traps in the same period (about 20 up to a maximum of 99 individuals per trap). For trial site 2 the mean SWD catches per trap were significantly different in the trial periods 2, 3 and 5 ($\alpha < 0.05$) and significantly higher in the Swiss cup trap than in the two other trap types.

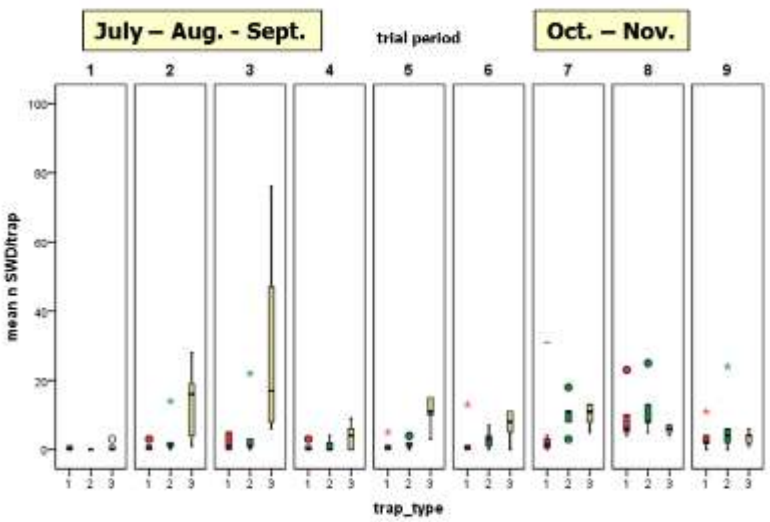

Fig. II-8: Mean number of caught SWD with traps (1 = DROSO-trap, 2 = green trap, 3 = Swiss cup trap) from July to November 2013. Trial period 1 to 10 (1 = week 27+29, 2 = week 29+31, …,10 = week 45+47) on site 2 (private garden, Eastern Tyrol).

Comparison of the 2 investigation sites concerning the 3 trap types

Statistically significant differences in the mean number of SWD trap catches between the 2 trials sites were found for the DROSO-trap in trial period 9, in trial period 2, 7 and 9 for the green trap and in trial period 2, 3, 5 and 9 for the Swiss cup trap.

2.3. Improvement of the monitoring (JKI)

2.3.1. Material and Methods

The research centre Fondazione Edmund Mach/IASMA, Centro Trasferimento Tecnologico, Unità Piccoli Frutti and the fruit grower Sant'Orsola in Pergine Valsugana in Italy (South Tyrol) discussed the monitoring trials as part of their cooperation and made soft fruit orchards available for the monitoring trials in 2012 and 2013. One strawberry, one raspberry and one blackberry orchard in 400 m, in 830 m and 1,200 m altitude, respectively, were chosen. The tests of the attractiveness of the traps were carried out on the same location and in the same fruit orchards near Pergine Valsugana from 3 to 7 October 2012 and from 2 to 16 October 2013. Nine traps per fruit were tested with 3 different lures: cider in combination with cherry juice, apple vinegar in combination with red wine (variety Trollinger) and apple vinegar in combination with white wine (variety Riesling). The ratio was in all three cases two third (50 ml per trap) to one third (25 ml per trap). Each trap with the same lure was tested with a band of three colors – yellow, blue and red (Fig. II-9a). The trap body is of plastic (prototype) with a snap-on-lid and a volume of 275 ml. The height of the trap is 9 cm and the lit has a diameter of 8 cm. The trap has a reasonable price with 0.47 Euro per piece. The cost for drilling the holes is not included in the price. The drilling of the holes did the JKI itself. It was 12 holes of 4 mm in the top of each trap circularly drilled.

The traps were deposited in a randomized way on the edges of the three fruit orchards in each second raw (raspberry and blackberry, Fig. II-9b) and in each third raw (strawberry). The traps were moved each day for one further position and rotated over the 14 days in their position to avoid influences of the location. 27 traps were emptied and refilled with new lure again. The insect material was collected with a sieve and removed with a soft brush to a tube with 70% ethanol. The containers are marked with the kind of lure, fruit, number of trap and date.

Under the investigation of the attractiveness of different traps and lure for the SWD 756 samples were collected - 378 in each year. The bycatch was taxonomically classified.

Fig. II-9a: Tested trap for the monitoring of *D. suzukii* with three different lures and colors.

Fig. II-9b: The position of the traps for the monitoring of *D. suzukii* in the blackberry orchard at 1,400 m altitude.

2.3.2. Results

All traps with different colors and lures were attractive to *D. suzukii* in general. In the two trapping weeks, 114 and 119 individuals of SWD were caught in total in 2012 and 2013, respectively. The traps caught 60 female and 54 males and 61 females and 58 males in 2012 and 2013, respectively. The sex ratio was 1.11 : 1 and 1.05 : 1 in the year 2012 and 2013, respectively. The sex ratios have shown that the females and males are balanced in number and both sexes are caught with the traps and lures.

The traps caught not only SWD but have also a high amount of bycatch. 3,858 individuals of *D. melanogaster* were caught besides the 114 adults of SWD (Fig. II-10). Furthermore, 119 species of the family Drosophilidae other than the mentioned species before were trapped. Furthermore, other insects were also trapped form the order of flies (Diptera) and of wasps (Hymenoptera) as bycatch in 2012. The ratio of SWD to other insects (bycatch) was 1:35 in the year 2012. In the year 2013 with 114 individuals of SWD, nearly the same number were caught as in the year before (Fig. II-11). But the amount of bycatch - with 1,714 *D. melanogaster*, 229 of *Drosophila* spp. and 351 other insects (Diptera, Hymenoptera) - was smaller. The ratio of SWD to other insects (bycatch) was 1:20 in the year 2013. The differences of bycatch in relation the different fruits (strawberry, raspberry, blackberry) were small in both years.

The number of catches of SWD in the different fruits were different in 2012 and 2013. In the year 2012, 75% were caught in blackberry, 20% in raspberry and only 5% in strawberry of all trapped SWD (Fig. II-12). In the year 2013, the most number of SWD were trapped in raspberry (52%) followed by strawberry (33%) and blackberry (15%) (Fig. II-13).

The different lures showed different attraction. In the year 2012, the most SWDs were trapped by the lure combination apple vinegar/red wine (45%) closely followed by the lure combination apple vinegar/white wine (43%) and by the lure combination cider/cherry juice (13%) (Fig. II-14). In the year 2013, the lure combination apple vinegar/white wine was most attractive and caught 38% (Fig. II-15). A little bit less attractive was the lure combination apple vinegar/red wine (34%) followed by the lure combination apple vinegar/white wine (28%).

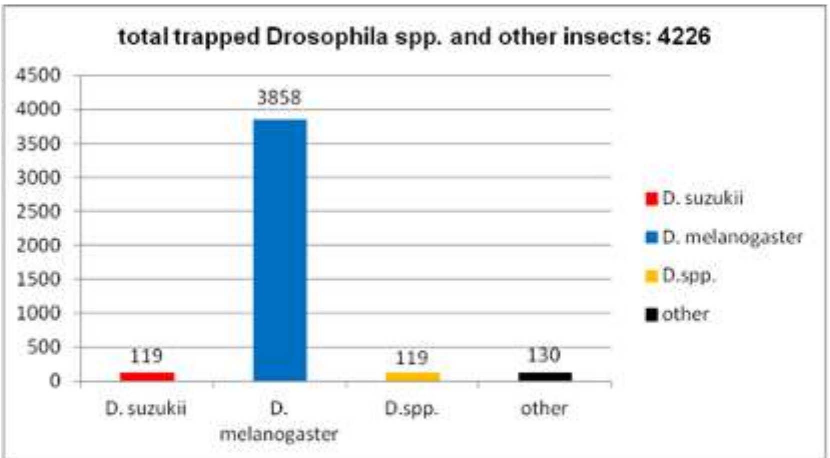

Fig. II-10: The total number of insects caught in 2012.

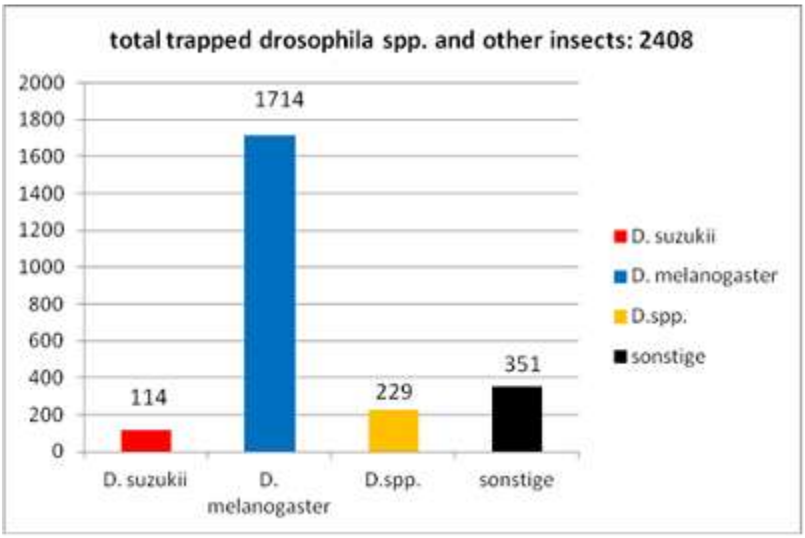

Fig. II-11: The total number of insects caught in 2013.

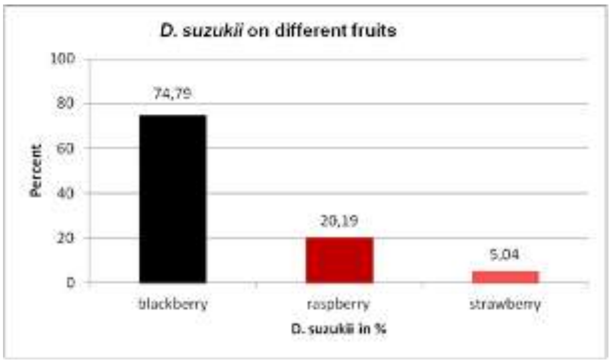

Fig. II-12: The distribution of the trapped *D. suzukii* according to fruit in 2012.

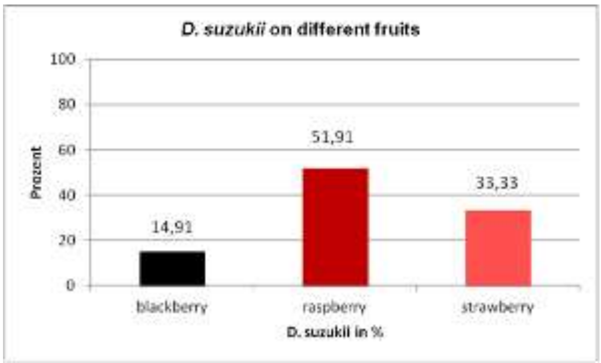

Fig. II-13: The distribution of the trapped *D. suzukii* according to fruit in 2013.

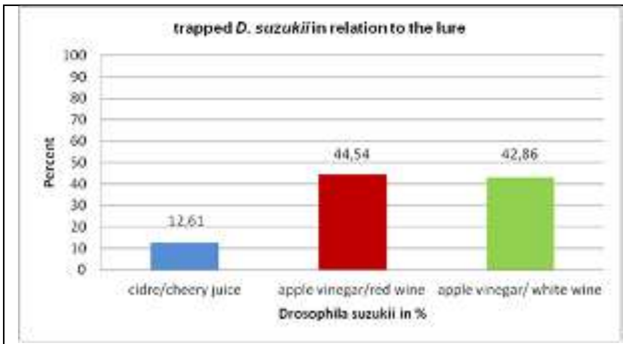

Fig. II-14: The percentage of trapped *D. suzukii* according to lure in 2012 (CK = cider/cherry juice, AR = apple vinegar/red wine, AW = apple vinegar/white wine)

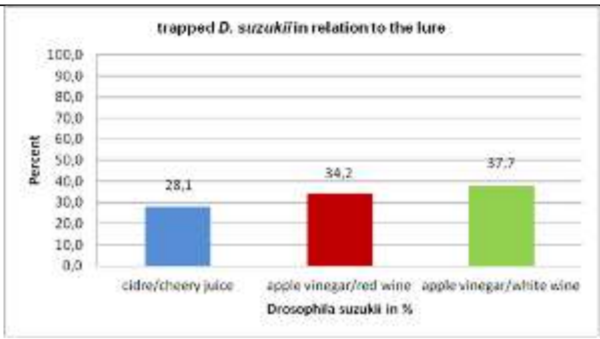

Fig. II-15: The percentage of trapped *D. suzukii* according to lure in 2013 (CK = cider/cherry juice, AR = apple vinegar/red wine, AW = apple vinegar/white wine)

In the year 2012, the red coloured traps were most attractive and caught 40% of all adults followed by the blue coloured traps with 35% and the yellow coloured traps with 25% (Fig. II-16). In 2013, however, the yellow coloured traps trapped most adults of *D. suzukii* with 38% followed by the red coloured traps with 33% and the blue coloured traps with 29% (Fig. II-17).

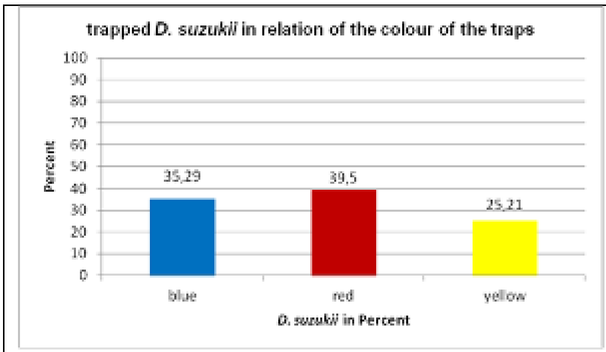	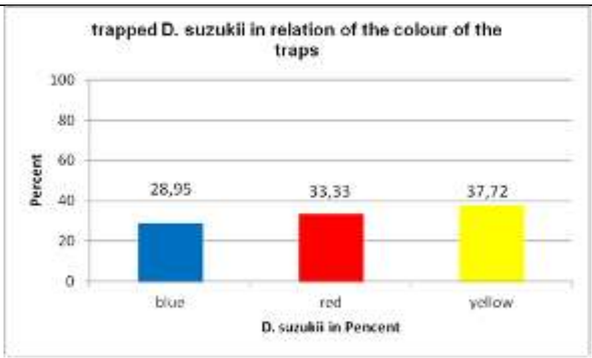
Fig. II-16: The percentage of trapped *D. suzukii* according to trap colour in 2012.	Fig. II-17: The percentage of trapped *D. suzukii* according to trap colour in 2013.

RESULTS OF MONITORING ACTIVITIES FOR INFORMATION OF SWD-DISTRIBUTION

CH-FOAG-RESULTS:

Distribution and population dynamics of D. suzukii *in Switzerland:*

- SWD is present from the plain to alpine regions in all Swiss regions.
- In 2012-2013: Individual captures of SWD were recorded over the winter as well as in spring. However, populations began to build up from the beginning of summer on to reach their peak in late October/early November.
- In 2013-2014, the mild winter allowed the SWD population to build up very early in the 2014 spring.
- In spring, the first individuals were not only captured in cherry, but traps in stone fruit orchards also caught most insects, followed by berry plantations and vineyards.
- Thus, cherry orchards and brambles seem to be particular favorable for the monitoring of the regional population dynamic over the season. Natural stands seem to be an important refuge for overwintering insects.

AGES-RESULTS:

Distribution of D. suzukii *in Austria:*

- SWD is present in most regions of Austria, mainly in the southern and western regions.
- 2012-2013: increased number of locations with SWD occurrence, but still very low number of caught flies in traps compared to other countries, especially in the northern and eastern parts of Austria (only single individuals).
- First single SWD caught in traps in July, number slowly increased in August and obvious peak from the end of September until November.
- 2012-2013: no visible damages reported from producers

JKI-RESULTS:

Distribution of D. suzukii *in Germany:*

- SWD is very rapidly spreading in Europe and has infested berries and stone fruits in eight Federal Lands of Germany.

DEVELOPMENT OF EFFICIENT TRAPS FOR MONITORING AND CONTROLLING SWD

CH-FOAG-RESULTS:

Comparison of lures and traps:

- Together with yeast baits, the commercial mixture of Riga AG captured most individuals.
- No differences in the selectivity of lures for *D. suzukii* versus vinegar flies could be observed.
- The ideal trap has a high number of small openings (= 2 to 3 mm), which are rain-proofed.
- Traps must be set in shady places (SWD needs humidity and shade)

Sanitation measures:

- Observations and a survey show that sanitation measures are the most important action to combat the development of *D. suzukii* in fruit plantations.

Mass-trapping:

- Mass-trapping is implemented on a broad scale in berry production and producers were satisfied with the achieved protection.
- A field study indicates that the deployment of mass-traps reduced the infestation level of raspberries by more than 60% compared to the previous week.

AGES-RESULTS:

Comparison of traps:

- Self-made traps tested in this study provide a potential alternative to commercially available traps to catch SWD.
- Technical improvements of the trap itself (e.g. number and size of openings, composition of the attracting lure) are advisable.
- Further development is required to improve the efficiency of traps in general, not only of self-made traps – e.g. the number and position of traps per site and/or the timing of control measures.

JKI-RESULTS:

Comparison of lures and traps:

Different traps and lures were investigated to improve the monitoring of *D. suzukii*. In South Tyrol (Pergine Valsugana), a new prototype of trap body was tested with three different lures (apple vinegar/red wine, apple vinegar/white wine and cider/cherry juice) and three different colours (yellow, blue and red) in three fruit cultures (strawberry, raspberry and black berry), giving 27 traps in total. The tests took place in the beginning of October 2012 and 2013. The traps were emptied and refilled with lure every day. The caught insects were identified later on in Kleinmachnow at the JKI. 114 and 119 SWD were caught in 2012 and 2013, respectively. All combinations of lures caught the fly in all three fruit cultures. The combination of apple vinegar and wine delivered the best results. Red and white wine were most favoured in year each, respectively. The combination of cider and cherry juice was less attractive in both years (13% and 28% of the total catch). The bycatch was, with a ratio of 1 : 35 (2012) and 1 : 20 (2013), quite high what made the identification more difficult. *Drosophila suzukii* was caught in blackberries (75%) in the first year and in raspberries (52%) in the second year. The distribution of the catch of the SWD differed in the individual years and no fruit was favoured. Furthermore, there was no preference as to colours since the catch results for blue and yellow were contradictory in both years. The red colour was of mean attractiveness in both years and could be a compromise.

RESULTS OF ANALYSIS OF BIOLOGICAL AND ECOLOGICAL INVESTIGATIONS ON SWD

CH-FOAG-RESULTS:

Cold chain:

- Laboratory trials indicate that the storage of fruits at temperatures below 5°C for at least 24 hours increases significantly the mortality of eggs and larvae within fruits and retards their development. However, the effect of cold storage seems to depend on the fruit species with insects being more vulnerable within berries than stone fruits.

RESULTS OF EXPECTED DAMAGES AND ECONOMIC CONSEQUENCES

CH-FOAG-RESULTS:

Economic damages:

- In 2013, although SWD larvae were also identified in cherry, plume and grapevine, economic damages were predominantly observed in raspberry, blackberry, blueberry and strawberry plantations. Economic damages appeared at the end of the season in berry cultures where producers neglected sanitation measures and mass trapping.
- In 2014, for the first time, the cherry orchards were severely attacked following the varieties and the regions in July.

Assessment of damages:

- Freezing fruits for 2 hours is a reliable detection method for small berries in order to estimate the infestation level by *D. suzukii* larvae.
- However, strawberries, stone fruits and probably also grapes should be squeezed and dissolved in salt water to assess the percentage of infestation.

JKI-RESULTS:

Situation in Germany:

- The pest does not get a quarantine status because of its rapid spread in Europe and Germany as well.
- Potential hosts are widely distributed throughout Germany. The best known orchard areas in Germany, with a total fruit growing area of nearly 73’000 hectare, are situated in the Altes Land in Lower Saxony at the river Elbe and in the Lake Constance region. Further traditional fruit growing areas are in the Rhine valley, in Hesse and Brandenburg.

WP3 *Susceptibility testing of different fruit varieties*

Lead: FEM (Gianfranco Anfora, Alberto Grassi, Claudio Ioriatti, Francesca Eccher, Valerio Rossi Stacconi)

Partners involved: CRA (Sauro Simoni, Elisabetta Gargani).

FEM activities

The olfactory responses of the adult flies for the odour released by intact host fruits (raspberry, blackberry, cherry, blueberry and strawberry) in behavioural assays (Y-shaped glass olfactometer) have been evaluated. *Drosophila suzukii* females were expected to choose between the test material (25 g of fresh fruits) and the control (holding the same amount of fruits wrapped in a transparent plastic bag). *Drosophila suzukii* females were significantly attracted to the volatiles emitted by the 5 tested fruit species. Volatiles released from the attractive host fruits have been extracted and identified (GC-MS), and their biological activity on *D. suzukii* females screened by means of electrophysiological analysis (GC-EAD) (Revadi *et al*., 2012). Electroantennographic (EAG) and behavioural assays (Y-shaped glass olfactometer) have been also carried out to test responses of *D. suzukii* mated females to isoamyl acetate, one of the most EAD-active volatiles. Indeed, this is the only compound released from all the fruit species analyzed and able to elicit always significant antennal responses in *D. suzukii* mated females. Analysis of the EAG responses to increasing doses of isoamyl acetate in hexane solutions (from 0.1 pg/µl to 100 µg/µl) showed a dose-response relationship. Moreover, in olfactometer experiments isoamyl acetate loaded in red rubber dispensers at the dosage of 10 µg elicited significant attraction in *D. suzukii* females. The release rate of those rubber dispensers was estimated as 2.4±0.6 ng/hr.

The results of the field survey (number of captures and fruit damages) on the same fruits analyzed in laboratory conditions are shown in the Fig III-1.

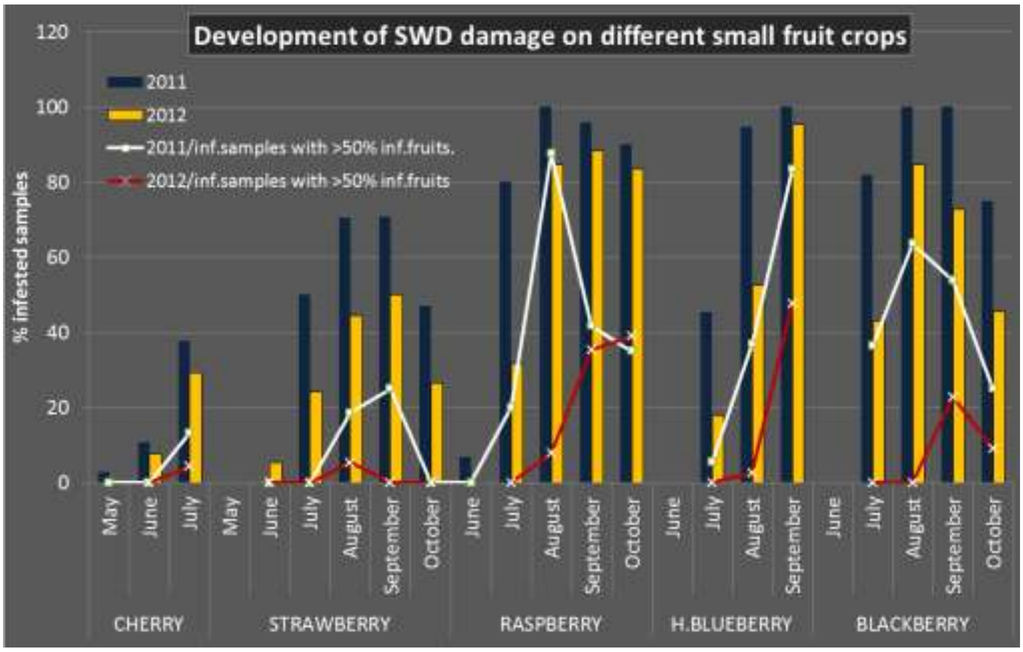

Fig. III-1. SWD infestation in different small fruit crops.

We have identified volatiles from host plants, which influence the olfactory behaviour of *D. suzukii*. We show that *D. suzukii* females are attracted to the volatiles emitted from intact fruits. Using gas chromatography coupled mass spectrometry (GC-MS) and GC-electroantennographic detection (EAD) we identified the biologically active suite of compounds released from ripe fruits that had been freshly picked. Mated *D.*

suzukii females were significantly attracted to rubber septa loaded with 10 µg of synthetic isoamyl acetate, one of the most EAD-active volatiles. The release rate of isoamyl acetate from attractive rubber septa, measured directly by solid phase microextraction (SPME), was comparable to that by fresh fruits. In addition, a genomic survey showed that *D. suzukii* possess the almost complete putative full repertoire of genes encoding odorant receptors normally activated by isoamyl acetate in *D. melanogaster*. The results indicate that *D. suzukii* uses olfactory cues to select oviposition sites. The identification of behaviourally-active volatiles emitted by host fruits of *D. suzukii* may therefore aid in the development of selective and efficient synthetic lures for use in control strategies such as mass trapping and attract and kill.
The results of the field survey (number of captures and fruit damages) on the same fruits analyzed in laboratory conditions are shown in the table below.

Table III-1. Number of captures and fruit damages on different small fruit crops

crop	month	weeks	n° of samples inspected in 2011	n° of samples inspected in 2012	n° of samples inspected in 2013	% infested samples in 2011		% infested samples in 2012		% infested samples in 2013	
						total	with > than 50% infest.fruits	total	with > than 50% infest.fruits	total	with > than 50% infest.fruits
SWEET CHERRY	*May*	*19-22*	**36**	**5**	**3**	2,8	0,0	0,0		0,0	
	June	*23-26*	**112**	**93**	**87**	10,7	0,0	7,5	0,0	18,4	0,0
	July	*27-30*	**53**	**48**	**143**	37,7	13,2	29,2	4,2	39,9	19,3
STRAWBERRY	*May*	*19-22*	**9**	**1**	**2**	0,0		0,0		0,0	
	June	*23-26*	**19**	**19**	**17**	0,0	0,0	5,3	0,0	0,0	
	July	*27-30*	**16**	**25**	**40**	50,0	0,0	24,0	0,0	40,0	12,5
	August	*31-35*	**27**	**43**	**52**	70,4	18,5	44,2	5,3	73,0	5,3
	September	*36-39*	**24**	**32**	**46**	70,8	25,0	50,0	0,0	73,9	11,8
	October	*40-43*	**17**	**19**	**35**	47,0	0,0	26,3	0,0	45,7	12,5
RASPBERRY	*June*	*23-26*	**15**	**4**	**3**	6,7	0,0	0,0		0,0	
	July	*27-30*	**20**	**19**	**12**	80,0	20,0	31,6	0,0	25,0	0,0
	August	*31-35*	**24**	**26**	**14**	100,0	87,5	84,6	7,7	92,8	46,1
	September	*36-39*	**24**	**17**	**18**	95,8	41,7	88,2	35,3	100,0	66,7
	October	*40-43*	**20**	**18**	**24**	90,0	35,0	83,3	38,9	91,7	50,0
H.BLUEBERRY	*June*	*23-26*	**28**	**8**	**2**	0,0		0,0		0,0	
	July	*27-30*	**55**	**39**	**33**	45,4	5,4	17,9	0,0	45,4	13,3
	August	*31-35*	**57**	**40**	**55**	94,7	36,8	52,5	2,5	50,9	3,6
	September	*36-39*	**6**	**21**	**42**	100,0	83,3	95,2	47,6	90,5	36,8
BLACKBERRY	*June*	*23-26*	**0**	**0**	**0**						
	July	*27-30*	**11**	**7**	**5**	81,8	36,4	42,8	0,0	80,0	25,0
	August	*31-35*	**22**	**26**	**15**	100,0	63,6	84,6	0,0	93,3	35,7
	September	*36-39*	**13**	**22**	**18**	100,0	53,8	72,7	22,7	100,0	22,2
	October	*40-43*	**4**	**11**	**12**	75,0	25,0	45,4	9,0	58,3	28,6

CRA activities
The most frequently identified yeast species from adult and larval *D. suzukii* were *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Pichia terricola*, and *P. kluyveri* (Hamby *et al*., 2012). *H. uvarum* seems to be the predominant yeast among the others specially on healthy fruits (Hamby *et al*., 2012). To investigate the role of olfaction in host recognition and selection and to identify biologically active volatile (kairomones), preliminary lab trials using Y-shaped glass olfactometer were carried out. Three lab *H. uvarum* strains, from the microorganism collection of Faculty of Agriculture at University of Florence, were tested in dual choices trials. The responses of the adult flies for the odour released by the three different yeast strains compared with a check in behavioural assays (Y-shaped glass olfactometer) have been evaluated.
Materials and methods
From the SWD population reared at ABP facilities, two-day-old adults were starved for 2 to 6 h prior to all experiments and kept at 25 °C. The yeast stored at 4 °C were kept at room temperature (25 °C) 1 h before conducting the experiment.
Y-tube olfactometer (stem: 30 cm; arm length: 20 cm, arm angle: 60°; internal diam.: 4 cm); each arm of the Y-tube was connected to a pyrex glass bulb (10 ml). One chamber held the test material (1cm square of agar with the yeast colony), the other chamber served as control holding the same amount of agar with the only rearing substrate (Saboraud). A constant airflow was maintained through each cylinder-olfactometer arm, using an air pump with flow adjusted with a flow-meter to 1 ml/min. The incoming air was passed through activated charcoal and humidified with distilled water. Single flies were introduced individually into the olfactometer at the entrance of the stem; they were observed until they had reached the end of an arm; they were discarded - recorded as 'no choice' - if no movement was registered within 5 mins. The samples were randomly assigned at the beginning of the bioassays, and they were reversed after having tested five individuals in order to minimize any spatial effects. After each trial, the Y-tube was washed with detergent, rinsed with distilled water and absolute ethanol. The responses of about 50-60 flies were tested. The experiments were conducted in a laboratory at a temperature of 25 ± 1 °C, a relative humidity of 60% ± 10%, 1000 lux.
Results
The first acquisitions were devoted to standardize and to improve the experimental set up. Among the three *H. uvarum* strains tested, SWD showed preference only for A strain (chi square = 4.28, P<0.05). Further investigations are in progress.

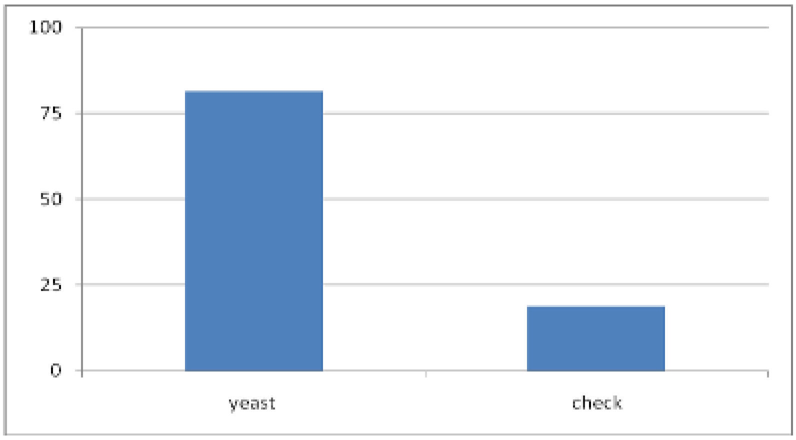

Fig. III-2. SWD choice response to olfactometer trials.

WP4 Survey on the infestation of grapes and grape varieties in Europe
Lead: CH-FOAG
Partners involved: AT-AGES, FEM, CRA.

Objectives and tasks
This work package aims to determine the real threat posed by *D. suzukii* to European table and wine grape production by assessing its distribution in European vineyards, by estimating fruit infestation levels and the economic damage in the different vine growing areas of Europe and by examining the susceptibility of the most important grape varieties (e.g. Chardonnay, Cabernet Sauvignon, Merlot, Pinot Noir, etc...).

Methods used and Results obtained
Importance of D. suzukii *for European vineyards*
An online survey on the threat that *D. suzukii* poses to European table and wine grape production was developed and emailed to phytosanitary services, industry partners, policy makers and scientists all over Europe in the winter 2013 and 2014. The questionnaire was written in English and contained a total of 20 questions on the distribution and abundance of *D. suzukii* in European vineyards, the level of fruit infestation, the susceptibility of grape cultivars, the economic impact on grapevine production as well as other related aspects.
For the vintage of 2012, we received 22 responses from 14 different vineyards that covered about 10% of Europe's viticultural area, whereas we got 26 responses from 19 regions covering 14% of the European viticultural area for the vintage 2013. The main finding were that at most locations the population of drosophilids consisted of an assemblage of *D. suzukii* and native drosophilids species. However, *D. suzukii* was nowhere dominating the vinegar fly community. Although *D. suzukii* was present, it did neither cause any major damages to table and wine grapes in 2012 nor in 2013. Its management was based on sanitation measures and winegrowers renounced nearly completely from the use of insecticides. Most respondents rated the potential of *D. suzukii* as an important viticultural pest therefore as low (Figure IV-1).

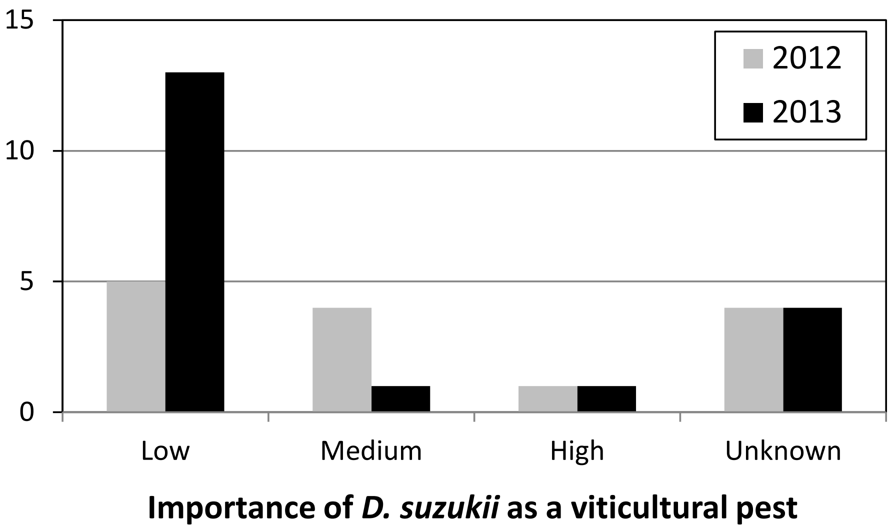

Fig. IV-1. Respondents' rating of the importance of *D. suzukii* as a viticultural pest insect in their region.

Development of D. suzukii *within vineyards:*
At a local scale, the development of *D. suzukii* population within Swiss vineyards was followed by using monitoring traps, by assessing grape infestation and by studying the drosophilid complex in wineries during vintage.
Although *D. suzukii* could be captured in most Swiss vineyards in 2012 and 2013, nearly no grape infestation could be recorded in both years and economic damages were the exception. In 2012 grape clusters infested by *D. suzukii* were only recorded in lateral valleys of the Ticino and we scrutinised some of these rare infestations. Nearly 90% of the investigated grape clusters showing symptoms of vinegar fly infestation came from red cultivars. Native drosophilids were observed in 88% of the examined grape clusters, whereas *D. suzukii* was identified in only 68% of the samples and with one exception, it was always associated with indigenous vinegar flies. The mean number of adults emerged per berry was 0.2 and 0.4 for *D. suzukii* and native drosophilids, respectively. Moreover, grapes showing sour rot infestations were only colonized by indigenous drosophilids. In 2013, just before harvest, we randomly picked three clusters of white and red cultivars in 14 vineyards of the La Côte region. The presence of *D. suzukii* within grapes could be confirmed in four of these vineyards and its adults emerged exclusively from red cultivars. Native vinegar flies were also present in 4 of these 14 vineyards, but they showed no preferences for white or red grape cultivars. Whereas the number of *D. suzukii* per grape cluster ranged between 0.7 and 37, 0.3 to 88 native drosophilids specimens emerged per cluster. Interestingly, *D. suzukii* and native drosophilids co-occurred only in one vineyard. Moreover, the presence of *D. suzukii* in the 14 vineyards was not correlated to the incidence of sour rot.
In 2012 and 2013, only a handful *D. suzukii* individuals were captured in the monitoring trap installed within the cellar of Agroscope at Nyon. Although this winery receives grapes from all over Switzerland, the proportion of *D. suzukii* consisted of less than one percent of all drosophilids captured in both years.

Susceptibility of grape cultivars to D. suzukii*:*
Since the level of grape infestation in Switzerland was negligible, the susceptibility of grape cultivars could not directly be observed in the field. However, findings from the Trento indicate that in particular thin-skinned cultivars, such as Lagrein and Schiava (=Vernatsch), are at higher risk since the force to penetrate their skin falls below 40-50 cN already several days before vintage (Burrack *et al*., 2013).
The susceptibility of the four red and two white cultivars was assessed in a laboratory “no-choice” test. Females of *D. suzukii* laid most eggs on the thin-skinned red cultivar Bondoletta followed by the other three red cultivars Gamay, Pinot noir, Divico and the two white varieties Chasselas and Müller-Thurgau (Figure IV-2). Adult emergence was considerably lower on grapes than on an artificial diet. Overall, adult emergence did not exceed 9% of the total number of eggs deposited. However, emergence increased with the maturation of grapes.

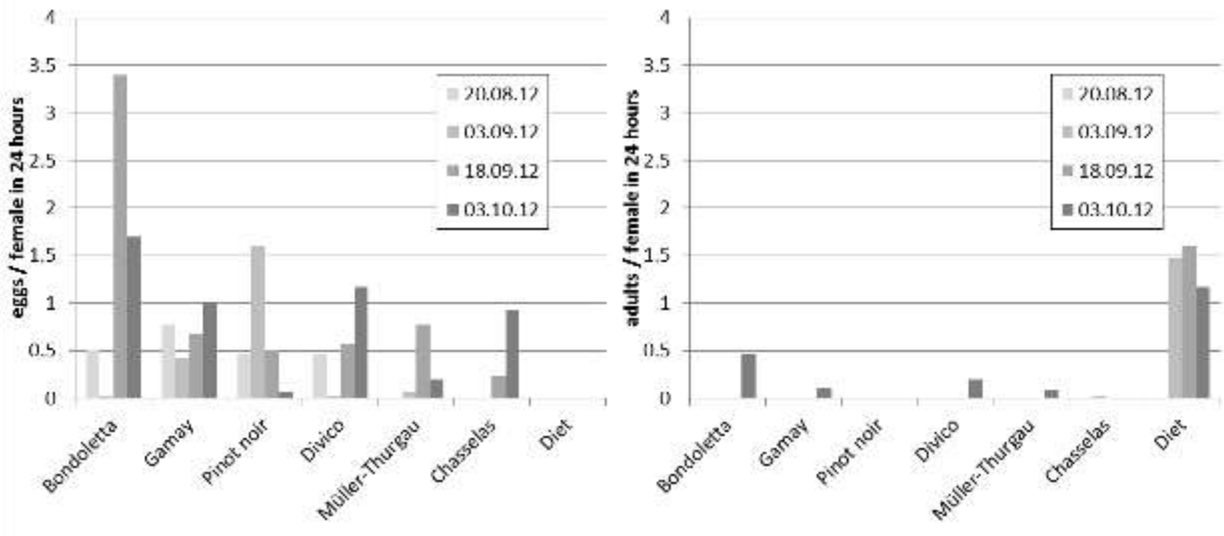

Fig. IV-2. Mean number of laid eggs (left) and emerged adults (right) per *D. suzukii* female on six grape cultivars.

Discussion

Drosophila suzukii is present in most European vineyards, but it did not cause any major damages on table or wine grapes in 2012 as well as 2013. Although we did not get any response from Spain, we are confident that the information acquired on *D. suzukii* during our survey is representative for its situation in European vineyards. First because the obtained responses cover more than 10% of the viticultural area of Europe, second we are not aware of any major damages in vineyards from our Swiss and European colleagues and third since the assumption that *D. suzukii* is of minor importance for grape production is also shared by our American colleagues. Nonetheless, we know that *D. suzukii* caused important losses in the Italian vineyards of the Trentino and of the South-Tyrol in 2011 (pers. comm. Claudio Ioratti & Roland Zelger). Their soft-skinned local cultivars Lagrein and Schiava seem to be particular prone to *D. suzukii* infestation; probably as a result that the force to penetrate their skin falls below the critical value 40 to 50 cN already several days before their vintage (Burrack *et al*., 2013). In general, soft-skinned cultivars seem to be preferred over thick-skinned ones, white grape cultivars seem to be less attractive than red ones and the suitability of grapes for larval development of *D. suzukii* increases with the maturation of grapes. Moreover, *D. suzukii* infestations in vineyards did neither favour native drosophilids nor sour rot on grapes.

Conclusions

Our various observations confirm that grapes can be damaged by *D. suzukii*, but they do not seem to be very suitable for larval development and the buildup of large adult populations. This is in strong contrast to the situation in other crops such as cherries or soft fruits. The interaction of *D. suzukii* with native vinegar flies and fungal pathogens is so far only partly understood. However, there are no clear evidences that its presence favors considerably the development of other drosophilids or sour rot. Nonetheless, such interactions cannot definitely be excluded and the development of *D. suzukii* within vineyards should therefore be monitored carefully. Overall, *D. suzukii* is currently considered as a minor threat to table and wine grapes in Europe, although soft-skinned cultivars might occasionally be attacked. Yet, time will tell if this first assessment is accurate or if this new pest is nonetheless able to cause sporadically severe damages in grapevines.

AGES activity

In 2012 no infestation with *D. suzukii* was detected in Austrian vineyards.
During the monitoring only single individuals were caught with traps in vineyards and in their vicinity but no infested grapes have been observed.

FEM activity

2012. Monitoring of the development of *D. suzukii* population and grape infestation in vineyards of Trentino Province has been conducted. First captures were recorded at week 32 (6-12 August 2012). The peak of captures ranging from 15 to 25 adults per trap per week was recorded in Schiava variety in correspondence of the grape harvest period (week 38-39). Overall Schiava variety showed the highest level of susceptibility to *D. suzukii*. However the percentage of Schiava grape bunches with *D. suzukii* eggs was always below 5% before harvest and reached considerable levels only on bunches remaining on plants after harvest.
Additional information is included in the questionnaire provided by CH-FOAG which was filled up and sent to the coordinator of the survey.
2013. Monitoring of the development of *D. suzukii* population and grape infestation in 2013 in vineyards of Trentino Province has been conducted (see Figure IV-3). SWD flight starts on week 32 and first egg infested berries were found on week 38 on cultivar Schiava and Pinot noir. 1089 eggs were found from week 38 to week 42: the great majority of eggs were found on Schiava (1040) followed by Pinot noir (35). Few eggs were also found on some of the other varieties, but they were generally laid on wounded berries.

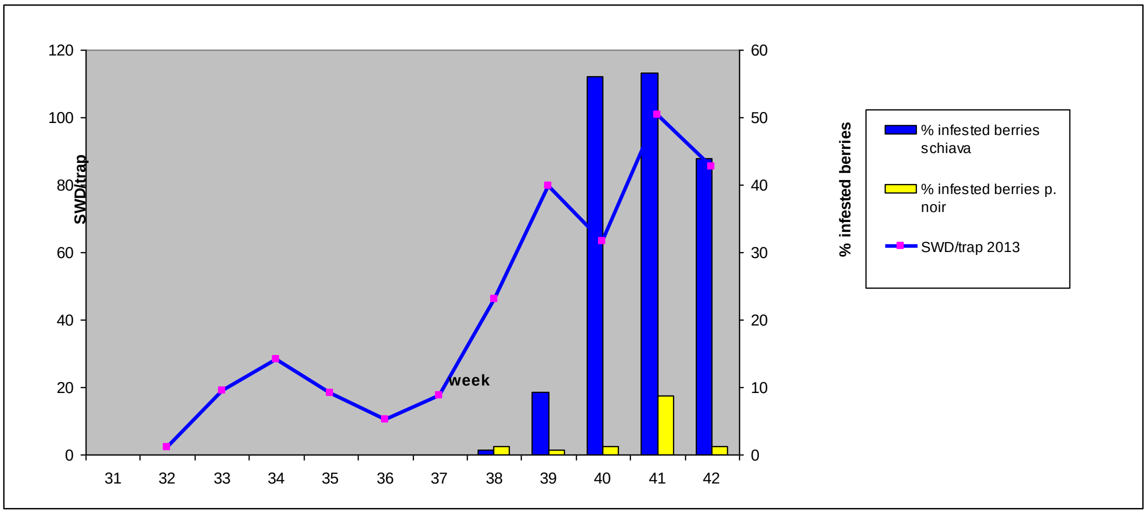

Fig. IV-3. Grape infestation in 2013 in vineyards of Trentino Province

CRA activity

In 2013 field survey were conducted to verify the presence of SWD in central Italy vineyards. In a 4 ha vineyard (Figure IV-4a), planted with Syrah and Cabernet Franc varieties and located in the Tuscan costal area, 16 traps were installed from the end of July to the end of September after the grape harvest.

Another survey was carried out in a very important vine growing area, that one for the production of Brunello of Montalcino (Siena) (Figure IV-4b), placing 8 traps from August to October. The captures of SWD are reported in Figures IV-5 and IV-6.

Fig. IV-4. Monitored vineyard areas in Tuscany , A) and B9 see the text .

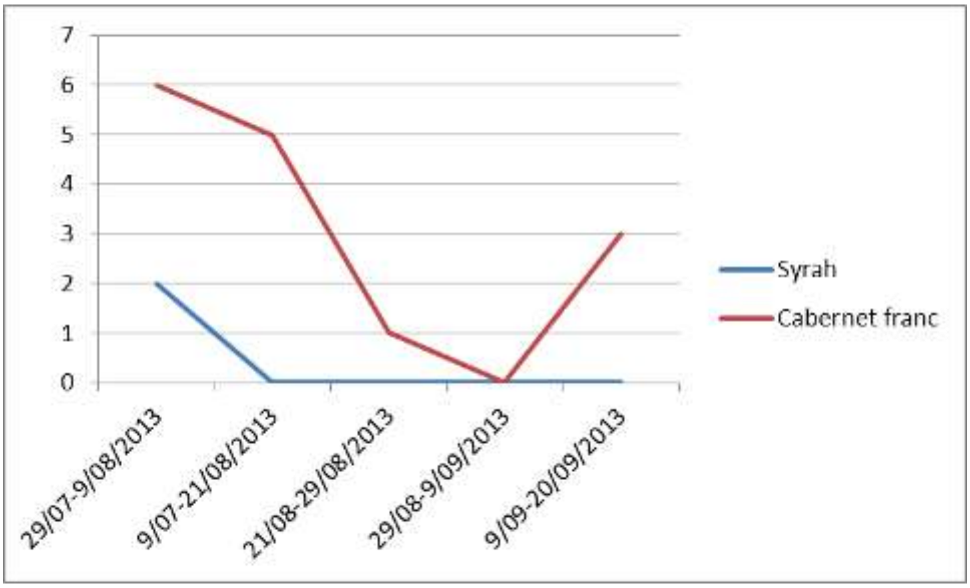

Fig. IV-5. Castiglione della Pescaia (Grosseto), *D. suzukii* weekly captures in 16 traps (blue line cultivar Syrah; red line Cabernet franc)

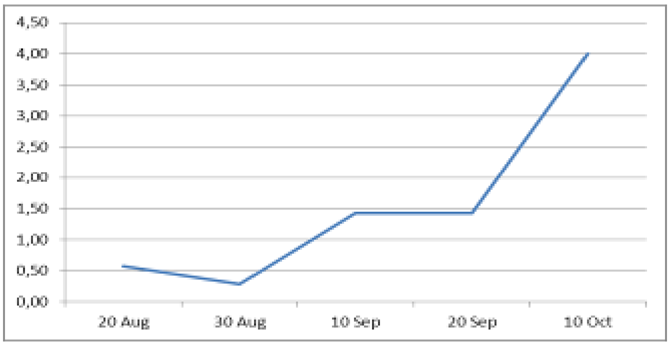

Fig. IV-6. Montalcino (Siena), *D. suzukii* captures/trap in 8 traps on Sangiovese vineyards.

Although *D. suzukii* was captured in the monitored vineyards, nearly no grape infestation was recorded.

The susceptibility of the three red cultivars was assessed in a laboratory "no-choice" test. First trials: 5 ♂♂ and 5 ♀♀ of SWD were free to mate and lay eggs for up to 72 hours on cluster of 20 berries (12 clusters for the 3 cultivars: Sangiovese, Merlot and Petit Verdot) and then removed. The same clusters were placed in glass jars for 14 days. At the end of the 14 days, emerged adults were counted. The clusters were immerged in a saturated NaCl solution, to obtain, by flotation, preimaginal stages developed inside the grapes. A second experiment was carried out on cluster with artificially damaged berries.

D. suzukii females were able to lay eggs on all the three grape cultivars.

A significant preference was observed for the damaged grape (trial 2) compared to intact ones (trial 1) (U=-3.25, n=18, P=0.0001).

Trial 1: grapes never used and 55% of adult mortality was recorded; no adult emerged (n° eggs=14). Trial 2: grapes used for oviposition, insects were active with a mortality of 5.5%; 3 ♀♀ and 2 ♂♂ (n° eggs=88).

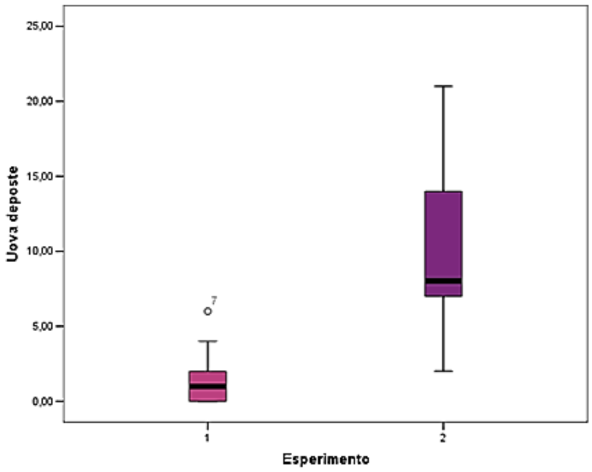

Fig. IV-7. Egg laid by SWD on healthy and damaged grape berries.

In laboratory, during the trials, *D. suzukii* females were able to lay eggs on all the three grape cultivars; however, a significant preference was observed for the damaged grape (trial 2) compared to intact ones (trial 1).

Probably the damages on grapes provide nutrients to mature females and reduce the resistance of the fruit peel, similarly to the observations for other grape cultivars.

WP5 *Actions for containment and control of D. suzukii by environmentally-friendly measures.*
Lead: FERA
Partners involved: CH-FOAG, FEM, CRA, JKI, AGES.

Objectives and tasks of the project:

Identification of:
- Appropriate BCAs from the roster of available and potentially available organisms.
- Assessment of appropriate (i.e. recommended) approved chemicals for fruit versus adults and/or larvae.
- Screening and first assessment of new control measures and management approaches.
- Assessment of candidate molecules (not currently approved but potentially available via mutual recognition/emergency SOLAs etc.) versus adults and/or larvae.
- Potential for oviposition deterrents for fruit protection to be used.

Materials and Methods:

Source of insects:

Drosophila suzukii used in the experiments originated from wild specimens from Northern Italy, collected in the autumn of 2012. Specimens were imported into the UK under a specific license required for importing non-indigenous invertebrates (Marris *et al.,* 2010). A colony was initiated within the secure Insect Quarantine Unit at the Food and Environment Research Agency, York, within bug-dorm (280 mm × 280 mm × 280 mm; *Watkins and Doncaster*, UK) insect cages at 25°C, 65% r.h. and 16:8 h L:D regime. The insects were maintained on a mixture of Drosophila diet (*Blades Biological*, UK) and organic blueberries (Cuthbertson *et al.,* 2014).

Control Products Selected for Investigation:

Bioassays were performed using formulated insecticide products. The biological agents tested are also commercially available in the UK. Products (along with their source and active ingredient (a.i.) dose rates tested) were: Coragen (DupPont; chlorantraniliprole; 0.0116g a.i./L), Movento (Bayer CropScience Ltd.; spirotetramat; 0.096 g a.i./L), Calypso (Bayer Cropscience Ltd.; thiacloprid; 0.216 g a.i./L), Conserve (Fargo Ltd.; spinosad; 0.096 a.i./L), Pyrethrum 5EC (Agropharm; pyrethrins; 0.02 g a.i./L), Decis Protech (Bayer CropScience Ltd., deltamethrin; 0.018 g a.i./L), Neem oil (Trifolio-M GmbH; 0.5% solution), Tri-Tek (petroleum oil based product; awaiting UK registration; 2% solution) and a new experimental product (coded: TA2674; 0.017g a.i./L); two entomopathogenic fungi (*Lecanicillium muscarium* as Mycotal (0.1% solution) and *Beauveria bassiana* as Naturalis (0.3% solution)); three entomopathogenic nematodes (*Steinernema carpocapsae*, *S. feltiae*, *S. kraussei*; all tested at 10’000 Infective Juveniles per mL).

Laboratory Bioassays to Investigate Effectiveness of Chemical Control Products

Blueberries (400 in total) were infested for 72 hours within four bug-dorm cages (100 berries per cage) each containing approximately 70 mixed-sex adult *D. suzukii*. Following this infestation period the blueberries were cleaned of adult flies and equal numbers were randomly dipped (full emersion) in field-rate concentrations of the following products: chlorantraniliprole, spirotetramat, thiacloprid, spinosad, pyrethrins, deltamethrin, Neem oil, Tri-Tek and TA2674. A water treatment acted as control. 40 berries were dipped in each control product. After dipping, berries were placed into 10 cm diameter ventilated plastic deli-pots and placed into a Controlled Environment (CE) cabinet and incubated for 10 days at 25°C. The pots were then assessed for presence of adult flies and the berries dissected to inspect for presence of larvae and/or pupae development.

Investigating Impact of Entomopathogenic Nematodes and Fungi on D. suzukii *Emergence:*

Berries were again infested for 72 hours (264 in total) in bug-dorm cages each containing approximately 70 adult mixed-sex flies. Forty four blueberries were then selected at random per treatment and, after full emersion in standard formulations of the treatment products (*L. muscarium, B. bassiana, S. carpocapsae, S. feltiae, S. kraussei*) were incubated in a CE cabinet for 10 days at 25°C. All larvae, pupae and adult flies that developed were counted. Berries were dissected for signs of larvae in treated dishes. Equal numbers of berries dipped in water acted as controls.

Impact of Direct Application of Chemical Products on Adult D. suzukii*:*

Spinosad, chlorantraniliprole and TA2674 were applied directly at standard field rates against equal numbers of male and female adult *D. suzukii* using an automatic-load Potter precision laboratory spray tower. Following treatment adult flies were maintained at 25°C and supplied with standard *Drosophila* medium as a food source within ventilated plastic deli-pots. Mortality was assessed following 24 and 48 hours. Direct application of all biological products (nematodes and fungi) was also undertaken and mortality was assessed after 7 days. For all treatments there were 5 replicates of 10 adult flies (5 male and 5 female; 50 adults in total). Individuals sprayed with water acted as controls.

Potential of Products to Act as Oviposition Deterrents:

To investigate the potential of the most efficient products from the current study to act as oviposition deterrents, blueberries were first dipped in the standard dose rates of Spinosad, Coragen and TA2674 (100 berries per product). They were then placed on 9 cm diameter Petri dishes (10 berries per dish) and allowed to air dry for 2 hours. Berries dipped in water acted as controls. The petri dishes were then placed into 10cm diameter plastic deli-pots with ventilated lids. Ten adult *D. suzukii* (5 males and 5 females) were then introduced to the berries contained within the deli-pots. All were maintained in a CE cabinet at 25°C. Mortality of the introduced adult flies was assessed over 48 hours. The berries were maintained at 25°C for a further 10 days at which time adult fly emergence was determined. Following this, the berries were dissected and examined for presence of any remaining larvae and/or pupae development.

Data analysis:

Data was statistically analysed where appropriate. Treatments were compared against the control. For analysis the numbers for each life stage in each individual treatment were combined. Assuming normality and constant variance, Analysis of Variance (ANOVA) was used to test any significant difference.

Results

Spinosad, deltamethrin and the new experimental product (TA2674) proved most effective in controlling *D. suzukii* (Figure V-1). Spinosad caused complete mortality of *D. suzukii*; no flies emerged from treated berries. Deltamethrin, TA2674, pyrethrins and thiacloprid all had significantly less *D. suzukii* numbers emerging from treated berries compared to the water control ($p < 0.01$). The products Neem oil and Tri-Tek would appear to have delayed population development of the flies as larger numbers of pupae were recorded in these treatments after 10 days incubation.

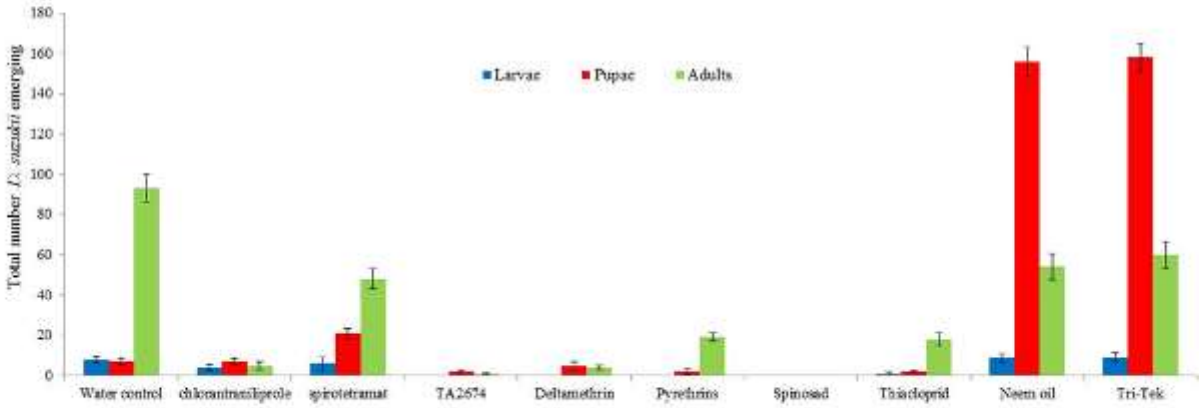

Fig. V-1. Impact of treatments on emergence of *D. suzukii* from infested blueberries (assessed after 10 days incubation at 25 °C). Total numbers from 40 berries per treatment.

Both the nematodes and fungi would appear to cause population decreases of *D. suzukii* (Figure V-2). However, neither would seem to have the potential to eradicate *D. suzukii*. There was no marked impact on fly emergence, with populations developing as normal (Figure V-2). There was no significant difference between the individual control agents or the water control ($p > 0.05$).

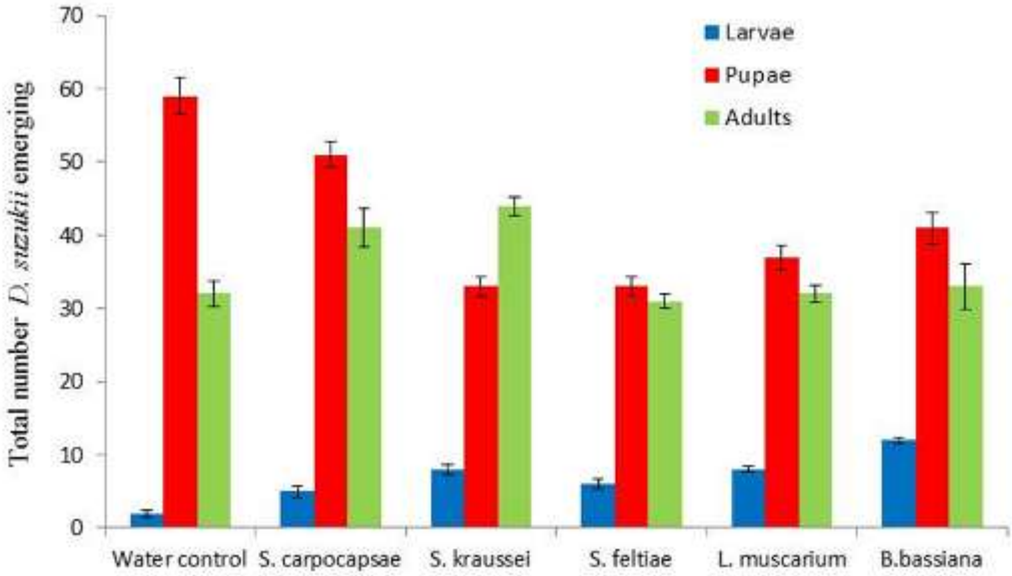

Fig.V-2**.** Impact of dipping infested berries in entomopathogenic nematodes and fungi on resulting fruit fly emergence following 10 days incubation at 25°C. Total numbers from 44 berries per treatment.

Direct application of all products had limited mortality on adult *D. suzukii*. Spinosad proved the best with 90% mortality being achieved, significantly higher than the water control ($p < 0.001$) (Figure V-3). Direct application of all the ‘green’ products (Neem oil, Tri-Tek and the entomopathogenic agents) all caused significantly higher mortality than the water control ($p < 0.05$) (Figure V-4). *Beauveria bassiana* caused 44% adult mortality after 7 days ($p < 0.01$). However, the entomopathogenic control products would not seem to be effective in controlling *D. suzukii* numbers as the next generation of larvae were already coming through in the feeding media following one week.

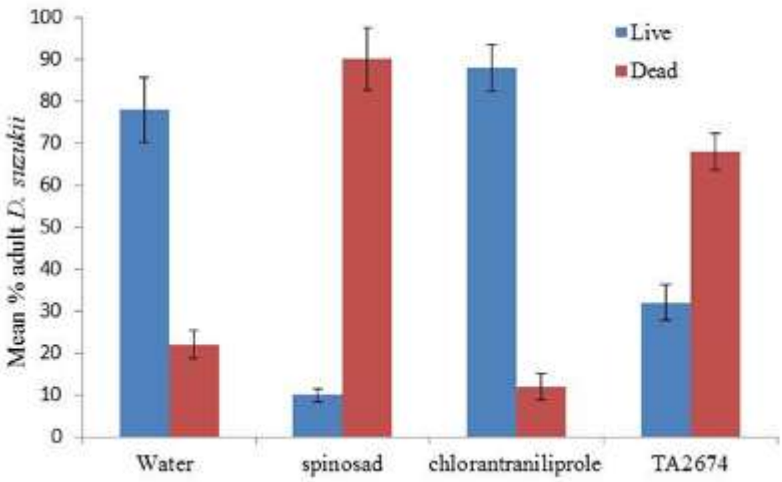

Fig. V-3**.** Impact of direct application of chemical control products against adult *D. suzukii*. Mortality assessed after 48 hours (50 flies per treatment).

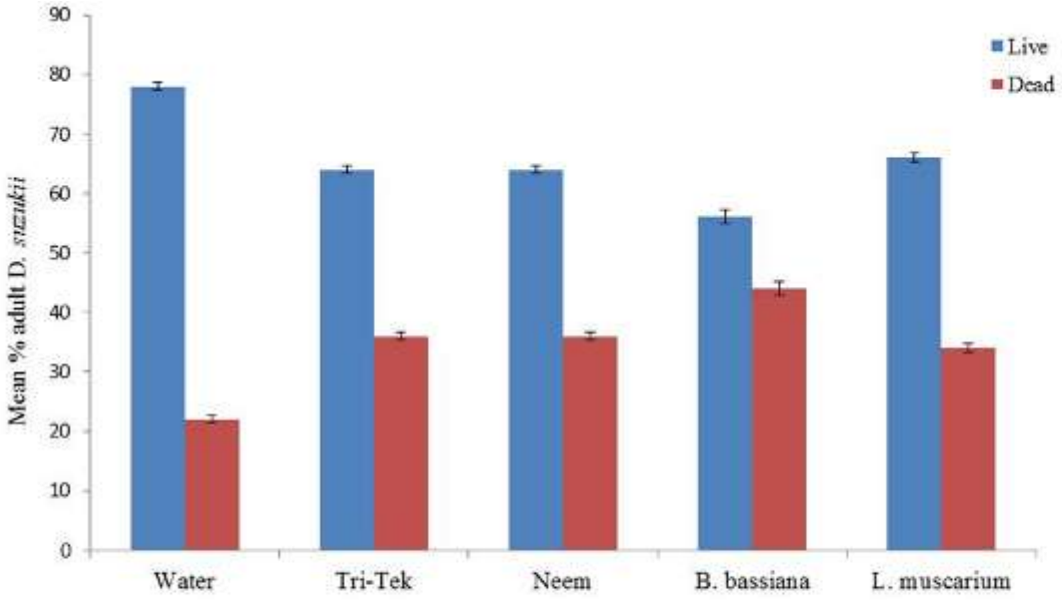

Fig. V-4. Impact of direct application of alternative products and entomopathogenic fungi against adult *D. suzukii*. Mortality assessed after 7 d (50 flies per treatment).

In assessing the potential of the most efficient products to act as oviposition deterrents, following 48 hours after the addition of the adult flies, 100 and 98% mortality was recorded in the spinosad and TA2674 treatments, respectively (Figure V-5). Subsequently, following a further incubation of 10 days, no flies developed from these berries (Figure V-6). Both spinosad and TA2674, therefore, provided complete protection from *D. suzukii* following the pre-treatment of fresh berries. Larvae, pupae and adults developed in the control berries as expected.

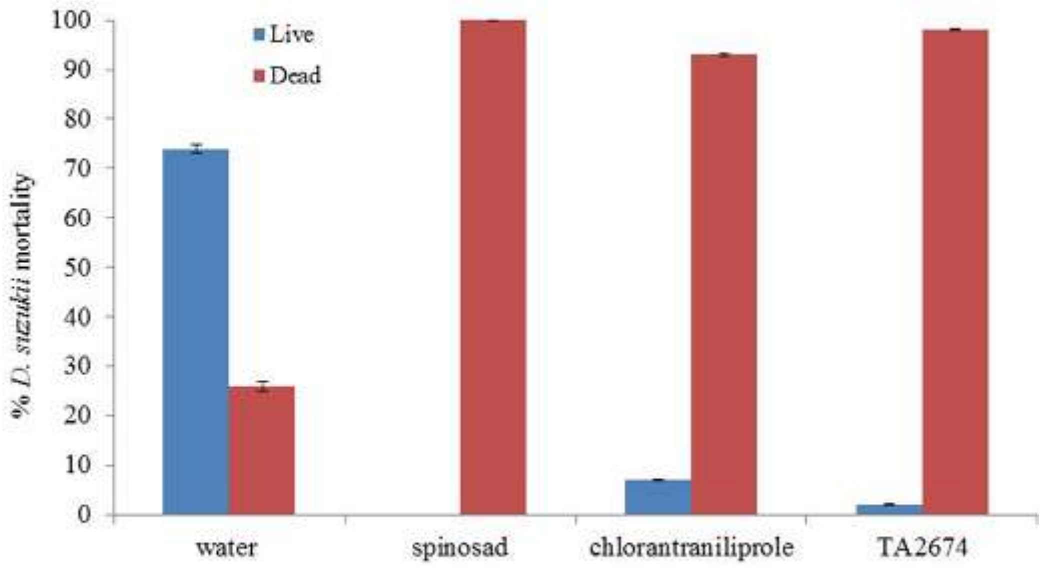

Fig. V-5. Mortality observed of adult *D. suzukii* flies following exposure to pre-treated blueberries. Mortality assessed after 48 hours (100 flies per treatment).

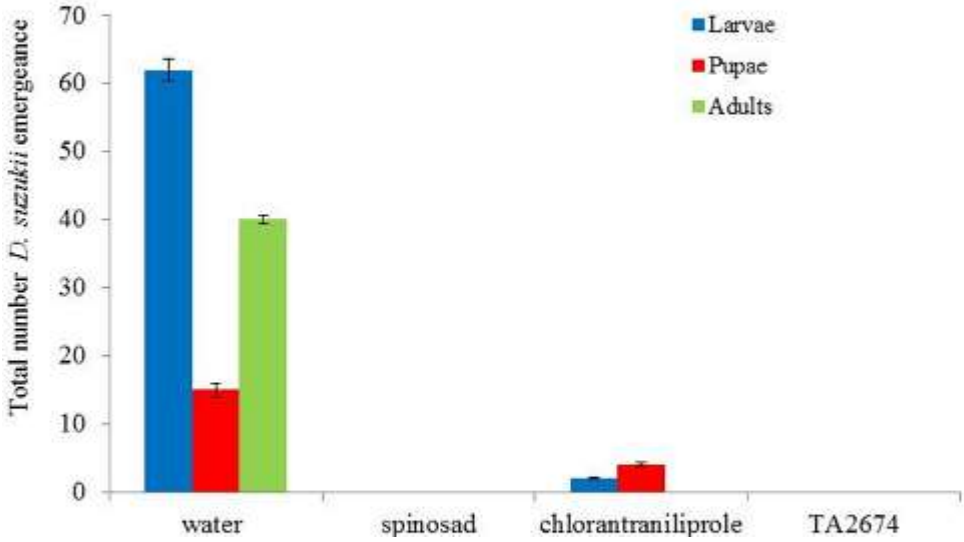

Fig. V-6**.** Impact of pre-treating blueberries with chemical insecticides on subsequent *D. suzukii* infestation and development (assessed after 10 days incubation at 25°C).

Discussion

This study has shown that currently available insecticides for use against *D. suzukii* in the UK can provide control, and in the case of spinosad and the experimental product (TA2674) eradication (Cuthbertson *et al.,* 2014). Spinosad, under laboratory conditions, has been shown to offer excellent control of *D. suzukii*. The results demonstrate that when uninfected berries are dipped in spinosad, and indeed TA2674, adult flies are prevented from ovipositing or if eggs are deposited the subsequent larvae/pupae do not develop; death occurs. In the current study we only tested the selected products' efficacy on blueberry fruit and, as *D. suzukii* readily feeds on various soft fruits (Steffan *et al.,* 2013) product efficacy still needs further testing on different host fruits. Our laboratory experiments provided little data for the support of the use of entomopathogenic fungi or nematodes as control agents against *D. suzukii* on blueberries. This, however, is in contrast to certain high levels of control obtained by similar products based on *B. bassiana*, reported by other studies (Gargani *et al*., 2013; Brust, 2014). This highlights the need to screen all available species and strains of fungi for their efficacy against a given pest species. Tri-Tek, a petroleum oil based product, proved disappointing in the current study. This product has shown excellent potential for incorporation into pest management strategies against adult whitefly, *Bemisia tabaci* (Cuthbertson *et al*., 2012). This study (Cuthbertson *et al*., 2012) reported that Tri-Tek acted through asphyxiation by trapping the flies while wet.

Laboratory data generally becomes more variable when treatments are transferred to the field. The findings from the current study, obtained under controlled conditions, must be tested on a broader scale before firm conclusions can be drawn. It has been shown that the efficacy of most insecticidal treatments is reduced greatly after exposure to just over 2 cm of rain and that after one week following treatment adult (*D. suzukii*) mortality is often not significantly different from the untreated controls for most insecticides that are exposed to rain (Bruck *et al*., 2011). In an outdoor situation, should berry protection be required but rain is forecast, the effectiveness of insecticides in this situation could possibly be enhanced by the addition of adjuvants to reduce loss due to rain.

Main conclusions

Drosophila suzukii presents a real challenge to the UK and European horticultural industry. Containment and/or eradication of this pest will prove difficult. Data from our trials indicates that spinosad along with chlorantraniliprole and the experimental product TA2674 show excellent potential as control agents of *D. suzukii* when used as either a pre or post-dipping treatment. None of the products tested provided complete mortality following direct application to adults, and larvae were seen to be much more susceptible following berry dipping. The biological agents (fungi and nematodes) caused reductions in population numbers of *D. suzukii* but are unlikely to control/eradicate populations. However, they should prove easy to incorporate into existing invertebrate control programmes as shown in other pest control strategies (Cuthbertson, 2013). Maintaining coverage of fruit clusters will be essential for effective protection against *D. suzukii*.

FEM activity

Natural enemies

2012. In order to evaluate the presence and the effectiveness of native generalist enemies, a survey for parasitoids of *D. suzukii* was set up. Petri dishes containing either standard medium diet for drosophilids, or banana slices or blueberries were previously exposed to mated *D. suzukii* and *D. melanogaster* females in order to be infested with eggs and used as attractant for parasitoids in traps. Traps baited with both *D. suzukii*- and *D. melanogaster*–infested substrates were deployed in three locations in Trento province: a forest environment (600 m a.s.l.), an organic blueberry orchard (900 m a.s.l.) and a vineyard (200 m a.s.l.). The experiment started at the end of July 2012 and traps were controlled and changed weekly until the end of October 2012. A generalist pupal parasitoid, *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae), was found to be able to attack both the fly species. After rearing it under controlled conditions, it was able to develop a second generation on *D. suzukii*.

2013. From May to October, in four locations of Trento Province (Italy). We conducted field and laboratory studies in order to determine the status of biological control agents utilizing *D. suzukii* as a host. Our study sites included a range of commercial soft fruit and natural non-commercial habitats. In each site, sentinel traps were baited with either *D. suzukii* or *D. melanogaster* larvae in different food substrates. In late season 2012, the generalist parasitoid, *Pachycrepoideus vindemiae*, was collected from both *D. suzukii* and *D. melanogaster* pupae in traps deployed in a selection of these sites. During the first part of season 2013 another species, the larval parasitoid *Leptopilina heterotoma* Thomson (Hymenoptera: Figitidae), hatched from both drosophila's species baited traps. Successive parasitism efficacy tests were set up under controlled conditions confirming the ability of both the species to attack *D. suzukii* (Rossi Stacconi *et al., 2013)*

Actions for containment
Preliminary trials were carried out in 2011 in order to develop a trap effective for the mass trapping method and, as a consequence, to set up sustainable management strategies. Results indicated that red colour increased the attractiveness of standard traps baited with apple cider vinegar. High captures of adults and a limited damage on fruits were obtained in a mass trapping trial on highbush blueberry using a mixture of apple cider vinegar and red wine (nicknamed “Droskidrink”) as a bait in standard traps. Based on these results, in 2012 we suggested to our local growers a large-scale application of mass trapping as a basic control method. About 45’000 red plastic jars baited with Droskidrink were distributed by Sant’Orsola Soft Fruit Grower Association to its members. The traps were exposed in small fruits and strawberry fields from April till November. A trial was carried out on highbush blueberry in one of the most infested areas of the region, with the aim to evaluate the effectiveness of the mass trapping method, comparing different layouts of the traps. Results confirmed the high attractiveness of the “Droskidrink” bait. Between the factors that contributed to limit the SWD damage and population development on our territory in 2012, it is likely that a role has been played also by the wide area application of mass trapping method. Particularly low damages were recorded in those farms where an optimal integration of sanitation practices, harvest procedures, mass trapping and insecticide application was applied. However, results of our trial indicate that where the pest pressure was very high, traps were not sufficient to significantly reduce the damage on the fruits to a tolerable level for the grower. The most effective arrangement was that with traps both on border and inside the field. Intensive trapping can be considered as a control method that can contribute to the reduction of SWD damage on small fruits, particularly if combined in an integrated management system.
The possible practical implications of this finding for the biological control of *D. suzukii* are discussed in the enclosed paper:

CRA activity
Material and methods
In 2013, lab trials were carried out testing two different commercial bio-insecticides, Naturalis and Botanigard, both based on living spores of naturally occurring strains of the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuillemin and other three products, without a known specific insecticidal activity, but with possible effects on plant defence mechanism. The vegetal based products were: “Agricolle” - composed of some natural polysaccharides -, “Duofruit” - emulsion of mustard oil added with defatted seed meals (DSM) able to release biological active compounds of Brassicaceae -, “Deffort” - liquid fertilizer based on *Sophora flavescens* Aiton -. In a second experimental round (2014), laboratory trials were carried out by adopting higher doses of Naturalis and “Deffort” and different rates and mixing of “Duofruit” components (DSM and oil).

Direct toxicity trials
Healthy blueberries were inserted in a climatic chamber at 25°C and exposed to SWD adults for 72 hours. After adults’ removal, a fixed number of blueberries for each tested product was immerged for 30 seconds into 100 ml of solution/emulsion. Air dried fruits were then kept in a cell at 25°C and 75±5% RH for 10 days; emerged adults were collected and counted under stereo microscope.

Residual contact toxicity test
Healthy blueberries were dipped into 100 ml of solution/emulsion for each product and then air dried for two hours. For each product, treated blueberries were placed into Petri dish, the whole material was kept in a cage with *D. suzukii* adults for 72 hours and stored in climatic chamber at 25°C and 75±5% RH. Inspections were made under stereo microscope in two steps, immediately after the removal of the adults in order to verify oviposition on fruits and 10 days later in order to count larvae, pupae and adults.

Results and discussion

During the first step of the laboratory trials (2013), with the exception of “Duofruit” and “Agricolle”, all the organically labelled products significantly reduced the vitality of SWD. In particular, Botanigard determined the highest Abbott mortality (84.6%), not significantly different from “Deffort” and Naturalis that reduced the population over 50% (Table V-1).

Table V-1. Lab direct toxicity against SWD.

Product	Number of adults (mean±SD)	Abbott index (%)
Botanigard ,125 ml/100l	2.00±0.89 c	84.62
«Deffort», 200 ml/100l	5.67±2.73 bc	56.41
Naturalis, 75 cc/100l	6.00±2.68 bc	53.85
«Agricolle», 300 ml/100l	10.00±3.90 ab	23.08
«Duofruit», 200 g flour + 1l oil on 100 l	11.33±2.74 a	12.82
Check (distilled water)	13.00±0.89 a	

As concerns the residual contact toxicity, the number of hatched eggs was reduced by “Duofruit”, Naturalis and Botanigard (Table V-2). The efficacy was calculated according to the Overmeer formula(1988): E=100-(100-M)*R - R = n medium eggs hatched in the treated/n medium eggs hatched in the check, M = mortality according to Abbott, E = Efficacy

Table V-2. Residual contact toxicity against SWD.

Product	Preimaginal stages (mean±SD)	Efficacy (%)
Botanigard ,125 ml/100l	7.88±5.74	84.62
«Deffort», 200 ml/100l	8.14±4.78 ab	56.41
Naturalis, 75 cc/100l	6.63±4.00 b	53.85
«Agricolle», 300 ml/100l	9.50±3.78 ab	23.08
«Duofruit», 200 g flour + 1l oil on 100 l	7.50±3.07 b	12.82
Check (distilled water)	16.13±4.67 a	

In the 2014 trials, with regard to the direct toxicity, the best results were obtained with Naturalis (used at the highest dose suggested on the label), which exceeded the

60% Abbott mortality, while "Deffort" settled around 50%. The product based on Brassicaceae seed meals, "Duofruit", has improved its performance by increasing the rate of DSM in the emulsion; the mortality, however, did not exceed 38% (Table V-3).

Table V-3. Lab direct toxicity against SWD.

Product	Number of adults (mean±SD)	Abbott index (%)
Naturalis, 150 cc/100l	9±6.38 a	62.89
«Deffort», 250 ml/100l	19.8±6.07 bc	0.00
«Duofruit», 400 g flour + 2.5 l oil on 100 l	14.6±6.26 ac	24.74
«Duofruit», 600 g flour + 2.5 l oil on 100 l	12±6.40 ac	38.14
«Duofruit», 800 g flour + 2.5 l oil on 100 l	16.4±1.79 ac	15.46
Check (distilled water)	19.4±1.48 bc	

Concerning the evidence of indirect contact toxicity, Naturalis once again showed good efficacy, reducing the SWD oviposition on treated fruits and confirming a coefficient of toxicity, E, around 80% as in the previous trials. For the other products, E settled in order of 50% (Table V-4).

Table V-4. Residual contact toxicity against SWD.

Product	Preimaginal stages (mean±SD)	Efficacy (%)
Naturalis, 150 cc/100l	12.4±4.72 a	81.24
«Deffort», 250 ml/100l	19.8±9.39 a	52.17
«Duofruit», 400 g flour + 2.5 l oil on 100 l	19.0±5.17 a	55.96
«Duofruit», 600 g flour + 2.5 l oil on 100 l	35.2±6.02 b	27.71
«Duofruit», 800 g flour + 2.5 l oil on 100 l	19.8±5.13 a	52.17
Check (distilled water)	41.4±6.46 b	

All the biological products determined reductions in the numbers of *D. suzukii* , but are not sufficient to control populations, however, they can be more effortlessly integrated into existing insect control strategies compared to conventional insecticides.

CH-FOAG activity

- *Insecticides*:
    - The application of sanitation measures and mass-trapping proves to be very effective against *D. suzukii* and nearly no insecticides were applied by commercial growers in 2013.
    - In 2014, with a higher pressure and earlier in the season, some insecticides treatments with spinosad were necessary to be combined with the mass trapping. Some insecticide treatments (Karate) were also necessary on finishing crops to avoid the migration of the existing SWD population on the neighbor crop in production
    - Field trials confirmed that there is a real risk that certain insecticides, such as spinosad, exceed the accepted level of residues on certain strawberry varieties.

JKI activity:
Literature research has shown that Chile uses equipment applying hot air to control harmful insects in fruit plantings (TPC – Thermal Pest Control). The procedure is held under patent more or less world-wide by the company *Lazo* from Chile. This applies to Europe, too. *Lazo* uses the procedure against harmful insects in fruit crops and viticulture. Thermal control is meant to be used on the following crops: vine, shrub berries, stone fruit (sweet cherry), strawberry, and, where applicable, tomato. The company has been contacted to obtain detailed information, especially on the control of Drosophilidae. Further knowledge on the control of Drosophilidae using this method is lacking and cannot be gained under the present project. However, the procedure offers an interesting approach to the control of the SWD and should be followed in future.

AGES activity:
Assessment of control measures and management approaches in areas with *D. suzukii*–infestation was not possible during 2012 due to the lack of *D. suzukii*-damage.

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