

Mitteilungen und Nachrichten

Sino-German Symposium on Integrated Management of *Drosophila suzukii*, Julius Kühn-Institut, Darmstadt/Dossenheim 26.06.–01.07. 2017

The Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae), is a highly polyphagous pest due to oviposition in undamaged ripening and ripe fruits and subsequent larval development, resulting in a fast decay of the fruits. This insect is considered a minor pest in the Asian area of its origin. However, since its arrival in North America and Europe, SWD has become an invasive species and has inflicted huge financial losses in stone and soft fruit production. While new control measures are still being developed in the world, there is an urgent need for the prompt development of sustainable control tools. German and Chinese experts had already met in June 2015 at Beijing to discuss current research and future cooperation on this topic during a workshop and a subsequent field excursion to Yunnan Province. Both events had been organized by the Department of International Cooperation of the Chinese Ministry of Agriculture and the National Agro-Technical Extension and Service Center (NATESC) as well as the German-Sino Agricultural Center (DCZ) as representative of the German Ministry of Agriculture (Vogt et al. 2017: Journal für Kulturpflanzen 69, 16-24). As a follow-up of this activity, the Symposium took place from 26th of June until 1st of July 2017 at the Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants at Darmstadt and Dossenheim, including a field excursion to concerned German fruit growing regions. The Symposium was organized by Dr. Annette HERZ and Dr. Heidrun VOGT (both JKI) and Dr. Yifan ZHAI (Shandong University), Prof. Dr. Pu-yun YANG (NATESC) and Dr. Feng ZHANG (East Asia Centre, CABI). A workshop with more than 30 contributions was held during the first two and half days at JKI Darmstadt and

JKI Dossenheim. A part of the group attended a following excursion to the LTZ Augustenberg (organized by Dr. Kirsten KÖPLER) and to the Ortenau fruit growing region, where participants were welcomed and guided by Mr. Hans-Dieter BEUSCHLEIN, Landratsamt Ortenaukreis, Offenburg, and Mrs. Susanne FRÜH, Obstgroßmarkt Mittelbaden eG, Oberkirch. The excursion included the visit of several fruit growing farms (blueberries, raspberries, cherries). All participants of the Symposium (workshop and excursion) agreed on the considerable progress which had been made regarding our knowledge on this particular pest insect and also the achievements in practice which help fruit growers to manage SWD until more sustainable pest management systems or natural control will keep population densities under damage thresholds. Nonetheless, further research is urgently needed to clarify many unsolved questions on the basic biology and ecology of SWD in both invaded and original regions of world. Furthermore, innovative approaches, e.g., bait and kill/attract and kill, push-pull strategies, exclusion netting, Sterile Insect Technique or selective breeding of resistant crop cultivars, need to be explored further. The Symposium has inspired the participants for new ideas on research and was successful in building a scientific consortium from both countries covering a multi-discipline approach. Abstracts of the presentations during the workshop are presented below for further information.

Acknowledgement

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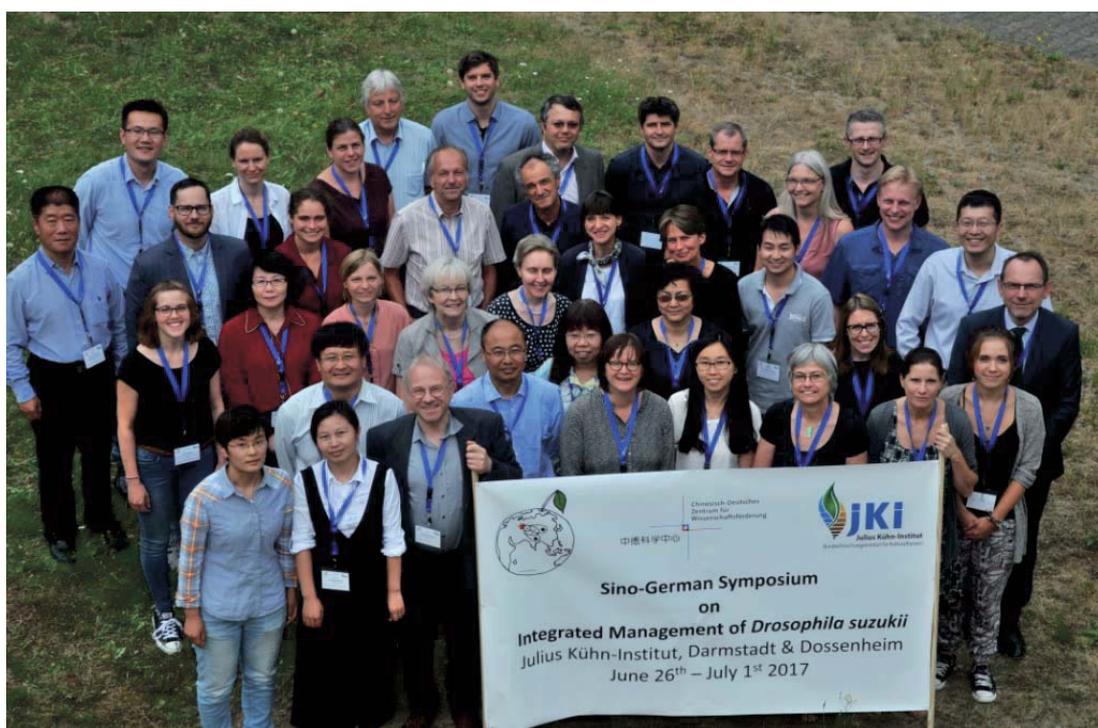


Fig. 1. Participants of the Sino-German Symposium

New insights into the ecology of *Drosophila suzukii* in Germany and its pest status

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Since its invasion to Germany in 2011, *D. suzukii*, commonly named Spotted Wing Drosophila (SWD), has become the most dangerous pest in stone and soft fruit. It is causing enormous economic losses. With the aim to develop sustainable control techniques, profound knowledge on SWD biology is indispensable. Main research areas at the JKI are: SWD phenology, GIS-based risk analysis, overwintering, feeding ecology, susceptibility of cherry varieties, impact of surrounding vegetation on pest pressure in orchards, effects of extreme heat and dryness, plant compounds, insecticides, bait mixes for traps, exclusion netting. Concerning SWD phenology, all-season higher captures occur in the landscape compared to orchards. Flies are active at mild days in winter. Lowest activity/population density is observed from April to June, highest in November to December. Our monitoring showed that in autumn and winter flies are preferably found in the canopy of evergreen trees like pines. In contrast, flies disappear with leaf fall in deciduous trees. Adults overwinter as winter morphs. We examined flies from our monitoring traps year round with regard to ovarian development, wing length and coloration. Compared to summer morphs, winter morphs are darker, have up to 0.5 mm longer wings and females do not produce eggs. In our region winter morphs occur from October to June, indicating their long life span. Egg production in winter morphs starts mid-March to April. Abiotic factors and food resources are most crucial for winter survival and early hosts in spring influence population built-up. We proved that mistletoe berries (*Viscum album*) are first host fruits in spring. Cherries play a major role for population built-up from May onward. Immigration into cherry orchards takes place in March to April each year, long before fruits are available. Oviposition starts in light red fruits and infestation increases quickly. SWD stays present in cherry orchards at least until leaf fall. Other cultivated fruits in the course of the year (raspberries, blackberries, blueberries, plums, elderberries, etc.) are used as reproduction hosts until late autumn. Temperatures above 30°C and low humidity have shown to slow down population built-up significantly. We demonstrated this by exposing flies to heat waves in a climate chamber. As a technical approach for SWD control the JKI is coordinating a demonstration project „Exclusion netting for managing Spotted Wing Drosophila in fruit crops“, which started in 2017.

Ecological status of Spotted Wing Drosophila (SWD), *Drosophila suzukii*, in Shandong Province, China

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Drosophila suzukii (Diptera: Drosophilidae) is able to lay eggs and feed on healthy ripening fruit. In this study, sugar-acetic acid-ethanol solution and sticky traps with different colours

were optimized and selected to monitor the phenology of *D. suzukii*. The results showed that flies had strong chemotaxis to sugar-acetic acid-ethanol solution after adding yeast and cherry fruits. Black sticky trap can attract more *D. suzukii* in the field; however, in the experiment of phobophototaxis we found that *D. suzukii* had stronger phobophototaxis to violet, blue and green light than to the other colours. *D. suzukii* and other *Drosophila* species were monitored in coastal (City of Weihai, Yantai, Rizhao) and inland region (City of Taian, Laiwu) of Shandong province. The number of flies in coastal region was higher than that in inland region due to the warm and humid climate. *D. melanogaster* and *D. suzukii* were the main *Drosophila* species and occurred at 13th May and 23th May, respectively. The number of *D. suzukii* was higher than *D. melanogaster* from 23th May to 1st June. In conclusion, the ecological niche of *D. suzukii* was different from other *Drosophila* species. Green and black can be the best selection for the colour of traps to monitor *D. suzukii* combined with the laboratory and field trial.

Using *Drosophila suzukii* as a comparative model for studying olfactory evolution.

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It has been demonstrated that *Drosophila suzukii* is capable of attacking ripening fruit, making it a unique species within a fly family named for their attraction towards the fermentation products associated with rotten fruits, vinegar, and yeast. It also has been hypothesized that *D. suzukii* is more attracted to the volatiles associated with the earlier ripening stages of fruit development, and in turn, that *D. suzukii* is less attracted to fermented food resources, especially when compared with *D. melanogaster*. Here, we demonstrate that *D. suzukii* and its close relative *D. biarmipes* are in fact more sensitive to volatiles associated with the fruit-ripening process. Our data also provide evidence for a similar evolutionary specialization to that observed within *Scaptomyza*, a close relative to *D. suzukii*, which possesses a serrated ovipositor and are leaf-miners. Moreover, we have unpublished evidence that the compound eye and visual activity of *D. suzukii* is far superior to that of *D. melanogaster*, a visual adaptation that may again support arrival at host fruit prior to ripening and the release of the full odour bouquet.

The attractivity of bayberry fruit volatiles to the vinegar fruit fly

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Chinese bayberry, *Myrica rubra*, is one of the most favourite hosts to vinegar fruit fly, *Drosophila suzukii*, which causes serious damage to Chinese bayberry fruits. The objective of our study was to identify new attractants for *D. suzukii* from Chinese bayberry volatiles. The bayberry fruit volatiles were collected by headspace absorption and identified by GC-MS, and the electro-physiological responses of *D. suzukii* to bayberry fruit volatiles were measured by coupled GC-EAD. The behavioural responses of the flies to bayberry fruits and their volatiles were tested in Y-tube olfactometer, and the attractivity of the synthetic compounds mixture was also confirmed by field trapping expe-

riment. The result showed that 46, 35 and 17 compounds were identified from ripe, half-ripe and raw bayberry volatiles, respectively. β -Caryophyllene was the dominant compound in bayberry fruits at the different ripening stages, and most esters were released from the bayberry fruits at the ripe stage. Five volatile compounds, namely methyl 3-hexenoate, methyl 2-hexenoate, ethyl 2-hexenoate, α -ylangene, α -caryophyllene, and an unknown chemical in the bayberry headspace samples, elicited GC-EAD responses from the female flies. Four synthetic compounds, namely 3-hexenoate, methyl 2-hexenoate, ethyl 2-hexenoate and α -caryophyllene and their mixtures could attract the females. The mixture was attractive to both males and females in a sandal wood orchard (*Osyris wightiana*, Santalaceae). It suggested that the mixture of methyl 3-hexenoate, methyl 2-hexenoate, ethyl hexenoate and α -caryophyllene is a good candidate for attracting *D. suzukii*.

The project was funded by DROPSA project „Strategies to develop effective, innovative and practical approaches to protect major European fruit crops from pests and pathogens“.

New approaches to apply plant compounds in control strategies: Screening for natural compounds against *Drosophila suzukii*

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Drosophila suzukii (Diptera: Drosophilidae) invaded Germany in 2011 and has become a major economic pest in soft and stone fruits. It is highly polyphagous and oviposits in ripe, undamaged fruits. Larvae feed on the fruit pulp, infested fruits collapse quickly, and leave no tolerance threshold for fruit infestation. Since the currently practiced calendar-scheduled treatment with insecticides is not a sustainable means of pest control, alternative monitoring and management options are urgently needed. Behaviour based strategies are often species-specific and require attractive or repellent substances. In order to identify potentially applicable compounds, we tested eight synthetic plant substances from different chemical classes and eight essential oils for toxic, feeding stimulant or – repellent effects on adult *D. suzukii*. Male and female flies with an age of 7–9 d were used in the assays. Compounds were sprayed on bottom and sides of test cups in 2%, 4%, 6%, 8%, and 10% emulsion in lecithin/water. Ten insects were released into the cups after complete evaporation of the spray cover. The lecithin/water solution served as control. Mortality through contact with treated surfaces was evaluated after 1 h, 4 h, and 24 h. Six replications were conducted for each concentration and compound. Capillary feeding assays were used to determine feeding stimulation or inhibition compared to a control without the test compound. Test insects were previously starved and kept with only water for 20 h. Five females (7–9 d old) were used in each test vial. Blue dye was added to the liquid diet to detect consumption of the food mix by each of the test insects. Substances were offered in 0.01%, 0.1%, and 1.0% solutions in the capillaries. After 4 h the amount consumed was measured for each group of flies. Ten replications were evaluated for each concentration and compound. During the assays, cups and vials with capillaries were maintained in a climate chamber. Purity of all substances was verified and composition of essential oils was analy-

zed by GC/MS (Hewlett Packard 890 II/Finnigan Mat SSQ 7000). Several potentially toxic and behaviour modifying compounds were identified. Farnesol and lemongrass oil had lowest contact toxicity and were strongest feeding stimulants, whereas cinnamon oil and eugenol showed highest contact toxicity and strongest feeding repellency. These substances will be further tested in behavioural assays. Their use as bait components in attract & kill or as volatiles in push-pull strategies will be investigated.

Behavioral rhythms of *Drosophila suzukii* and *Drosophila melanogaster*

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Drosophila suzukii and *Drosophila melanogaster* feed on various fruits, causing great economic losses. In order to find the optimum time for controlling *D. suzukii* and *D. melanogaster*, the daily rhythms of oviposition, egg hatch, pupation, adult eclosion, copulation, and feeding of these two pests were studied. We found the circadian rhythm of *D. suzukii* oviposition to have a single pattern with a peak from 20:00–24:00, while the peak oviposition of *D. melanogaster* was from 16:00–4:00 (the next day). Neither *D. suzukii* nor *D. melanogaster* showed a daily pattern of egg hatch. The single peak of egg hatch for *D. suzukii* occurred 24–32 h after oviposition, while that for *D. melanogaster* followed a bimodal pattern with the first peak of egg hatch from 0–4 h after oviposition and the second from 32–36 h after oviposition. Pupation in *D. suzukii* showed a single peak from 8:00–16:00, while in *D. melanogaster* pupation followed a bimodal pattern, with peaks from 4:00–8:00 and 12:00–20:00. Eclosion of *D. suzukii* adults followed a unimodal pattern and generally took place from 0:00–8:00, while that of *D. melanogaster* also showed a single peak, generally from 0:00–12:00. Meanwhile copulation of *D. suzukii*, which showed a bimodal pattern, was concentrated from 0:00–12:00 and 20:00–24:00 (the next day), while copulation of *D. melanogaster* showed a single peak, generally from 0:00–12:00. Both *D. suzukii* and *D. melanogaster* had a preference for feeding in light, and in a 24 h photoperiod the percentages of feeding insects were 80.8 and 81.1, respectively.

Effect of selected fungi from diet on the growth and development of *Drosophila suzukii* (Diptera: Drosophilidae)

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Drosophila suzukii (Diptera: Drosophilidae) is one of the very few *Drosophila* species which are able to lay eggs and feed on healthy ripening fruit. Adults and larvae are all able to obtain the nutrition in the decaying food, which will generate many microorganisms. However, the relationship between *D. suzukii*

and microorganism are poorly understood. In this study, 13 species of fungi were identified in an artificial diet fed by *D. suzukii*. 8 and 7 species of fungi were identified in cherry and grape fed by *D. suzukii*. Short-term and continuously life table experiments were conducted to determine the impact of three of these fungus species including *Geotrichum candidum*, *Talaromyces minioluteus* and *Actinomucor elegans* on the growth and development of *D. suzukii*. Results revealed that, compared to the control, *G. candidum*, *T. minioluteus* and *A. elegans* increased the mortality of *D. suzukii* adults in the short time, while extended the developmental time of pupal *D. suzukii* by 18.00%, 16.22% and 26.44%, respectively in the life table experiment. *T. minioluteus* reduced the total longevity of *D. suzukii* by 15.52%, while *A. elegans* enhanced the total longevity by 25.96%. *G. candidum* and *A. elegans* increased the fecundity by more than two folds. *T. minioluteus* elongated the mean generation time (T) by 31.34%, whereas *G. candidum* shortened it by 15.26% but increased the net reproductive rate (R0) by 217.76%, intrinsic rate of increase (r) by 88.89% and finite rate of increase (λ) by 9.17%. It was concluded that *G. candidum* and *A. elegans* significantly had a beneficial effect on the growth and reproduction of *D. suzukii*, however, *T. minioluteus* had a negative effect on *D. suzukii*. Our results could provide a new integrated pest management strategy for *D. suzukii* which would be discussed in this study.

Adult reproductive diapause in *Drosophila suzukii* females

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Drosophila suzukii (Diptera: Drosophilidae) is an emerging pest of soft fruits, but in this species diapause has not been thoroughly explored. We examined the effects of different temperatures and photoperiods on diapause induction and termination under laboratory conditions. There was variation in ovarian development and oviposition rate under different photoperiods at 10±1°C, and the percentage of adults with immature ovaries was higher during the short photoperiod (8L: 16D) than other photoperiods at 10±1°C. Adults were most sensitive to photoperiod within three days of eclosion. The optimal combination of photoperiod and temperature for diapause termination was long photoperiod (16L: 8D) at 25±1°C. The supercooling point was significantly reduced in reproductive diapause females, and trehalase, pyruvate kinase, sorbitol dehydrogenase, hexokinase and phosphofructokinase enzyme activities were significantly reduced (36.46%, 57.85%, 32.64%, 54.68% and 24.59%, respectively), and glycogen and triglyceride were significantly increased (42.17% and 120.36%). We conclude that *D. suzukii* is a typical short-day diapause species within a certain photoperiod range. This information might contribute to a more fundamental understanding of adult reproductive diapause for this important pest.

Developing a molecular approach to analyze the diet of *Drosophila suzukii*

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Drosophila suzukii is overwintering as adult fly in a reproductive diapause, getting active at mild days in winter and spring. It has a wide range of host plants, mainly identified by oviposition and developmental experiments in fruits. Unfortunately, identifying the nutrition hosts by those methods is challenging, especially in winter. Observations of feeding activity in the field are problematic due to limited access (e.g. forest canopy, shruberies). The correct identification of moving flies in the field without a stereo microscope is difficult, too. A third problem is that in laboratory feeding-trials insects often feed and/or oviposit on host plants which are not typically used in-field. Thus, there is a big risk of overestimating the range of host plants used by *D. suzukii*. We developed an approach to examine ingested plant DNA in *D. suzukii*. Feeding experiments were established where adult *D. suzukii* from a laboratory colony were fed with raspberry (*Rubus idaeus*), allowing them to digest for up to 72 h. Applying PCR with species-specific primers, the DNA of *R. idaeus* was detectable for up to 48 h post-feeding in whole body extracts of *D. suzukii*. Further, a bleach experiment was conducted to exclude the risk of false-positive detection due to DNA sticking on the flies' body surface. Females fed with *R. idaeus* were contaminated at their tibia or wing-tip with mistletoe (*Viscum album*). Then half of the flies were washed with a bleach-solution and all individuals were tested for DNA of *R. idaeus* and *V. album* again using diagnostic PCR. While the DNA of *V. album* was successfully removed from the fly's body surface, the gut content was not negatively affected by decontamination. In a next step field trapped individuals will be used for next-generation sequencing for further analysis.

Potential geographical distribution of *Drosophila suzukii* based on MaxEnt

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Drosophila suzukii is an important fruit insect pest and its global spread and damage has been paid more attention in recent years. In order to prevent invasion of *D. suzukii* and protect fruit production in China, we studied potential geographical distributions of *D. suzukii* in the world and in China under current and future climate conditions. MaxEnt combined with ArcGIS was applied as technical mode which based on collecting global geographical distributions information and screening main climate variables. Under current climate conditions, *D. suzukii* presented a relative wide potential geographical distribution in the world and 61.68% areas in China with suitability including 7.59% areas of high level, 27.81% areas of medium level and 2628% areas of low level. Under RCP26 and RCP85 climate conditions in 2050, potential geographical distribution of *D. suzukii* enlarged and northern borderline of suitable region expanded northward in the world as well as in China. We suggested related organizations in China to strengthen surveil-

lance, integrated pest management and export quarantine measures of *D. suzukii* systematically.

Landscape effects on *Drosophila suzukii* dispersal and migration

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The presence and spatial distribution of various landscape elements play an important role in pest occurrence and damages. While high-resolution landscape data is widely available, sampling data are often not gathered with landscape studies in mind. In this presentation, we explore methods to analyze trap-capture data of *Drosophila suzukii* from south-west Germany with the objective of mapping spatio-temporal risk for their dispersal and migration based on landscape characteristics. We also present DROSOMON, a centralised, harmonized web platform for data gathering and easy access to trap-capture data and related parameters. The traps monitored by Julius Kühn-Institut over three years were all concentrated in three clusters, 75 km being the maximum distance between the traps. The trapping periods, baiting mixture and local environment vary between the traps. We study the correlations between the capture rates and landscape elements at local and landscape scales using buffer widths ranging from 10 m to 5000 m. In particular, we look into theorized hypothesis concerning *D. suzukii* captures to forested area, edge densities, residential areas and water bodies. The challenges in analyzing this dataset are the relatively homogeneous landscape distribution and the fact that trap captures probably do not reflect pest damage. With data from a wider region at German or European-scale, we expect to minimize the effects of these shortcomings.

Ecological adaptation of *Drosophila suzukii* in northern China

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Drosophila suzukii was first collected in 1935 in China and widely spread at least to 22 provinces. This pest was reported to damage berry, blueberry and bayberry. In order to understand the occurrence, distribution and damage of *D. suzukii* population in Beijing, we have carried out research on the ecology adaptability of this pest. The development of *D. suzukii* Beijing population was tested at different temperatures (15, 19, 23 and 25°C) in the lab. Population dynamics were monitored by sugar-vinegar traps in orchard and forested semi-natural area near the city at five different elevations (from 300 to 1100 m) in Beijing from May 2014 to September 2015. Ovarian maturity and diapause stage of female *D. suzukii* were assessed by dissection. The cold resistance of *D. suzukii* adults was studied by measuring the supercooling point. The results showed that *D. suzukii* completely developed in the range of 15–25°C by fee-

ding on artificial diet and the best temperature was 23°C. Population dynamics trends were similar among all monitoring sites and the population peak was observed from mid-July to early August. *D. suzukii* entered diapause stage from late September to next May. The flies enter reproductive stage one month after terminating diapause stage. The supercooling point of different day-old females was not significantly different, but there was a significant difference for different day-old males. The range of supercooling points of female and male adults was -17.27°C to -18.89°C and -17.59°C to -21.09°C respectively. These results provide a theoretical basis for forecasting and control of this pest.

SIMKEF – Development of a Decision Support System for *Drosophila suzukii*

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Within the project SIMKEF, a decision support system (DSS) which depicts the complex pathosystem host (fruit/grapevine) – *Drosophila suzukii* will be developed. The DSS is expected to predict the population dynamics of *D. suzukii* as well as the actual pest infestation risk for different berries, stone fruits and grapes. For this reason, the interaction of the entire life cycle of *D. suzukii* with the most important meteorological factors as well as the influence of the different host fruits on the biology and behavior is described or functionally determined. Laboratory tests, monitoring activities and already published data will be combined within the DSS. SIMKEF is divided into three different modules. Regarding the habitat, the potential infestation risk will be analysed in the first module. Orchards and vineyards nearby to forest or hedges could implicate a higher risk for an early occurrence of *D. suzukii* as isolated ones. In addition, in this first module a second factor is the temperature gradient during the winter period. Temperature has a significant influence on the survival and overwintering rate of adult flies. For the quantification of the initial population after winter period, laboratory experiments and monitoring data are analysed. As a result, the first module of the DSS identifies higher risk areas/habitats for fruit infestation and estimates the potential risk as an initial value in the DSS for the beginning of the season. In a second module of the DSS, phenological models describe the availability of host fruits. These ontogenetic models are based on the calculation of a daily growth rate of host plants depending on the daylight period and temperature. The summation of these growth rates will be correlated with monitoring data of growth stages of the different host fruits. In a third module depending on the current weather the population dynamics of *D. suzukii* will be calculated per host plant and over the fruit development period (from its beginning of attractive stage to its ripeness). Here, a multiplication factor per host plant passage and thus a risk factor for the infestation of the following host plant can be determined during growing season. The investigations on population dynamics as a function of temperature and relative humidity are carried out in laboratory trials. The provided output of SIMKEF should improve the timing of monitoring of fruit infestation and pest control of *D. suzukii*.

The results can be useful for both organic and conventional fruit and grapevine production and will be available at www.isip.de.

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The occurrence and management of *Drosophila suzukii* in Hubei Province, China

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Spotted wing drosophila (*Drosophila suzukii* Matsumura) is an economically important pest damaging to soft skinned berries and stone fruits over the world. In the past years, the population remained relatively low in the main production areas of cherries in Hubei province, China. However there were still several cases of outbreak reported, yet the main cause is still elusive. We monitored the population dynamics using sugar/vinegar liquid traps in cherry orchards and analyzed the relationship of population dynamics with climatic factors. The population dynamics showed very similar tendency between early and middle varieties which have approximately two weeks delay of fruit maturation without exhibiting an obvious correlation with the fruit maturation. The overall dynamics showed two peaks throughout the experimental season, which coincided with the phenology of cherry and neighbouring strawberry, suggesting a potential shift across host plants. The correlation analysis indicated that temperature is the main factor positively affecting the mean number of captured flies whilst precipitation strongly negatively influenced them. The results suggest that the *D. suzukii* population fluctuation was significantly associated with host phenology and climate. Over past years, demonstration blocks were established nationwide under the support of the National Agro-Tech Extension and Service Centre. The various control strategies were integrated to fight against *D. suzukii* including (a) attracting and killing strategies – sugar/vinegar liquid together with sticky traps with different shapes and colors, (b) cultural control – habitats/orchards sanitation, deep plowing or rotary tillage, (c) recommended chemical spray in the case of outbreaks. In addition, strategic research such as gene mining and candidate selection targeting reproductive processes to facilitate SIT were carried out in the laboratory to support the pest control. Those inputs aim to provide the theoretical basis and develop effective and sustainable suppression approaches against *D. suzukii* in the long run.

Assessment of mortality factors for *Drosophila suzukii* in Switzerland

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A life table study for *Drosophila suzukii* was conducted under semi-natural condition at a forest site in Delémont, Switzer-

land. The aim of the study was to examine mortality factors of all developmental stages, such as natural mortality, predation and parasitism. Fruits infested with eggs, L1/L2, and L3 were exposed over a short period of time using potted blueberry plants (*Vaccinium corymbosum*). Groups of pupae were exposed on the ground underneath the bushes. These were then collected for examination and rearing in the laboratory, to quantify mortality factors in the different developmental stages. For each exposure period, controls consisted of non-attacked fruits and fruits attacked by *D. suzukii* kept in screened cages. To estimate the generational mortality of *D. suzukii* in Switzerland, life tables were constructed using the data from the exposure experiments and following the methods described by Bellows et al. (1992). For the egg and L1/L2 stages, mortality was not much different if they were caged or not (respectively 36.05% and 31.25% for the egg stage and 60.64% and 62.43% for the L1 stage). For the L3's, exposed larvae were more attacked (76.12%) than the caged ones (63.75%). The same tendency was observed for pupae in the soil (25.63% for the non-caged and 18.67% for the caged ones). Based on these mortality rates, the total generational mortality was 95.57%, which provided a realistic R0 of 4.7. This mortality cannot be explained by parasitoids because none of them emerged from the larvae and pupae exposed. Mortality was only caused by two parameters here: predation or abiotic mortality. Mortality in the larval and pupal stage could be increased by introducing classical biological control agents from Asia, such as larval parasitoids in the genus *Ganaspis*, or using native pupal parasitoids (e.g. *Trichopria drosophilae*) for inundative biological control. To drop the population growth below 1 (= declining populations), 30% larval parasitism would be necessary (R0 = 0.92), whereas increasing pupal parasitism to 30% would had a much lower effect on the fly populations (R0 = 2.8).

Natural Enemies of *Drosophila suzukii* in Yunnan Province

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Drosophila suzukii is one of the most important pests of many fruit crops. It has a wide variety of natural host plants. Current control measures for *D. suzukii* depend on pesticides to great extent. Biological control using parasitoids plays an important role in population regulation of *D. suzukii*. The objective of our study was investigating the parasitic natural enemy species of *D. suzukii* and studying their biological characteristics in Yunnan Province. More than 45 plant species were collected from 23 sampling sites in Yunnan province, among them there were 15 host plant species of *D. suzukii*. There were four parasitic wasp species in Yunnan province, namely *Ganaspis brasiliensis*, *Leptopilina japonica*, *Trichopria drosophilae*, and *Pachycrepoideus vindemmiae*, which emerged from five host plants. The species, populations and parasitism rates by the parasitic wasps varied with different sites and host plant species as well as with the same plant species at different sites. The highest rate of natural parasitism was 38.5 % in a *Myrica rubra* orchard. *G. brasiliensis* occurred in four generations in a year, the female population was higher than male (sex ratio ♀:♂= 2:1). This parasitoid parasitized 2nd larval instar of *D. suzukii*. Its average lifetime was about 49 days and the duration from egg to adult emergence was about 29 days. The peak period of oviposition was from the 7th to the 22nd day after emergence of females.

The project was funded by DROPSA project „Strategies to develop effective, innovative and practical approaches to protect major European fruit crops from pests and pathogens“.

Prospects for biocontrol of *Drosophila suzukii* by invertebrates in Germany

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Ongoing research aims to identify native parasitoids of Drosophilidae in Germany and tries to assess their ability for natural control as well as their suitability for being used as biological control agents in pest management of *Drosophila suzukii*. Due to the lack of adapted and specific native antagonists of *D. suzukii*, collection of parasitoids attacking native *Drosophila* species took place in different regions in Germany during 2015 and 2016. Over several months naturally infested fruit were collected from plants and soil. We captured seven different *Drosophila* parasitoid species. First experiments illustrated that the pupal parasitoids *Trichopria drosophilae*, *Spalangia erythromera* and *Pachycrepoideus vindemniae* successfully parasitize *D. suzukii*. The two larval parasitoids *Leptopilina heterotoma* and *Asobara rufescens* were not successful in parasitizing the new host species. Lifetime fecundity, progeny production and sex ratio of the progeny during the life period of female parasitoids were investigated in two German populations of *T. drosophilae*. Females and males of both strains lived on average more than 30 days. The number of eggs produced over the whole lifespan was higher in the Central German population (85.5 ± 2.7 eggs/female) than in the South German population (80.7 ± 3.4 eggs/female). Compared to this the female offspring was slightly superior in South German population (61%) than in Central German population (46%). Progeny development usually lasted about three weeks in both strains.

Evaluation of the potential biological control of *Drosophila suzukii* with Asian parasitoids

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Native to Asia, the Spotted Wing Drosophila, *Drosophila suzukii* (Diptera: Drosophilidae) has successfully invaded the Americas and Europe since the late 2000s, followed by rapid range expansion and increased damage records within these continents. Despite a few indigenous larval and pupal parasitoids being recorded and attacking *D. suzukii* in Europe, the control effects of these European parasitoids are rather limited due to lower parasitism rates and/or impossible to complete development within the host body of *D. suzukii*. Classical biological control is strongly considered as a sustainable approach to control *D. suzukii* in invaded regions by introducing natural enemies from its native region. In the framework of the EU-funded project DROPSA, we conducted extensive surveys in varied habitats, locations and time of the season in 2015 and 2016 in China and Japan, where *D. suzukii* causes little damage and very little is known on its natural enemy complex. Three Asian larval parasitoids were recovered from field surveys, e.g. *Lepto-*

pilina japonica, *Ganaspis brasiliensis* and *Asobara japonica*. Efficiency of Asian larval parasitoids was studied in the quarantine laboratory to understand whether the parasitoids are able to forage and parasitize *D. suzukii* reared with artificial diet or fresh blueberry. Single mated female parasitoid was used and allowed to forage for 48 h with 10–30 24 h old fly larvae in a tube. Then the number of emerging flies and parasitoids were counted and the dead host larvae were dissected to record encapsulation events. Three Asian parasitoids can attack and successfully develop in *D. suzukii* larvae in both rearing substrates, while as a control the European *Leptopilina heterotoma* eggs were all encapsulated by *D. suzukii*. Moreover, *G. brasiliensis* strains showed a strong preference for the host larvae in blueberries in comparison with the diet, indicating a high degree of behaviour response of the parasitoid to fruit cues. No-choice host specific tests of three Asian parasitoids were also conducted with *D. suzukii* and another four non-target *Drosophila* species in Europe. Our preliminary results showed that *G. brasiliensis* Japan strain was more host specific than other two Asian parasitoids. Further research is still required to fully understand the efficiency and host specificity of the selected Asian larval parasitoid.

Pathogens to control *Drosophila suzukii*

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Drosophila suzukii MATSUMURA (Spotted Wing Drosophila, SWD) is an invasive pest that causes enormous damage to soft ripening fruits. It appeared first in Germany in 2011. During the last years, investigations on potential natural enemies and microbial antagonists have been intensified. The widely used biocontrol agent *Bacillus thuringiensis israelensis* (B.t.i.) and the plant extract Azadirachtin from the Neem oil tree have been tested in the laboratory. Whilst B.t.i. did not show any significant efficacy against SWD, Azadirachtin increased mortality rates up to 100%, but only when applying a concentration ten-times higher than recommended for other pest insects, whereas recommended concentrations could only achieve up to 20% mortality. Furthermore, SWD samples from affected countries are continuously screened for microbial and viral pathogens. Recently, microsporidia have been detected. These are obligate intracellular parasites infecting many organisms up to vertebrates. Microsporidia often cause chronic diseases with low mortality rates, mostly decreased fertility and fecundity, retarded developmental time and reduced fitness of host individuals. For biological control, these characteristics can be advantageous if a pest insect has rapid reproduction time and large offspring numbers or when control of the pest is complicated by its preference of hardly accessible habitats. In our screening, a microsporidium was detected in SWD samples obtained from USA. Its introduction to our German breeding line of SWD and its re-isolation was possible. Its characterization was initiated with molecular markers based on SSU rDNA that were amplified by PCR using universal primer pairs. Sanger sequencing of the PCR fragments suggested that the isolated microsporidium belongs to the genus *Tubulinosema* (Tubulinosematidae). For further species characterization, morphological examinations on tissue tropism and life cycle are conducted by light and electron microscopy. Infection experiments to evaluate median lethal concentration (LC50) as well as possible impacts on developmental times are ongoing.

The invasive *Drosophila suzukii* and native parasitoids in Central Europe

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Introduced into Europe in 2008, the invasive vinegar fly *Drosophila suzukii* has caused large economic damage in stone fruit, berry and vine cultivation during recent years. Control of this pest remains challenging due to its high mobility, the large number of crop and non-crop hosts, and its development inside fruits shortly before harvest.

To investigate the potential role of hymenopteran parasitoids for *D. suzukii* control, we conducted a field survey in various regions of Switzerland. Using *D. melanogaster* sentinel hosts we collected a total of eight hymenopteran parasitoid species. Capture of particular species varied among regions, time of the growing season, and habitat type. Laboratory no-choice assays with the field-collected species demonstrated that the larval parasitoids *Leptopilina boulandi* and *Leptopilina heterotoma* (Figitidae) were able to parasitize and kill *D. suzukii*, but did not develop with this host. In contrast, the pupal parasitoids *Pachycrepoideus vindemmiae*, *Vrestovia fidenas*, *Spalangia erythromera* (all: Pteromalidae) and *Trichopria drosophilae* (Diapriidae) could all successfully utilize *D. suzukii* for reproduction. Thereby *P. vindemmiae* and *T. drosophilae* produced most offspring. Thus, native parasitoids could contribute to the control of *D. suzukii* and information on their phenology and habitat preference is particularly important in this context.

Current status of the *Drosophila suzukii* management in Trentino (Italy), research achievements and perspectives for sustainable control

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In September 2009 in Trentino, for the first time in Europe economically important damage by *Drosophila suzukii* on soft fruits was reported. The consequent increasing use of insecticides, augmented the pesticide residues on the harvested fruits and jeopardised the results obtained with IPM on soft fruits. Development of alternative control methods appeared urgent to ensure an economic future for the concerned fruit industry and researchers and technicians of Fondazione Edmund Mach responded rapidly to this new threat. We considered that possible solutions would only arise from a coordinated and international network of diverse expertise, from molecular biology and neurophysiology to pest management techniques, aiming at understanding the fundamental aspects of the ecology of this pest. Accordingly, we have determined the genome sequence of Italian *D. suzukii* in order to assist both basic and applied research

and to provide information about genes involved in processes such as intra- and inter-specific communication and overwintering. The mechanisms of fruit recognition and selection mediated by the olfactory and/or gustatory systems are being investigated. The population dynamics and seasonal migrations in different agroecosystems have been followed taking into consideration the population bottleneck after the winter reproductive diapause. Development, comparison and selection of the most reliable attractants have been performed to provide the growers with effective tools to track fly activity over time and properly time control measures. Mass trapping, physical crop protection by using anti-insect nets as well as botanicals and synthetic insecticides are under experimental evaluation in order to set up sustainable control strategies for the local soft fruit industry. Because of the legal preclusion from developing classical biocontrol, several indigenous parasitoids of larvae and pupae are under investigation as possible biocontrol agents in the framework of augmentative biocontrol. Pros and cons of the present control strategies will be discussed as well as the future directions of research on pest management of *D. suzukii*.

Integrated Pest Management strategy for fruit flies in China – a successful case of area-wide Integrated Pest Management Programme for controlling *Bactrocera minax*

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The conventional Integrated Pest Management (IPM) Strategy for Fruit Flies in China is resorted to the implementations of key technologies such as sterile insect technique (SIT), fruit bagging, insect pheromone trapping and spraying chemical insecticides etc., but each key technology of the conventional IPM strategy used in China has constraints which are especially difficult to overcome in small holder fruits farming systems. From the year 2007 to 2009, there was a sharp decline in citrus production in China due to the infestation and outbreaks of *Bactrocera minax* (Diptera: Tephritidae). In the year 2010, a national area-wide IPM programme was launched for controlling *B. minax* across major citrus regions in China. The specially designed fruit fly protein bait (FFPB) products based on biological behaviour of tephritid fruit flies was developed and used by mass baiting in accordance to accurate determined time of applications under field pest monitoring. Field demonstrations indicated the citrus fruit damage rates were drastically reduced from over 10% to less than 1% before and after the implementation of the Area-Wide IPM strategy. Our innovative Area-Wide IPM strategy for the fruit fly involved systematic planning, technical support, proper farmer training, organization, and involvement of all of the key stakeholders in the citrus production including citrus farmers, farmer associations and local governments. From 2010 to 2014, over 370 IPM demonstration plots were established, while over 160 IPM farmer field school (IPM-FFS) had been held with 5,120 farmers graduated from the IPM-FFSs in the programme. Farmer cooperatives or specialized associations were organized to ensure collective and coordinated actions of small holder farmers in the programme areas by contracting. 2,300 contracts were signed and enforced covering 616,200 acres of citrus orchards between the farmers and the farmer pest control cooperatives in the programme period.

Control strategies for *Drosophila suzukii* management in fruit crops in Germany

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The Spotted Wing *Drosophila* (SWD) with its highly broad host spectrum is one of the most serious pests in Germany in fruit crops since its first record in 2011. The most susceptible crops are all stone fruits particularly cherries and almost all berry crops particularly raspberries and blackberries. Infestation levels can reach up to 100 % without any regulation or control measures. Even if control measures were applied, losses can reach an economical level, where harvest must be ceased. Depending on population development and climate, infestation pressure varies between different periods during one season or between different years. For most threatened crops in Southern Germany potential control periods of SWD are from May (strawberries and cherries) until October (raspberries, blackberries). Hence, growers cultivating different crops have to face a huge challenge in plant protection. For short term control, different insecticides are registered. In stone fruits, only annual authorizations for emergency uses are allowed (article 53 EU-regulation 1107/2009). In 2017, spinosad (SpinTor) and lambda-cyhalothrine (Karate Zeon) could be used in all stone fruits. In sweet and sour cherries as well as plums cyantraniliprole (DuPont Exirel) could be applied additionally. In all berries, spinosad is registered according to a regular authorization against SWD. Furthermore, the application of Karate Zeon is possible in raspberries, blackberries, currants and blueberries. DuPont Exirel can be used only in currants and blueberries according to an article 53-authorization. Pros and cons of chemical control have to be taken into account. This includes varying efficacies against certain SWD stages, different maximum residue levels and following pre-harvest periods. Additionally rain fastness as well as side effects on honey bees or predatory mites vary between the different insecticides. Therefore, other measures are necessary to decrease the population and infestation pressure of SWD in fruit crops. The general recommendations to growers focus on preventive measures as well as cultivating techniques such as netting, regular monitoring of SWD, consequent pruning, regular mulching, short harvesting intervals, complete harvest, consequent sanitation measures and quick refrigerating and processing of fruits. All these control and preventive measures increase cost for fruit production which can be a risk for the economic feasibility of farms. This requires an economically as well as environmentally sustainable control strategy against this serious invasive pest.

Field demonstration of control techniques of *Drosophila suzukii* in China

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From year 2015 to 2017, four pilot sites (two for cherry and two for bayberry) were selected as demonstration areas for controlling *Drosophila suzukii*. The techniques demonstrated were monitoring of *D. suzukii* in orchards including seasonal fluctuation and peak periods, techniques of attract-and-kill adult flies and removal of dropped fruits. In addition, two field experi-

ments were conducted with the aim to test the efficiency of different wine lures trap, different colour trap and chemical control on population of *D. suzukii* in orchards. The following conclusions can be given based on the field demonstrations: (1) Using the sugar and vinegar liquid is effective for the control of *D. suzukii*. Farmers can use simple traps for monitoring, predicting, controlling and trapping fruit flies. (2) The rotted fruit can attract the vinegar flies, so it can be added to the sweet and sour wine solution to increase the control effect. (3) The tests with colour traps for *D. suzukii* in red bayberry orchard show that blue and yellow are more effective than green and red, but this result needs further verification. (4) Attraction by different wine lures for *D. suzukii* in red bayberry orchard show that there was no significant difference between the different ratios of white wine and vinegar. (5) The larvae can pupate in the fruit and soil, so the removal of dropped fruits on the ground can effectively reduce the number of fruit flies in the orchard. (6) It is suggested to suppress the population of *D. suzukii* around the orchards. (7) The field test with the chemical compound of ethyl polybacteria show that chemicals can reduce the populations of *D. suzukii* in the orchard, but in general chemical sprays should not be used except really necessary. (8) Spray treatments of chemical pesticides can be applied at the population peak of the flies or after harvest, and it is necessary to improve spray methods in order to lower environmental pollution, including to minimize spray amounts and frequency so as to protect natural enemies.

Evaluation of soft fruit genetic resources for resistance to the Spotted Wing *Drosophila* (*Drosophila suzukii*)

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The Spotted Wing *Drosophila* (SWD) is an invasive insect which infests in particular thin-skinned berries as strawberries, raspberries, blackberries, blueberries, gooseberries and currants. Females of *Drosophila suzukii* attack fresh, ripe fruit by using their saw-like ovipositor to lay eggs under the fruits skin. The infestation occurs shortly before the harvest and the subsequent larval development makes the fruit unusable for marketing. A sustainable control of SWD can be only assured by the cultivation of resistant or at least less susceptible soft fruit cultivars. The aim of the project is the detection of genotypic differences in the susceptibility against the SWD in genetic resources of raspberry and strawberry. Resistant or less susceptible cultivars can then be used for breeding or recommended to growers in infested areas. In total, 19 summer-fruiting and 9 autumn-fruiting raspberry cultivars were evaluated for susceptibility against SWD in laboratory experiments with ten replicates per cultivar. For the infestation assay, 10 *D. suzukii* females and 5 males of an age between 4–6 days were released into a 125 ml plastic beaker with an aerated lid containing three fruits. The incubation temperature was 23°C with a relative humidity of 65% and a light:dark regime of 16:8 h. After 24 h flies were removed and larvae were counted 5 days after infestation. There were significant different infestation rates between the cultivars evaluated. The summer-fruiting cultivars 'Cascade Delight' and 'Reflamba' showed the highest infestation rates with an average of 4.8 larvae per fruit and female. The lowest infestation rate were determined for the cultivars 'Dorman Red' and 'Glen Ample' with an average of 1.2 and 1.8 larvae per fruit and female, respectively. The autumn-fruiting cultivars 'Polana'

and 'Polka' showed an infestation rate of 2.9 larvae per fruit and female. Here, the infestation rate was significantly higher compared to the cultivars 'Autumn Best' and 'Aroma Queen'. However, these results were received from annual data and further research is needed. The evaluation of existing genetic resources is the first step of the identification of possible donors for further breeding purposes.

Natural compounds and their effect against *Drosophila suzukii*

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The recent worldwide spread as well as the polyphagous nature of the Spotted Wing *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) calls for efficient and selective control strategies. The use of insecticides is one option for management of this invasive pest insect. However, problems associated with the application of insecticides on ripening fruits include the consideration of preharvest intervals and pesticide residue levels as well as an exposure of nontarget organisms present on fruits. Biopesticides based on natural plant extracts offer an alternative to synthetic insecticides. Here, we report on laboratory bioassays using three different types of substrates allowing a thorough screening of four biopesticides (NeemAzal-T/S: a.i. azadirachtin; SpinTor: a.i. spinosad; Spruzit: a.i. pyrethrine; Piretro Verde: a.i. pyrethrine) and one synthetic insecticide (Mospilan, a.i. acetamiprid) for their effects against *D. suzukii* eggs, larvae and adults. An application of all products except for NeemAzal-T/S on water-apple juice agar before oviposition significantly reduced the number of eggs laid since adults died within the first 24 h after contact with the treated medium. A similar effect was visible if grape berries were treated with the products SpinTor and Mospilan. NeemAzal-T/S significantly reduced the number of larvae hatching out of eggs. Treatment of apple-nutrition medium after oviposition significantly reduced the number of individuals reaching the adult stage for all insecticides except for Spruzit. Moreover, the use of biopesticides in an attract-and-kill strategy is currently assessed in different set-ups. One option is an application of the biopesticide as small droplets onto leaves with the aim of adult feeding and an accordingly lower female fertility. A second option are traps containing a substrate for egg deposition, the biopesticide and an attractant. First results show that trap design matters and point to various avenues for the design of attract-and-kill strategies.

Sterile Insect Technique for *Drosophila suzukii*

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The Spotted Wing *Drosophila* or cherry vinegar fly (*Drosophila suzukii*) is native to Asia but has invaded other continents since 2008 and has spread throughout Europe. The females have a serrated ovipositor allowing them to penetrate the skins of intact ripening fruits to deposit their eggs, and the developing larvae rapidly destroy the fruits close to harvest. *D. suzukii* has

a rapid life cycle and the larvae develop well beneath the fruit surface. This means that the use of pesticides is problematic and often not effective, first due to their restricted use close to harvest to protect consumers, and second because the larvae are deep enough inside the fruit to avoid contact. There are currently no cost-effective and environmentally sustainable pest control methods for this species available, resulting in extensive damage to fruit crops. The potential of new technologies as a basis for the urgently needed specific and long-term control of this species should be considered. In this respect, molecular technologies for eco-friendly control of agricultural pests have been developed for other species already and can be transferred to *D. suzukii*. Different technologies, from the development of transgenic conditional lethal systems to new genome editing methods like CRISPR/Cas, are compared and benefits and risks discussed.

Low temperature survival of German populations of *D. suzukii* in relation to food availability and *Wolbachia* infection

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Drosophila suzukii was first recorded in Southern Germany in 2011, including the viticultural area of Palatinate in the Southwest of Germany. Since 2012, regular monitoring is carried out at Neustadt/W., located within this region. Overwintering was studied at a hot-spot site in the following winters of 2013/2014, 2014/2015, 2015/2016 and 2016/2017. The results showed that overwintering does not take place in human shelters or in the ground. Active flies are readily captured when temperatures rise above 8°C during day and it is assumed that they hide on conifer trees. This way, they were able to survive conditions of -10°C and up to 11 ice days within a winter period. Males and females overwinter equally well. Therefore, we addressed the questions in laboratory trials whether German populations adapted to colder climates and whether sex or food supply have a major impact. For this we worked with two different populations: one old "laboratory" line (KEF4W) established in 2013 and one recent line (KEF8) established from the hot spot site just before starting the trials. PCR testing showed that KEF4W individuals were 100% infected with *Wolbachia* whereas all tested KEF8 individuals were negative. Flies were caged individually or in groups of 5 in 50 ml culture tubes. They did not survive without food supply for longer than 6 days at 10°C. However, on artificial diet they survived for more than 260 days at 10°C without loss. Therefore, all further experiments were carried out with food supply. At 3°C at constant darkness flies were still able to feed and survived more than 100 days (lethal time 80). Chill coma was observed at temperatures below 1°C and flies were no longer able to feed. At constant 0.5°C and -1°C maximum survival dropped to 20-25 days. Whereas cold hardened flies (1 week at 10°C) were still regarded as summer morphs, we also induced winter morphs experimentally by letting them develop entirely at 10°C. These winter morphs survived longer at -5°C (up to 6 days) than summer morphs. In conclusion, we observed neither a difference between *D. suzukii* populations nor an influence of *Wolbachia* on cold tolerance. At temperatures below 0°C females survived longer than males and winter morphs longer than summer morphs.