Monitoring and risk assessment of *Drosophila suzukii* in soft fruit in Sweden

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Introduction

The invasive fruit fly, *Drosophila suzukii* (spotted wing drosophila, SWD), is an economic threat in Sweden to soft fruit and berry production. Since its detection in Europe in 2008, SWD has rapidly spread across Europe, infesting a wide range of cultivated and wild fruits and berries, with up to 100% damage reported in berries and cherries (Asplen et al. 2015). From its first detection reported in 2014 in southern Sweden (Jordbruksverket, JBV), the population had spread with damage reported in post-harvest raspberries in 2015, and a significant increase in the number of flies throughout Skåne in 2016 and 2017 (Jordbruksverket). In addition, SWD was found in almost every fruit and berry crop in southern Sweden, including raspberries, strawberries, blueberries, plums, elderberries and red grapes, and was detected for the first time north of Skåne (Jordbruksverket).

Our research proposal aimed to analyze the risk of SWD to soft fruit and berries grown in Sweden, and to conduct research that would aim to minimize the economic impact of this pest. Our project goals were: 1) to optimize monitoring tools for SWD; 2) to assess the occurrence and infestation of SWD in commercial crops and in natural hosts throughout the year; and 3) to optimize information and communication flow with and between stakeholders. To accomplish these goals, extensive research was conducted in the laboratory and in raspberry cultivations in Skåne, members of our collaboration at SLU, JBV, and Hushållningssällskapet (HIR Skåne) regularly organized and participated in berry grower meetings in Skåne, and contributions from the research conducted under this grant were disseminated at two international conferences. An extensive assessment of our research and communication with stakeholders within this project was conducted, which has led to further interactions with growers and advisors. This project has cemented our network of researchers, advisors and growers, working together to come up with economically and environmentally sustainable measures for monitoring and population control of SWD.

Details of the Work Performed

1. Optimizing monitoring tools

While mated female SWD are highly attracted to fresh fruit for oviposition (Revadi et al. 2015a), females have pre-reproductive period of at least 2.5 days during which they feed and mate (Revadi et al. 2015b). SWD is associated with the yeast *Hanseniaspora uvarum*, and adults are attracted to this yeast for feeding (Hamby et al. 2012, Mori et al. 2016). Females increase yeast feeding immediately after mating, and behavioural response increases to yeast and to blueberries after mating (Mori et al. 2016). This pre-reproductive period when females are actively feeding is a window to optimize monitoring and control measures to target females prior to oviposition in fruits.

In laboratory experiments, we found that mated and virgin female SWD are highly attracted to the yeast *H. uvarum* (Fig. 1), and in a closely-related species, *Drosophila melanogaster*, response is lower and females do not actively contact this yeast (Fig. 2). This supports the hypothesis that SWD and *H. uvarum* have a specific association.

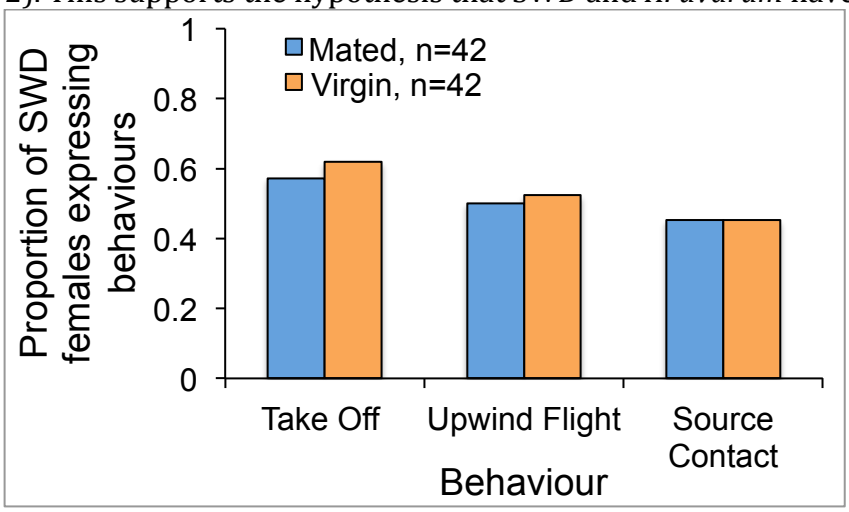


Fig. 1. Mated and virgin female SWD are highly attracted to the odour of the yeast *H. uvarum* in a laboratory wind tunnel experiment. Behaviours in the wind tunnel are measured as "take off" from the insect release vial, "upwind flight" of the fly in the wind tunnel towards to odour source, and "source contact" of the fly touching or landing on the glass pipette or jar that is emitting the odour.

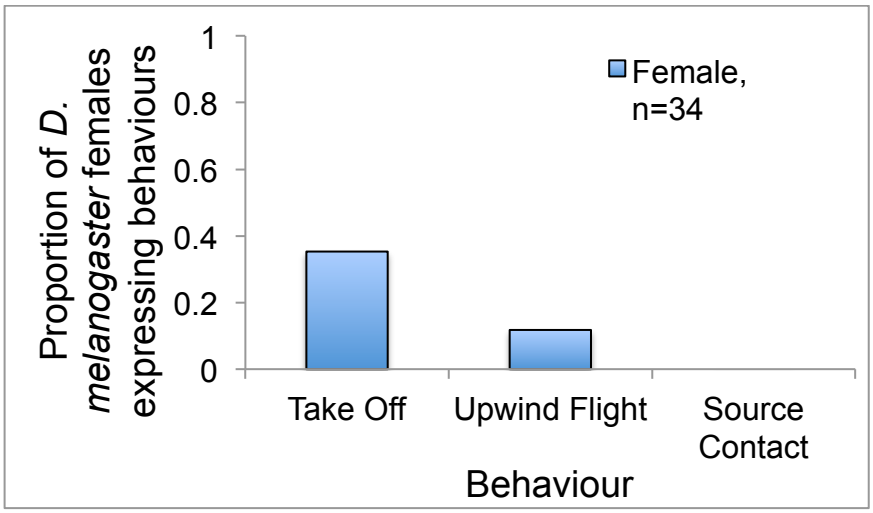


Fig. 2. Mated female *Drosophila melanogaster* do not express source contact behaviour to the odour of the yeast *H. uvarum* in the wind tunnel experiment. Behaviours in the wind tunnel are measured as "take off" from the insect release vial, "upwind flight" of the fly in the wind tunnel towards to odour source, and "source contact" of the fly touching or landing on the glass pipette or jar that is emitting the odour.

Our collaborator, Sebastian Håkansson (SLU, Uppsala), prepared freeze-dried *H. uvarum*, that we were be able to test in the laboratory and in the field in a monitoring experiment. In the laboratory wind tunnel experiment, SWD mated and virgin females were highly attracted to the freeze-dried yeast that had been re-suspended into a liquid minimal medium (Fig. 3).

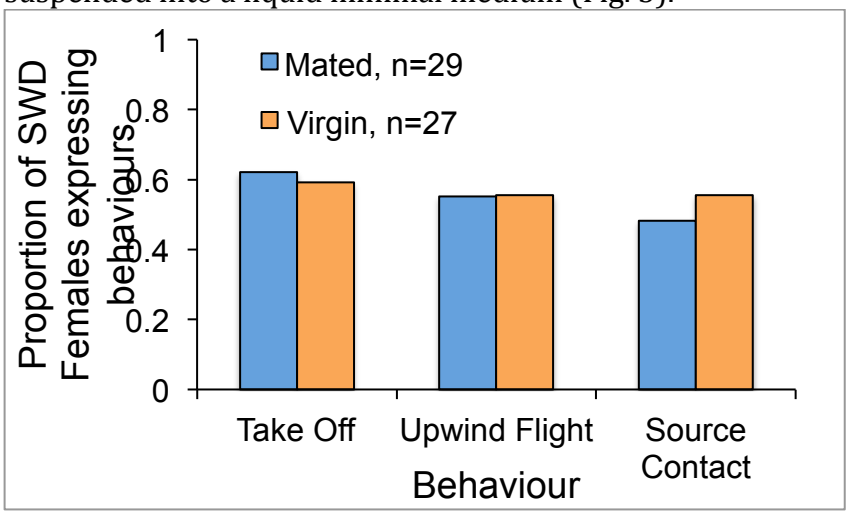


Fig. 3. Mated and virgin female SWD are highly attracted to the odour of the freeze-dried yeast *H. uvarum* in a laboratory wind tunnel experiment. Behaviours in the wind tunnel are measured as "take off" from the insect release vial, "upwind flight" of the fly in the wind tunnel towards to odour source, and "source contact" of the fly touching or landing on the glass pipette or jar that is emitting the odour.

In a commercial raspberry farm in Skåne, we prepared a monitoring experiment in 2017 to compare the attraction and specificity of *H. uvarum* to a commercial lure and to apple cider vinegar, which is commonly used in monitoring traps for SWD. The commercial lure caught the highest number of individuals, but in terms of specificity, especially for female *D. suzukii*, *H. uvarum* was the most selective attractant (Fig. 4), which demonstrates the stage-specific attraction that we were hoping to achieve. These results indicate that *H. uvarum* would be a very good tool to pursue further in terms of improving monitoring traps, and also in the development of specific and environmentally-friendly attract-and-kill technology.

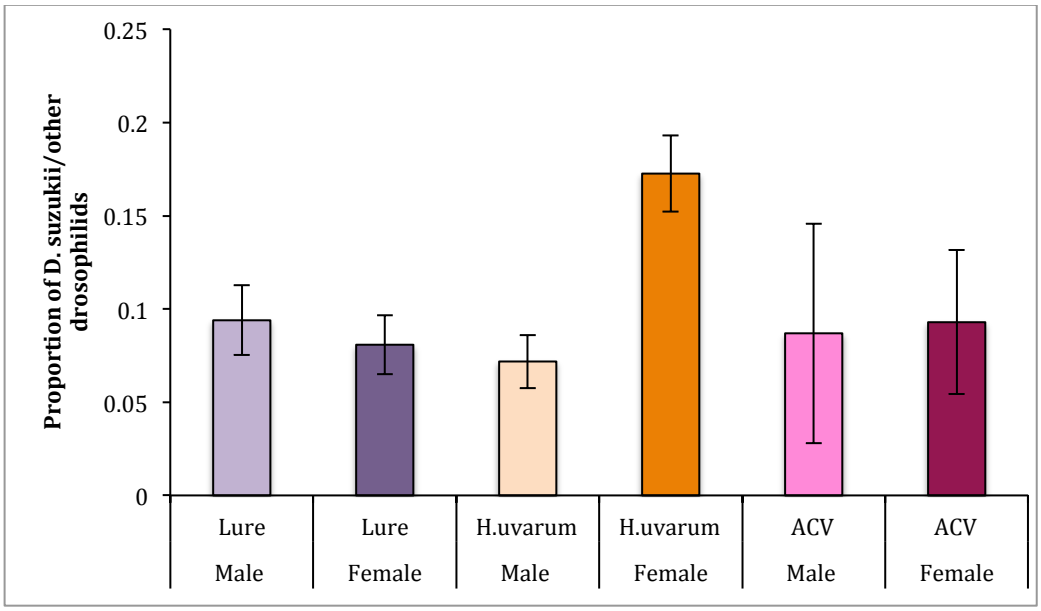


Fig. 4. Stage-specific attraction for female SWD in a field monitoring experiment, by the yeast *H. uvarum*. The odour tested were: a commercially-available SWD lure (Lure), the yeast *H. uvarum* (H,uvarum) and apple cider vinegar (ACV).

The commercial lure also caught the highest amount of non-SWD individuals, compared with almost no by-catch in the *H. uvarum* bait (Fig. 5)

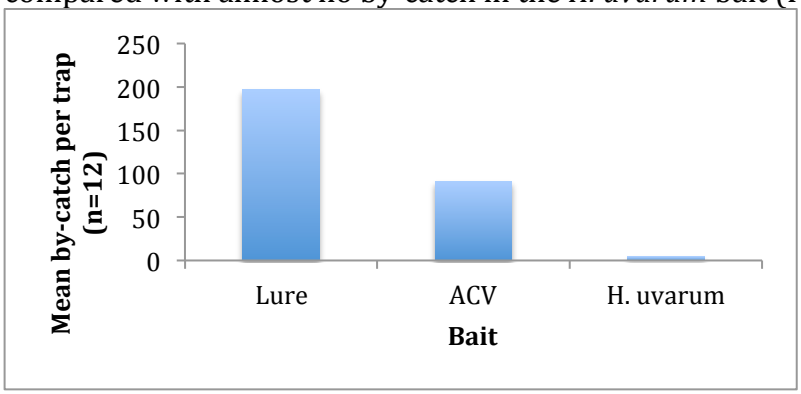


Fig. 5. Mean number of non-target individuals caught with different baits. The odour tested were: a commercially-available SWD lure (Lure), the yeast *H. uvarum* (H,uvarum) and apple cider vinegar (ACV).

2. Assessment of *Drosophila suzukii* in Sweden

Adult trap Catch: To assess the presence and number of *D. suzukii* in Sweden, we collaborated with Jordbruksverket to set out traps and check them regularly throughout the spring, summer, fall and winter seasons. Traps were set out at various soft fruit farms (raspberries, blueberries, plums, grapes, strawberries) throughout Skåne (Fig. 6), and also in typical wild hosts (elderberry, blackthorn and blueberry) (Fig. 7). Traps were baited with a standard white wine and apple cider vinegar blend recommended by Jordbruksverket and is recommended to growers to check for *D. suzukii*. Adult flies were caught from cultivations throughout Skåne from September-mid-October, when traps were no longer checked because the cultivation season was finished (Fig. 6). Traps from wild hosts surrounding a cultivation demonstrated that although the cultivation season finished in October, adults were caught until January 2017 (Fig. 7). This demonstrates that SWD are capable of moving from one suitable host to another, and have established populations that likely overwinter in Sweden.

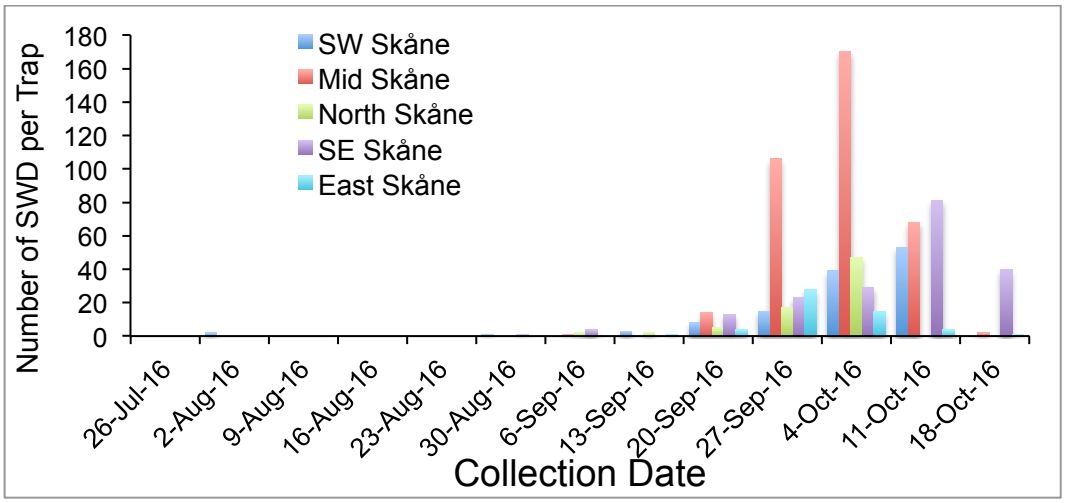


Fig. 6. Monitoring data throughout Skåne from July-October 2017. These are representative data, and each bar indicates the total number of *D. suzukii* caught in one trap, in one location, in one week.

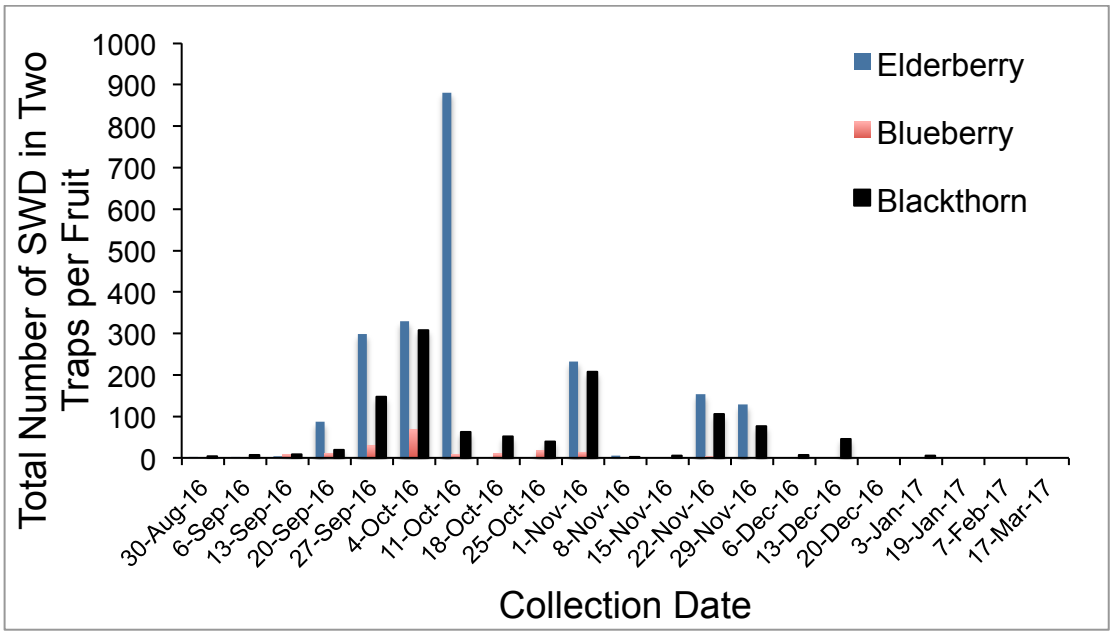


Fig. 7. Collections of *D. suzukii* from in wild hosts surrounding a raspberry cultivation. Flies were caught until 3 January 2017 in Blackthorn bushes. There were two monitoring traps in each wild host fruit.

Larval estimates: The larval stages cause the damage in the fruit by eating the inside. We collected larvae in two different ways, through salt-water extraction of 100 sample berries per week, and also by placing individual berries in vials, and recording the number of adults that emerge from them. In this way, we could assess whether our larval samples were, in fact, SWD (larvae from many drosophilids are indistinguishable for one another), and how our larval counts related to the actual number of berries in a cultivation that are infested. The different types of larval collections reported similar numbers of larvae in each week, and we conclude that either method gives a good representation on the level of infestation in a crop. The larval counts seem to be predictive, and the higher the number of larvae found in one week leads to an even higher number of adults in the subsequent week. Larval checks would be a good way for growers to assess damage and infestation level in their crops.

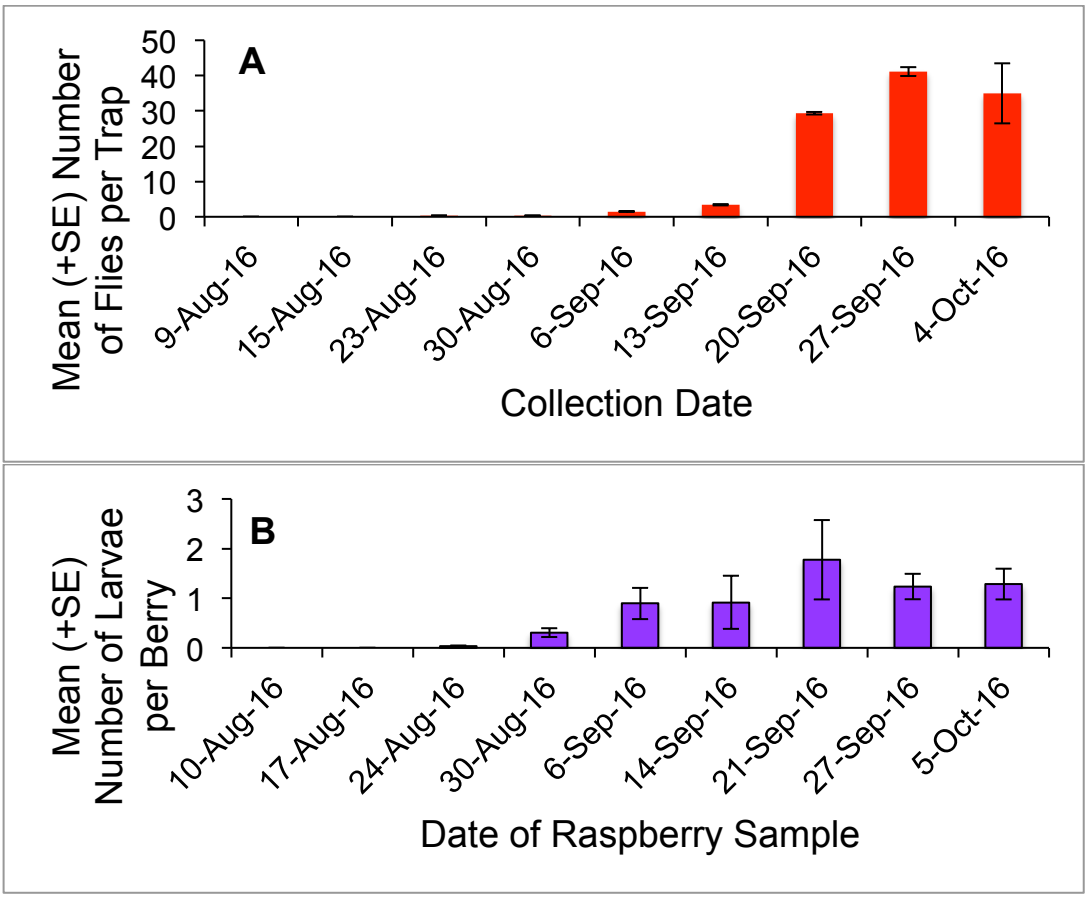


Fig. 8. Mean number of (A) adult SWD collected in traps inside of a raspberry cultivation, and of (B) larvae per berry estimated from samples of 200-800 berries collected from the same raspberry cultivation each week. Berries were soaked in a salt-water solution, and the larvae that emerged were counted. Both adults and larvae were collected for the first time in the same week.

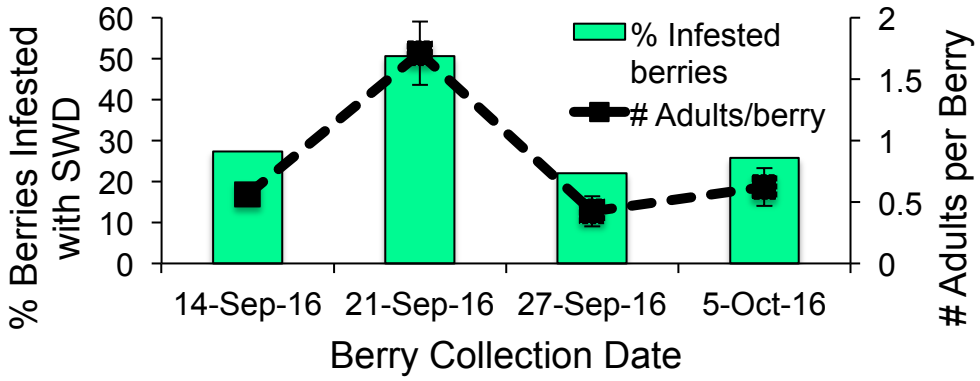


Fig. 9. From individual berries, the percentage of the total number that were infested with SWD, and the number of adults that emerged from individual berries each week.

Fruit was also collected throughout Skåne with our collaborators at JBV, and they found that every fruit-type they sampled contained SWD larvae or adults, including raspberries, strawberries, blueberries, plums, elderberries and red grapes.

3. To optimization of information and communication flow with and between stakeholders

Web-based platform

We wrote, developed and launched a web-based platform (www.drosophila-suzukii.se) that contains information related to SWD for stakeholders in Sweden, as well as international. This website provides information about SWD, its biology, lifecycle, presence, monitoring and control measures. This website also provides information and links to current meetings and opportunities to learn about SWD, news, and updated information about the current status of SWD in Sweden. The website was written in English, and the Swedish translation is complete, and is currently in the stages of being updated on the website. The website also has contact information for researchers and advisors with JBV and HIR Skåne, and a message system for anyone reading the website who would like more information to write a message to us. Our aim is to continue to keep the website up-to-date with current information, news and communications.

Scientific Meetings

Results from the experiments conducted within this grant were presented at two international conferences, the Orchard Pest and Disease Management Conference in Portland, Oregon, USA (January 2017), and the Entomological Society of America meeting in Denver, Colorado, USA (November 2017). There was great interest and feedback from other international researchers around the world who are also studying SWD. This research conducted in Sweden will contribute valuable information to the global knowledge of this pest, and will provide future opportunities for collaborations with international institutions.

Berry Meetings with stakeholders

It was very important to us that we had direct ties with the soft fruit and berry growers in Sweden, which was facilitated by our collaborations with HIR Skåne and Jordbruskverket. We had the opportunity to attend and contribute to three meetings with the berry growers during this time (Field Days at Orelund, 10 May 2016; Hallongården, 30 September 2016; Bröderna Sibbesson, 6 October 2016), where we were able to directly speak with the growers and advisers, update on the current status of *D. suzukii,* and discover the interests of the growers. Through this communication, the growers were made aware of the timing of SWD infestation and levels in 2016 in Sweden, and several growers of raspberries chose to have more of their cultivation ripen earlier in the season in 2017, before the onset of SWD, so that when the flies were first caught in 2017 (11 August 2017), much of the growing

season was already finished, and there were no larvae found in fruit that were being picked and sold. This highlights one of the most significant outcomes from this research project, that results from our research in the laboratory and the field have already contributed to grower decision-making, and in reducing the opportunity for infestation of SWD in cultivations.

Assessment of our collaboration and communication with stakeholders

An assessment of our research, collaborations, and grower knowledge and interest was conducted. Interviews with representatives from SLU, HIR Skåne and JBV assessed the collaboration in this project; an online grower survey was designed and completed by 37 growers of soft fruit and berries in Skåne, to assess their knowledge of *D. suzukii*, and to identify the types of information they are interested in knowing, and how this pest fits into their planning and assessment of their own crops (Mühlhäuser 2017). Out of those growers, 4 were selected for follow-up in-depth interviews to gain a better understanding of their perception of *D. suzukii*, and what types of research we should continue to focus on in order to contribute to the soft fruit and berry industry.

We received a lot of feedback, with some highlights in Figs 10-11 (Mühlhäuser 2017). These include information on measures that growers had taken and were planning on taking against SWD, which demonstrated that SWD is becoming more important for the growers, and they are more aware of this pest species (Fig. 10). How the growers prefer to receive communications about pests, including SWD, which has informed how we have chosen to continue disseminating our results so that growers have access to this information (Fig. 10). Also valuable for our project was to see that the majority of growers would consider participating in future research of SWD (Fig. 11), and that there are a variety of ways they are willing to provide feedback and information (Fig. 11).

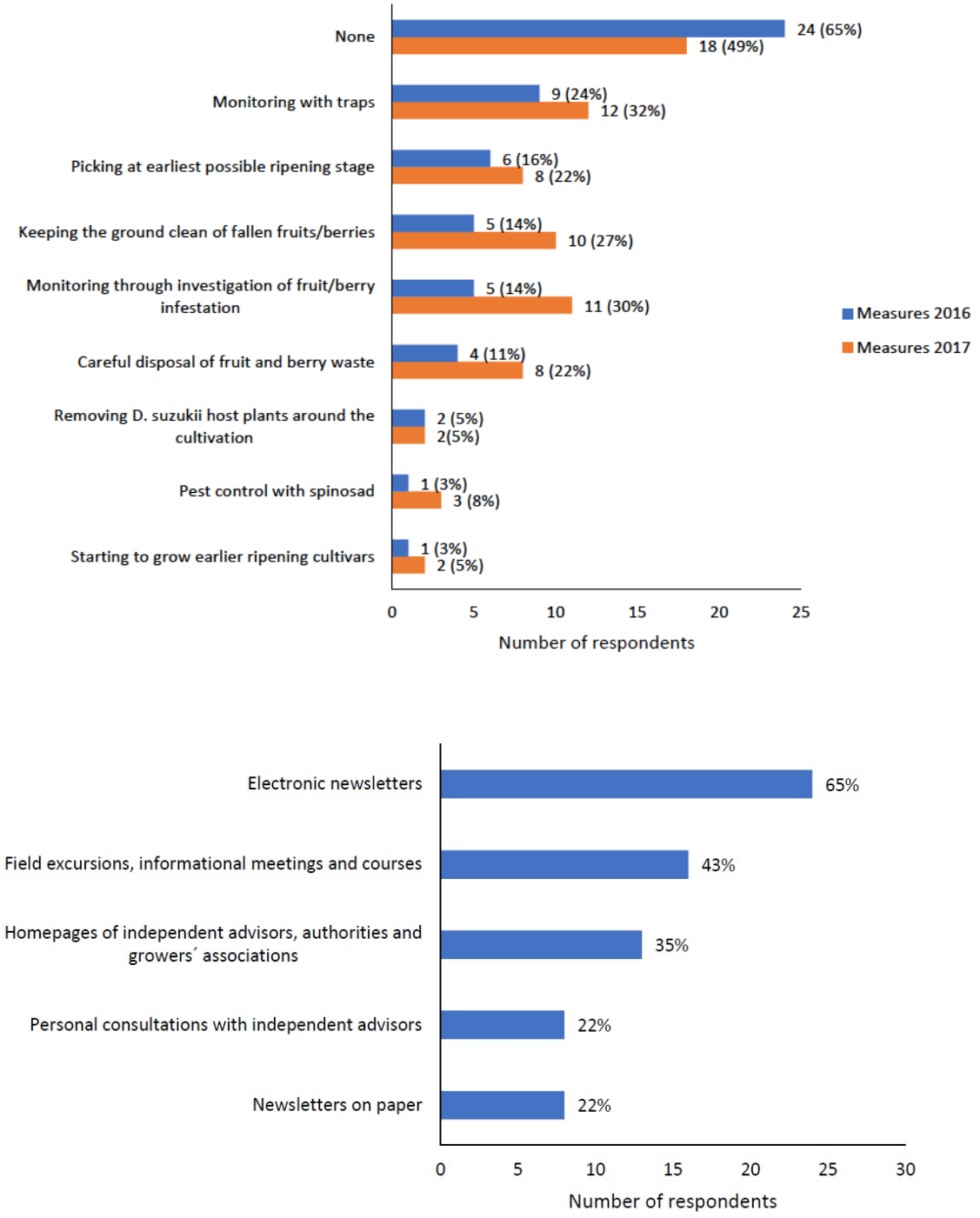
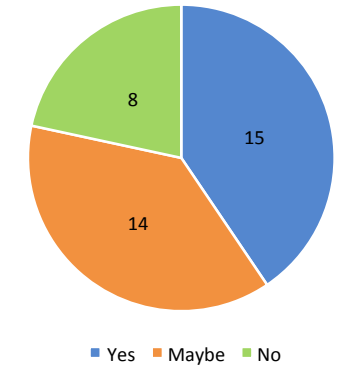
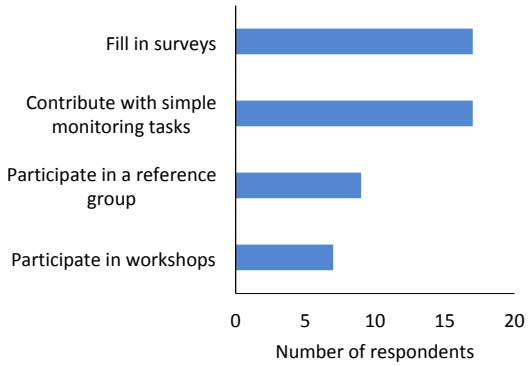


Fig. 10. Top panel: Measures that growers took against SWD in 2016, and measures they were planning for 2017. Bottom panel: how growers prefer to receive information about pests, including SWD (from Mühlhäuser 2017).



Q: Would you consider participating in the national *D. suzukii* work?



Q: In the case of "yes", which activities would you consider participating in?

Fig. 11. Left Pane: the proportion of growers who would consider participating in future research on SWD. Right panel: the types of feedback that growers are willing to provide (from Mühlhäuser 2017).

Summary of Outcomes and Conclusions

We have accomplished all of the goals set-out in this research project, and have made great progress optimizing monitoring tools for SWD, also leading into possible control measures. We have assessed the occurrence and current infestation levels of SWD in commercial crops and in natural hosts throughout the year and find that it is very likely that this population has become established, and can overwinter in Skåne. Finally, considerable effort has been invested in optimizing information and communication flow with and between stakeholders, and we are already seeing positive results and collaborations coming from this investment in communication.

From the results of the assessment of our collaboration and communication with stakeholders, we find that we are on the right track, and our research objectives meet the growers needs for this pest. We have also been given some future directions for how to communicate with growers, which we are currently acting on. A fact sheet on SWD will be published in an upcoming issue of Viola newsletter, and distributed to growers, which will include information that we have found during this project. Growers and advisors are very concerned with having a reliable control measure for SWD available to Swedish soft fruit and berry growers. For the last two years, there has been emergency dispensation for the use of Spinosad (Jordbruksverket) application for some soft fruit and berries, but no permanent registration, and not all fruit and berries are eligible for this dispensation. An alternative control measure it of the utmost importance for Swedish soft fruit and berry growers.

Future research will focus on further testing of the specificity of *H. uvarum* for SWD, and will need to be tested for attraction to non-target and beneficial insects that are also found in soft fruit and berry crops, including bees, butterflies and pollinating flies, before being implemented in a large-scale attract-and-kill experiment. The development of a specific lure based on *H. uvarum* is also in the research stage, and would be useful in monitoring for SWD, as the by-catch is minimal when *H. uvarum* is used as a bait, compared with a commercial lure or apple cider vinegar.

Acknowledgments

We would like to acknowledge everyone who has contributed to the success of the project. Included in this list are the named individuals in the project consortium from SLU including Teun Dekker, Paul Becher, Birgitta Svensson, and postdoc (Joelle Lemmen-Lechelt); Stakeholders including Thilda Håkansson (HIR Skåne) and Sanja Manduric; and international advisors Gianfranco Anfora and Vaughn Walton. In addition, we would like to acknowledge the contribution from Victoria Tönnberg (HIR Skåne), and MSc graduate in Agroecology, Emilia Mühlhäuser. We would like to thank Sebastian Håkansson (SLU, Uppsala), for his collaboration on the work with *H. uvarum*. We would also like to greatly acknowledge the soft fruit and berry growers throughout Skåne would kindly allowed us to collect samples and set out traps, who participated in meetings and who filled out our grower survey. We look forward to continued collaborations and new, innovative research.

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