CHANGES IN PARTICIPANTS

The initial leader of the project, Zsolt Kárpáti, resigned from the project in an early phase. With contract modification, Balázs Kiss, a senior project participant, became the project's principal investigator in the middle of 2017. The competence of Zsolt Kárpáti was irreplaceable for the insect neuroanatomical investigation. This relatively small but promising part of the research plan was thus omitted from the project. On the other hand, the agronomical aspects of the work were expanded. The main reason for this was that spotted wing drosophila had caused severe economic damages in 2016. The pest status of the species in Hungary became thus more relevant than at the time of the submission of the proposal.

For all colleagues, an irretrievable loss was the passing away of Dr. Gábor Vétek with tragic suddenness in December 2020. Dr. Gábor Vétek has supervised several students. Most of his contributing results related to the project are published in MSc thesis works; these items were added to the list of publications (the project support was mentioned in all cases).

I also note the changing of Ferenc Deutsch's role, who joined our group in 2017 as a technical assistant with a BSc degree. During the years of the project, Ferenc Deutsch has graduated as an engineer of agronomy (MSc) and continued his carrier as a PhD student. The topic of his PhD research is also based on the research issues of the present project.

FIELD SURVEYS

General overview of the phenology of SWD in the project period

In Hungary, the abundance of spotted wing drosophila (SWD) is very low until mid-summer; hardly any specimen can be found in spring. They appear in numbers in July or August, and suddenly they reach high densities in a couple of weeks, especially in rainy conditions. The peak of trap catches is in October or November when they are the dominant Drosophilids in the traps until winter. Within this basic phenological pattern, the variation between years in the abundance of the pest is high.

The seasons of the project period had markedly different weather scenarios, which were heavily reflected in the yearly catch results of SWD. In 2016, a mild winter was followed by a humid summer, which was highly favourable for the pest. That was the first year where SWD caused economically significant damages in Hungary. We have observed close to 100 % infection rates in some raspberry blackberry plantations. In 2017, an unusually cold period occurred in January, followed by a hot and dry summer. The first SWD imagoes were detected late, at the end of July, and the catches remained low until September. Subsequently, economically significant damages were not observed in that year. In 2018, due to the mild temperatures, SWD imagoes were captured until the end of January, but frosty periods cut the catches for February. After this, similarly to other years, no catches occurred before June.

The rainy June in 2018 was a new scenario since the mass spreading of SWD in Hungary (2014). Consequently, at the beginning of July, high catch results were obtained relatively early in the season. For the first time in Hungary, economic damages were also reported before August, mainly in the western part of the country. On the other hand, the rainy June was followed by hot and dry summer months, and the catch results were decreasing or stagnant until the end of August when they started to increase similarly to other years. 2019 can be considered a warm but relatively stereotypical year, with average catch results. In 2020, the mild winter and the humid summer months led to the highest abundance of SWD in Hungary since the beginning of the surveys. Finally, in 2021, the hot summer months were accompanied again by low catches of SWD.

Countrywide survey of SWD in orchards

SWD imagoes were monitored by bottle traps with apple vinegar as a lure in orchards by counties from June until November. In 2016, more than 80 000 individuals were caught, with the highest catch results in Nógrád and Somogy, Zala and Komárom counties. The catches started at the beginning of July, reaching their maximum in the second part of October. The highest catch results were observed in plum, elderberry, blackberry and cherry (and sour cherry) plantations; on the other hand, and the catches were strikingly low in vineyards. Interestingly, high catch results of imagoes were not always related to damages, e.g. in cherry plantations, the imagoes appeared only after harvest. In 2017 and 2018, the catches were lower, but they have sown similar phenological patterns. 90 % of SWD individuals were caught after August. (This survey was designed to monitor the species' expansion in Hungarian orchards. It was stopped in 2018 because the efforts with numerous collaborators (local plant protection inspectors) were no more justified, as the species became generally established).

Countrywide survey of SWD in highway rests areas

SWD was firstly found in Hungary in 2012 within the framework of a countrywide trapping survey in highway rest areas (HRA) representing two transects across the country (highways M1, M5, M7, M3, M0). Since then, the same trapping survey was repeated every year with the same methodology to ensure comparable data on population densities of SWD. Bottle traps with apple vinegar as lure were installed in 45 sites along highways at the beginning of September each year, and they were emptied after three weeks. The number of SWD males and females and the number of other Drosophilids were identified in the catch results.

The catches of SWD has shown high variations among the years (Table 1.). Hot and dry summer months resulted in lower densities of SWD in September. The effect of harsh winter can not be proved because only one such period has occurred since the beginning of the surveys. The catches have shown marked differences by geographical areas. The highest catch results occurred in the west-southern region, while they were the lowest in "Tiszántúl" region. The catches were outstandingly high in Táska rest area (Fig.1). The proportion of the two sexes was close to equal (females 53,4 %). The ratio of SWD within all drosophilids has had an increasing tendency since the beginning of the invasion of the species. It is continuously above 50 % since 2018, and it has reached its maximum with 73,8 % in 2020.

(n=30 575)	2014	2015	2016	2017	2018	2019	2020	2021
SWD/trap mean (±SE)	**154,8** (±37,2)	**1,1** (±0,4)	**122,5** (±54,5)	**13,0** (2,9)	**105,8** (±14,8)	**150,0** (±24,7)	**262,4** (±63,5)	**59,5** (±13,9)
SWD/other drosophilids	**15,6 %**	**14,9 %**	**42,4 %**	**28,2 %**	**63,0 %**	**67,3 %**	**73,8 %**	**53,3 %**
summer precipit. [mm	250	130	253	164	202	178	261	132
Nb. of days (T_{max}>35 °C)	0	13	0	7	0	2	1	2
Nb. of cold da (T_{min} < -10 °C)	1	3	5	14	2	3	0	2

Table 1: SWD caches by years in September in highway rest areas (source of meteorological data: www.met.hu)

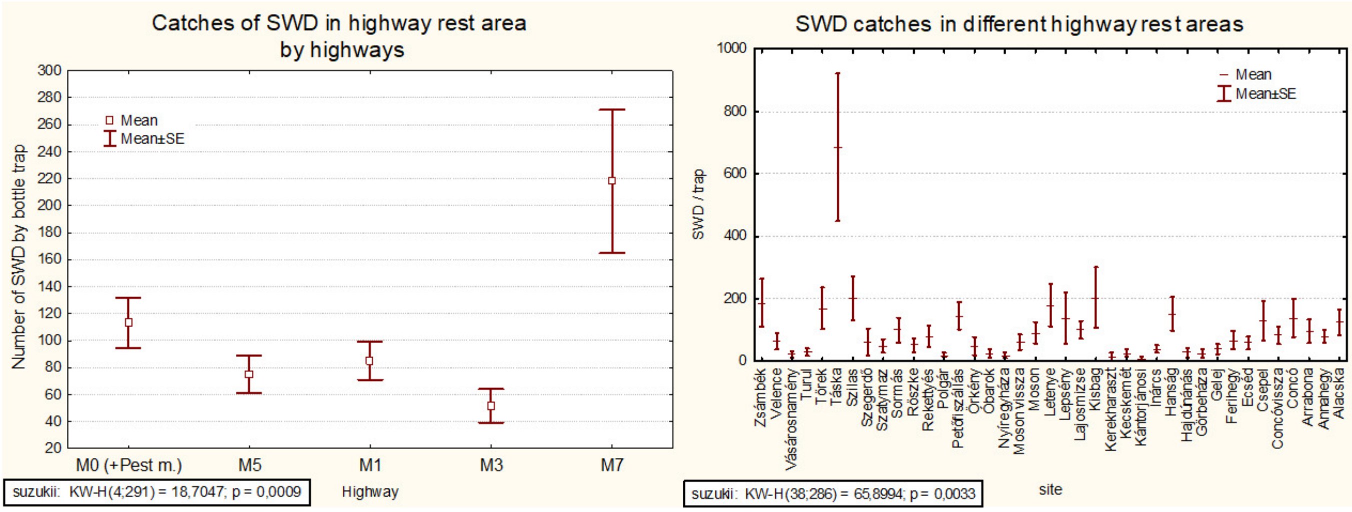

Fig. 1: SWD caches in September in highway rest areas in different regions

The above-detailed survey was supplemented from 2017 by monthly trapping on the most extended highway transect (M3-M7). In the framework of this trapping program, bottle traps were used pairwise from 2019, one with apple vinegar (AV), and another with a mixture of apple vinegar+red wine (3:1) (AVR) as the lure. Out of the total number of SWD imagoes trapped in this survey (n=151473), only 74 were caught before June and 656 before July (mainly in 2020 and 2021). More than 90 % of the early catches (592/656) occurred in sites on the southwestern highway (M7) and in Pest county. Wintermorphs were present until May (19/74), after which they disappeared from the catches from June. In autumn, the first wintermorphs appeared in September (0,03 %), and their proportion reached 12,9 and 26,9 % for October and November, respectively. The traps with added red wine caught 1,80 fold more SWD than AV traps. The same ratios were 1,87 and 1,50 for summermorhs and wintermorphs, respectively. The proportions of SWD in Drosophilidae were under 0,5 % before July, while they were 6,6%, 40,9% and 67,7 % in July, August and September and over 70 % in the last three months. The proportion of sexes was similarly close to equal (males: 50,7 % and 48,0 % in AVR and AV, respectively). There was no striking difference in the selectivity of the two lures; the overall proportion of SWD was 67 % and 70 % in the traps with or without added red wine.

The survey was also supplemented with trappings in 3 sites in the neighbouring range of Táska highway rest area (HRA), in a landscape dominated by wet meadows (Nagy-Berek), between 2017 and 2021 (but in 2021, Táska HRA was closed for public from April to September). The SWD catches in Táska HRA (mean 482±131,7) were not significantly different from the catches in the neighbouring habitat (417±118,6) (t=0,36; d.f. 281; p=0,72). The catches in Nagyberek have shown similar characteristics as highway catches in terms of yearly fluctuation, monthly changes, the proportion of sexes and winter morphs, the relative effectiveness of AV and AVR. The only remarkable difference was in early catches in 2020, where 55 SWD (out of which 17 wintermorphs) were caught in April and May, while only two summermorphs were caught in Táska HRA in the same period, and only one SWD was caught before June in the other four years.

Parallel multi-year survey in neighbouring habitats

The main goal of this study was to compare the population dynamic of SWD in different adjacent habitats. The trapping spots were appointed near two villages in Nógrád county (Berkenye and Romhány) in the similar four habitat types: built-up area (village centres), (sour) cherry and blackberry plantations and forested areas. Two bottle traps (16 traps in all) were operating from April 2017 until October 2021; the traps were changed monthly.

More than 71000 SWD were caught in the survey. Most SWD-s were caught in cherry plantations (41,30 %) and forested areas (33,8 %), while the catches were lower in blackberry plantation (12,95%)

and built-up areas (11,95%). Only two SWD imagoes were caught before July, which may reflect the colder winter climate of this mountainous region compared to the southwestern part of the country. The lower overall catches in blackberry plantations, where the ripening fruits are available until mid-autumn, probably are due to the open structure of the plantation exposed to sun and dryness. In the cherry plantations, the high catches occurred in periods where no suitable fruits were available for SWD, implying that cherry plantations provide suitable microclimatic conditions for SWD, the same way as forested areas, irrespectively of fruit production. In contrast with the absolute numbers, we could not find any substantial differences in the seasonal changes of trap results between the different habitats, and we could not demonstrate mass migrations between the territories (Fig.2)

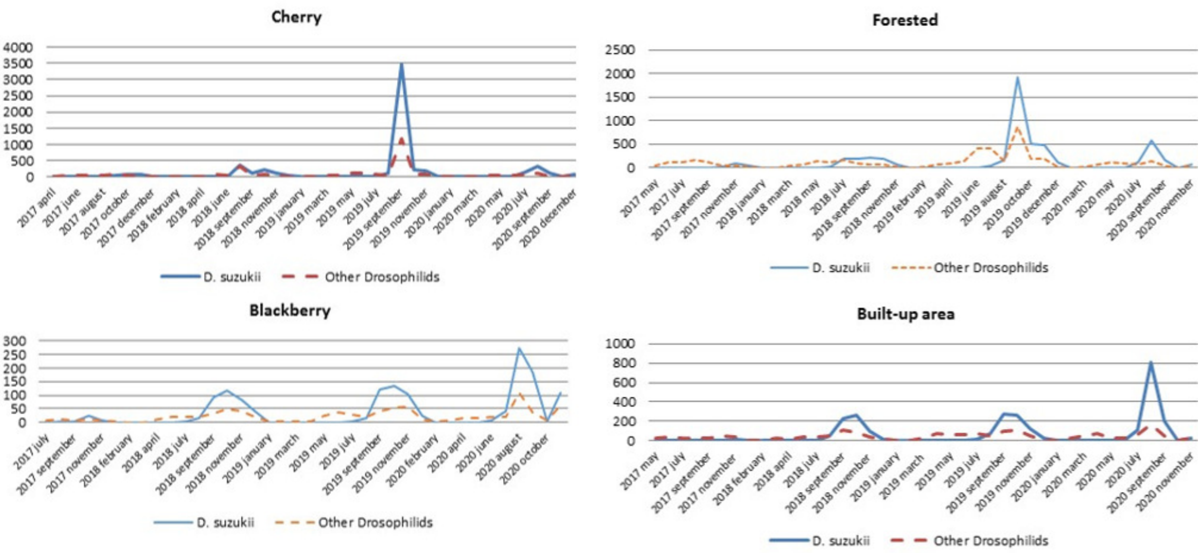

Fig.2 Catches of SWD in neighbouring habitats (catch/bottle trap)

A parallel monthly survey was also effected near Pilisvörösvár to compare pine and deciduous forests (near Iluskaforrás), forest edge, and village edge habitats. The catches of SWD (n=20523) were similar in deciduous and pine forest, and forest edge (30,4 %, 28,6%, 24,5 %, respectively), while in village edge they were lower (17,0%), but the difference was not significant. Ten SWD (6 wintermorphs) were caught before June. The proportion of SWD within all drosophilids is 16% and 53% in July and August and reaches 70 % in the autumnal period: the same pattern as in orchards or highway rest areas.

Multi-year survey of SWD abundances in Budapest (Buda side)

The abundance of SWD was monitored in three sites of Budapest in 2019-2021 by two bottle traps changed monthly. The project's main aim was to test the overwintering capacity of the species in urban conditions. The three sites provided different conditions: Városmajor is a typical urban park in a highly built-up area close to the city centre, the trapping site in Aquincum is in a relatively humid area dominated by gardens, while the site in Adyliget is at the border of Budapest, in a rather cold hilly area, in the forest edge.

51 % of total SWD catches (n=20625) were in Aqincum, while 26% and 23 % of captures were in Városmajor and Adyliget, respectively (Fig). 2,9 % of catches (n=593) occurred before June. The contrast between Adyliget (1,8%) and the two more urban sites was more pronounced in early catch results (58,8%, 39,4%). Similarly, out of the total number of wintermorphs caught before June (341), only two catches occurred in Adyliget (Fig.3).

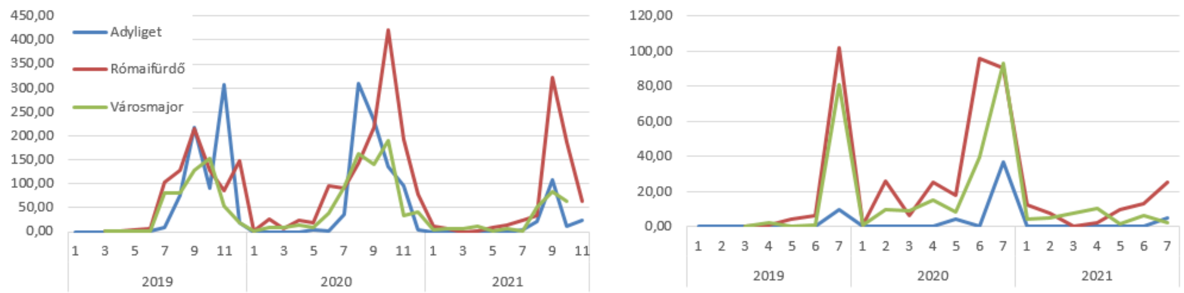

Fig.3 SWD catches by bottle trap at three trapping sites in Budapest

Effect of thermal water flow on abundances of SWD

A trap survey was conducted to show the effect of specific microclimate conditions on SWD abundances near two thermal watercourses (Tapolca patak, Hévízi-elvezető). Bottle traps with AV were installed in groups of five at different distances from the outflow points (warmest water temperature) along and away from the watercourse. The 90 traps in 18 groups were changed monthly from March to October in 2021.

As in the other surveys, most SWD (n = 17977) were caught in autumnal months (November: 69 %, September: 25 %), and less than 1% were captured before June. However, in the case of the two thermal outflow points, the proportion of early catches was much higher (3,8 %). In another approach, in March, April and May, 46 %, 41 % and 42 % of the catches occurred in the traps at the outflow points (fig. 4). The statistical analysis has shown the significant negative effect of distance from outflow point on SWD catches in the case of early catches. The catches in Hévíz were higher than in Tapolca (GLM whole model test F=5,27 p<0,001; effect of distance F=18,54, p<0,001, effect of watercourse F=10,99, p<0,0001, effect of Months N.S., interaction: N.S.). The proportion of wintermorphs within early SWD catches was high, 81 %, 78% and 39% in March, April and May, respectively, while 13 % in September. These results suggest that thermal waters help the overwintering of *D. suzukii*; however, it is not clear how much this phenomenon contributes to the mass population of the pest in late summer.

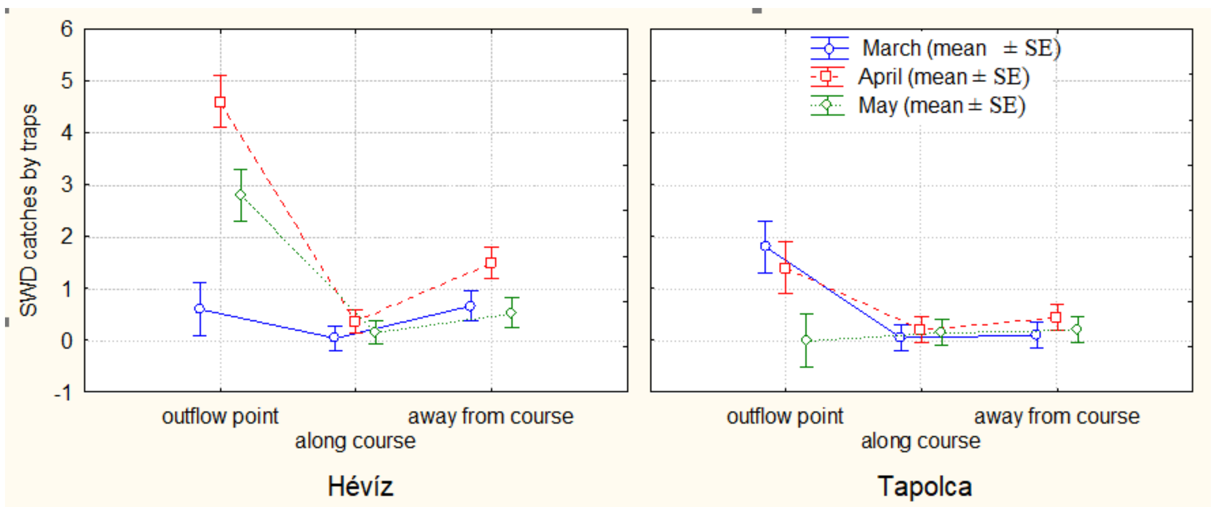

Fig. 4 Early catches of SWD close to thermal watercourses

Spin off side project: analyses of catch results of Phortica spp.

The information on the occurrence of *Phortica* spp. (Steganinae, Drosopilidae) is very deficient in Hungary because the group had no practical relevance until recently. The situation has changed, since the parasitic oriental eye worm, *Thelazia callipaeda,* was introduced into Europe, and in the last years, it has also been rapidly spreading in Hungary. In Europe, the lachryphagous fly, *Phortica variegata* is the only known vector of this parasitic nematode, causing agent of thelaziose of dogs, cats and wild carnivores. As *Phortica spp.* were caught in noticeable numbers in our survey for SWD, we used the opportunity to improve our knowledge of these species.

Summarising the multi-year trapping surveys for 2018-2020, 390 *Phortica* individuals were caught. Both of the two species known from Hungary were present in the traps: *Ph. variegata* (♀:81 ♂:65), *Ph. semivirgo* (♀:134 ♂:110). Surprisingly, most of the *Phortica* flies were caught in March and April (17,7% and 35,4 % of yearly catches), although the lachrymophagous males appear more commonly at the human face on hot summer days. In three incidences, we found the mite species *Blattisocius mali* (Oudemans, 1929) attached to *Ph. semivirgo* imagoes. *B. mali* is new to the Hungarian fauna. The association of *B. mali* with Drosophilinae is known, but this is the first report on its association with species belonging to the subfamily Steganinae. It should be noted that no such attached mite was found on SWD individuals.

Evaluation of damages in blackberry and raspberry

In 2016, the first essential damages of SWD were detected in several country regions. We have determined the infection rate by rearing imagoes from 50-50 raspberry or blackberry fruits in separately (Table 2).

Location	Variety	Date	Ratio of infected berries	SWD/berry
Nógrád (Nógrád county)	Loch-Ness (blackberry)	VIII.30.	100 %	12,4 (±10,0)
Kisgöbrő (Zala county)	Loch-Ness (blackberry)	IX. 14.	98 %	12,6 (±5,6)
Berkenye (Nógrád county)	Sugana (raspberry)	VIII. 23.	100 %	6,1 (±3,5)
Berkenye (Nógrád county)	Polka (raspberry)	VIII. 23.	98 %	5,0 (±4,1)
Kisgöbrő (Zala county)	ZewaIII (raspberry)	IX. 14.	98 %	8,7 (±4,8)
Ecséd (Heves county)	Sugana (raspberry)	X. 6.	60 %	3,5 (±3,1)

Table 2. SWD infections in different plantations (2016)

We followed the SWD infection evaluation in 2016 at two sites (Dejtár/raspberry, Nógrád/blackberry). (The berries were treated in pooled groups for the rearings). Surprisingly, the infestation of the fruits decreased without chemical treatment from September at both sites. The average number of larvae per fruit has changed as follows: blackberry: 12,4 (30-VIII); 6,6 (14-IX); 2,4 (5-X.); 1,8 (13-X); 0,6 (20-X); 0,4 (26-X), in raspberry: 6,0 (14-IX); 0,8 (5-X.); 0,0 (13-X); 0,0 (20-X)). Apart from the direct effect of lower temperatures, the reproductive diapause of winter that morphs may also contribute to decreasing infections.

We proved that blackberry is extremely exposed to SWD because of the gradual ripening of different parts of the individual berries (Fig.5). We compared SWD infections of 30-30 unripe, partly ripened, and completely ripened berries. In the case of partly ripened berries, the two parts (light red and darkened) were separated before the rearing. No SWD left the unripe berries, while 1,4±1,3 and 5,2

(±4,3) SWD were reared from unripe (light) and ripened (darkened) part of partly ripened berries (mean±SD) respectively, while 11,0 ±7,8 SWD left the completely ripened berries.

Fig 5. Gradual ripening of blackberries and symptom of damage caused by SWD larvae

Susceptibility of buckthorn and sour cherry cultivars to SWD

The susceptibility of four sea buckthorn cultivars (' Askola',' Habego',' Leikora' and' Sirola') to SWD was assessed in laboratory choice tests. The fruit samples originated from organic products from a farm located in Budapest, where there was evidence of SWD occurrence from previous years, though without any reports on damage to fruits. The samples were taken in August and October. One intact fruit of each cultivar (four fruits per plastic dish) was put together with a single 10–13 days old SWD female in 36 replicates. The females were allowed for oviposition for two days, and then the fruits were examined for the presence of eggs. The emergence of adults was checked four weeks later. The results showed that all the cultivars were suitable for SWD oviposition and development, though the number of emerged adults was relatively low. The thickness of the skin of the fruits, based on the results of parallel measurements of penetration force, seems to be an essential factor that determines the susceptibility of sea buckthorn cultivars to oviposition by SWD, as it has been proved in the case of other cultivated plants (e.g., grapes).

The susceptibility of three sour cherry cultivars ('Cigánymeggy C7', 'Kántorjánosi' and 'Újfehértói fürtös') to the SWD was also assessed. Fruit samples originated from a farm of organic production at Felsőörs (Veszprém county), where there was evidence of SWD occurrence from previous years, though without reports on damage to fruits. On 19 June 2017, 50-50 ripe/ripening fruits per cultivar were picked and carried to the laboratory to examine natural infestation by SWD. In the laboratory, no-choice tests were carried out in 8 replicates per cultivar, in which one single fruit of each cultivar was put together with a female and a male SWD. The females were allowed to lay eggs for two days. The fruits were then examined for the presence of eggs, and the emergence of SWD adults was checked 2–3 weeks later. No SWD adults could be reared from fruits collected in the orchard. In contrast, in the laboratory no-choice tests, all cultivars were found to be suitable for oviposition and development of SWD.

Potential role of SWD in sour cherry production

Contradictory new findings in the literature concerning the potential damage of SWD in sour cherry in Hungary led us to a more intensive investigation of this issue. Our study took place in large scale plantations in two localities (Gárdony –Fejér county (warmer climate); Berkenye – Nógrád county (colder climate) in the same two varieties ("Újfehértói fürtös" and "Debreceni bőtermő") from 18 June to 19 August. Sour cherry fruits (100-100) were collected from the trees weekly. The number of flies hatching from subsamples of 20-20 fruits was determined in the following 15 days in the laboratory. 3-3 vinegar bottle traps were operated on the spots in parallel with fruit sampling. In Gárdony, only 5 SWD were caught by the traps in the first two weeks. In mid-July, the catches started to increase and reached 58,8 SWD /trap for 14 August. In Berkenye, SWD catches increased later, but they got higher values than in Gárdony from August. No SWD imagoes were reared from the sour cherry fruits collected before harvest; on the other hand, close to two thousand SWD were raised from the fruit samples collected later. In Gárdony, the harvest by shaking machine took place on 1-2 ("Újfehértói") and 13-14 ("Debreceni") of July. The number of SWD developing in fruits of "Újfehértói" started to increase in the fruits collected on 10 July (36 SWD imagoes/100 fruits). It reached its maximum (158) on 24 July, while in "Debreceni", the infestation rate started to increase on 31 July (52) and reached its maximum (150) one week later. In Berkenye, the situation was similar, with slightly higher numbers (maximum: 244 SWD/100fruits, "Debreceni" 7 August). An important proportion (37,5 %) of the drosophilids developing in sour cherry fruits were other species than SWD (Tephritid imagoes were not found). The results confirm our previous conclusion that SWD has no direct economic effect on cherry production in Hungary. However, cherry plantations may play a role in the seasonal propagation of the pest.

The abundance of SWD in elderberry production with nearby sour cherry plantation

Although producers often mentioned important damages caused by SWD in elderberry in Hungary, no published evidence was available until the present study. The study took place in 2021 near Alsószentiván (Fejér county) in an elderberry plantation (6 ha), with a direct transition to a sour cherry plantation ("cigánymeggy") on one side, and a row of locust trees with wild elderberry plants on the other. The bottle traps with AV were installed in groups of five in rows. The traps were in the 5th row of sour cherry trees, in 6 rows in the elderberry plantation, and the one row in the wild elderberry plants. The traps were changed every second week from 8 July to 29 September (Table 3). The SWD infection of the fruits was also determined in 100 cherry fruits on three dates (21/7, 04/8, 17/8) and 30 elderberry and ten wild elderberry umbrellas of average size at five dates (21/7-15/9).

In accordance with our previous experiences, SWD appeared in significant numbers in the traps at the beginning of August, only after the harvest of sour cherry. After that, until the last date, the catches were the highest in sour cherry, and they were higher in the traps closer to sour cherry plantation than in the rows which were farther. For the last trapping period, the catches in elderberry rows became homogenous. The SWD infection was determined in 100 sour cherry fruits. The number of SWD reared from the cherry berries was the highest in the case of 4 August (n=21). In elderberry, SWD was reared only from the samples collected on 15 September (elderberry plantation: n=388/30 umbrellas, wild elderberry: 211/10 umbrellas, note that the two infection rates are not directly comparable because of numerous factors (shadow, insecticide treatment, ripening status etc.).

date	sour cherry	e.b. 1	e.b. 2	e.b. 3	e.b. 4	e.b. 5	e.b. 6	w. e.b.
2021.07.08	0	0	0		1		1	0
2021.07.21	1	0	0	0	0	0	0	0
2021.08.04	45	0	3	3	1	1	2	1
2021.08.17	79	28	28	12	6	3	12	24
2021.09.01	207	90	65	43	34	27	32	46
2021.09.15	351	208	200	151	129	73	92	130
2021.09.29	682	431	449	476	529	329	407	393

Table 3. Distribution of SWD catches in space and time in elderberry plantation. (e.b.=elderberry, w.e.b.=wild elderberry).

Spin off side project on *Phortica spp.*

Our traps for SWD have caught a significant number (n=319) of Phortica spp. (Drosophillidae). These Diptera got eminent attention in the last years in Europe as vectors of an invasive nematode, the oriental eyeworm (Thelazia callipaeda), which causes veterinary problems, mainly in predators (dogs, cats). We use the opportunity to analyse the faunistic data of the two *Phortica* spp. known from Hungary.

In the framework of trapping programs, we caught and identified 246 specimens of *Phortica semivirgo* and 169 specimens of *Phortica variegata*. In both species we found a slight female dominance (>60%). (The males of *Ph. variegata* are the only known vectors of *T. callipaeda*). Three specimens of phytoseiid mite *Paragarmania mali* were found attached to *Ph. semivirgo* individuals, the first record for this mite species from Hungary. It is notable that no incidence of such attached mites was observed on *D. suzukii* during the trapping surveys.

Development of a more specific yeast-based lure for SWD

As drosophilids are known to be attracted to yeasts and various volatile components formed during microbial fermentation, we tested the possibility of using live yeast cultures for baits in field trapping experiments. The mutualistic relationship between SWD and *Hanseniaspora uvarum* is known and provides an opportunity to create a highly effective and species-specific bait for monitoring or even mass trapping SWD.

The effectiveness and the selectivity of apple vinegar+redwine (AVR) bait were compared with a yeast-based (YB) lure formulation in a field trapping experiment conducted in a sour cherry plantation near Berkenye (Nógrád county) in October 2018. Yeast baits were prepared using 4 grams of lyophilised yeast inoculated in liquid yeast broth. The bottle traps containing 200 ml of lure were changed weekly during the four-week trial period to mitigate the possible alterations in the volatile emission of the traps. In total, we have caught close to 10.000 SWD individuals with a roughly equal sex ratio. The traditional AVR lure attracted twice as many SWD as the YB lure. The proportion of SWD in YB traps was 69% in all dipteran specimens and 89% in all drosophilids. In comparison, the same proportions in AVR traps were 52 % and 79 %, respectively. (See in details: Erdei et al. NÖVÉNYVÉDELEM 2019, 80(6)).

Field trapping experiment was carried out in a commercial cherry orchard to evaluate the attractiveness of bottle traps baited with liquid culture (150 ml) of four fruit epiphytic yeast species: *Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Pichia terricola* and *Saccharomyces cerevisiae* as well as the mixed culture of the first two species. To determine the importance of fruit substrate, we also completed experiments using apple juice inoculated with *H. uvarum*. The traps were placed in a sour cherry orchard near Berkenye. The experiment was completed from 9 September to 21 October. We have compared the efficiency and selectivity of yeast culture baits to that of apple cider vinegar. The side catches of all drosophilids were also counted to compare the specificity of the lures. Apple cider vinegar was less specific for SWD but more attractive for all examined drosophilid species. The *H. uvarum*-containing lures were more specific for SWD and the attractivity was significantly increased if it was inoculated in apple juice. *H. uvarum* in synthetic media caught winter morph females with a higher probability than the same yeast species in fruit substrate. This finding supports earlier data on the behavioural shift towards fruit substrates upon egg maturation.

(The results of this work are presented in a submitted MS which is under review in Journal of Applied Entomology ID: JEN-2021-0473: A.L. Erdei, M.O. Szelényi, F. Deutsch, P. Rikk, B. P. Molnár "Trapping Drosophila suzukii with liquid yeast cultures: Is variability of microbial emission a benefit or drawback?")

Field trial using SPLAT technology

The Specialized Pheromone & Lure Application Technology (SPLAT) is a proprietary base matrix formulation of biologically inert compounds used to control the release of added volatile compounds. This product is developed by ISCA technologies and it is a valuable IPM tool so far against several lepidopteran species. We have cooperated with a developer team from SLU Swedish University of Agricultural Sciences to test the efficiency of the field intervention with the technology. The intervention experiment was conducted from 14 August to 30 September in a blackberry orchard in Nógrád municipality. Insecticide was mixed in the SPLAT lures, and 1250 SPLAT-traps were put at approximately 1-meter distances from each other on two experimental plots, and the application was repeated after two weeks. Fifty-six bottle traps containing apple cider vinegar were placed out on the field and changed every week. The population of SWD and other Drosophilids was monitored and mapped based on the weekly catches of the traps. The experiment results showed that the application had no statistically significant effect on the population density of SWD adults based on monitoring ACV bottle trap catches. The lack of significant effect may be due to multiple factors, like the high initial SWD infestation of fruits on the field or the high temperatures causing rapid ageing of dispensers (Fig 6).

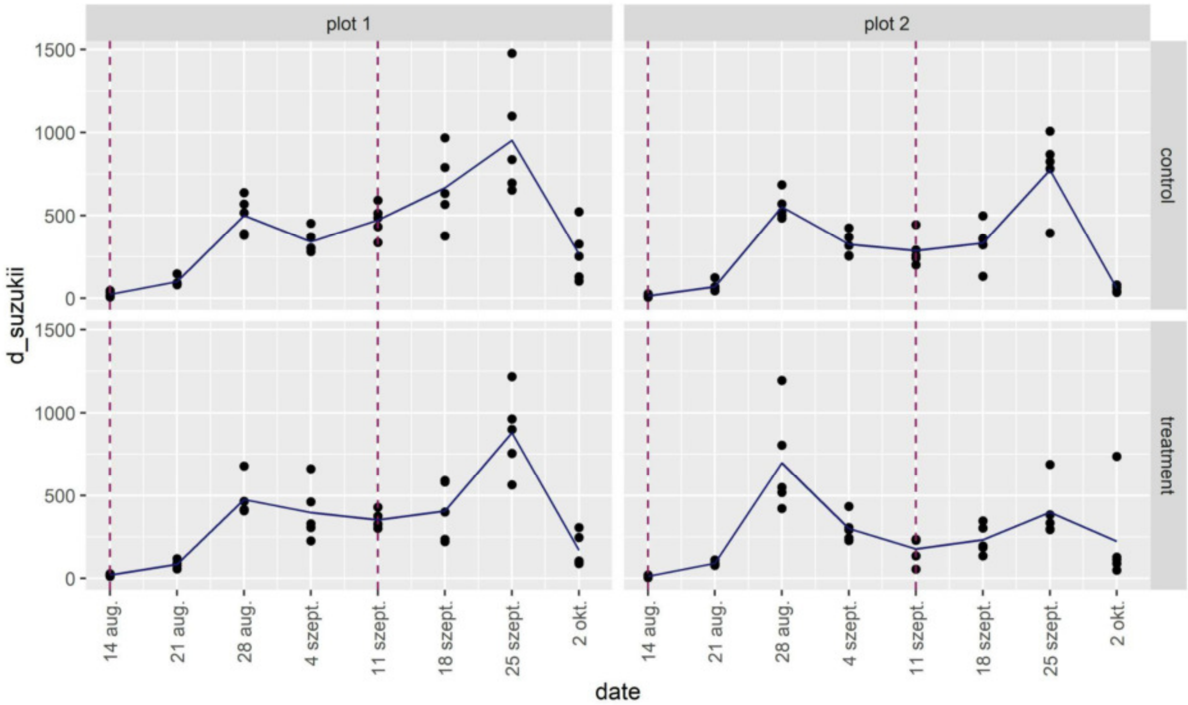

Fig. 6 The number of SWD individuals captured by apple vinegar traps on control and treated segments of the experimental fields. The intermittent lines show the time of field application

Non-lure based collecting methods and quest for overwintering individuals of SWD

During the project, we have unsuccessfully quested for overwintering imagoes of SWD. Several buildings (inside walls) were visually inspected in wintermorphs in urban areas and orchards.

We have searched for overwintering SWD also in leaf litters. Leaf litter samples were collected in three years (2017, 2019, 2020) in January in a forested area and cherry plantations in Berkenye end Nógrád county. The samples (n=36) contained ca 0,5 kg of leaf litter in plastic bags, which were deep-frozen for killing the predators and potential SWD imagoes before visual inspection of the samples under stereomicroscope. Despite the efforts, no overwintering SWD was found in leaf litter.

Suction sampling machine (D-vac) and sticky traps were unsuitable for catching SWD images. The catches of 30 sticky traps (20x30 cm, yellow, carrot and red colour) and 10 bottle trap AV was compared. The sticky traps captured 4 (!) individuals in all (0,13 SWD/trap), while the average bottle trap catch of SWD was 266,0$_{\pm 76}$. The SWD proportion within all Drosophilids was 4,0 % and 75,3 % in the case of the sticky traps and bottle traps, respectively. This difference in the proportions shows that bottle traps AV, apart from effectivity, is more selective to capture SWD than sticky traps. This also means that

SWD is overrepresented in bottle trap AV samples within Droshilids concerning the given habitats' absolute dominance values.

LABORATORY STUDIES

Maintenance of stock population of SWD

The pool populations of SWD for the experimentation was maintained at 23 °C (l:d 14:10) in standard fruit fly media. In these conditions, successive generations of summer morphs of SWD were produced over the years. The typical time for newly hatched adults is 18 days (egg stage 2 days, larval stages 10 days, pupa: 6 days).

The medium of our SWD stock population became infected by *Geotrichum candidum* (a yeast species), which led to early mortality and heavily reduced reproducing capacity of SWD imagoes. After identifying the problem and sequencing the extraneous microbe (thanks to our colleague Alexandra Pintye), we could get rid of *G. candidum* by adding living commercial yeast to newly prepared rearing media.

Factors inducing the formation of wintermorphs

The literature on factors influencing the dimorphism of SWD became very rich in the last five years, but at the starting point of the experiments, very little was known on this issue. From a practical point of view, it was crucial to be able to shorten the time needed for the formation of wintermorphs in the laboratory, for which three months were required by the method published at that time (rearing SWD from egg stage on 10°C).

A series of rearing trials were conducted to determine the role of light and temperature regime in the formation of winter or summer morphs of SWD. The progenies of summer morph imagoes were kept at 13 different light/temperature regimes, including regime changes between different developmental stages. Most importantly, we have found that low temperature (12 °C) at pupal stage induces in itself winter morph imagoes in 100%, irrespectively of the light regime, and irrespectively of the light/temperature regime, the individuals met in their previous developmental phases. In the treatment where the development of SWD eggs and larvae took place on 23 °C (16:8 l:d) until pupation, and then the pupae were exposed to stable 12 °C until hatching, the formation of the winter morph imagoes needed $34{,}2_{(\pm 2{,}9)}$, $35{,}7_{(4{,}9)}$ and $38{,}6_{(\pm 3{,}4)}$ days (mean $\pm$SE) in 0:24, 24:0 and 12:12 (l:d) respectively (n=306; 84; 44).

Our next experiment has demonstrated that temperature during the last period of the pupal stage is decisive for the development of winter morph adults. The average time from pupation to the hatching of imagoes is $5{,}9_{\pm 0{,}3}$ days at 23 °C (control). The pupae transported from warm to cold (12 °C) condition before the fourth day (n=78) became wintermorph with no exception. The pupal period of individuals transported to cold after 3 days is 16 days on average, which means that these wintermorphs reached the imago status in less than 28 days. From the pupae transported to cold on the 4^{th} pupal day, one third became wintermorphs, while the pupae transported to cold on the fifth day became summermorphs. It should be noted that much less time was needed to become summermorph than wintermorph (Table 4).

The average time from pupation to the hatching of imagoes was $23{,}2_{\pm 5{,}5}$ days at 12 °C (control). The pupae transported from cold to warm temperature after 6, 10, 14 days (n=40; 45; 50) became summer morphs in 100 %, while the pupae transported on 18^{th} day (n=46) became summer morphs in 87 %.

pupal days at 23 °C	pupal days at 12 °C	summermorphs (n)	wintermorphs (n)
1	21,9±1,67		15
2	19,9±4,11		31
3	13,0±1,51		32
4	3,42±2,61 (sm) 12,8±3,48 (wm)	12	25
5	2,5±2,20	35	

Table 4: Time to hatching and distribution of summer- and wintermorph imagoes of SWD after transportation of pupae to cold temperature in different pupal phase

Comparison of traits of the two morphotypes

One of the project's objectives was to identify adaptive traits in the two morphs of SWD facing different ecological constraints. We hypothesised that SWD wintermorph imagoes might have more energetical reserves, thus survive longer without feeding. However, a contradictory hypothesis is also viable: as the morphotype is decided after pupation, the larger and darker wintermorphs may have less energetical reserves than summermorphs. Survival time of summer and winter morph imagoes (n=283) were compared in case of complete starvation (with water supply) and in case of added sugar supply at standard laboratory conditions (23°C, RH: 70 %) from hatching from the pupae. We had no significant differences by sexes. In case of complete starvation, winter morphs survived significantly longer than summer morphs ($70_{\pm 7,1}$ and $57_{\pm 7,4}$ hours in average, respectively). In contrast, in the case of sugar supply, the situation was the opposite: summer morphs lived longer ($187_{\pm 7,9}$ vs $138_{\pm 7,8}$ hours).

We compared the mating preference of different morphotypes toward each other. Virgin individuals were assigned into four groups of pairs (summermorph female (SF) – sumermorph male (SM), SF-WM, WF-SM, WF-WM). The pairs were put together after one acclimatisation day at 23 °C into petri dishes for mating for one hour. Unmated couples were put together again on the following days until the mating occurred. After the mating, females were isolated on their standard medium, and the number of offspring was determined. The mating groups were significantly different in the main mating characters. Summermorphs needed fewer days after hatching from pupa to mate, which is in accordance with our present knowledge on the reproductive diapause of wintermorphs. Wintermorph males mated significantly longer. The offspring was significantly lower in the case of wintermorph couples (Fig. 7). Summer females had viable offspring in all cases with only one exception (SF-SM: 32/32, SF-WM: 71/72), while a considerable number of females mated with winter males has not put fertile eggs (4/54, 10/70).

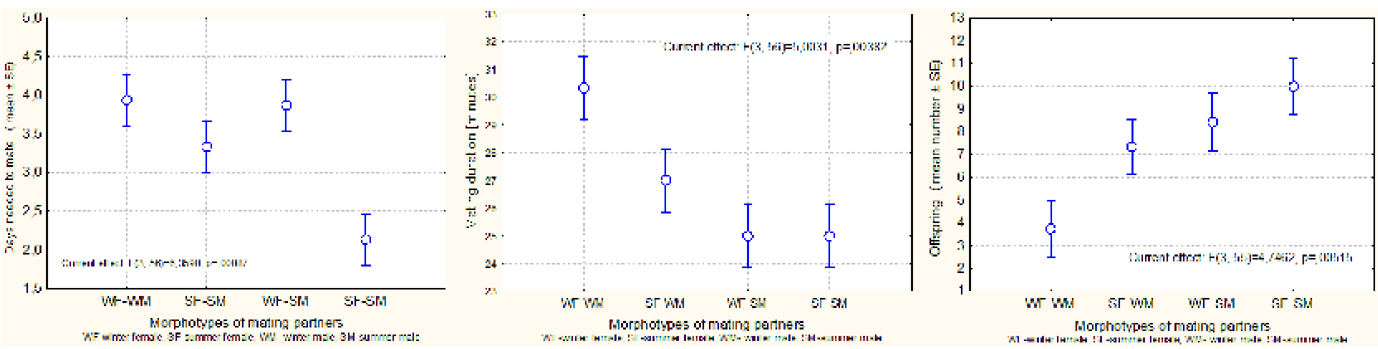

Fig. 7. Mating characteristics of SWD couples of different morphotypes

Volatile collections

The headspace volatiles emitted in closed airspace (odour-free, special polyester oven bag) by the different potential attractive or repellent objects (host fruits, microbial agents, other plant materials etc.) have been collected with an open based volatile collection device. The collected odours were trapped with an adsorbent (charcoal or Porapak Q) filter inserted into the system. The volatiles were collected for 24 or 4 hours. The odours were eluted with n-hexane and/or n-pentane solvent from the filters and have been stored at –40°C.

In parallel with the solvent-based sampling, we collected headspace volatiles using the Solid Phase Micro-Extraction (SPME) technique. The SPME headspace volatile collection was prepared prior to the electrophysiological measurement (GC-EAD). This method does not require solvent; the trapped samples have been eluted by thermal desorption. Therefore the most volatile fractions could also be examined. In connection with the trapping experiments with yeast-based lures (2018-2019) the volatile headspace of bottle traps was sampled with solid-phase microextraction (SPME) fibres and analysed by gas chromatography coupled mass spectrometry to identify the most abundant components (Fig 8).

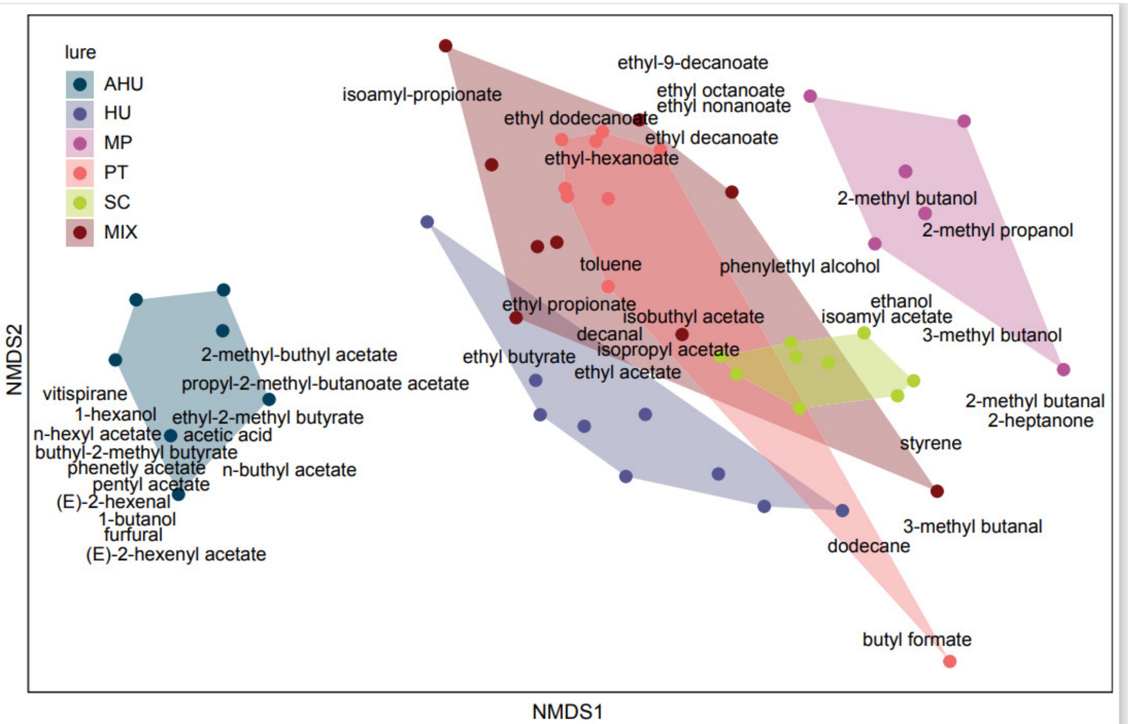

Fig. 8 Nonmetric multidimensional scaling (NMDS) analysis of the volatile emission of yeast-culture lures before field incubation. The volatile profile of *Hanseniaspora uvarum* inoculated apple juice (AHU) and *Metschikowia pulcherrima* inoculated synthetic medium (MP) groups separately form the volatile profile of the three other yeast lures. Stress value of the NMDS plot is 0.1642. (HU- *Hanseniaspora uvarum,* PT- *Pichia terricola,* SC- *Saccharomyces cerevisiae*)

Peripheral electrophysiology (GC-EAD)

The GC-EAD can be used to determine the antennaly active volatile headspace components. In this case, the antenna of the SWD is used as a biosensor to detect the important and behaviourally active volatile components. We identified the retention times (RT) of the active components, which can attract or repel the SWD. We used the antennae of 2 days old females. Before the sample injection into the GC we stimulated the antennae with 100 ng dose of E2-hexenal to find out the physiological state of the antennae and to know if the antenna was alive and not partially damaged during the preparation. When we received a positive response to the stimulus, we injected the volatile collection sample from raspberry into the GC. We used on-column injection mode and the GC's temperature was programmed for: 1 minute at 50 °C at 10 °C /min, then 10 minutes at 250 °C, separating column HP-5. The HP-5 column in the GC then separated the components from the mixture based on the mass and polarity of the components. The GC column was separated into two parallel columns using a 4-way-cross splitter, which leads half of the sample to the flame ionisation detector (FID) and the other half to the antenna preparation. Therefore, we can see the simultaneous response of the FID and the antenna. As a result, we succeeded in determining the retention time of the components that showed biological activity. In the raspberry volatile collection extract, we found seven active components with a RT of 1.) 4.43, 2.) 4.65, 3.) 4.81, 4.) 5.10, 5.) 6.16, 6.) 6.52, 7.) 7.15 min. We also executed SPME-GC-EAD measurements to see if the solvent peak (n-pentae) in the volatile collection extract can cover some important, highly volatile components. We placed two raspberry fruits into a 1.5 mm diameter glass cylinder, and the cylinder was covered with parafilm and aluminium foil. We then inserted the SPME fibre into this cylinder to collect the headspace volatiles for half an hour. After the collection, we immediately inserted the SPME fibre into the GC inlet to thermally desorb the components. Meanwhile, the SWD antenna was prepared. Eleven active RT were determined: 1.) 1.13, 2.) 1.29, 3.) 1.63, 4.) 2.11, 5.) 2.31, 6.) 2.79, 7.) 2.92, 8.) 3.07, 9.) 3.37, 10.) 3.44, 11.) 4.70. These components and their proper blend are likely involved in the attraction of the females and/or males to the raspberry fruit. However, not only the presence of the components but their relative proportion could also be important in the attractiveness.

The olfactory receptor sensitivity of SWD to various volatile compounds was examined. To acquire this dataset, we developed a list of compatible ligands for all the known olfactory receptors of *D. melanogaster* based on the Database of Odorant Responses (http://neuro.unikonstanz.de/DoOR/-default.html), which includes all the currently known receptor ligands. These 71 compounds were combined into seven mixtures (all completed with an internal standard compound), which can be chromatographically separated using capillary column by GC-EAD. These mixtures were tested on females and males of winter and summer morphs of SWD and, for comparison, on *D. melanogaster*, *Phortica variegata* (Drosophillidae). Each synthetic mixture was tested on males and females of each species at least three times (Fig. 9). Our result suggests that there are only minor differences in the peripheral level of olfactory detection between winter and summer morph individuals of SWD. However, we cannot rule out the possibility that the central processing and behavioural effect of those compounds are different.

The SPME volatile collection of the headspace of *H. uvarum* liquid culture was analysed with gas chromatograph coupled electroantennography to understand which components can be detected by the antennae of SWD. We identified nine antennaly active volatile components (ethyl acetate, ethyl proprionate, isoamyl alcohol, 2-methyl-1-butanol, ethyl isobutyrate, isoamyl acetate, 2- methyl-1-butyl acetate, 2-heptanone, 2-phenylethanol).

We used synthetic compounds in different dilutions to verify the results of peripheral electrophysiological measurements with natural volatile mixtures. Our results indicated that even trace contaminations of synthetic standards could falsify the outcomes. Therefore, we investigated this phenomenon in a spinoff project on the widely used *D. melanogaster* model system and its transgenic mutants. Our results were published in the journal Progress in Neurobiology in 2019.

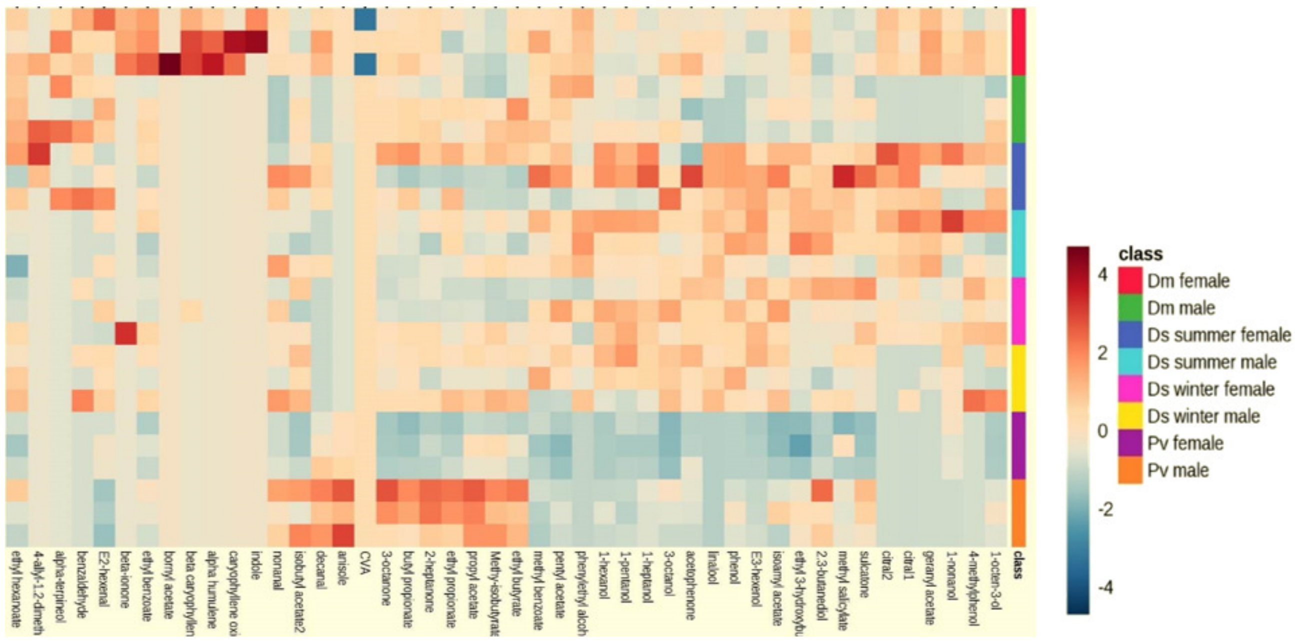

Fig. 9 Comparison of peripherial sensitivity of *Drosophila melanogaster*, *D. suzukii* winter and summermorphs and *Phortica variegata* (heatmap)

6-choice olfactometer experiments

We have identified behaviorally active volatiles emitted from two environmentally-relevant volatile sources: yeast cultures and fruit substrates as well as from AVR traps. We have formulated synthetic blends of these compounds and made 9 different mixtures of them. These mixtures were tested on female summer morph SWD individuals in a competitive 6-choice olfactometer setting. The volatile blends lacking acetic acid and ethyl lactate had significantly lower short-range catch efficiency in the competitive 6-choice olfactometer assays than the complete blend. The attractivity of the complete blend was not significantly different from that of acetic acid.

In cage test of lures based on different yeast species

The SWD catches of bottle traps with different yeast-based liquid lures were compared in cages in a multichoice experiment. The experiment showed that *P. terricola* had the highest attractivity for males and females (Fig 10.). In field experiments the attractivity of *H. uvarum* clearly exceeded that of *P. terricola*. From these differences, we hypothesise that some volatiles emitted from *P. terricola* liquid cultures act as short-range attractants. Still, long-range attractants present in H. uvarum cultures are more important in overall trap efficiency in a field setting.

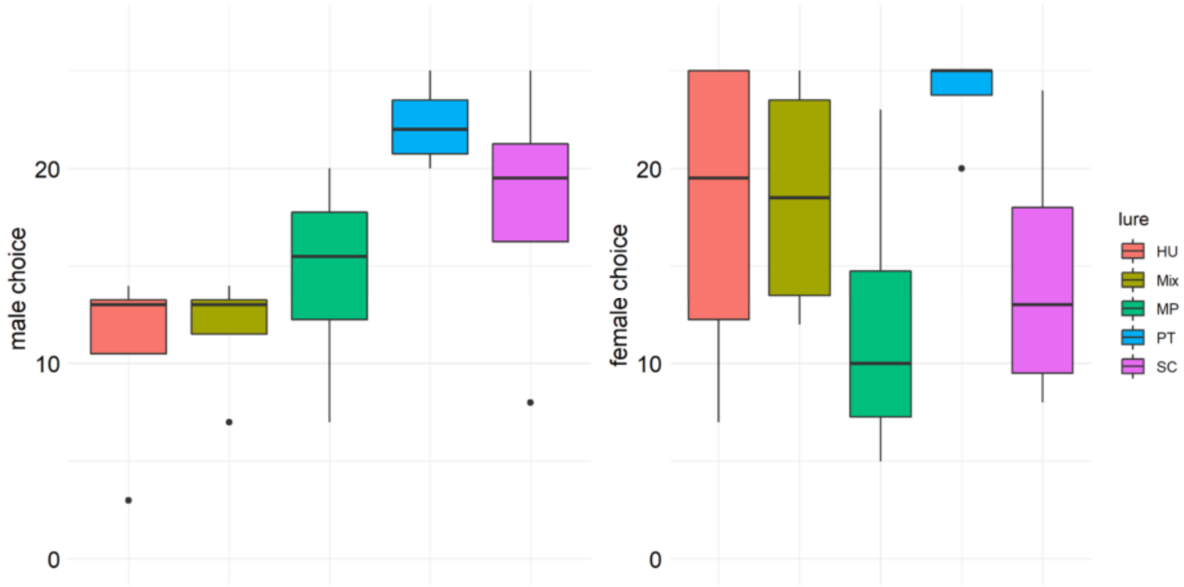

Fig. 10 Short-range attractivity of yeast-lure formulations for SWD imagoes in cages (*Hanseniaspora uvarum (HU), Metschnikowia pulcherrima (MP), Pichia terricola (PT), Saccharomyces cerevisiae (SC)*)

CLOSING REMARKS

At the date of submitting the research proposal, the scientific knowledge about SWD was highly incomplete, even in fundamental issues. That was even more true for the future role of the new invasive pest in Hungary. In the framework of the project, we have explored the main phenological traits of the species in our region. These results explain several differences in the experience of Hungarian fruit producers compared to producers in certain other European countries (e.g. damaged cultures). We have also made some non-insignificant progress in understanding the olfactory characteristics of SWD, with the aim of developing more effective trapping methods and lures.

In the last years, SWD has become one of the most studied pest species worldwide. With the help of the research support, we have also acquired necessary competencies for further scientific works with the species.