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ORIGINAL PAPER

A simple and cost-effective molecular method to track predation on *Drosophila suzukii* in the field

Sarah Wolf[1] · Christiane Zeisler[2] · Daniela Sint[2] · Jörg Romeis[1] · Michael Traugott[2] · Jana Collatz[1]

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Abstract
The vinegar fly *Drosophila suzukii* (Matsumura) is an invasive species that attacks ripening fruits and berries, leading to considerable losses in fruit production. So far, management mainly relies on chemical and cultural control, but additional measures such as biological control are needed. Hence, for the development of sustainable control measures of this pest it is important to identify potential natural enemies such as generalist predators that feed on *D. suzukii*. Here, we established a simple and cost-effective assay to specifically detect *D. suzukii* DNA in the guts of arthropod predators. Furthermore, we developed a general *Drosophila* spp. primer pair to identify predators of *Drosophila* species in general that might also feed on *D. suzukii* and to compare predation rates on *D. suzukii* to those of other *Drosophila* species. We applied the assays to field-collected predators and identified three predator taxa—earwigs, spiders and predatory bugs—that had fed on *D. suzukii*. The assays provide a first step towards unravelling the predator community attacking *D. suzukii* that should be considered as biological control agents but also as non-targets potentially affected by other measures to control this invasive pest.

Keywords Spotted-wing drosophila · SWD · Molecular gut content analysis · PCR · Trophic interactions · Biological control

Key message

- *Drosophila suzukii* causes large economic losses in fruit and berry production. However, little is known about predators that might feed on this pest.
- We developed molecular assays to specifically detect DNA of *D. suzukii* and of *Drosophila spp*. in the guts of arthropod predators.
- Up to 40% of field-collected earwigs, spiders and bugs had consumed *D. suzukii*.
- The approach allows to further investigate the role of predators in controlling *D. suzukii* populations.

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✉ Jana Collatz
jana.collatz@agroscope.admin.ch

[1] Agroscope, Research Division Agroecology and Environment, Reckenholzstrasse 191, 8046 Zurich, Switzerland

[2] Mountain Agriculture Research Unit, Institute of Ecology, University of Innsbruck, Technikerstrasse 25, 6020 Innsbruck, Austria

Introduction

The spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), a vinegar fly native to South East Asia, has recently invaded Europe, North- and South America and is spreading rapidly (Asplen et al. 2015). *Drosophila suzukii* has a high dispersal ability, fast life cycle and shows rapid population growth (Hamby et al. 2016), which allows it to quickly migrate and establish stable populations in new habitats (Cini et al. 2012). In contrast to native frugivorous *Drosophila* species that use decaying fruit for oviposition, females of *D. suzukii* lay eggs into ripening fruits and berries of numerous crop species and initiate a process of rapid fruit decay while larvae develop. Therefore, *D. suzukii* has become a major problem in fruit production leading to substantial economic losses (Mazzi et al. 2017).

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Currently, the most widely adopted strategy to cope with *D. suzukii* in crop production is chemical control (Cini et al. 2012; Haye et al. 2016; Mazzi et al. 2017). Since insecticides target mainly adult *D. suzukii,* which have a high reproductive capacity and overlapping generations, multiple applications are needed and there is a risk of resistance development. Moreover, residues may remain on fruit at harvest and chemicals can have non-target effects on natural enemies and other beneficial organisms (Roubos et al. 2014; Haye et al. 2016). Mass trapping, sanitary measures, netting and kaolin/chalk applications are further measures to protect fruit and berry cultures (e.g. Baroffio et al. 2016), but most of these procedures are labour intensive and do not necessarily reduce fly populations.

On top of these limitations of current control strategies comes the wide distribution of *D. suzukii* at the landscape level (Wang et al. 2016). It infests a broad spectrum of host plants including fruit crops, but also wild plants (Lee et al. 2015; Poyet et al. 2015; Kenis et al. 2016), and very likely uses semi-natural habitats for overwintering and as refuges in times of weather extremes or food shortage (Lee et al. 2015). However, semi-natural habitats often accommodate a high diversity of predatory arthropods such as earwigs, spiders, bugs and beetles (Geiger et al. 2009), and these might play an important role in the regulation of *D. suzukii* populations. The impact of such natural enemies inhabiting orchards and semi-natural habitats could be enhanced by providing them with necessary resources such as shelters, overwintering sites and alternative food sources (e.g. pollen and nectar), by protecting them from the adverse effects of plant protection strategies (e.g. chemicals and sanitary measures) (Gurr et al. 2017), or by the supplemental release of natural enemies into the habitat. In order to support natural enemies of *D. suzukii* according to their needs, they first have to be identified.

Parasitic wasps are the best studied natural enemies of *D. suzukii* in the invaded areas because they are relatively easy to collect with sentinel larvae and pupae of vinegar flies (e.g. Chabert et al. 2012; Mazzetto et al. 2016; Knoll et al. 2017). As potential classical biological control agents, parasitoids from *D. suzukii*'s native range are being investigated as well (Daane et al. 2016). Besides parasitoids, predatory arthropods might reduce fly populations by feeding on eggs, larvae, pupae and adults, and as generalists they can provide background-level control when pest densities are still low (Symondson et al. 2002).

So far, most research on potential arthropod predators of *D. suzukii* has been limited to laboratory no-choice feeding trials where the anthocorid species *Orius insidiosus* (Say), *O. laevigatus* (Fieber), *O. majusculus* (Reuter) and *Anthocoris nemoralis* (Fabricius) (Hemiptera: Anthocoridae), the earwig *Labidura riparia* (Pallas) (Dermaptera: Labiduridae), the staphylinid *Dalotia* (*Atheta*) *coriaria* (Kraatz) (Coleoptera: Staphylinidae) and the mite *Hypoaspis miles* (Berlese) (Acari: Mesostigmata: Laelapidae) were fed different *D. suzukii* life stages (Cuthbertson et al. 2014; Malagnini et al. 2014; Gabarra et al. 2015; Renkema et al. 2015; Woltz et al. 2015). While these studies provide valuable information on the general suitability of *D. suzukii* as a prey and the feeding rates of the predators, they do not allow concluding whether *D. suzukii* is utilized as a prey in the field and how frequently the pest is consumed by specific predator taxa.

Observations from the field are very rare and represent mostly indirect evidence of *D. suzukii* predation: Arnó et al. (2012) collected *O. laevigatus*, *Cardiastethus nazarenus* Reuter, *C. fasciiventris* (Garbiglietti) (all Hemiptera: Anthocoridae) and *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) from potentially *D. suzukii*-infested fruit samples (strawberry, *Solanum luteum* Mill s.l. and *Arbutus unedo* Linnaeus, while Gabarra et al. (2015) collected *O. laevigatus* from raspberry samples. Recently, predator-exclusion experiments revealed that fewer *D. suzukii* developed in straw- and blueberries that were accessible to predators than in berries that were protected by a bag (Woltz and Lee 2017). The predators collected from the accessible berries included species belonging to the Staphylinidae, Araneae, Dermaptera and Formicidae suggesting biological control of *D. suzukii* by these taxa. However, there is only little direct evidence for predators attacking *D. suzukii*. This includes observations of *Orius* spp. feeding on larvae in raspberries (Walsh et al. 2011), ants and spiders feeding on larvae and pupae in blueberries on the ground (Woltz et al. 2015), and video observation of prey removal by ants digging up and carrying away pupae (Woltz and Lee 2017). Since many arthropods are fluid feeders, prey remains in the gut cannot be investigated microscopically, and therefore, molecular methods are needed to identify the consumed prey.

Molecular methods have proven to be a powerful and convenient way to unravel trophic interactions under natural conditions (Traugott et al. 2013). Several primer-based assays have been developed to identify *D. suzukii* DNA, some of which can be used to track predation (Dhami and Kumarasinghe 2014; Kim et al. 2014; Murphy et al. 2015; Renkema et al. 2015). However, these assays require real-time PCR systems and/or the use of restriction enzymes, which makes them cumbersome to apply. Moreover, the primers designed in Murphy et al. (2015) target single-copy genes, reducing the sensitivity of this assay to detect small quantities of degraded prey DNA (King et al. 2008).

The aim of this study was thus to develop and test a simple and cost-effective PCR assay that allows to specifically detect *D. suzukii* DNA in laboratory experiments as well as in field-collected predators and that is suitable for mass-screening. Additionally, a general *Drosophila* spp. primer pair was developed to identify predators of *Drosophila* species that might also feed on *D. suzukii* if available. The

comparison of results obtained with the two primer pairs can serve as a proxy for specific predation on *D. suzukii* in relation to other *Drosophila* species. By applying the molecular approach in a first case study to a range of field-collected invertebrate predators, we could show that different taxa of generalist predators had consumed *D. suzukii.* The new methods and gained knowledge can now serve as basis for developing further studies to investigate interactions between *D. suzukii* and its natural enemies in more detail.

Materials and methods

Arthropods

Adult drosophilids were collected alive with banana-baited traps (0.8-L plastic vials provided with a row of holes (4 mm diameter) and covered with a plastic lid), which were distributed in different habitats in Zurich-Affoltern, Switzerland, in July 2016. Flies were identified morphologically to species level according to Bächli and Burla (1985) using a stereomicroscope and individually stored in 80% ethanol. Additional species were provided by other researchers and the Drosophila Species Stock Center (University of California, San Diego). A list of all drosophilid species and their origin is provided in Online Resource 1.

Predatory arthropods were collected in the vicinity of Zurich, Switzerland, in July 2016 at the following sites: an untreated and highly *D. suzukii*-infested cherry culture in Zurich-Wädenswil, blackberry and raspberry cultures from two organic farms in Zurich-Affoltern (Bärenbohl and Riederhof), and hedgerows and feral blackberry in Zurich-Affoltern (Reckenholz). The presence of *D. suzukii* at the time of collection was verified non-quantitatively using custom-made cylindrical fly-traps as described by Knoll et al. (2017). Predators were caught by shaking the trees and shrubs and collecting the fallen predators from a towel or net placed below. All predators were stored individually in tubes and kept on ice until reaching the laboratory. They were subsequently frozen and stored at − 24 °C. Online Resource 2 provides a detailed list of the number of predators caught at each site and culture.

Since the cherry culture in Zurich-Wädenswil was highly infested with *D. suzukii* and therefore contamination of the predator's body surface with *D. suzukii* DNA was likely, the predators were washed with bleach to remove such contamination prior to analysis (Greenstone et al. 2012; Wallinger et al. 2013). The tube with the predator was filled with 1–1.5% bleach solution (1 part 10–15% sodium hypochlorite (Sigma-Aldrich, St. Louis, Missouri, USA), 9 parts Milli-Q water, Tween (detergent) 0,2%) and shaken for 30 s. The predator was then transferred twice to a fresh tube with 1 mL of Milli-Q water and shaken for 15 s. Finally, the predator was stored in a fresh dry tube at − 24 °C.

DNA extraction

DNA was extracted from whole specimens of all fly species and from 2 to 3 legs per predator (from 1 to 7 individuals per taxon, see Online Resource 3). The tissue was submerged in 180 µL TES buffer (0.1 M TRIS, 10 mM EDTA, 2% SDS, pH8) and 20 µL Proteinase K (20 mg/mL) and then homogenized with 3–4 3-mm glass beads in a Precellys® 24 Tissue Homogenizer (Peqlab, Erlangen, Germany) for twice 35 s at 5500 rpm. The samples were centrifuged for 5 min at 14,000 rpm and incubated overnight on a rocking platform at 56 °C. DNA was extracted with the BioSprint 96 instrument using the BioSprint 96 DNA blood Kit (both QIAGEN, Hilden, Germany) following the instructions of the manufacturer's manual except for the modification of the lysis described above and elution in 1× TE buffer.

Primer design and assay development

To track the consumption of *D. suzukii* on a species-specific level and the consumption of *Drosophila* spp. on a genus-specific level, two primer pairs were developed. The target region for DNA amplification with the *D. suzukii*-specific primers was part of the mitochondrial cytochrome *c* oxidase subunit 1 gene (COI), a region often used to distinguish species (Hebert et al. 2003). The DNA of flies and predators was amplified in an Eppendorf Mastercycler Nexus (Eppendorf, Hamburg, Germany) with 1.5 µL DNA extract, 0.2 mM dNTP mix (Genecraft, Köln, Germany), 0.5 µM of each primer (LCO1490 and HCO2198, Folmer et al. 1994), 1x reaction buffer with magnesium (NEB, Ipswich, USA), additional 1.2 mM $MgCl_2$ (to reach a final concentration of 3 mM $MgCl_2$), 5 µg bovine serum albumin (BSA), 0.25 U oneTAQ polymerase (NEB) and PCR grade water to a final volume of 10 µL. The following conditions were used: 2 min of initial denaturation at 94 °C followed by 35 cycles of 20 s at 94 °C, 30 s at 50 °C and 60 s at 68 °C. The final elongation was run for 3 min at 68 °C. Amplicons were visualized with QIAxcel Advanced (QIAGEN; QIAxcel DNA Screening Kit, method AM320 with 30 s injection time, ScreenGel v1.4). PCR products from all drosophilid species and from one predator per family were enzymatically cleaned with Exo (NEB) and TSap (Promega, Mannheim, Germany) and sent for sequencing to Eurofins MWG Operon (Munich, Germany). Drosophilid sequences were submitted to GenBank (accession numbers in Online Resource 1).

Sequences were aligned in BioEdit (Hall 1999) together with additional sequences obtained from GenBank. A primer pair targeting *D. suzukii* was designed with Primer Premier 5 (PREMIER Biosoft International, Palo Alto, USA).

Due to the high variability in the COI sequences among the different *Drosophila* species, it was impossible to design a group-specific primer on this gene. Based on the alignment of 18s rDNA sequences used to design group-specific primers in Sint et al. (2014) and additional *Drosophila* sequences from GenBank, the already existing primer pair Cal-gen-S263 and Cal-gen-A264 (Sint et al. 2014) was modified to receive a genus-specific primer pair for *Drosophila* spp. using BioEdit and Primer Premier 5.

Both the specific and the general primer pairs were tested separately in singleplex PCR to identify the optimal annealing temperatures.

To test the sensitivity of the primer pairs, standardized DNA templates with known numbers of DNA copies/µL were generated as described in Sint et al. (2012). A stepwise dilution series of the resulting DNA templates was tested with both primer pairs to determine the minimal number of DNA copies needed for successful amplification (minimum signal strength on the QIAxcel of 0.1 relative fluorescent units (RFU)). Both primer pairs target so-called multi-copy genes to increase the detection probability in dietary studies (King et al. 2008). However, as they are amplifying a mitochondrial (COI) and a nuclear gene (18s rDNA), respectively, comparing solely the number of DNA molecules needed for successful amplification does not take the differences in copy numbers for the two genes per cell into account. Therefore, also the total DNA concentration of a *D. suzukii* DNA extract was determined, using Quant-iT™ PicoGreen® (Invitrogen, Paisley, UK) on a VICTOR™ X4 Multilabel Plate Reader (PerkinElmer, Waltham, USA). A dilution series was tested with both primer pairs to determine the minimal amount of total DNA detected by the primers.

Specificity of the diagnostic PCR assays was assessed with DNA from 79 and 88 taxa with the *Drosophila* spp. and the *D. suzukii* primer pairs, respectively (including 24 of 35 known *Drosophila* species of Switzerland according to Bächli (1998) and DNA from different dipteran families that was available from an earlier study by Sint et al. 2014, see Online Resource 1).

Screening of field-collected predators

Six taxonomic groups of predators were collected in the field: bugs (Hemiptera: Heteroptera), earwigs (Dermaptera), spiders (Araneae), harvestmen (Opiliones), ladybird beetles (Coleoptera: Coccinellidae) and rove beetles (Coleoptera: Staphylinidae). Earwigs, predatory bugs and ladybirds were identified to species, spiders and rove beetles to family and harvestmen to order level. Most bugs belonged to the species *Himacerus mirmicoides* (O. Costa) (Nabidae) (147/155) and were mainly collected in black- and raspberries. *Forficula auricularia* Linnaeus (Forficulidae) made up the largest part of the earwigs analysed (137/143) with 114 originating from the cherry cultures. The spiders belonged to 11 different families that were assigned to two different functional groups: web-building spiders (Agelenidae (16), Araneidae (25), Linyphiidae (12) and Theridiidae (20)) and hunting spiders (Clubionidae (2), Lycosidae (3), Philodromidae (20), Pisauridae (1), Salticidae (12), and Thomisidae (24)). Not identified spiders (17, mainly spiderlings) and the only specimen of the family Tetragnathidae were not assigned to either group. Only small numbers of the other predator groups were collected (14 harvestmen, 9 ladybirds and 8 rove beetles). The different field sites and cultures were not analysed separately because of the uneven distribution of the samples among sites and cultures (Online Resource 2). In total, 482 arthropod predators were screened with each primer pair (i.e. specific *D. suzukii* and general *Drosophila* spp.). Lysis of small predators (e.g. juvenile earwigs and predatory bugs) was done in 200 µL lysis buffer (190 µL TES buffer and 10 µL Proteinase K), of large ones (adult earwigs, large spiders) in 400 µL. Otherwise, lysis and DNA extraction followed the same procedure as described above. All samples were tested in singleplex PCR assays with the two primer pairs. Two positive (*D. suzukii* DNA) and two negative controls (molecular water) were included per 96-well plate to check for correct amplification and cross-contamination, respectively. DNA was separated and visualized with QIAxcel, a capillary electrophoresis system enabling objective evaluation of PCR results compared to standard agarose gel. The detection threshold was set to 0.1 RFU (i.e. all amplicons of the respective fragment size and a strength of $\geq$ 0.1 RFU were counted as positive), which is well above potential background noise of the capillaries.

To verify that the PCR products consist of the targeted DNA (*D. suzukii* DNA for the specific primers and *Drosophila* spp. DNA for the general primers), PCR products of 1–5 individuals per predator taxon were sent for sequencing (Eurofins).

Data analysis

The 95% confidence intervals of the mean number of positive samples were obtained for each predator group and primer pair, separately, using the adjusted bootstrap percentile (BCa) method (package BOOT: Canty and Ripley 2016) with R version 3.3.3 (R Core Team 2017). Non-overlapping confidence intervals served as a conservative proxy for significant differences (Payton et al. 2003).

Results

Primers and assay validation

The species-specific primer pair Dro-suz-S390 and Dro-suz-A380 is targeting *D. suzukii* DNA and results in an amplicon

of 171-bp length (Table 1). The *Drosophila* spp. primers, Droso-S391 and Droso-A381, are targeting several *Drosophila* species and generate an amplicon of 240-bp length. PCR conditions were identical for both primer pairs: each 10 µL reaction included 2 µL of DNA extract, 1x Multiplex PCR Master Mix (Qiagen), 0.5 µM each primer, 5 µg BSA and PCR grade water. Thermocycling was 15 min at 95 °C, 35 cycles with 30 s at 94 °C, 90 s at 62 °C and 60 s at 72 °C, and the final elongation for 10 min at 72 °C.

The primers for *D. suzukii* were highly specific and did not show DNA amplification in the tested non-target fly and predator taxa, except in one species of Heleomyzidae (unidentified species collected at an altitude of 2400 m, Sint et al. 2014). The *Drosophila* spp. primers amplified DNA of all tested *Drosophila* species (including *D. suzukii*), but also of *Cacoxenus indagator* Loew and *Scaptomyza* sp. (Diptera: Drosophilidae), of *Geomyza apicalis* (Meigen) (Diptera: Opomyzidae), and one winter crane fly (Diptera: Trichoceridae). However, the amplification of the latter remained with 0.08 RFU below the threshold for positive detection. Details of the specificity tests are shown in Online Resource 3.

Assay sensitivity is high and very similar for both primer pairs. DNA amplification was achieved starting from 75 double-stranded templates per reaction (*D. suzukii* primers: 0.08 RFU, *Drosophila* spp. primers: 0.1 RFU) and as little as 0.02 pg total DNA was sufficient for DNA amplification with the species- and group-specific primer pairs (0.27 RFU and 0.11 RFU, respectively).

The sequences generated from the PCR products obtained by testing field-collected predators were compared to GenBank entries using BLAST to verify amplification of target DNA. All 25 sequences from DNA fragments amplified with the specific *D. suzukii* primers matched 100% to *D. suzukii*-GenBank entries. All matches for the *Drosophila* spp. primers consisted of *Drosophila* sequences with a similarity of 98%–100%, except for two sequences that matched *Thaumatomyia notata* (Meigen) (Diptera: Chloropidae) with 99%–100% similarity.

Screening of field-collected predators

Drosophila suzukii and *Drosophila* spp. DNA were mainly amplified from three taxa of field-collected predators (Fig. 1). In earwigs, DNA of *D. suzukii* was amplified from 43.4% and *Drosophila* spp. from 44.1%. While 48.7% of the tested individuals from the highly infested cherry culture had consumed *D. suzukii*, it was only 21.4% for the other field sites. In bugs, 15.5 and 8.4% and in spiders 14.4 and 26.8% of the individuals, were positive for *D. suzukii* and *Drosophila* spp., respectively. In all three predator taxa, some individuals were found that tested only positive for DNA of *D. suzukii* but not *Drosophila* spp., however, only in bugs this was a relevant proportion of the detections. Furthermore, three out of eight rove beetles tested positive for *D. suzukii* DNA and two of these also for *Drosophila* spp. DNA. No *Drosophila* DNA was amplified from the nine ladybirds and 14 harvestmen examined.

Taking the functional groups of the spiders into account (web-building vs. hunting), most of *D. suzukii* DNA detections originated from web-building spiders, of which 20.5% tested positive for DNA of *D. suzukii* and 35.6% for DNA of *Drosophila* spp. (Figure 2). In contrast, only 8.1 and 16.1% of the hunting spiders tested positive for *D. suzukii* and *Drosophila* spp. DNA, respectively.

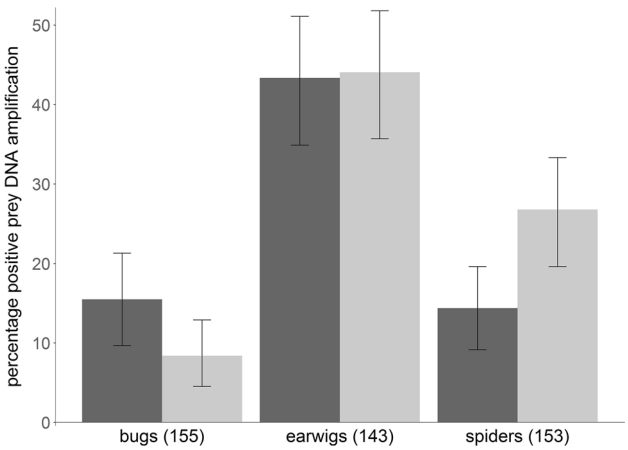

Fig. 1 Percentage of field-collected predators (bugs, earwigs and spiders) from which DNA of *D. suzukii* (dark bars) and *Drosophila* spp. (light bars) was amplified. Numbers in parenthesis represent the number of individuals tested. 95% BCa confidence intervals were obtained from 10,000 bootstrap replicates [R, boot.ci(type="bca")]

Table 1 Primer sequences and resulting amplicon sizes

Target genes	Primer	Primer sequence (5´-3´)	Size (bp)
Drosophila suzukii (COI)	Dro-suz-S390	TTGAACTGTTTACCCACCTCTT	171
	Dro-suz-A380	GGTATTCGGTCTAATGTAATACCC	
Drosophila spp. (18s)	Droso-S391	AAATAACAATACAGGACTCATATCc	240
	Droso-A381	gTAATACGCTTACATACATaAAGGTATA	

S denotes the forward and A the reverse primer. Lower-case letters in the group primer sequences indicate modifications of the original primers (Sint et al. 2014)

Discussion

The developed assays are easy to apply, highly suitable to detect *D. suzukii* predation, and revealed four taxa of generalist predators that had consumed *D. suzukii* in the field. Our findings show that earwigs, spiders and predatory bugs feed regularly on *D. suzukii* and should therefore be considered when developing control approaches, both as potential biological control agents and as non-target organisms possibly affected.

The specificity of the *D. suzukii* primer pair was tested against 24 *Drosophila* species that occur in Switzerland as well as against one species which is not present in Switzerland but which is closely related to *D. suzukii* (*D. biarmipes* Malloch). None of these species were amplified by the *D. suzukii* primers. *Drosophila subpulchrella* Takamori and Watabe (a species not tested) shows a high sequence similarity to *D. suzukii* in the amplified COI fragment with only one mismatch at the 3'-end of the reverse primer and it thus cannot be ruled out that this species will be amplified, at least to some extent, with the *D. suzukii*-specific primer pair. While *D. subpulchrella* is not present in Europe, it needs to be considered in South East Asia where it occurs together with *D. suzukii*. A species of Heleomyzidae that was collected at an altitude of 2400 m (Sint et al. 2014) was amplified with the *D. suzukii* primers and has a high sequence similarity to *D. suzukii* in the primer regions. Heleomyzidae is a small fly family with a mainly boreo-alpine distribution (Soszyńska-Maj and Woźnica 2016). Many species are cold-adapted, live in caves, burrows of mammals or nests of birds and their larvae feed on fungi, dung, decaying plant and animal matter (Lo Guidice and Woźnica 2013). It is therefore highly unlikely to find Heleomyzidae at the same time and place as *D. suzukii*. In this study, all the sequences from gut contents amplified with the *D. suzukii* primers matched 100% to *D. suzukii*. The *Drosophila* spp. primer pair amplified the DNA of all tested *Drosophila* species, of few other species from the family Drosophilidae and of one Opomyzidae species. The opomyzid fly was a single individual net-caught below apple trees. Opomyzidae are associated with grasslands and cereal fields (van Zuilen and Roháček 2009) and do not share the habitat with *D. suzukii*. Two sequences from gut contents amplified with the *Drosophila* spp. primers had the closest match to a single sequence of *T. notata* (Chloropidae). However, from this genus only this single 18s rDNA sequence is available in GenBank and when blasted its closest matches are *Drosophila* and *Delia* (Diptera: Anthomyiidae) sequences. Neither Chloropidae nor Anthomyiidae were amplified with the *Drosophila* spp. primers in the non-target test, so that also the possibility of misidentification of the available *T. notata* sequence cannot be ruled out. All things considered, it is unlikely that in this study non-target DNA was amplified to a larger extent by the two primer pairs.

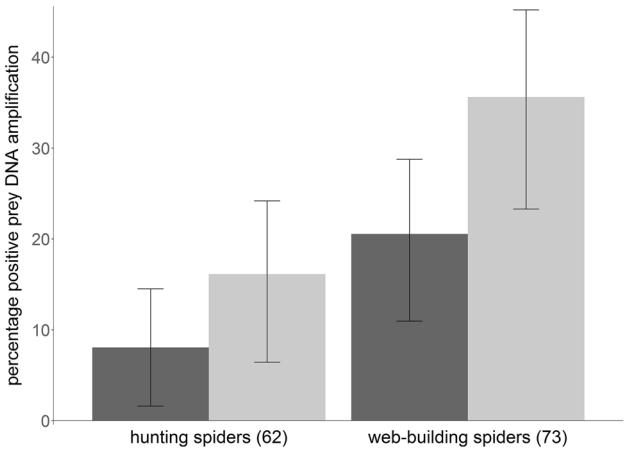

Fig. 2 Percentage of hunting and web-building spiders from which DNA of *D. suzukii* (dark bars) and *Drosophila* spp. (light bars) was amplified. Numbers in parenthesis represent the number of individuals tested. 95% BCa confidence intervals were obtained from 10,000 bootstrap replicates [R, boot.ci(type="bca")]

In this study, both primer pairs were run separately in singleplex PCRs, although at the same PCR conditions. Thus, as the amplified DNA fragments differ 69 bp in length, they could be combined in a duplex assay, especially as relatively little interference between the primers is expected as they target different genes (mitochondrial COI vs nuclear 18s rDNA). However, multiplex reactions are in general more complex compared to singleplex reactions, so that the PCR would have to be optimized regarding PCR conditions (e.g. primer concentrations, annealing time and additives) to obtain comparable results as with singleplex reactions.

There are existing molecular assays to identify DNA of *D. suzukii*. Dhami and Kumarasinghe (2014) developed a real-time PCR assay that can differentiate between *D. suzukii* and the closely related *D. subpulchrella* if coupled with high-resolution melt analysis. But the necessity for specific PCR systems and dyes increases expenses and effort. Another assay is based on standard PCRs combined with restriction fragment length polymorphism analysis, hence adding an extra analytical step (Kim et al. 2014). The qPCR-based assay by Renkema et al. (2015) was developed for gut analysis of predators, but besides depending on qPCR cyclers, it also amplified DNA of *Drosophila immigrans* Sturtevant, a vinegar fly that is distributed worldwide, very common in Switzerland and could thus interfere with the specific detection of *D. suzukii*. This assay also amplified DNA of *D. nepalensis* Okada, a species occurring in Asia, where it would have to be considered when applying the assay. The primers designed by Murphy et al. (2015) target single-copy genes and might therefore lack the necessary sensitivity for

gut content analysis, where prey DNA is usually degraded and semi-digested (King et al. 2008). Compared to the existing systems, our assay is simple and able to detect minute amounts of low-quality DNA. It is cost-effective and thus suitable for high-throughput gut content analysis.

To validate the applicability of the primer pairs developed in this study, common predator taxa were collected in habitats where *D. suzukii* was found to be present. The highest detection rates of *D. suzukii* DNA were found in earwigs where nearly half of them had been feeding on the spotted-wing drosophila, most of which were collected in a highly *D. suzukii*-infested cherry culture. *Drosophila* spp. also served as prey since 25% of earwigs only tested positive for *Drosophila* DNA, but not *D. suzukii* DNA. Although earwigs are omnivorous feeding on a large variety of plant- and insect-derived foods, their predatory potential is increasingly being recognized. While in citrus orchards their pest status is still discussed, their beneficial impact on aphid reduction was demonstrated (Romeu-Dalmau et al. 2012a, b). Earwigs have also been identified as important predators of lepidopterans through video and gut content analysis (Frank et al. 2007; Unruh et al. 2016), and reported to feed on woolly aphids and psyllids (Nicholas et al. 2005; Höhn et al. 2007).

Detection frequency in spiders was higher with the general *Drosophila* spp. primers than with the specific *D. suzukii* primers. This indicates that these predators also consumed *D. suzukii*, but additionally fed on other *Drosophila* species. Twice as many detections resulted from web-building spiders compared to the hunting spiders. This is likely due to the fact that adult flies get caught in webs. As web-building spiders do not necessarily consume all prey items caught in their webs, they can induce mortality to more flies than they actually feed on. Earlier findings showed that Diptera in general are a more important food source for web-building spiders compared to hunting spiders (Nyffeler 1999; Michalko and Pekar 2016).

Predatory bugs showed similar detection rates for *D. suzukii* DNA to the spiders. However, the detection rate appeared to be higher for *D. suzukii* than for *Drosophila* spp., which seems contradictory at first sight. As the sensitivity of both primer pairs was comparable, this might be explained by the different target regions of the primer pairs. The *D. suzukii* primers target the COI region in mitochondrial DNA, while the general primers bind to the nuclear 18s rDNA. In cells of adult *Drosophila* individuals, the copy number of the two target genes seems to be comparable, as the two assays showed a similar sensitivity for both the number of template molecules and the amount of total DNA needed for successful amplification. By contrast, eggs are known to have a much higher density of mitochondria compared to somatic cells and a single *Drosophila* egg contains up to 15 Mio copies of mtDNA (Tourmente et al. 1990), but only one nucleus with a few hundred copies of 18s rDNA (Long and Dawid 1980). Since most of the predatory bugs were collected in berries with no obvious *D. suzukii* infestation (personal observation), their prey probably often consisted of *D. suzukii* eggs, resulting in an excess of *D. suzukii* mitochondrial DNA in the guts and therefore in an increased detection probability for the *D. suzukii*-specific assay compared to the *Drosophila* spp. primers targeting nuclear DNA. This explanation is supported by a laboratory feeding trial, where bugs of the species *O. laevigatus* fed on eggs of *D. suzukii* within strawberries and significantly increased egg mortality compared to the control treatment (Gabarra et al. 2015).

Beyond the predators tested in the present study other taxa are worth considering. In this study, three out of eight rove beetles tested positive for *D. suzukii*, and in laboratory trials rove beetles fed on different larval stages of the fly (Cuthbertson et al. 2014; Renkema et al. 2015). Carabid beetles are successful predators of fruit fly pupae in citrus orchards (Urbaneja et al. 2006; Monzó et al. 2011) and might prey on *D. suzukii* pupae as well. Ground-dwelling predators actually deserve special attention, since the majority of *D. suzukii* pupate on the ground and in the soil (Woltz and Lee 2017). Furthermore, larvae of hoverflies (Diptera: Syrphidae), ladybirds and lacewings (Neuroptera) are successful predators of small and soft-bodied prey (e.g. Moser et al. 2011; Tschumi et al. 2016) and could thus putatively also feed on *D. suzukii*. The here included testing of field-collected predators served only as proof-of-concept study to show the general applicability of the developed assays and to gain some first insight into the potential predator community of *D. suzukii*. Thus, future laboratory and field studies need to be conducted to assess the predator complex attacking *D. suzukii* in detail, taking into consideration the species' feeding modes (e.g. chewers and fluid feeders), feeding habits (e.g. predation, scavenging, secondary predation and consumption frequency) and digestion rates, all of which can influence the time span during which prey DNA stays detectable post-consumption (Harwood et al. 2001; Greenstone et al. 2014; Mansfield and Hagler 2016). In addition, the impact of the predator community on *D. suzukii* populations in the field needs to be elucidated.

The assays presented here offer the opportunity to quickly and inexpensively screen the communities of potential generalist predators of *D. suzukii* in different habitats. Although the assays do not quantify the feeding on *D. suzukii*, they can serve as a tool to reveal the *D. suzukii* consumption frequency within a predator community. This way, the importance of predators as natural enemies of *D. suzukii* can be estimated. Identifying natural enemies of this pest is an important step towards better understanding how *D. suzukii* is utilized as prey in newly invaded areas and thus towards efficient control measures for this pest in orchards and semi-natural habitats.

Author contributions

JC, MT and JR conceived and designed research. SW conducted the field survey. SW and CZ conducted the laboratory work. DS advised the laboratory work. All authors contributed to data analysis and interpretation. SW wrote the manuscript with supplementary input from all authors. All authors approved the final version of the manuscript

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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