SUSCEPTIBILITY OF WINE GRAPES TO *DROSOPHILA SUZUKII* - A THREE YEAR FIELD AND LABORATORY STUDY IN AUSTRIA

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The infestation dynamics of *Drosophila suzukii* in vineyards and the vulnerability of a wide range of grapevine varieties to this pest in Austria were studied in field and laboratory studies. Oviposition of *D. suzukii* in the field was investigated on 19 vine varieties at two locations from calendar weeks 33 to 42 in the years 2015 to 2017. The field studies were complemented by laboratory no-choice and free-choice oviposition experiments. In the field in 2015 virtually no oviposition was recorded, in 2016 and 2017 oviposition was detected from calender week 36 and 37 onwards. Significant differences between varieties in regard to oviposition were noticed, 'Blauer Portugieser', 'Frühroter Veltliner' and 'St. Laurent' were the most sensitive ones and their vulnerability increased with progressed ripening. Medium susceptibility was recorded for 'Zweigelt' ('Rotburger') and 'Roesler'. No or almost no eggs were recorded on berries of 'Cabernet Sauvignon', 'Pinot Gris', 'Roter Veltliner', 'Blaufränkisch', 'Blauer Wildbacher', 'Pinot noir', 'Syrah', 'Cabernet franc', 'Chardonnay', 'Grüner Veltliner' and 'Welschriesling'. In laboratory no-choice experiments with visually undamaged berries the flies penetrated the skins of all 12 varieties in the test already from calendar week 33 and 34. Selection behavior in laboratory free-choice experiments confirmed the field results as the flies laid significantly more eggs on 'Frühroter Veltliner', 'St. Laurent', 'Blauer Portugieser' and sometimes also on 'Zweigelt' ('Rotburger'). All in all the data indicate that in cooler wine producing areas such as Austria and the neighboring countries *D. suzukii* poses a threat to vulnerable varieties in unfavorable years.
Keywords: Spotted wing drosophila, pest control, field trial, laboratory study

Anfälligkeit von Weinreben gegenüber Kirschessigfliege (*Drosophila suzukii*) Feld- und Laborstudien über drei Jahre in Österreich. Die Befallsdynamik der Kirschessigfliege und die Anfälligkeit verschiedener Rebsorten gegen dieses Schadinsekt in Österreich wurde im Rahmen von Feld- und Laborstudien untersucht. Eiablagen wurden in den Jahren 2015 bis 2017 an insgesamt 19 Rebsorten in zwei Weingärten von Kalenderwoche 33 bis 42 erhoben. Diese Feldstudien wurden durch Free-choice- und No-choice-Experimente im Labor ergänzt. Im Freiland wurden 2015 so gut wie keine Eiablagen festgestellt, 2016 und 2017 traten Eier ab den Kalenderwochen 36 und 37 auf. Es gab signifikante Unterschiede im Hinblick auf die Anfälligkeit verschiedener Sorten. Die Sorten 'Blauer Portugieser', 'Frühroter Veltliner' and 'St. Laurent' waren am anfälligsten, danach folgten 'Zweigelt' ('Rotburger') und 'Roesler'. Keine oder kaum Eiablagen wurden an Beeren der Sorten 'Cabernet Sauvignon', 'Grauer Burgunder', 'Roter Veltliner', 'Blaufränkisch', 'Blauer Wildbacher', 'Blauer Burgunder', 'Syrah', 'Cabernet franc', 'Chardonnay', 'Grüner Veltliner' und 'Welschriesling' beobachtet. In No-choice-Versuchen war die Kirschessigfliege in der Lage, bereits ab den Kalenderwochen 33 und 34 in unbeschädigte Beeren aller zwölf untersuchten Sorten Eier abzulegen. Das Auswahlverhalten in Free-choice-Versuchen bestätigte die Freilandergebnisse, die Fliegen legten in Beeren der Sorten 'Frühroter Veltliner', 'St. Laurent', 'Blauer Portugieser' und manchmal 'Zweigelt' ('Rotburger') am meisten Eier. Insgesamt zeigte die Studie, dass die Kirschessigfliege in kühleren Weinbaugebieten wie Österreich und seinen Nachbarländern in ungünstigen Jahren für anfällige Sorten eine Gefährdung darstellt.
Schlagwörter: Kirschessigfliege, Schädlingsbekämpfung, Feldversuch, Laborstudie

Drosophila suzukii Matsamura (Diptera: Drosophilidae) is a polyphagous commercially relevant pest of thin-skinned fruits such as berries, stone fruits and grapevines. Initial reports about the fly originate from Japan where it was observed as early as 1916 (Kanzawa, 1935 and 1939). Over the last 10 to 15 years the distribution area of the insect has dramatically expanded and the fly is now present in large parts of North America and Europa and hence also in Austria (Hauser, 2011; Cini et al., 2012, for review).

D. suzukii females possess a strong, serrated ovipositor enabling them to slit the skin of sound, unwounded fruit and lay their eggs thereunder. The larvae developing from these eggs feed on the flesh beneath the skin and cause soft, sunken areas on the fruit and extensive fruit decay. Infestation of fruit by *D.suzukii* can entail large secondary damage due to other pests e. g. other vinegar fly species, fungi and bacteria. The tremendous reproductive potential of the fly is of particular concern. Up to 10 to 15 generations per year have been observed and on average each individual female lays 380 eggs. Under favorable climatic conditions very large populations can build up in a short time (Walsh et al., 2011). Attractiveness for the flies, susceptibility for oviposition and suitability for larval development vary widely between host species, varieties and ripening stages (Burrack et al., 2013). The probability of oviposition on a fruit increases, when skin penetration force decreases and pH increases during ripening (Lee et al,. 2016). Apart from crop species a wide range of wild plants and cultivated ornamentals serve as hosts for the flies, which is an essential consideration for appropriate management strategies (Kenis et al., 2016). Tiefenbrunner et al. (2017) observed a higher abundance of *D. suzukii* adults at forest margins as compared to vineyards. Wild host plants in the woods could play a role for overwintering (Pelton et al., 2016), for example fly reproduction in mistletoe berries in winter and spring has already been proven (Briem et al., 2016).

In case of grapevines (wine and table grapes) observations of Saguez et al. (2013) and Van Timmeren and Isaacs (2014) suggested that the fly is able to complete its life cycle on this host species. Lee at al. (2011) reported that in comparison to other small fruits few eggs were laid on wine grapes and that only a low percentage developed into adults. Ioriatti et al. (2015) investigated the potential of *D. suzukii* to impact wine grapes and found that the risk of oviposition increased during ripening with increased sugar content and decreased levels of acidity, penetration force and, in coincidence with the latter, skin hardness. Skin hardness was identified as a critical component of *D. suzukii* host selection between grape varieties. The authors concluded that although grapes are in most cases not an ideal host for *D.suzukii* development the species poses a serious risk to grape and wine production. In accordance with this finding Entling et al. (2018) identified berry skin resistance as key factor for oviposition preferences between varieties and found varieties such as 'Dornfelder', 'Trollinger' ('Schiava', 'Vernatsch'), 'Portugieser', 'Roter Elbling' and 'Cabernet Dorsa' particularly vulnerable.

In Swiss laboratory tests oviposition was highest on the red and thin-skinned wine grape varieties ('Bondoletta' and 'Gamay') but the development rate did not exceed 9 % of the deposited eggs. The authors therefore inferred that grapes can be infested by *D. suzukii* but the crop is not suitable for the build-up of large pest populations (Linder et al., 2014). In South Tyrol (Northern Italy) in 2011 and in 2014 the wine grape varieties 'Vernatsch' ('Schiava', 'Trollinger') and 'Lagrein' were significantly affected by the pest resulting in large economic losses (Sinn, 2015). Weissinger et al. (2019) observed that the flies prefer red varieties over white ones.

Ioratti et al. (2018) pointed out that the presence of *D. suzukii* but particularly the oviposition of *D. suzukii* exponentially increased the concentration of acetic acid bacteria on undamaged and especially on artificially incised berries. Volatiles produced by spoilage and sour rot microorganisms on infested berries could initiate a decay process by attracting further *D. suzukii* and also other Drosophilids initiating additional spoilage. Moreover, the authors emphasized the risk that accumulation of acetic acid due to *D. suzukii* infestation could cause significant alterations of grape berries, rendering them unsuitable for wine making. Similar observations were made by Rombaut et al. (2017). Their study indicated that grape infestation by *D. suzukii* facilitated *D. melanogaster* reproduction by making the berries accessible to

D. melanogaster oviposition and thus promoted sour rot outbreaks.
Control of *D. suzukii* is based on several strategies. Cultural practices reducing air moisture and removal of any infested, ripe, overripe and rotten fruit aim to keep fly development low during the ripening and harvest period. Insecticides from several classes have proven effective and several compounds are homologized for control. Appropriate and sustainable chemical control, however, must consider infestation risks and times. These are influenced by factors such as fruit coloration, sugar levels, pH, and firmness (Walsh et al., 2011; Lee et al., 2016). This applies in particular to vineyard management and requires knowledge on the susceptibility of grapevine varieties to the fly (Jarausch et al., 2017). Thus, the aim of the current study was to enlarge knowledge on infestation dynamics of *Drosophila suzukii* in Austrian vineyards and on vulnerability of a wide range of international and traditional Austrian grapevine varieties to this pest.

MATERIAL AND METHODS

ABBREVIATIONS OF VARIETIES

BB	'Blauer (Spät-)Burgunder', 'Pinot noir'
BF	'Blaufränkisch', 'Lemberger'
BL	'Blauburger'
BP	'Blauer Portugieser'
BW	'Blauer Wildbacher'
CF	'Cabernet Franc'
CH	'Chardonnay'
CS	'Cabernet Sauvignon'
FV	'Frühroter Veltliner', 'Früher Roter Malvasier'
GR	'Grauer Burgunder', 'Pinot Gris', 'Ruländer'
GV	'Grüner Veltliner'
ME	'Merlot'
RL	'Roesler'
RV	'Roter Veltliner'
SB	'Sauvignon Blanc'
SH	'Syrah'
SL	'St. Laurent', 'Saint Laurent'
WR	'Welschriesling'
ZW	'Zweigelt', 'Rotburger'

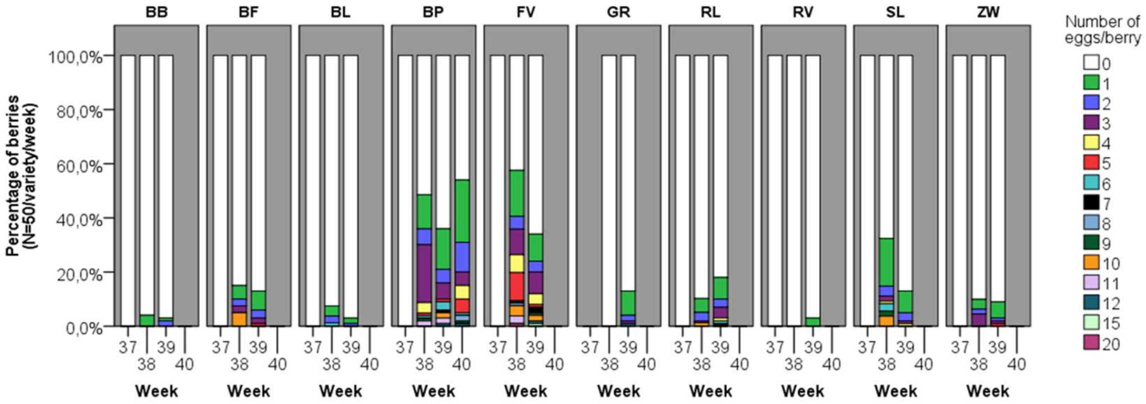
EXPERIMENTAL VINEYARDS AND COLLECTION OF SAMPLES

The field observations were carried out in two experimental vineyards (Federal College and Research Institute for Viticulture and Pomology Klosterneuburg) in Klosterneuburg (KL) and Langenzersdorf (LE) (Lower Austria) from 2015 to 2017. Both vineyards are maintained according to integrated pest management strategies.
Presence of adult flies in the vineyards was monitored by bait traps made of 1.5 l transparent plastic bottles with 20 holes (diameter 3 mm) containing 200 ml of liquid bait ("droskidrink"; Grassi et al., 2015) hung in the vine canopy for one week each (10 traps per location and week).
The vineyards in KL and LE are experimental vineyards in which a relatively high number of varieties is cultivated in small plots in close distance leading to comparable infestation pressure over all varieties. Grape berries were sampled weekly from calendar week 33 (2015 and 2016) or 34 (2017) until calendar week 42, in case of early ripening varieties in 2016 only until calendar week 40. Each week one sample per cultivar and vineyard (KL and LE) was collected. Each sample comprised randomly taken parts of ten to fifteen randomly selected bunches (in total around 150 berries) from one plot (of 10 to 20 vines). These berry samples were used both for determination of oviposition in the field and for laboratory tests. All year round in both experimental vineyards meteorological data were recorded daily by means of a weather station (Adcon, Klosterneuburg, Austria).

OVIPOSITION IN THE FIELD

Field infestation was monitored by means of a dissecting microscope on 50 berries per sample (2 to 5 berries per part of a bunch randomly cut off). Numbers of eggs per berry (based on spiracles protruding from the berry surface) were recorded. In 2015 125 samples (50 berries each) from 12 varieties per location (in LE: BB, BF, BL, BP, BW, FV, GR, RL, RV, SL, TR. ZW from calendar week 33 to 42; in KL: BP, BW, CH, SL, FV, GR, GV, RL, RV, SL, TR, ZW from calendar week 33 to 42) were analyzed. Samples analyzed in 2016 and 2017 (varieties per calendar week per location) are presented in Figures 1 to 4.

A

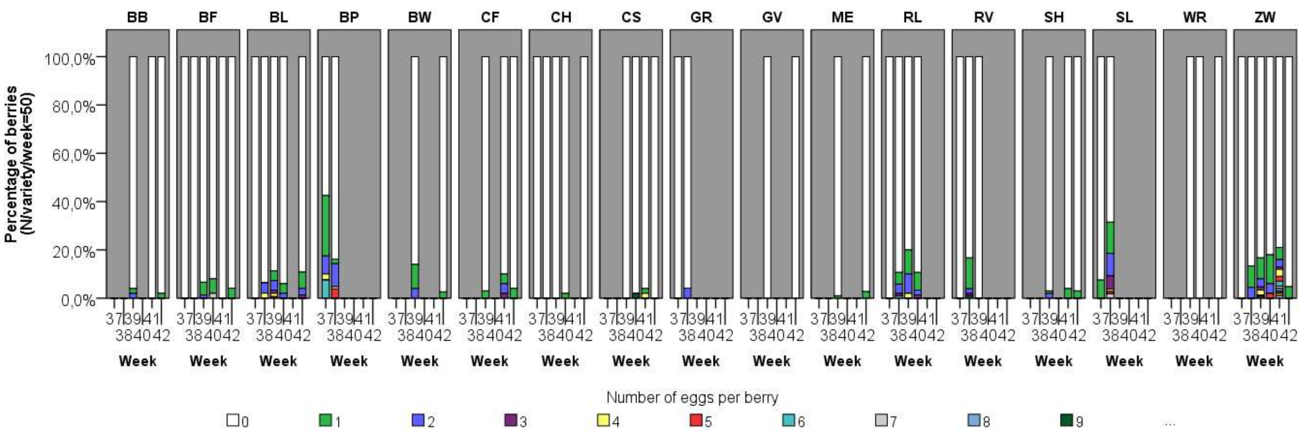


B

Calendar week	Kruskal Wallis Test			Analyzed varieties; result of post hoc analysis, significant differences between varieties indicated by different letters (p≤0.05).
	DF	H	Sign.	
37			n.s.	
38	9	197,8	***	GR^{a} ,RV^{a}, BB^{a}, BL^{a}, ZW^{a}, RL^{a}, BF^{ab}, SL^{bc}, BP^{cd}, FV^{cd}
39	9	110,2	***	GR^{a} ,RV^{a}, BB^{a}, BL^{a}, ZW^{a}, RL^{a}, BF^{a}, SL^{a}, BP^{b}, FV^{b}

Fig. 1: Infestation of berries in the field, Langenzersdorf, 2016 (calendar weeks 37 to 39, variety BP weeks 37 to 40); A: Percentages of infested berries (N = 50/calendar week/variety) and numbers of eggs per berry; B: Result of corresponding Kruskal Wallis analysis

A

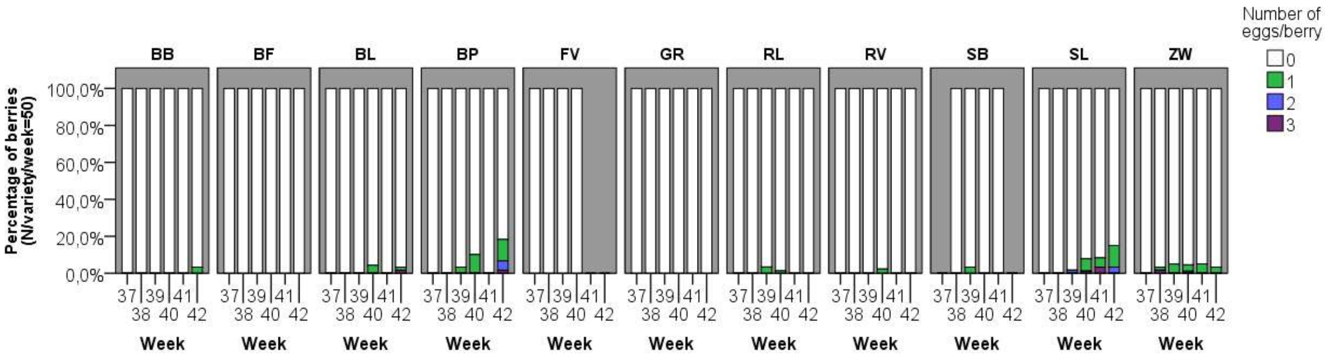


B

Calendar week	Kruskal Wallis Test			Analyzed varieties; result of post hoc analysis, significant differences between varieties indicated by different letters (p≤0.05) .
	DF	H	Sign.	
37	8	121.16	***	BF^{a}, BL^{a}, CH^{a}, GR^{a}, RL^{a}, RV^{a}, ZW^{a}, SL^{a}, BP^{b}
38	8	49.96	***	BF^{a}, CH^{a}, GR^{a}, BL^{a}, RL^{a}, ZW^{a}, RV^{a}, BP^{ab}, SL^{b}
39	13	83.64	***	RV^{a}, WR^{a}, CH^{a}, CS^{a}, GV^{a}, ME^{ab}, CF^{ab}, SH^{ab}, BB^{a}, BF^{ab}, BL^{abc} BW^{abc}, RL^{bc}, ZW^{c}
40	6	18.40	**	WR^{a}, CH^{ab}, CS^{ab}, BL^{ab}, BF^{ab}, RL^{ab}, ZW^{b}
41	5	41.50	***	BB^{a}, BF^{a}, CF^{a}, SH^{a}, CS^{a}, ZW^{b}
42	8	17.29	*	WR^{a}, CH^{a}, CS^{a}, GV^{a}, BB^{ab}, BW^{ab}, ME^{ab}, SH^{ab}, CF^{ab}, BF^{ab}, ZW^{ab}, BL^{b}

Fig. 2: Infestation of berries in the field, Klosterneuburg, 2016 (calendar weeks 37 to 42); A: Percentages of infested berries (N = 50/calendar week/variety) and numbers of eggs per berry; B: Result of corresponding Kruskal Wallis analysis

A

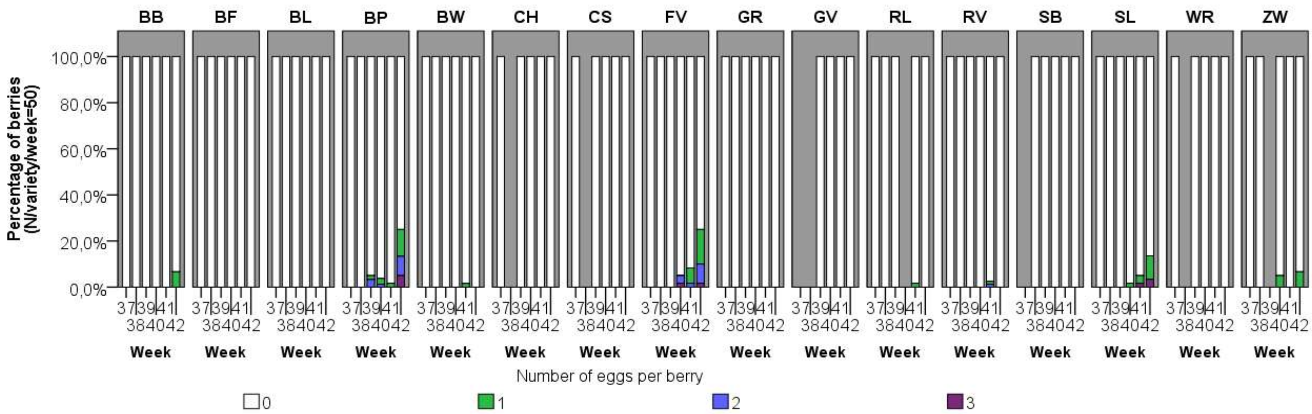


B

Calendar week	Kruskal Wallis Test			Analyzed varieties; result of post hoc analysis, significant differences between varieties indicated by different letters (p≤0.05)
	DF	H	Sign.	
37			n.s.	
38	10	20,43	*	BB^{a}, BF^{a}, BL^{a}, BP^{a}, FV^{a}, GR^{a}, RL^{a}, RV^{a}, SB^{a}, SL^{a}, ZW^{b}
39			n.s.	
40	10	40,50	***	BB^{a}, BF^{a}, GR^{a}, RL^{a}, SB^{a}, FV^{ab}, RL^{ab}, RV^{ab}, BL^{ab}, ZW^{ab}, BP^{b}
41	9	30,87	***	BB^{a}, BF^{a}, BL^{a}, BP^{a}, GR^{a}, SB^{a}, RL^{a}, RV^{a}, ZW^{a}, SL^{b}
42	9	50,56	***	BF^{a}, GR^{a}, RL^{a}, RV^{a}, BB^{ab}, ZW^{ab}, BL^{ab},SL^{b}, BP^{c}

Fig. 3: Infestation of berries in the field, Langenzersdorf, 2017 (calendar weeks 37 to 42); A: Percentages of infested berries (N = 50/calendar week/variety) and numbers of eggs per berry; B: Result of corresponding Kruskal Wallis analysis

A



B

Calendar week	Kruskal Wallis Test			Analyzed varieties; result of post hoc analysis, significant differences between varieties indicated by different letters (p≤0.05) .
	DF	H	Sign.	
37			n.s.	
38			n.s.	
39	14	41.10	***	BB^{a}, BF^{a}, RL^{a}, RV^{a}, SB^{a}, SL^{a}, WRa, BL^{a}, BW^{a}, CH^{a}, CS^{a}, FV^{a}, GR^{a}, GV^{a}, RL^{a}, RV^{a}, BP^{b}
40	14	29.90	**	No significant differences in post hoc analysis
41	15	37.65	**	BB^{a}, BF^{a}, SB^{a}, WR^{a},ZW^{a}, BL^{a}, CH^{a}, CS^{a}, GR^{a}, GVa, BW^{ab}, RL^{ab}, BP^{ab}, RV^{ab}, SL^{ab}, FV^{b}
42	15	152.67	***	BF^{a}, RL^{a}, RV^{a}, SB^{a},WR^{a}, BL^{a}, BW^{a} CH^{a}, CS^{a}, GR^{a}, GVa, BB^{a}, ZW^{a}, SL^{ab}, BP^{b}, FV^{b}

Fig. 4: Infestation of berries in the field, Klosterneuburg, 2017 (calendar weeks 37 to 42); A: Percentages of infested berries (N = 50/calendar week/variety) and numbers of eggs per berry; B: Result of corresponding Kruskal Wallis analysis

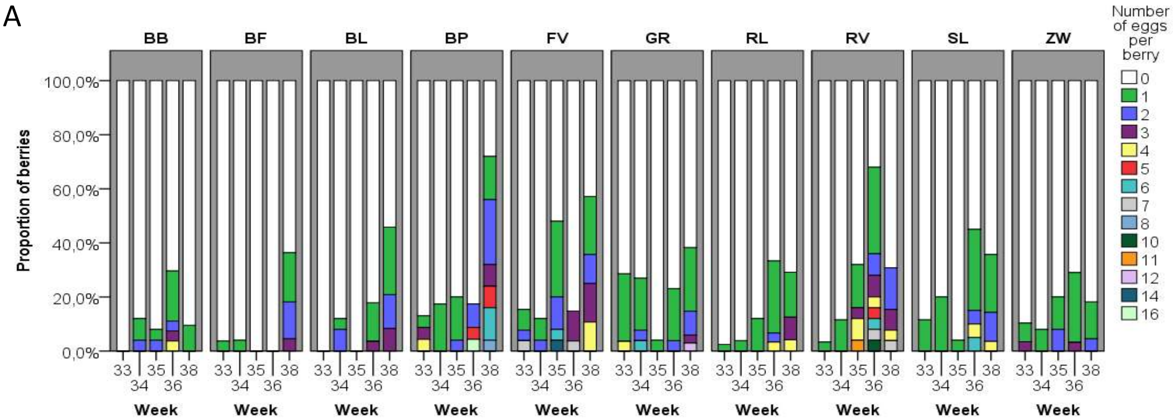
LABORATORY TESTS

Randomly selected non-infested (microscopically determined as described above) and visually undamaged berries with pedicel were used for subsequent laboratory analyses. *D. suzukii* used in the laboratory study were offspring of insect populations naturally present in the experimental orchard at Federal College and Research Institute for Viticulture and Pomology Klosterneuburg. The flies were raised in our laboratory in closed glass containers with moist filter paper on the cage floor (replaced regularly) at 23 ± 2 °C under long-day (L16:D8) conditions. Artificial cornmeal diet served as food and as medium for egg laying (Belutti et al., 2018). Adults used for the experiments were 1 to 3 weeks old.

NO-CHOICE ASSAYS

The tests were carried out in transparent plastic boxes (15 × 15 × 12 cm). The bottom of each box was covered with wet filter paper. Per test 25 to 30 berries (depending on berry size) of the same variety were placed on the bottom of a 10 cm petri dish and inserted into a box. 26 *D. suzukii* adults (13 males, 13 females) were introduced into each box. A cotton ball soaked with 5 % (w/v) sucrose solution was also included. The boxes were closed tightly and kept at 23 ± 2 °C and a photoperiod of L16:D8. After 48 h the flies were removed and eggs on the berries were counted under the dissecting microscope. In 2016 and in 2017 per calendar week, vineyard (KL and LE) and variety one experiment was carried out (Fig. 5 and 6).

A

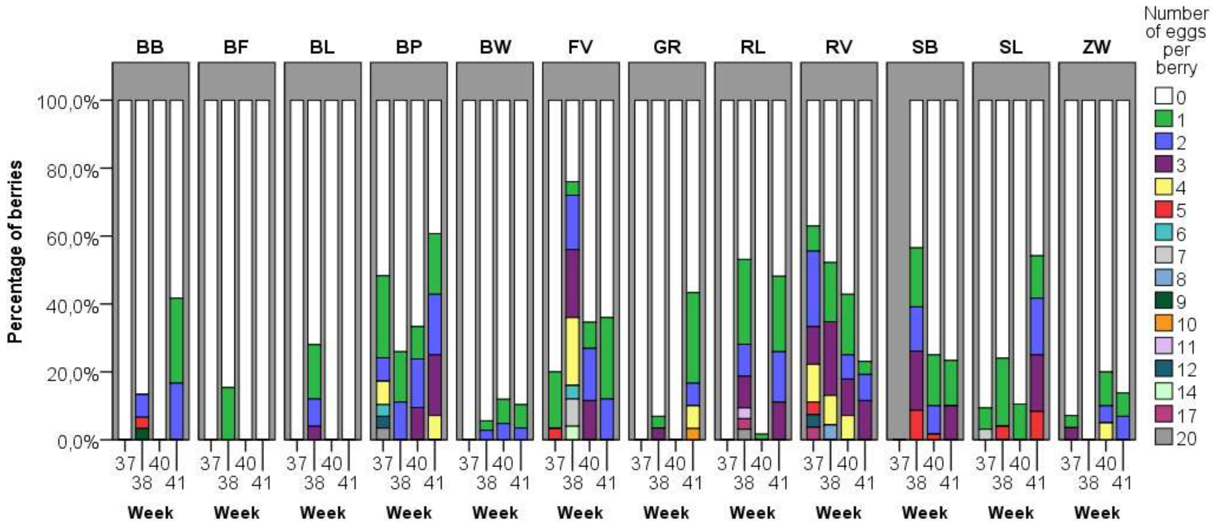


B

Calendar week	Kruskal Wallis Test			Analyzed varieties; result of post hoc analysis, significant differences between varieties indicated by different letters (p≤0.05)
	DF	H	Sign.	
33	9	29.90	**	BB^{a}, BL^{a}, RL^{a}, RV^{a}, BF^{a}, ZW^{ab}, SL^{ab}, BP^{ab}, FV^{ab}, GR^{b}
34			n.s.	
35	9	44.24	***	BF^{a}, BL^{a}, GR^{ab}, SL^{ab}, BB^{ab}, RF^{ab}, BP^{abc}. ZW^{abc}, RV^{bc}, FV^{c}
36	9	39.92	***	BF^{a}, BL^{ab}, FV^{ab}, BP^{ab}, GR^{ab}, ZW^{ab}, BB^{ab}, RL^{abc}, SL^{bc}, RV^{c}
38	9	36.17	***	BB^{a}, ZW^{a}, RL^{ab}, SL^{ab}, BF^{abc}, GR^{ab}, RV^{abc}, BL^{abc}, FV^{bc}, BP^{bc}

Fig. 5: Infestation of berries in no-choice experiments, Langenzersdorf, 2016; A: Percentages of infested berries (N = 25 to 30/calendar week/variety) and numbers of eggs per berry; B: Result of corresponding Kruskal Wallis analysis

A



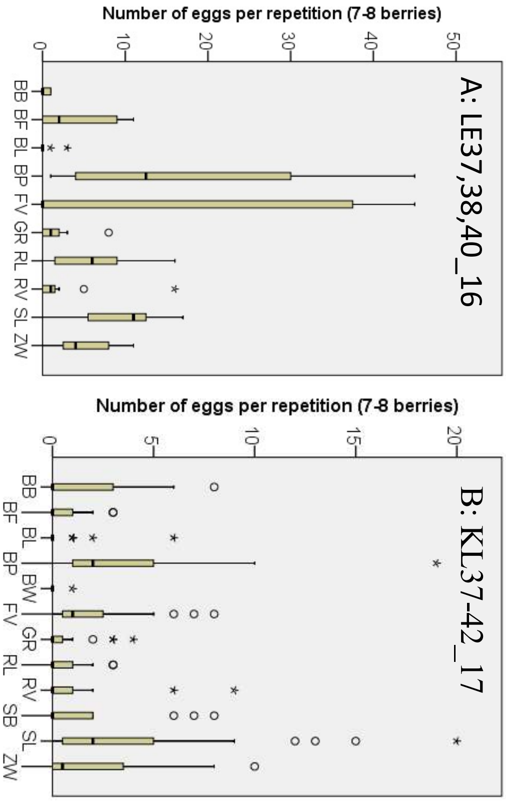
B

Calendar week	Kruskal Wallis Test			Analyzed varieties; result of post hoc analysis, significant differences between varieties indicated by different letters (p≤0.05)
	DF	H	Sign.	
37	10	117.7	***	BB^{a}, BF^{a}, BW^{a}, BP^{a}, RL^{a}, ZW^{a}, SL^{a}, FV^{ab}, BP^{bc}, RV^{c}
38	11	94.79	***	ZW^{a}, BW^{a}, GR^{a}, BF^{ab}, BB^{abc}, SL^{abc}, BP^{abc}, BL^{abc}, RL^{bcd}, RV^{bcd}, SB^{cd}, FV^{d}
40	11	65.27	***	BB^{ab}, BF^{abc}, BL^{abc}, GR^{abc} RL^{a}, SL^{abcde}, BW^{abcd}, ZW^{abcde}, SB^{bcde}, BP^{cde}, FV^{de}, RV^{e}
41	10	64.92	***	BF^{a}, BL^{a}, BW^{ab}, ZW^{ab}, SB^{abc}, RV^{abcd}, FV^{abcd}, BP^{abcd}, GR^{bcd}, RL^{bcd}, SL^{cd}, BP^{d}

Fig. 6: Infestation of berries in no-choice experiments, Klosterneuburg, 2017; A: Percentages of infested berries (N = 25 to 30/calendar week/variety) and numbers of eggs per berry; B: Result of corresponding Kruskal Wallis analysis

FREE-CHOICE ASSAYS

For each test repetition seven to eight berries of each variety were placed on the bottom of a 5 cm petri dish. Petri dishes with all tested varieties (one petri dish per tested variety; 10 varieties for LE, 12 for KL) were simultaneously arranged in plastic containers (23 × 13 × 6 cm). 26 *D. suzukii* adults were inserted into each box and allowed to freely move around and select between the varieties in the arena. Experimental conditions and analysis were the same as for no-choice experiments. The studies were carried out in 2016 and 2017, and per test (calendar week/location/year) 4 individual repetitions were conducted (Fig. 7).



Experiment (Location, calendar week, year)	Kruskal Wallis Test			Analyzed varieties; result of post hoc analysis, significant differences between varieties indicated by different letters (p≤0.05)
	DF	H	Sign	
LE37,38,40_16	9	35.35	***	BL^{a}, BB^{a}, GR^{ab}, RV^{ab}, FV^{ab}, BF^{ab}, FV^{ab}, RL^{ab}, ZW^{ab}, BP^{b}, SL^{b}
KL37-42_17	11	65.14	***	BW^{a}, BL^{a}, GR^{ab}, BF^{ab}, RL^{ab}, RV^{abc}, BB^{abcd}, SB^{abcd}, ZW^{abcd} FV^{bcd}, SL^{cd}, BP^{d}

Fig. 7: Analysis of oviposition data from laboratory free-choice analyses; A: LE 2016 (N = 12; data from calendar weeks 37, 38, 40; 4 independent repetitions per calendar week with 7 to 8 berries each per repetition); B: KL 2017 (N = 24; data from calendar weeks 27 to 42; repetitions as in A); Boxplots show median, box boundaries mark the 25th and 75th percentiles of each distribution. Outliers (values between 1.5 and three times the interquartile range) are identified with an O. Extreme values (more than three times the interquartile range) are marked with a *. Numbers of eggs per repetition were compared by Kruskal Wallis H tests. Significant differences between varieties in post hoc analysis (pairwise comparisons p ≤ 0.05) are indicated by different letters.

STATISTICAL ANALYSIS

All statistical analyses were performed by means of the statistics program SPSS 22.0 (SPSS, Chicago, Illinois, USA).

Both in field examinations and in laboratory studies the majority of berry samples and single berries were free of eggs, thus data distributions skewed and were not normal even after transformation. Analyses of oviposition were therefore carried out by aid of non-parametric tests, namely Kruskal Wallis H tests. In case of significant Kruskal Wallis tests, post-hoc analyses by pairwise comparisons (Mann-Whitney test for each pair of groups using a Bonferroni multiple comparison procedure) were performed as offered by the program at a significance level of $p \leq 0.05$.

Egg numbers recorded for each individual berry were used to analyse the difference between the test years (2015 to 2017). The susceptibility of varieties both in field and in no-choice laboratory studies was also compared based on egg numbers per single berry. As illustrated in Figures 1 to 6 data for each week were analysed individually by Kruskal Wallis H test. The same data were used to elucidate a possible effect of progressive ripening on oviposition. Numbers of eggs in the berries of the individual varieties were correlated with progressing harvest (calendar) weeks by aid of Kendall's tau c coefficients.

In case of free-choice assays egg numbers obtained from each repetition (consisting of 7 to 8 berries) were analysed and data from several weeks were analysed together (Fig. 7).

RESULTS

FIELD DATA

In both vineyards over all calendar weeks and varieties presence of eggs in the berries varied significantly between all three observation years (KL: Kruskal Wallis H = 151.22, DF = 2, $p \leq 0.001$; LE: H = 199.18, DF = 2, $p \leq 0.001$).

In 2015 virtually no oviposition in the field was recorded (data not shown). Only one egg was detected in a FV originating from LE sample in calendar week 39.

The highest egg numbers and highest proportions of infested berried were recorded in 2016 (Fig. 1 and 2). In this year first eggs were found in calendar week 37 (September 12) on SL and BP at the location KL. In LE in 2016 the highest numbers of eggs per sample were observed on the varieties FV, BP and SL, relevant infestation was recorded from calendar week 38 onwards. Infestation rates ranged up to 54 % in case of BP, up to 57.5 % in case of FV and 32 % in case of SL, weekly statistical analysis revealed a significant difference between these varieties and less susceptible ones such as GR, RV, BB, BL, ZW and RL (Fig. 1). In KL in calendar week 37 42.5 % of the analyzed BP berries and 31.5 % of the SL berries showed eggs, from calendar week 38 onwards more than 17 % of the ZW berries were infested. Weekly statistical analysis revealed significantly higher rates of oviposition in BP, SL and ZW as compared to most other studied varieties (Fig. 2).

In 2017 the first eggs were detected in week 36 in LE (1 egg per 50 berries September, 8th, on SL (data not shown) and in week 38 on ZW (Fig. 3)). Significant deposition of eggs was not recorded before calendar week 40 in any of the varieties at both locations and numbers of eggs per berry never exceeded 3 eggs per berry. Notable numbers of eggs and proportions of infested berries were found for the varieties BP and SL at both locations, in KL also for the variety FV and in LE also for ZW. Depending on the respective calendar week in LE a statistically higher vulnerability of SL and BP as compared to e. g. BF, GR, RL, RV was recorded (Fig. 3), in KL the varieties FV and BP differed significantly from most of the other varieties in the test (Fig. 4).

Statistical analysis also revealed a small to moderate correlation of egg numbers on the berries and calendar weeks, especially in vulnerable varieties in 2016 (e. g. 2016 BP: $\tau c = 0.34^{***}$, FV: $\tau c = 0.23^{***}$ SL $\tau c = 0,13^{***}$; 2017: BP: $\tau c = 0.08^{***}$, FV $\tau c = 0.075^{***}$, SL $\tau c = 0.06^{***}$).

Captures of adult flies in the bait traps are illustrated in Figure 8. In 2016 relevant numbers of flies were observed from the second half of August until the beginning of October, average weekly captures (10 traps) reached up to 112.6 ± 219.77 individuals in Klosterneuburg and 36.25 ± 19.55 in Langenzersdorf. In 2015 and 2017 at both sites captures were much lower.

Daily maximum temperatures and precipitation from the beginning of April until the end of October 2015 to 2017 are illustrated in Figure 9. The hottest temperatures were recorded in 2015. From August onwards 18 days with temperatures above 33 °C were noticed in Klosterneuburg and 15 days in Langenzersdorf. Hot temperatures lasted unusually long, at both locations temperatures above 33 °C were recorded until day of the year 260 (calendar week 38). In 2016 temperatures exceeded 33 °C only on one day in Klosterneuburg (day of the year 241, calendar week 35) more than 30 °C were recorded on 11 days in Klosterneuburg and on 7 days in Langenzersdorf. In 2017 daily maxima above 33 °C were noticed six times in Klosterneuburg and five times in Langenzersdorf, last on day of the year 239 (calendar week 34).

In 2015 the cumulative amount of rainfall from beginning of April until end of October reached 367 mm in Klosterneuburg and 321 mm in Langenzersdorf, in 2016 it was 571 mm in Klosterneuburg and 423 mm in Langenzersdorf. In 2017 for Klosterneuburg 382 mm were measured, for Langenzersdorf 352 mm (data not shown).

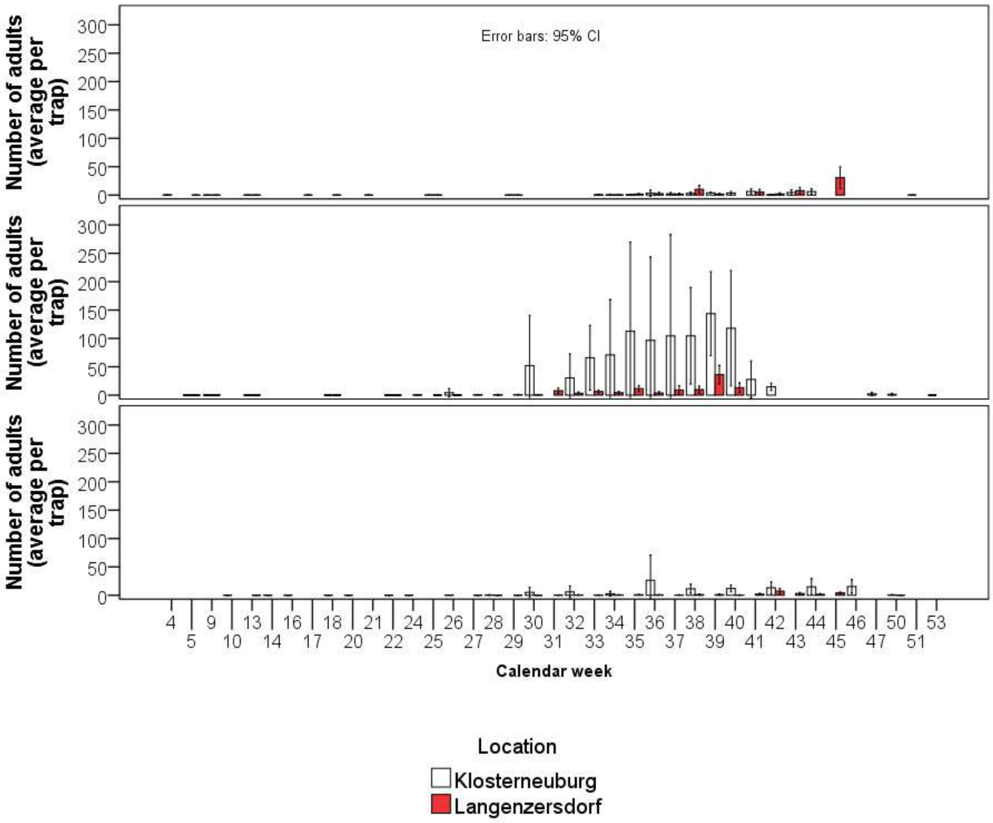


Fig. 8: Catches of adult flies in bait traps (10 traps per calendar week, location and year)

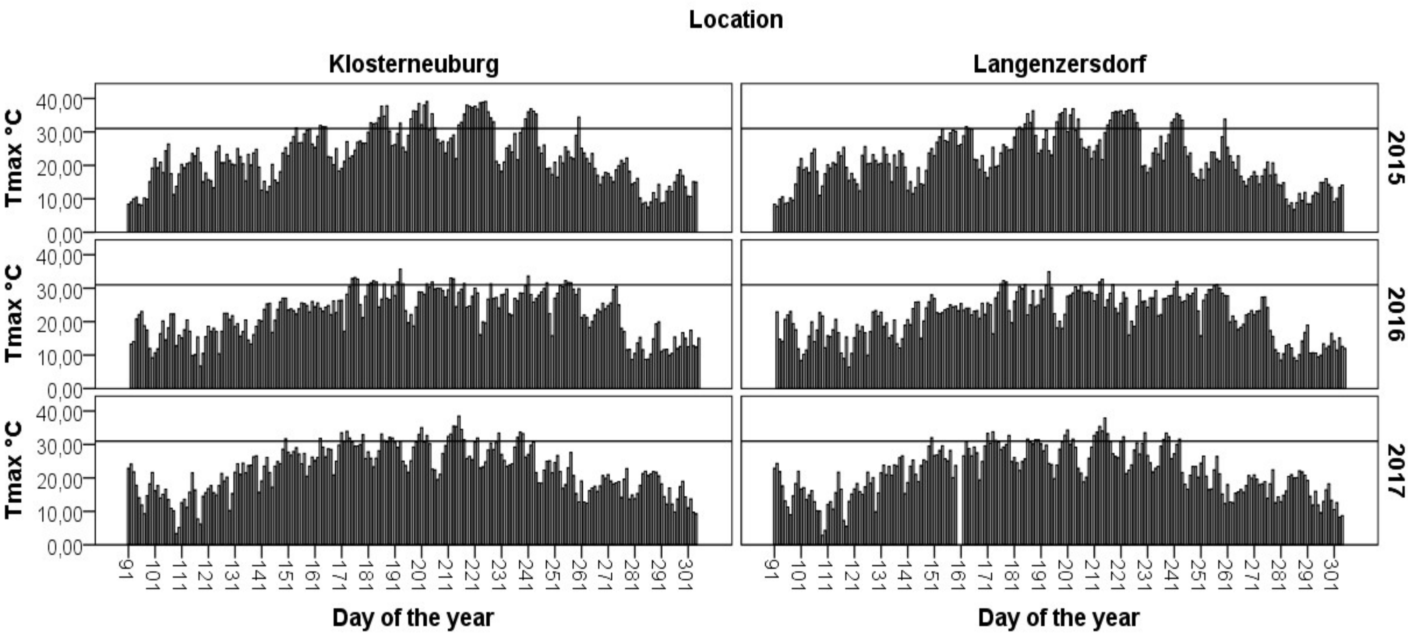


Fig. 9: Profiles of daily maximum temperatures at both test locations, continuous line showing 31 °C

LABORATORY NO-CHOICE ASSAYS

All varieties in the test were vulnerable to damage by *D. suzukii,* eggs were recorded in all tested cultivars in all years (LE, 2016 Fig. 5; KL 2017 Fig. 6; data KL 2016 and LE 2017 not shown). In 2016 both in KL and in LE already in calendar week 33 and 34 the insects were able to penetrate the skin of almost all varieties included in the trial. In some varieties such as BP, FV, GR, RV, SL or ZW 10 to 20 % of the berries, in case of SL in KL in calendar week 33 even 36.7 % of the berries were infested with eggs. In LE in 2016 the highest percentages of infested berries were observed for RV and SL in calendar week 36 and for FV and BP in week 38. Weekly statistical analysis revealed significantly higher numbers in eggs in RV, FV, SL and BP than in less susceptible varieties such as BB or BF (Fig. 5).

In 2017 weekly assessments in LE showed the highest proportions of infested berries and the highest numbers of eggs per berry for the varieties BL, BP, RV, SL and ZW, in KL for the varieties BP, FV, RV, SL and RL. Statistical analysis of the weekly data for KL 2017 revealed significantly higher numbers of eggs in the varieties RL, RV, SB, FV, BP SL and in week 41 also in GR than in e. g. BB, BF, ZW and BW (Fig. 6).

LABORATORY FREE-CHOICE ASSAYS

In 2016 in experiments carried out with berries harvested in LE the highest number of eggs was recorded for the varieties BP, RL, SL and ZW, significant differences were observed for SL and BP as compared to BL and BB (Fig. 7). In KL in 2016 highest numbers of eggs were recorded in BP, RL, SL and ZW (data not shown). In 2017 in tests with berries from KL flies preferred FV, SL and BP for egg laying, differences were statistically significant as compared to e. g. BW and BL (Fig. 7). Regarding berries from LE in 2017 the highest numbers of eggs were recorded in BL, BP, SL and ZW (data not shown).

DISCUSSION

Regarding all the findings obtained in the current study the main conclusion is that there is no general threat of Austrian vineyards by *D. suzukii* but that the risk strongly varies between years and vine varieties. Appropriate and sustainable management of *D. suzukii* in vineyards therefore requires an individual assessment for each year and each vineyard.

Our field observations revealed significant differences between the observation years with regard to oviposition and proportions of infested berries. Several literature reports indicate unanimously that temperatures at or above 30 °C significantly delay or abolish pupation and adult eclosion (Kinjo et al., 2014; Tochen et al., 2014) and also enhance mortality of adult flies (Eben et al., 2017). It is therefore reasonable to relate the observed differences in *D. suzukii* infestations to temperature profiles of the respective years (Fig. 8). The hottest temperatures from August onwards were recorded in 2015 and temperatures above 33 °C lasted until calendar week 38. Presumably, only a very limited extent of fly development occurred during this period and heat stress enhanced adult mortality. As a result more or less no oviposition in the field was recorded and numbers of insects in the bait traps were negligible. Also in Germany in 2015 fly populations remained low, an observation that was linked to record temperatures and dryness (Eben et al., 2017). By contrast in 2016 conditions, especially during the month of August (day of the year 214 to 243), were probably more suitable for the flies, temperatures exceeded 33 °C only on one day in Klosterneuburg (day 241, calendar week 35). Correspondingly important infestations on susceptible varieties were recorded from calendar week 37 to 38 onwards and comparatively high numbers of flies were captured in the bait traps. In 2017 weather conditions were likely not conducive to fly multiplication, daily maxima above 33 °C were frequently recorded until day of the year 239 (week 34). Subsequently notable berry infestations were not observed before week 40 on any variety and individual numbers in the bait traps were low again. These data indicate that in the future a relevant danger for the crop will predominantly occur in cooler vegetation periods with daily temperature maxima rarely exceeding 30°C.

Knowledge of the susceptibility of the different grapevine varieties is a fundamental requirement for planning of appropriate management strategies. Our field studies showed considerable differences between vulnerable varieties such as BP, FV and SL sometimes also ZW and less or hardly threatened varieties such as e. g. BB, BF, BW, GR, BL, CH, GV and WR. All in all the flies seemed to prefer early ripening red or flesh pink varieties (Fig. 1 to 4). Significant differences in view of susceptibility to *D. suzukii* have also been observed in Germany, where, as in the current study, early ripening red varieties such as 'Portugieser' were particularly prone to oviposition (Entling et al., 2018) and in Northern Italy, where the varieties 'Vernatsch' and 'Lagrein' were particularly affected (Sinn, 2015).

Both in 2016 and in 2017 numbers of eggs significantly correlated with maturation (calendar week), an observation that coincides with literature reports (Ioratti et al., 2015; Baser et al., 2017; Entling et al., 2018). The increase of oviposition during ripening is primarily seen in connection with decreased skin penetration force (Ioratti et al., 2015; Lee et al., 2016; Baser et al., 2017). In our study the increased vulnerability for egg laying during ripening was mainly observed for soft-skinned varieties such as FV, BP, SL (Fig. 1 to 4), whereas other red or flesh pink varieties such as BB, CS, BF or GR remained hardly infested over the entire ripening period.

No-choice tests excluding selection behavior of the flies were carried out to elucidate the extent to which the flies penetrate the intact berry skin and lay eggs into undamaged berries of a given variety at a given time point. In our study the insects were capable of depositing at least some eggs into all analyzed varieties, in many varieties already in calendar week 33 (middle of August). In consequence significant infestations of berries at early ripening stages and damage to a wide range of varieties in future unfavorable years with high pest pressure cannot be ruled out. In this regard, however, it must be considered that the no-choice experimental design did not allow any freedom of choice, neither between grapevine varieties nor between grapevines and other possible hosts. This situation, however, never happens under field conditions. Well validated conclusions will therefore require long-standing field observations. Incidence of laid eggs in no-choice experiments varied significantly between the varieties with the varieties RV, BP, FV, BL, SL and in 2015 also ZW being most affected. This confirms the suspicion that the berry skin of these varieties can be penetrated by the flies relatively easily.

In contrast the free-choice experimental design allowed the flies to freely select between berries of different varieties. Therefore in this experimental design oviposition was determined both by the resistance of the berry skin against penetration and the preference behavior of the flies. The results largely confirmed the field experiments with FV, SL, BP and in some cases also ZW as most preferred varieties for egg laying. The congruence between free-choice and field results suggest that appropriate laboratory setups can be a valuable tool not only for variety testing but presumably also for testing management strategies, e. g. the effect of repellents.

The field observations made during this study were confirmed by numerous grower reports from the surrounding region (Weinviertel, Lower Austria). In 2016 serious infestation of the must with larval stages of *D. suzukii* and/or volatile acidity were frequent in the variety BP, for other varieties only few or no notifications of damage were received. In 2015 and 2017, in contrast, no significant damage was announced in any variety.

From a practical viticultural point of view the results of the current study show that sustainable management of *D. suzukii* requires a correct assessment of the infestation risk in a given vineyard. This depends on the climatic conditions of the year influencing the fly populations, on the susceptibility of the variety and also the geographic position and the vineyard surroundings (other host plants, forest). Likely important is the point in time at which the grapes become infested. In the current study (2015 to 2017) infestations of berries in the field were not detected before September. At this point in time the early (and vulnerable) varieties in the experiments were shortly before harvest (but they were left in the experimental vineyard beyond the actual harvest date for observation purposes). This indicates that in future years with similar weather conditions and comparable development of grapes and flies infestations in most cases will mostly occur some days before planned harvest only. In consequence regular inspection of endangered varieties from beginning of ripening or at least from berry softening onwards will be the crucial point for sustainable *D. suzukii* management. A prompt detection of sudden infestations with *D. suzukii* will allow to react quickly e. g. by advancing the harvest or, if the grapes are not mature enough for harvest, by application of homologized insecticides, provided that the legal waiting time required after a treatment can be observed.

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