Integrated Protection and Production in Viticulture
IOBC-WPRS Bulletin Vol. 128, 2017
pp. 28-36

Geographical area extension of *Drosophila suzukii* (Diptera: Drosophilidae) in Bordeaux vineyards

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Abstract: The invasive fruit fly *Drosophila suzukii* (Diptera: Drosophilidae) was recorded for the first time in 2011 in the Bordeaux vineyard through a trap network located in the Sauternes subregion (south of Bordeaux). This species is now regularly present in our food traps surveys. In 2013, we observed complete reproductive cycles of this species on grapes in vineyards located on the left bank of the Garonne river both in the Sauternes (South) and the Médoc (North) subregions (ca. 70 km from the 2011 first observation). In 2014, almost all the Bordeaux vineyard was affected by *D. suzukii* and this was concomitant with unusual widespread development of sour rot. This was particularly the case for black cultivars (Merlot and Cabernet-Sauvignon). This invasive drosophila is now considered as part of the regular drosophila community on grapes. Ongoing studies examine the variability of behavioral traits of *D. suzukii* on different cultivars, the damages induced by this species, and the evolution in space and time of fruit flies communities on grapes.

Key words: fruit fly, drosophila communities, grapevine, *Drosophila suzukii*, monitoring

Introduction

In 2011, we reported the invasive species, *Drosophila suzukii*, at the adult stage in the Bordeaux vineyard (*i.e.*, in Sauternes and Barsac area) (Rouzes *et al.*, 2012), but did not detect it on clusters at harvest.

Behavioral and morphological characteristics of this species make it a real threat to many fruit crops (Walsh *et al.*, 2011) including grapes (Ioriatti *et al*., 2015). Unlike most other fruit fly species infesting ripe fruit, rotten fruits or fruits fallen to the ground, *D. suzukii* is able to attack intact fruit during ripening (Mitsui *et al.*, 2006). The female is equipped with a large and sharp ovipositor allowing laying eggs in healthy fruit (Attalah *et al.*, 2013). After larval development, the fruit collapses around the feeding site and then decays rapidly. In parallel, the bruises and the small injuries left by the ovipositor of the female provide many pathways to secondary infections (insects and pathogens) accelerating the decaying process.

A first assessment of the general situation of the threat that *D. suzukii* poses to European vineyards in 2012 considered this pest a minor problem to grapes (Kehrli *et al.*, 2014). However, an updated evaluation of the situation is needed in European vineyards and especially in the Bordeaux area. The objective of this work was a survey of the distribution and abundance of the species since its introduction in the Bordeaux vineyard. Is it associated with other drosophila and damage? To tackle these questions, we initiated a study on the population dynamics of fruit flies and sour rot in the Bordeaux vineyard.

Material and methods

Subregions from the Bordeaux vineyard used

Six subdivisions from the Bordeaux vineyard (Figure 1) were selected, each corresponding to geographical area and type of vine production:

- "Côtes-de-Blaye/Bourg": located east of the Gironde estuary and north of Bordeaux, this area produces white and red wines using mostly Sauvignon-Blanc and Merlot cultivars,
- "Côtes-de-Bordeaux/Entre-Deux-Mers": between the Dordogne and the Garonne, this area is very diversified in terms of landscape, produces all types of wines using all Bordeaux cultivars,
- "St-Emilion/Pomerol/Fronsac": on the right bank of the Dordogne, this area is essentially a production zone of red wines with Merlot as dominant cultivar,
- "Médoc": located west of the Gironde estuary and north of Bordeaux, this area produces red wines with Cabernet-Sauvignon as dominant cultivars and Merlot,
- "Pessac-Léognan/Graves": on the left bank of the Garonne and south of Bordeaux, this area produces white and red wines using Sauvignon-Blanc and Merlot as dominant cultivars,
- "Sauternes": on the left bank of the Garonne and south of Bordeaux, this area is essentially the production zone of botrytized white wines with Semillon as dominant cultivar.

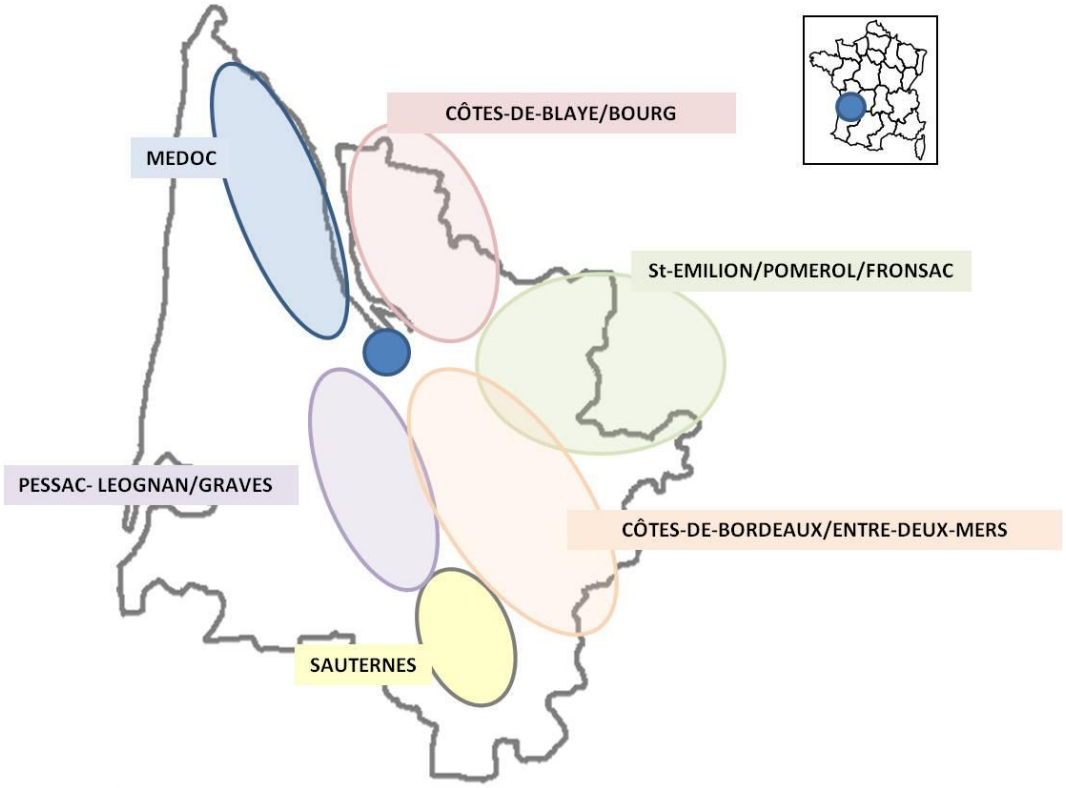

Figure 1. Location of the six subregions surveyed in Bordeaux vineyard.

Drosophila presence/absence in traps

During the season, *D. suzukii* adults were checked in food traps used for *L. botrana* monitoring. Each trap was filled with apple molasses diluted in water, as described in Thiéry *et al.* (2006), and hanged in the grapes foliage: one trap per plot was used.

Drosophila emergences from bunches

The presence of *D. suzukii* on grape bunches was also monitored since 2011 by collecting 10 bunches taken randomly in each surveyed plot in the vineyard close to harvest. Presence/Absence of damage associated on bunches (sour rot, grey mold, grapevine moth larvae) is noted from 2011 to 2013, and severity in 2014.

Bunches were isolated each in plastic boxes closed with perfored lids and tulle and stored in lab during at least seven days to obtain adults (Delbac *et al.*, 2014). Boxes were then placed in freezer less than two hours in order to facilitate the adults collection. Adults drosophila were determined using a binocular microscope at a 50 fold magnification. *D. suzukii* were differentiated from other drosophila species using the taxonomic determination key of Seguy (1934), updated by Seguy (1938) and Vlach (2010).

2014 survey conducted by the french Ministry of Agriculture

In 2014, the unusual presence of foci of sour rot was suspected to be due to *D. suzukii*. The Ministry, through the "Service Régional de l'Alimentation" (SRAL) of our region (Aquitaine), wrote a questionnaire organized in two major parts to obtain responses of the winegrowers. The first part targeted the presence of sour rot in the vineyard with evaluation of the frequency and the severity on bunch. The second part targeted the presence of *D. suzukii* when confirmed by technicians. The electronic survey was published by the regional agricultural warning services to winegrowers and technicians of the region.

Statistical analysis

Statistical analyses were done on the 2014 dataset owing to the large sampling size collected. For a normalized residuals distribution, as described by Southwood (1978), we applied a square-root transformation on count data [number of *D. suzukii* per bunch] and a logarithmic transformation on percentage evaluation [% of bunches with *D. suzukii*, % *D. suzukii* per other drosophila, and frequency or severity of sour rot on bunches]. We used the ANOVA model for pairwise comparisons of means and Pearson correlation to test linear link between variables. Systat® 11 software (Systat Software, San Jose, CA, USA) was employed for all statistical tests, which were performed with a type-I error rate of 0.05.

Results and discussion

Drosophila traps

The first detection of *D. suzukii* in Bordeaux vineyard was confirmed in 2011 in three traps in the Sauternes subregion (Table 1). The catches were detected on September 14, 2011. In 2012, the insect was not recorded in Pessac-Léognan/Graves subregion. This year was characterized by few catches (less than 10 adults). In 2013, adult catches were more important and effectives in all the traps followed (more than 100 adults). In 2014, the catches were confirmed in Pessac-Léognan/Graves area with more than 50% of positive traps.

Table 1. Presence of adults *Drosophila suzukii* in food traps (moth or fruit flies) in grapevine per Bordeaux subregion and year.

Year	Subregion	Number of traps	*D. suzukii* detected	% of positive traps
2011	Sauternes	3	YES	100
2012	St-Emilion/Pomerol/Fronsac	3	YES	100
	Médoc	2	YES	50
	Pessac-Léognan/Graves	1	NO	0
	Sauternes	3	YES	33.3
2013	St-Emilion/Pomerol/Fronsac	4	YES	100
	Médoc	1	YES	100
	Sauternes	1	YES	100
2014	Médoc	7	YES	100
	Pessac-Léognan/Graves	14	YES	57.1
	Sauternes	1	YES	100

A specific survey of one trap conducted in our INRA experimental vineyard (Pessac-Léognan/Graves subregion) in 2014, revealed that *D. suzukii* was accompanied by other drosophila (Figure 2). *D. suzukii* represented the third of the total drosophila catched (N = 176). Three other species being found: mostly *Drosophila melanogaster*, few *Drosophila subobscura* and *Drosophila simulans*.

These other species were earlier found in the Bordeaux vineyard 20 years ago (Capy *et al.*, 1987; Gravot, 2000). Our results, both on numbers of inviduals captured and species present are consistent with other studies of fruit flies trapping in the vineyard (Ioriatti *et al.*, 2015; Justrich, 2013; Marchesini *et al.*, 2014).

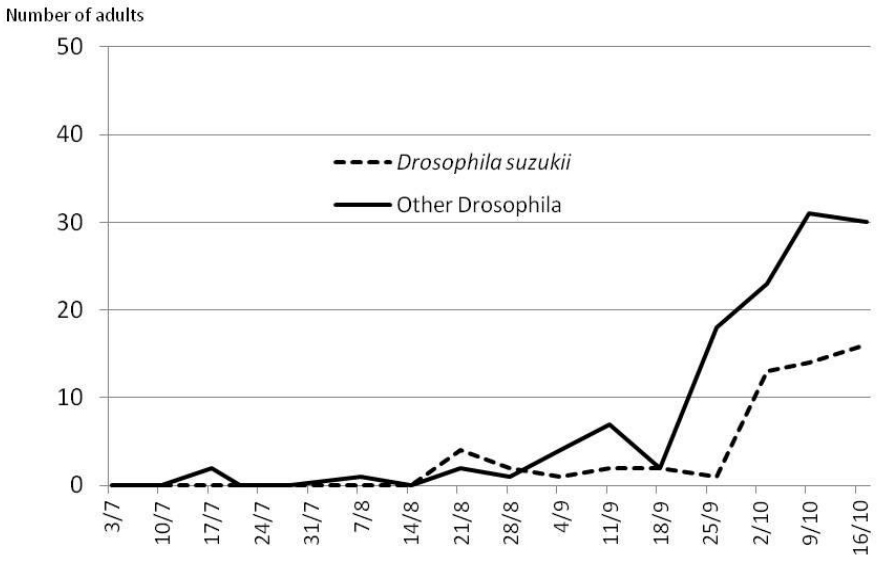

Figure 2. Captures of *Drosophila suzukii* adults in one trap in INRA site in 2014.

Drosophila emergences from bunches

In 2011 and 2012 (Table 2), no *D. suzukii* was found on bunch and drosophila were essentially *D. melanogaster*. The year 2013, corresponded to the first occurence of *D. suzukii* in grape bunches. *D. suzukii* was founded in 27.3% of the surveyed vineyards, *i.e.* one plot, respectively, in Sauternes, Médoc and Pessac-Léognan/Graves subregions. Each time, *D. suzukii* emerged from bunches, sour rot symptoms were observed in these bunches.

Table.2. Emergences and identifications of drosophila on bunch per Bordeaux subregion at harvesttime.

Year	Subregion	Sampling plots	% of plots with *D. suzukii*	% bunches with *D. suzukii* (mean ± S.E. in 2014)	% *D. suzukii*/other *Drosophila* (mean ± S.E. in 2014)	*D. suzukii*/ bunch (mean ± S.E.in 2014)	Other damage on bunches (% of severity ± S.E. in 2014)
2011	Sauternes	2	0	0	0	0	*Lobesia botrana* & sour rot
2012	Sauternes	1	0	0	0	0	*Lobesia botrana*
2013	Côtes-De-Bordeaux/ Entre-Deux-Mers	6	0	0	0	0	grey mold, hail
	St-Emilion/Pomerol/ Fronsac	2	0	0	0	0	grey mold, hail
	Medoc	1	100	80.0	82.2	3.7	sour rot
	Pessac-Léognan/Graves	1	100	30.0	1.1	0.3	sour rot
	Sauternes	1	100	90.0	29.4	13.4	sour rot
2014	Cotes-De-Blaye/Bourg	2	100	95.0 (± 7.1)a	13.4 (± 11.2)abc	3.4 (± 1.1)ab	sour rot (23.5 ± 4.2)a
	Côtes-De-Bordeaux/ Entre-Deux-Mers	9	66.6	48.9 (± 43.4)a	18.3 (± 30.2)bc	0.9 (± 1.1)b	sour rot (17.8 ± 14.9)a
	St-Emilion/Pomerol/ Fronsac	15	100	67.1 (± 30.0)a	27.7 (± 25.2)ab	4.0 (± 4.4)a	sour rot (15.8 ± 12.8)a
	Médoc	8	100	49.5 (± 20.8)a	49.9 (± 38.8)a	1.6 (± 1.1)ab	sour rot (7.2 ± 8.6)a
	Pessac-Léognan/Graves	11	81.8	24.5 (± 20.5)a	15.6 (± 17.5)abc	0.5 (± 0.6)b	sour rot (19.5 ± 17.9)a
	Sauternes	8	87.5	52.0 (± 39.1)a	1.6 (± 2.8)c	0.6 (± 0.8)b	sour rot (37.2 ± 8.7)a

S.E.: Standard Error; Different letters indicate a significant difference between subregion for the year 2014 for each variable tested ($P \leq 0.05$) as determined by Fisher's test and pairwise comparison

In 2014, plots sampling were realized in all Bordeaux area. A large part of these plots (86.8%) was infested by *D. suzukii*. The proportion of *D. suzukii* in the drosophila emerged and the number of *D. suzukii* per bunch was statistically higher on black cultivars subregions (ANOVA, $p = 0.001$ and $p < 0.001$ respectively). All these bunches presented sour rot symptoms that was unusual for a presence as widespread in Bordeaux on black cultivars (Merlot and Cabernet-Sauvignon). We however, did not observed statistical differences between subregions for this variable (ANOVA, $p = 0.098$).

Since 2013, on infested plots, *D. suzukii* was associated with other native *Drosophila* species in an increasing of grape bunches collected. We noted mainly *Drosophila melanogaster* and few *Drosophila simulans*. The number of *D. suzukii* per bunch was always more important on black cultivars than in white ones (mean on black cultivars = 2.3 ± 3.1; mean on white ones = 0.6 ± 0.7; ANOVA, $p = 0.024$) which seems to be an important trend for *D. suzukii* in vineyards. This is consistent with other studies on fruit flies emerging from grape (Ioriatti *et al.*, 2015; Van Timmeren & Isaacs, 2014) or lab study (Linder *et al.*, 2014).

In our study, when sour rot severity was heavy, the proportion of *D. suzuki versus* other drosophila species was less. This was confirmed in 2014 when we obtained a significant negative correlation on the sour rot severity and the proportion of *D. suzuki versus* other drosophila species variables (Pearson's r = -0.624, $p < 0.001$) ; this is in contradiction with the study of Linder *et al.* (2014). But, as specified by these authors, their occurrence of the rot disease was very low, while in our study, the occurrence of the disease was greater. The decreasing proportion of *D. suzukii* could be explained by its behavior: as probably a pioneer species, it seems to be less adapted to rotten berries than other fruit flies probably because of the evolution of the chemical composition of the berries.

Investigation of the 2014 vintage by a survey to winegrowers

One hundred twelve growers answered the survey and their responses are reported in Table 3. In total 62.5% positively reported sour rot damage. The sour rot was observed in all Bordeaux subregions with an average occurrence of 20.9% of infected bunches. No statistical difference was observed by ANOVA mean comparison on frequency or severity of sour rot ($p = 0.639$ and $p = 0.263$, respectively). First symptoms of the disease were noted in August for white wine areas of production and later (before mid of September) for the red ones.

An investigation made in Bordeaux related to the beginning of the 90's on the sour rot symptoms (Vible & Stockel, 1997) reported that the disease was historically associated to white cultivars and to the Sauternes subregion. A 33 years analysis of the regional agricultural extension services reports (source: "Avertissements Agricoles" Vigne Aquitaine 1982 to 2009 and "Bulletin de Santé du Végétal" Aquitaine Viticulture 2010 to 2014) confirms that sour rot presence on black cultivars is very unusual as only noticed once in 1997 in the Médoc subregion. This was associated with skin bursting of berries after heavy rainfall during long dry conditions, which was not the case in the 2014 vintage. The other years with significant sour rot epidemics (1987, 1989, 1994, 1998 and 1999), only concerned the white cultivars, mainly in Sauternes, with few occurrences in Pessac-Léognan/Graves and the Côtes-de-Bordeaux/Entre-Deux-Mers subregions.

Table.3. Results of 2014 per Bordeaux subregion appellation at harvesttime.

subregion	number of answers	% presence of sour rot in parcel	% bunches with sour rot (± S.E.)	% severity of sour rot on bunches (± S.E.)	% of positive plots with *D. suzukii* confirmed by technicians
Côtes-De-Blaye/ Bourg	14	21.4	30 (± 20)a	5.8 (± 2.9)a	66.7
Côtes-De-Bordeaux/ Entre-Deux-Mers	42	71.4	22 (± 18.6)a	4.7 (± 3.9)a	26.7
St-Emilion/ Pomerol/Fronsac	31	64.5	17 (± 11.7)a	3.9 (± 2.4)a	45
Médoc	8	50	15 (± 10)a	3.4 (± 2.8)a	25
Pessac-Léognan/ Graves	7	85.7	20 (± 16.7)a	3.7 (± 3.0)a	50
Sauternes	10	70	27.1 (± 21.4)a	7.5 (± 4.1)a	28.6

S.E.: Standard Error; Same letters indicate no significant difference between subregion for each variable tested ($P > 0.05$) as determined by Fisher's test

Conclusion

D. suzukii seems now well established in the Bordeaux vineyard since 2011.The species is found regularly and in inceasing amounts in traps placed inside the vineyards and also in landscapes close to vineyards (our unpublished data). In several places, *D. suzukii* attacked grapes between veraison and harvest time but was always associated with other drosophila species, mostly *D. melanogaster*.

The proportion of each species varied as a function of the cultivar with more *D. suzukii* found on black cultivars (Merlot and Cabernet-Sauvignon) as compared to white ones (Sauvignon-Blanc and Sémillon). This proportion was dependant of the sour rot severity. Integrating our data with previous studies, we can now draw a chronology of the evolution of the health situation of the vineyard over the acid rot and fruit flies associated in the Bordeaux vineyards over the last 33 years (Figure 3). How will evolve the situation in the future remains unknown, especially with potential climate changes. Further studies will be conducted to understand the relation between *D. suzukii* and sour rot on grapes, the impact on the fruit flies communities in vineyards and the incidence of the surrounding landscapes.

Acknowledgements

We are grateful to all the winegrowers who have participated to this study. We would also like to thank SRAL Aquitaine for providing their survey data, the technicians of "Fédération Régionale de Défense contre les Organismes Nuisibles" Aquitaine, Laure Trapateau (Ets Sallelas), Nathalie Poppe (Ets Touzan) and Nadège Bidou (Ets Soufflet-vigne) for contributing to data acquisition. A special thanks to Jean-Marie Brustis (INRA SAVE) and Malika El-Mir (Entomo-Remedium) for their precious technical assistance. The study was partly funded by the research project CASDAR "BIOCONCONTROL" IP 2012 N°1218 from French Ministry of Agriculture.

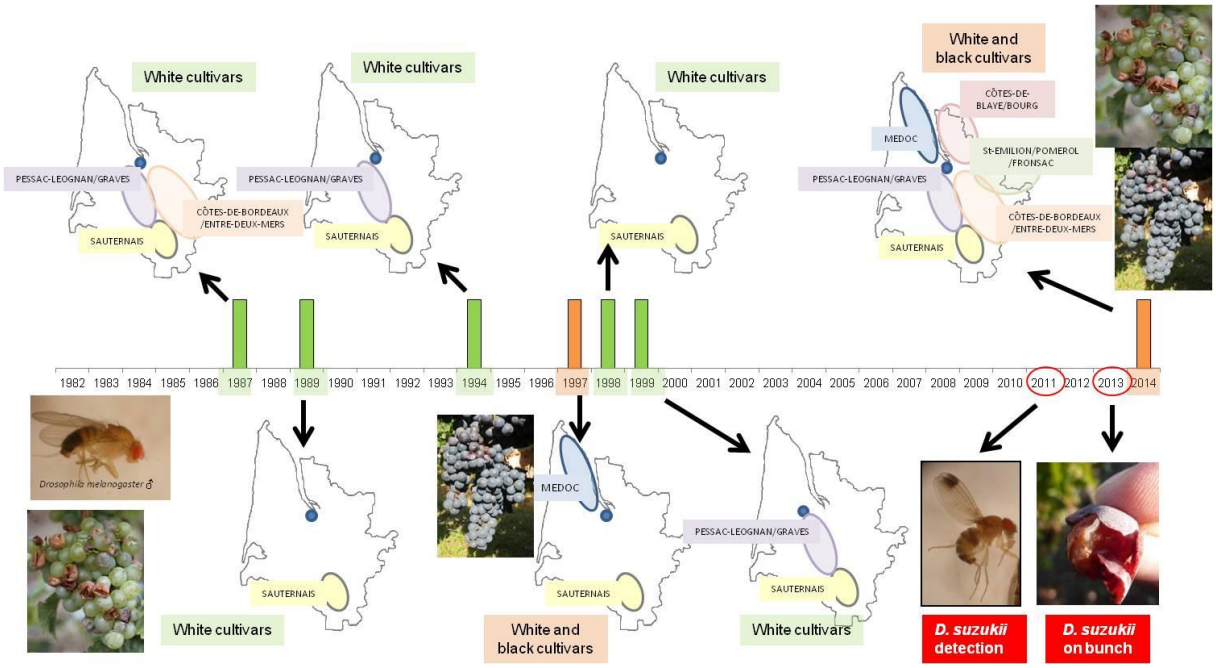

Figure 3. Chronological situation of *Drosophila* and sour rot in Bordeaux vineyard during the 33 past years (green bars: sour rot detected on white cultivars; orange bars: sour rot detected on black and white cultivars).

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