Integrated Protection in Viticulture
IOBC-WPRS Bulletin Vol. 154, 2020
pp. 93-100

Maturity indicators to predict grape skin strength for controlling *Drosophila suzukii*

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Abstract: *Drosophila suzukii* has become a pest of economic importance ever since it has spread around the world through the soft fruit trade. In vineyards, it is thought that the risk of infestation is correlated with the strength of the grape's skin. There is an idea that there might be a common threshold in skin strength when a grape passes from being safe from infestation to being susceptible to infestation. The relationships between *D. suzukii* infestation rate and grape properties were explored. Also, the relationships with grape skin strength (= penetration force) and other grape properties related to total soluble solids, acidity, nitrogen content, and grape seed color were compared at a single site and across different sites. Cultivars were found to be infested by *D. suzukii* across a wide range of penetration forces. Therefore, the idea of a common threshold is not supported by our study. Factors relating to grape maturity were overall more successful in predicting risk of infestation. Penetration force was not able to be successfully correlated with any single tested grape property or with a statistical combination of them. Moreover, cultivars behaved quite differently depending on their location. Thus, the study was not able to discover any general relationships that might be used to indicate a grapevine's susceptibility to *D. suzukii* infestation.

Key words: sour rot, sugar content, pH, acidity, nitrogen, ammonia

Introduction

The spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae), has become a major pest of great economic importance throughout Europe, Asia, and the Americas in recent years. Recent research has indicated that there is a direct relationship between the strength of the grape skin, or penetration force, and the risk of infestation by *D. suzukii*. It has been shown that there might be a threshold, about 40 cN, for when a berry passes from being unattractive to *D. suzukii* infestation to becoming susceptible (Ioriatti et al., 2015). Thus, it follows that if one were able to accurately predict the penetration force of a given berry than one would be able to assess the risk of infestation. With this in mind, this research was undertaken to see if there might be some way in which penetration force could be correlated with any other property that is commonly measured in grapes.

Material and methods

At the Agroscope research vineyard in Pully (Switzerland) 17 different cultivars were followed throughout the 2017 growing season. Five of these cultivars (Gamay, Garanoir, Mara, Merlot, and Pinot Noir) were also tracked at five other sites in Western Switzerland. Beginning on

August, 75 berries were collected from each of the cultivars every one to two weeks. Of the 75 berries, 50 were chosen at random and examined under a microscope. A berry was deemed infested by *D. suzukii* when a healthy filament tube was observed emerging from the grape skin. Moreover, 50 berries were crushed in order to measure with an infrared spectrophotometer (FOSS WinescanTM) their value of Brix, pH, titratable acidity, tartaric acidity, malic acidity, yeast assimilable nitrogen (YAN), ammonia (NH_3), and alpha amino nitrogen (AAN). In addition, 20 seeds were chosen at random in order to determine their maturity. The remaining 25 berries from each cultivar were measured for penetration force using A Universal Testing Machine TAxT2i Texture Analyser (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 2 mm blunted needle (Letaief et al., 2013).

The data were examined through correlation matrices and linear regressions. Principal component analyses were used to examine the percentage of impact of the major correlations on the grapes. All statistical analyses were done using R statistical software.

Results and discussion

Relationships with infestation across cultivars

The correlation matrix for all cultivars at the Pully site indicated that infestation is significantly correlated with days until harvest ($R = -0.39$, $P < 0.001$), Brix ($R = 0.39$, $P < 0.001$), total acidity ($R = -0.27$, $P < 0.01$), tartaric acidity ($R = -0.38$, $P < 0.001$), malic acidity ($R = -0.29$, $P < 0.01$), seed color rating ($R = 0.29$, $P < 0.01$), and penetration force ($R = -0.26$, $P < 0.01$). Having a closer look, infestation rate increased with increased sugar content and seed color rating. It decreased with increasing total acidity and penetration force. Oviposition could be observed from 19 Brix on and a seed color rating of at least 4. No infestation was observed at total acidity levels higher than 10 g/l. However, egg deposition could be observed at a penetration force from 46 to 108 cN (Figure 1). However, it is not clear whether these factors are actually playing a role in *D. suzukii* infestation or whether they just happen to correlate with grape maturity since the days until harvest relationship correlates strongly with the infestation rate.

There is a higher likely hood that cultivars with lower penetration forces are infested (Figure 2). Nine out of the ten cultivars with the lowest average penetration force had some level of infestation during the growing season. The other three factors, Brix, total acidity and seed color rating, were much less grouped with infestation indicating that these factors are probably not directly related to infestation, but rather with maturity. Thus, it seems that the correlation seen between penetration force and infestation rate is not merely due to the maturity of grapes.

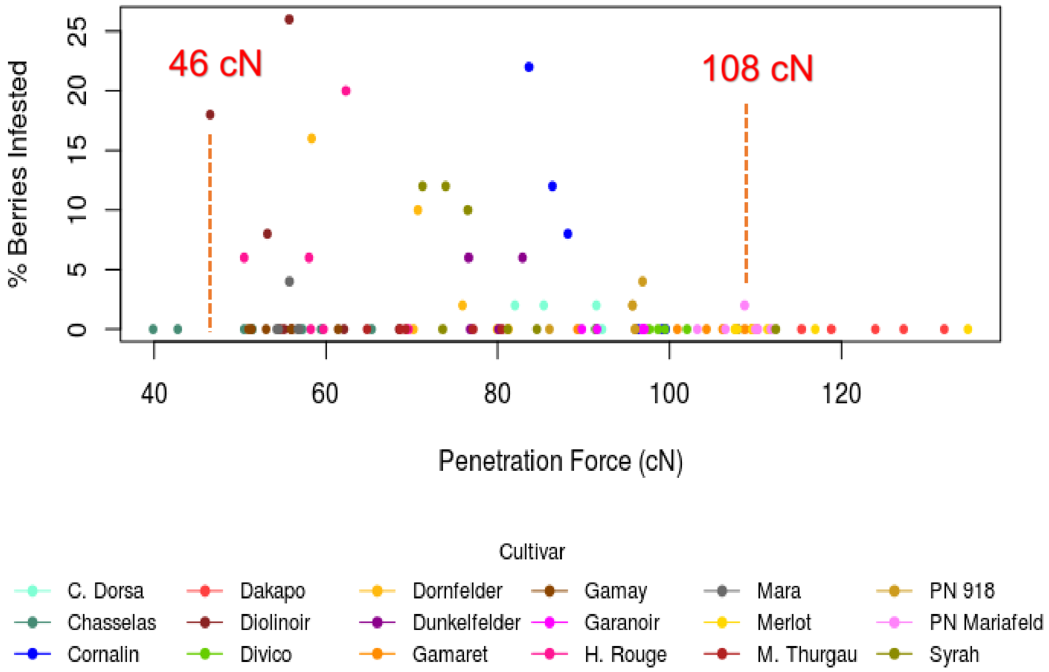

Figure 1. Penetration force graphed against the amount of berries infested with *D. suzukii* for all cultivars studied at the Pully vineyard in 2017.

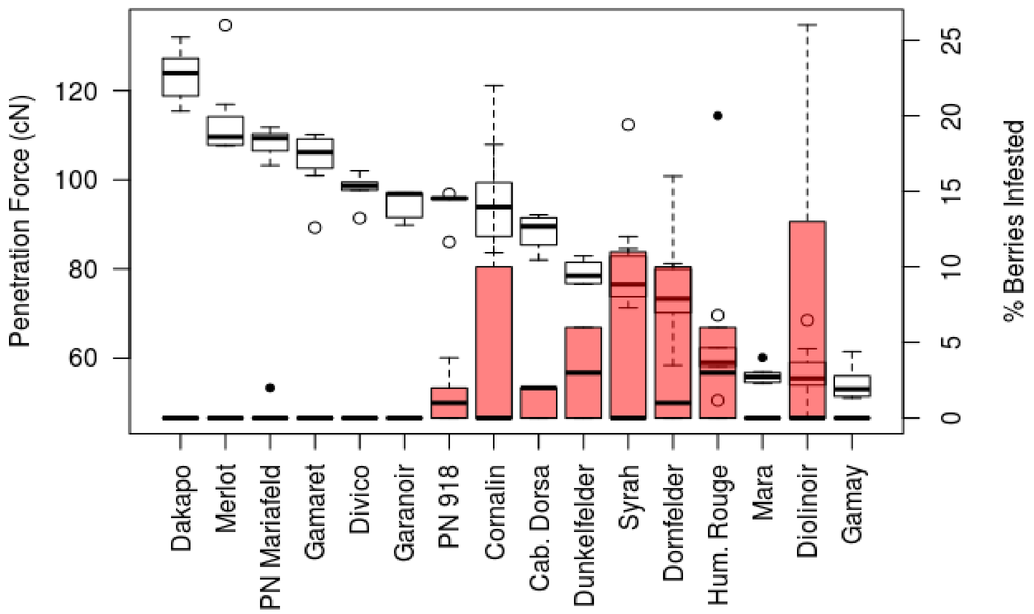

Figure 2. The range of penetration forces measured throughout the 2017 growing season for every red cultivar at the Pully vineyard with each cultivars' range of infestation rates graphed on top in red.

Relationships with infestation within cultivars

Having established the relationships between infestation rate and the physical and chemical properties of the cultivars as a whole, let us go ahead and look at the relationships of these factors within individual cultivars. Compared to when the data was looked at as a whole, the number of significant relationships decreases. Days until harvest was significantly correlated with infestation for Cornalin, Dornfelder, Humagne Rouge, Pinot Noir 918 and Syrah. Brix was significantly correlated with infestation for Cabernet Dorsa and Syrah. pH was significantly correlated with infestation for Cabernet Dorsa. Tartaric acidity was significantly correlated with infestation for Cabernet Dorsa, Cornalin, Humagne Rouge, and Pinot Noir Mariafeld. Malic acidity was significantly correlated with infestation for Syrah. Yeast assimilable nitrogen was significantly correlated with infestation for Syrah. Penetration force was significantly correlated with infestation for Cornalin and Dornfelder. Total acidity and seed color rating saw no significant correlation with infestation for any of the cultivars. What is also seen is that particular cultivars seemed to have better overall correlations than others. Cabernet Dorsa and Syrah, in particular, were responsible for six out of the eleven total significant correlations when not accounting for days until harvest correlations. The days until harvest was the factor with the most significant correlations, having five out of the nine. However, this is not extremely helpful beyond understanding that grapes become more susceptible with maturity later in the season. Of the chemical and physical properties, tartaric acidity had the most significant correlations with four cultivars. Thus, the relationships that we saw in the cultivars when looked at all together breakdown when these cultivars are examined individually. Brix, total acidity, malic acidity, seed color rating, and penetration force, properties that saw significant correlations with the entire data set, are almost entirely nonsignificant within the individual cultivars. This makes it difficult to use this information in any meaningful way when tracking a particular cultivar. The fact that the significant correlations decrease between penetration force and infestation for the individual cultivars is quite interesting. It seems to highlight the idea that there is not an exact threshold for when grapes become susceptible. Rather, it is more of just a general trend that cultivars with weaker skin see higher rates of infestation. Thus, infestation susceptibility might be better thought of as a broad range rather than a threshold to explain differences between cultivars. A second problem with the penetration force data is that initially it was thought that penetration force will decline throughout the growing season, although maybe not in a directly linear way (Ioriatti et al., 2015). However, the variation between penetration force measurements week by week was higher than expected (Figure 3). While there is definitely an overall trend that the penetration forces are becoming weaker throughout the season, as seen by the abline in black, individual cultivars varied drastically from one another and four cultivars saw their penetration force even to increase throughout the growing season (Divico, Dunkelfelder, Mara and Pinot Noir 918).

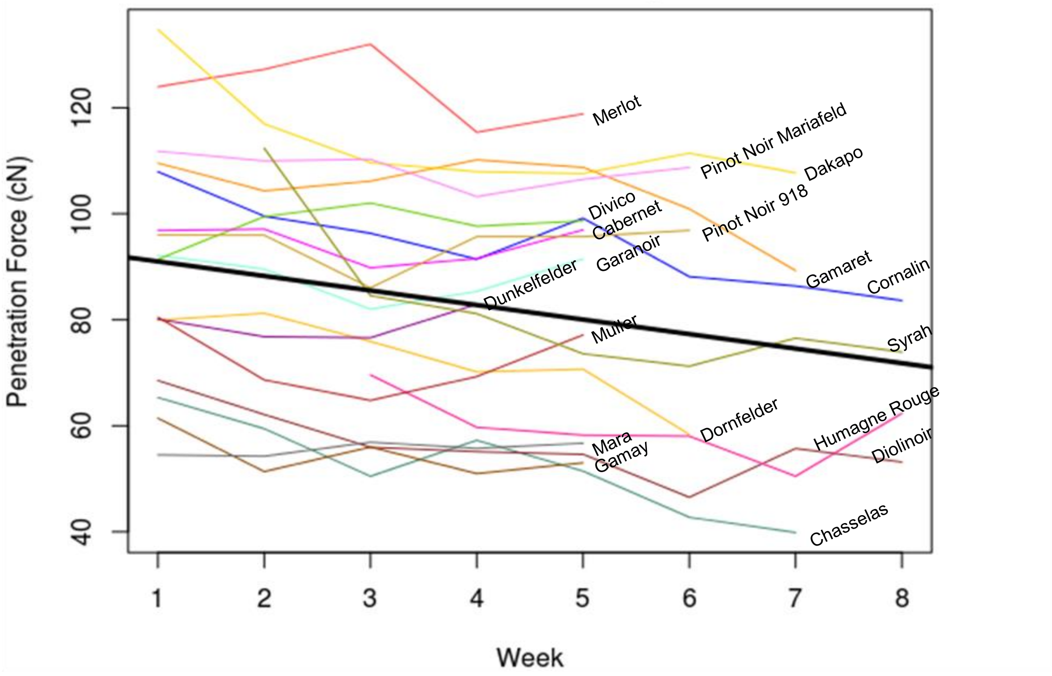

Figure 3. Penetration force by week for all cultivars studied at the Pully vineyard in 2017.

Relationships between penetration force and other grape properties

Over all cultivars penetration force was significantly correlated with pH ($R = -0.29$, $P < 0.01$), total acidity ($R = 0.38$, $P < 0.01$), tartaric acidity ($R = 0.28$, $P < 0.01$), malic acidity ($R = 0.41$, $P < 0.01$), yeast assimilable nitrogen ($R = 0.25$, $P < 0.01$), ammonia ($R = 0.20$, $P < 0.05$), alpha amino nitrogen ($R = 0.23$, $P < 0.05$), and the seed color rating ($R = -0.27$, $P < 0.01$). However, the relationships that were seen when all cultivars were looked at together did not continue when the cultivars were analysed individually. Days until harvest was significantly correlated with penetration force for Chasselas, Cornalin, Diolinoir, Dornfelder, and Syrah. Brix was significantly correlated with penetration force for Chasselas, Cornalin, Diolinoir, Merlot, and Syrah. pH was significantly correlated with penetration force for Chasselas, Cornalin, Diolinoir, Merlot, and Syrah. Total acidity was significantly correlated with penetration force for Chasselas, Cornalin, Diolinoir, Merlot, and Syrah. Tartaric acidity was significantly correlated with penetration force for Cornalin, Diolinoir, Dornfelder, and Merlot. Malic acidity was significantly correlated with penetration force for Chasselas, Cornalin, Diolinoir, Merlot, and Syrah. Yeast assimilable nitrogen was significantly correlated with penetration force for Merlot. Ammonia was significantly correlated with penetration force for Merlot. Alpha amino nitrogen was significantly correlated with penetration force for Dornfelder. Seed color rating was significantly correlated with penetration force for Chasselas, Cornalin, Diolinoir, Divico, and Merlot. Similar to the correlations with infestation, correlations with penetration force were dominated by a few particular cultivars. Chasselas, Cornalin, Diolinoir, Merlot, and Syrah were responsible for 33 of the 37 significant correlations between penetration force and the berry's properties. Not surprisingly, these five cultivars were among those that saw their penetration forces decrease at a relatively linear slope throughout the growing season. From these five cultivars, Brix, pH, total acidity, and malic acidity were the properties that consistently significant. At this point all that can be said with the data is that cultivars when looked at as a whole have a higher predictive ability than when examined separately. To test if there might be a combination of factors that can lead to a more accurate prediction of penetration force two-way stepwise regressions were run on the data. The best model provided a R2 value of 0.4276 and a p-value of 2.51×10^{-9} and was based on the variables Brix, pH, total acidity, tartaric acidity, malic acidity, yeast assimilable nitrogen, alpha amino nitrogen, and days until harvest. However, it remains unclear how helpful such a model would be for the average winegrower. Thus, we are unable to uncover a meaningful relationship that would allow us to accurately predict penetration force and even if we were able it is unclear on how helpful it would ultimately be.

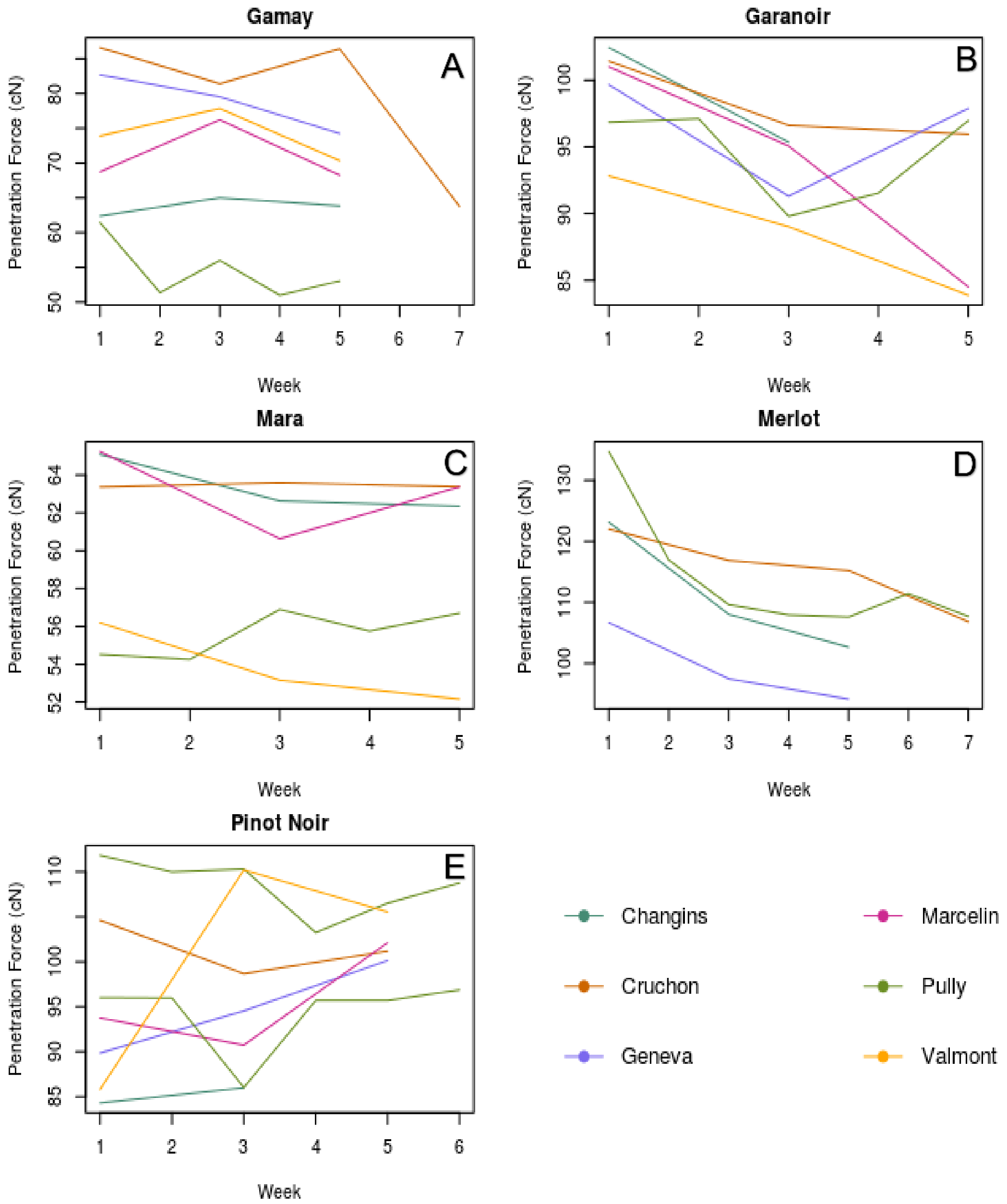

Figure 4. The progression of penetration force by week for the six vineyard sites of a) Gamay b) Garanoir c) Mara d) Merlot e) Pinot Noir in 2017.

Relationships with penetration force across sites

Displaying the progression of penetration force by week for each cultivar at the six sites shows that, for the most part, penetration forces did not progress similarly either across cultivars or within vineyards (Figure 4). Of the cultivars, Mara and Merlot seem to have the most consistent slopes between sites. However, both cultivars have wide variation in starting points between the different sites. Gamay and Garanoir are roughly similar to one another in that their vineyards follow similar progressions of penetration force throughout the season with a few individual samples that do not follow the pattern. Pinot Noir had the least similar behavior between vineyard sites and actually had five of its seven samples increase in penetration force throughout the growing season. Sites had a maximum difference of around 25 cN. However, the samples all converged towards a common penetration force by week, with a final range of about 10 cN. One might attribute these differences to there being different clones of some of these varieties. Pinot Noir and Gamay for instance are usually selected down to be clonally specific. However, the recently breed cultivars Garanoir and Mara were not any more consistent across sites. Therefore, one is unable to say that the site differences seen were due to clones rather than location. In conclusion, the sites behave so differently from one another throughout the cultivars that one can have little confidence that penetration force will progress similarly in different locations. The relationships with penetration force and other grape properties that do exist in particular vineyards can in consequent not be applied broadly.

Conclusions

Similar to previous studies infestation rate was correlated with maturity, time or days until harvest, Brix, total acidity, and penetration force (Lee et al., 2011; Ioriatti et al., 2015). This study also determined that tartaric acidity, malic acidity, and the seed color rating were significantly correlated with infestation rate as well. When cultivars were examined individually these relationships were not as evident. Tartaric acidity was the most represented property for significant relationships within the cultivars, but even it was only significant in just half of those that saw infestation. From this data, we can only confirm the general conclusion of Ioriatti et al. (2015) that cultivars with lower penetration force are more likely to be infested than those with higher penetration force. However, the 40 cN threshold of grape susceptibility to *D. suzukii* infestation was not supported by this research. Cultivars saw infestation at a range of penetration force measurements from 46 to 108 cN. Our research therefore indicates that the penetration force of grape skin cannot be considered an absolute driver for whether a particular grape is at risk or not. It is together with vineyard location, climate, cultural practices and the phytosanitary condition of the berries only one of many factors that have to be considered. There was less success in correlating penetration force with any of the other properties of the berries. Previous studies had found correlations between time and Brix with penetration force (Ioriatti et al., 2015). When all cultivars were examined together, there was not a significant correlation with either of these two properties and penetration force. However, five of the 17 cultivars/clones tracked did have significant correlation between these properties when examined individually, but on the same time, ten of the 17 cultivars had zero correlations for any property with penetration force. With the inability to correlate any property with penetration force, and the fact that berries were infested over such a large range of penetration forces, it became impossible to build any useful model from this data that might predict the risk of infestation by *D. suzukii*. Thus, there does not seem to be an exact time that a cultivar switches from not being susceptible to being susceptible. Rather, it is more like a gradual scale based on probabilities. Thus, the idea of tracking the penetration force throughout the season as a mean to monitor threat levels of infestation is not very useful. Moreover, cultivars behaved quite different according to their location. These differences made it impossible to apply the progression of penetration force throughout the season from one vineyard to another and cultivars at different sites experienced differences in penetration force of up to 35 cN from samples collected the same date. Thus, we conclude that cultivars cannot be expected to behave similarly when compared at different vineyard sites, at least for their progressions of penetration force and its relationship with other properties. Thus, it is first nearly impossible to predict penetration force within a particular vineyard by relating it with other grape properties and second such attempts seem to be site specific and are not generalizable.

Acknowledgements

The authors are grateful to the winegrowers who participated in the study. This work received financial support from the Federal Office for Agriculture FOAG within the framework of the Taskforce *Drosophila suzukii*.

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