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Efficacy of lime treatments against *Drosophila suzukii* in Swiss berries

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Abstract: *Drosophila suzukii* is an invasive pest that lays eggs in healthy fruits with a serrated ovipositor, resulting in considerable economic losses, mainly in berry crops. It was first recorded in Switzerland in 2011, causing considerable damage in all small fruit crops, especially in late-developing crops (autumn raspberries, blackberries, blueberries and elderberries). Control methods have been implemented to minimize the use of insecticides. Greenhouse trials were conducted in 2015 to test lime (calcium hydroxide) treatments efficiency as a substitute or complement to insecticide treatments. Fourteen blueberry bushes were individually placed in insect-proof cages: seven of them were treated weekly with a solution of lime, while seven others did not receive any treatment. In each cage, 10 *D. suzukii* were released per week. Ten blueberry fruits per bush were harvested each week, and the number of larvae and eggs per fruit was determined for each cage. The pH, strongly suspected to contribute to the efficiency of lime against *D. suzukii*, has been measured in 2015 on the treated and non-treated strawberry epidermis during three days. The semi-field test on blueberry showed that after several treatments, lime significantly reduced the number of *D. suzukii* eggs in fruits. The pH was consistently higher in treated strawberries than in non-treated strawberries. Our greenhouse trials showed the potential efficiency of lime treatments. However, they need to be repeated on-farm in order to provide producers with an efficient management strategy (treatments, mass trapping, nets, hygiene), ensuring a high quality and residue-free Swiss berry production.

Key words: spotted-wing drosophila, integrated control, calcium hydroxide, small fruit

Introduction

As a consequence of the dramatic expansion of the international food trade, numerous exotic insects have been unintonentially introduced in many regions, causing severe damage to the agro-ecosystems (Bacon *et al.*, 2012). Native to Southern Asia, *Drosophila suzukii* (Diptera: Drosophilidae), also known as spotted wing drosophila, represents a considerable danger with its capacity to lays eggs in healthy, ripening fruits via a serrated ovipositor, unlike other drosophilae that can only lay eggs in rotten, fallen fruits (Cini *et al.*, 2014; Depra *et al.*, 2014; Hamby *et al.*, 2016; Hauser, 2011; Hauser *et al.*, 2009; Walsh *et al.*, 2011). The fruit is damaged by the larvae feeding on the flesh and causing wilting of the tissues (Baroffio *et al.*, 2012; Bolda *et al.*, 2010; Hauser *et al.*, 2009; Walton *et al.*, 2010). The wound is an entry door for secondary infections by fungi, bacteria and other drosophilidae (Baroffio & Fischer, 2011; Kehrli *et al.*, 2012). *Drosophila suzukii* is highly polyphagous, developing in the fruits of various cultivated and wild plants, with a preference for soft skin fruits (Kanzawa, 1939; Walsh *et al.*, 2011). In recent years, *D. suzukii* rapidly expanded its distribution area via the food trade. In autumn 2008, it appeared almost simultaneously in both California (Santa Cruz) (Hauser *et al.*, 2009) and Spain (Calabria *et al.*, 2012), spreading later on to most temperate areas in North America and Europe (Cini *et al.*, 2012). In Switzerland, *D. suzukii* was first detected in July 2011 (Baroffio & Fischer, 2011). It has now become a widespread pest for stone and small fruit in all countries where it has been introduced, especially for late maturing crops . Significant crop losses can result from *D. suzukii* invasion. In the U.S., they were estimated at $ 500 million for three economically important States in terms of fruit production in 2008 (Bolda *et al.*, 2010). For the same year, crop losses ranging from 30 to 40% have been observed on blueberry, blackberry and raspberry in Italy, and up to 80% on strawberry in France (Lee *et al.*, 2011). Economical consequences caused by the spotted wing drosophila are not limited to crop losses: increasing production costs and potential market losses might be expected, as well as additional time, money and workforce in order to correctly apply control methods against *D. suzukii* .

Most invaded countries have established an integrated pest management (IPM) strategy in order to control *D. suzukii* (Asplen *et al.*, 2015). Switzerland has its own functional approach based on a national monitoring and control strategy which combines hygienic measures, mass trapping and, as a last resort, insecticide applications (Baroffio *et al.*, 2015). The Swiss IPM strategy has to be improved, because it does not guarantee sufficient protection of crops in critical situations. Research on alternative cultural practices is ongoing, with the objective of acquiring a better knowledge and consequently of improving the common control methods. As a complement to the current strategy, new methods are being tested and combined to develop a holistic approach to protect small fruit against *D. suzukii*. This study mainly focuses on lime treatment, which seems to be effective against *D. suzukii*.

Material and methods

Plant material

The fruits were sampled from the Swiss centre for agricultural research Agroscope in Conthey (canton of Valais, Switzerland). The blueberry bushes of the variety ‘Liberty’ were produced soilless in black-round plant pots (45 l). The strawberries of the variety ‘Joly’ were cultivated in soilless system under tunnel since 2015.

Insect rearing

Drosphila suzukii individuals were obtained from a laboratory colony (Agroscope in Changins, canton of Vaud, Switzerland) established from a mixture of strains from France and from locally collected wild insects. The insects were kept in a climate chamber at 23 °C, 70% r.h. and with a 16:8 (light: dark) photoperiod. The insects were placed in 1300 cm^3 plastic containers, closed with a fine-meshed tulle lid. The bottom was replaced with a 0.5 × 0.5 mm grid and placed on a banana-based diet-cube. With this setup, the adults can easily feed and lay eggs on the diet cube which can subsequently be replaced without directly manipulating the insects. The nutrient medium is then placed in a 200 cl plastic cup under the same rearing conditions.

Lime and metallic colloidal

Nekapur®2 hydrated lime (calcium hydroxide; Kalkfabrik Netstal AG KFN, Switzerland) at a concentration of 1.8 kg/ha in 1000 l/ha of water was completed with 0.2 l/ha of foliar fertilizer Cuprum (2% copper, 0.02% molybdenum; PlantoSys GmbH). The products were added to the tank that had been previously filled with water, and the pH of the solution was measured with a portable pH-meter Seven2Go^{TM} (Mettler Todedo). The solution was sprayed onto fruits in the evening or in early morning with a knapsack sprayer hobby star, 5 liter and 3 bar.

Lime - metallic colloidal treatment in semi-field on blueberries

Fourteen blueberry bushes were individually placed in insect-proof cages: seven of them were treated weekly with the solution of lime and metallic colloidal, while seven others did not receive any treatment. The cages were 1.5 × 1.5 × 3 m and were hanged on the greenhouse structure by the use of straps. The mesh wall size was 300 × 200 µ and one side had a plastic zipper door. The drip irrigation system passed through the cage by a tulle sleeve, which prevented the insects from escaping.

In each cage, 10 *D. suzukii* (5 males and 5 females) were addded weekly, one day after the lime application. One week later, just before the application of the treatment on half of the blueberry bushes, 10 fruits per plant were collected. The fruits were then individually observed under a binocular magnifier, and larvae and eggs number per fruit was counted for each modality. This was repeated weekly during 4 weeks.

pH evolution of strawberries epidermis

One strawberry line was treated with the solution of lime and metallic colloidal, while the other did not receive any treatment. The application was made once in the morning. 18 fruits were selected per modality and those same 36 fruits were used throughout the test. The pH of the 18 fruits per modality was measured in the afternoon during three days with the portable pH-meter Seven2Go^{TM} (Mettler Todedo). Before the measurement, one drop of demineralised water was applied on each fruit.

Statistical analysis

Datasets were analysed for each week with a non-parametric test with XLSTAT. For the semi-field study on blueberries, the number of eggs and larvae per plant and per week were analysed with the non-parametric Kruskal-Wallis test of ranks with a 95% confidence interval. The sum of infested fruits per plant was compared (and not the 10 fruits individually) between the seven repetitions and the two modalities (treated and non treated). For the study on the pH evolution of strawberries epidermis, the pH of treated fruits was compared with the pH of non treated fruits for each day with the non-parametric Kruskal-Wallis test of ranks with a 95% confidence interval.

Results and discussion

Lime - metallic colloidal treatment in semi-field on blueberries

As the datasets were heterogenous, the medians were used to compute the tests. Table 1 gives the median value of *D. suzukii* egg number per date for both modalities (treated and non-treated), the p-values of Kruskal-Wallis tests per date as well as for the total study period. For both modalities given in Table 1, no significant difference in median egg number was observed for the trial period within the same modality. Per date, the two modalities were statistically different for the week of the 13.08.2015 and 20.08.2015. For both dates, the median egg number was systematically higher in non-treated fruits. The median egg number found in the treated fruits has gradually decreased until becoming inexistent at the end of the study. For the non-treated blueberries, the median egg number was higher.

Table 1. Median value of *D. suzukii* egg number per date and modality, with p-value of Kruskal-Wallis tests for the egg number per date and for the total study period.

Date	**Treated: median egg number**	**Non-treated: median egg number**	**p-value**
29.07.2015	7	9	0.400
05.08.2015	2	4	0.243
13.08.2015	1	10	0.024
20.08.2015	0	2	0.023
p-value	0.093	0.874	-

Table 2 gives the median value of *D. suzukii* larvae number per date for both modalities (treated and non-treated), the p-values of Kruskal-Wallis tests per date as well as for the total study period. The two modalities were never statistically different at a given date. On the whole trial period, the median larvae number was however statistically different, and was systematically higher in non-treated fruits than in treated fruits where it remained low and even null for the first and last dates. Larger number of larvae were found for the non-treated modality.

Table 2. Median value of *D. suzukii* larvae number per date and modality, with p-value of Kruskal-Wallis tests for the larvae number per date and for the total study period.

Date	**Treated: median larvae number**	**Non-treated: median larvae number**	**p-value**
29.07.2015	0	1	0.087
05.08.2015	2	7	0.274
13.08.2015	1	0	0.237
20.08.2015	0	7	0.062
p-value	0.063	0.039	-

Table 3 gives the median value of *D. suzukii* individual (eggs and larvae) number per date, the p-values of Kruskal-Wallis tests per date as well as for the total study period. For the first three periods, the median individual number was not statistically different within the same modality. For the fourth period (20.08.2015), with a p-value of 0.034 at a 95% confidence interval, both modalities were statistically different, the median individual number being systematically higher for the non-treated modality. Between modalities, the median individual (eggs and larvae) number was substantially larger for the non-treated than for the treated fruits. The median individual number for the treated modality slightly increased until the 05.08.2015 and then dramatically dropped, while for the non-treated modality, it remained important and constant.

Table 3. Median value of *D. suzukii* egg and larvae number per date and per modality, with p-value of Kruskal-Wallis tests for the individual number per date and for the total study period.

Date	Treated: median larvae and egg number	Non-treated: median larvae and egg number	p-value
29.07.2015	7	16	0.272
05.08.2015	8	12	0.275
13.08.2015	2	11	0.063
20.08.2015	0	9	0.034
p-value	0.134	0.968	-

Table 1 and 3 show that after several applications, the lime treatment was able to significantly control *D. suzukii* on blueberries. It seemed to be more efficient against eggs (Table 1) than larvae (Table 2). The lime applied on the fruit, may deposit on the respiratory filaments of the eggs and cause their suffocation. The larvae living in the berry are thus less exposed. This observation supports laboratory test results whereby the relationship between emerging adults and the number of eggs laid was lower for treated blueberries than for untreated blueberries (Fischer, 2015). Indeed, the Kruskal-Wallis values given in Table 1 shows a significant difference between the two treatments. The efficiency of lime application during the first week is debatable, because treated plants could be influenced by another factor than lime. It would have been appropriate to make a first fruit sampling one week after adding *D. suzukii* but without any lime application. The possibility of another factor influencing the results would have been rejected.

pH evolution of strawberries epidermis

The pH evolution of strawberries that were treated or not with lime was significantly different for both modalities (Figure 1). The pH of the treated strawberries was always higher than the pH of the untreated fruits, but the difference between these two modalities progressively decreased over time: for the first measure the pH of the treated strawberries was 46% higher than for the untreated strawberries, while for the last measure it came down to 23%. The pH of the treated strawberries for the first measure (06.10.2015) was significantly different from the other two measures, while no difference was statistically evidenced for the untreated strawberries. The efficiency of the treatment is evidenced by the pH of treated fruits being always statistically higher than the pH of untreated strawberries. This could be explained by the modification of the environment surrounding the fruit surface by the lime application, resulting in the reduction of the fruit's attractiveness or the repulsive effect for the drosophila. The duration of the effect increased over time, because the further from the application date it was, the higher the pH difference between the treated and untreated was. This observation confirms the comments of Gerber (2015) whereby the calcium hydroxide is quickly reduced by the CO_2 of the air. This reduction was however not as quick as it was assumed, because after three days the difference was still perceivable. It would therefore have been interesting to continue the test until the pH of the treated strawberries was equal to the pH of the untreated fruits. This would have enabled to determine the efficiency time and to adapt the application intervals for the lime treatment accordingly. Although the measuring accuracy was questionable, due to the addition of demineralised water which modified the pH, a difference was nevertheless observable for the whole study. Considering that the method was similar for all measurements, its lack of accuracy was identical for all fruits. Therefore, we can conclude that the observed difference was due to the applied treatment.

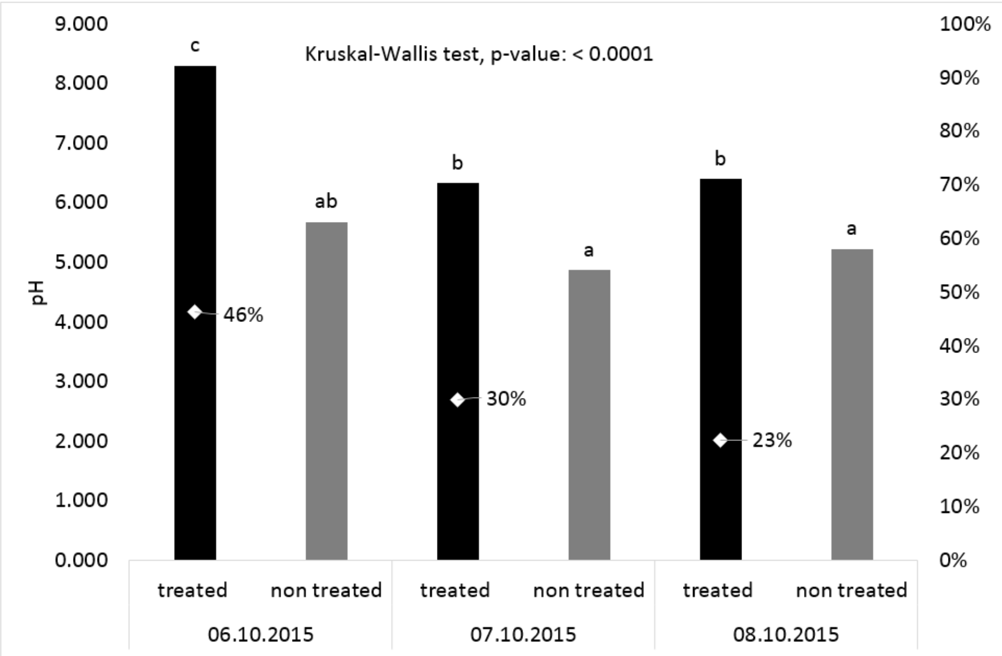

Figure 1. pH evolution of strawberries that were treated or not with lime, and relative pH increase percentage from treated strawberries compared to non-treated strawberries

Conclusion

The trial in semi field on blueberries confirmed that lime-metallic colloidal treatments reduce infestation rate after several applications. The contamination of non-treated blueberries was significantly higher than for the treated blueberries. Such treatment has to be tested on farm and, if the results are positive, lime treatment might be an effective method complementing the control strategy of *D. suzukii*.

The pH of treated fruits was systematically higher for the treated strawberries, but other mechanisms might play a role in the repulsive effect of lime against *D. suzukii*. This trial should be combined with a trial in semi-field in order to determine if the increase of pH does really have an effect on *D. suzukii* behaviour. This would make it possible to verify if, when the pH of the treated fruits becomes similar to the one of non-treated fruits, *D. suzukii* infestations increase.

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