Fruit Growing Research, Vol. XXXIX, 2023 DOI 10.33045/fgr.v39.2023.15
https://publications.icdp.ro/index.php

PRUNUL TRANSGENIC HONEYSWEET ARE EFECT NEUTRAL ASUPRA DIVERSITĂȚII ȘI DINAMICII POPULAȚIILOR DE AFIDE ÎN CONDIȚII DE CLIMAT TEMPERAT- CONTINENTAL

HONEYSWEET TRANSGENIC PLUM HAS A NEUTRAL EFFECT ON DIVERSITY AND DYNAMICS APHID POPULATION UNDER TEMPERATE CONTINENTAL CLIMATE CONDITIONS

Zagrai Ioan, Zagrai Luminiţa Antonela, Guzu Georgeta Maria
Research Station for Fruit Growing Bistrița, Romania
Corresponding author:Zagrai Luminița Antonela; e-mail: luminita.zagrai@scdp-bistrita.ro

Abstract

'HoneySweet' is a genetically engineered plum resistant to *Plum pox virus*. Potential risks such as its impact on the diversity and dynamics of indigenous aphid populations was assessed. The study compared winged population of aphids visiting transgenic and conventional plums under continental temperate climatic conditions of Romania. The experimental analysis was conducted during three years (2016-2018) within an experimental plot containing transgenic ('HoneySweet') and conventional ('Reine Claude d'Althan' and 'Stanley') plums. This study revealed a similarity of both flight curves and the number and species of aphids landing on the two types of plum. Overall results showed no statistically differences between aphid species visiting the three cultivars, respectively the two types of plum. Consequently, our results support the hypothesis of the neutral effect of the transgenic plum on indigenous populations of aphids.

Cuvinte cheie: virusul *Plum pox*, prun transgenic, risc, afide.
Key words: *Plum pox* virus, transgenic plum, risk, aphids.

1. Introduction

It is well known that systemic pathogens such as viruses may strongly affect pome and stone fruits (Hadidi et. al., 2011). *Prunus domestica* L., commonly known as European plum, is among the most valued fruit-bearing species worldwide. The plum is the dominant fruit species in Romania ranking the second place after China in the top of major plum growing countries (FAOSTAT 2021 data base). This means that Romania has paid special attention to this specie, which has gained great importance in the national agricultural economy. Unfortunately, plum production is seriously affected by infections with Plum pox virus (PPV), the causal agent of Sharka disease. PPV has agronomic and political consequences because it causes serious economic losses resulting from severe yield declines (Cambra et al., 2006). Due to its economic impact, PPV infection is considered one of the main limiting factor for the profitability of stone fruits crops in many European countries (Dunez and Sutic, 1988; Cambra et al., 2006; Kamenova et al. 2010; Barba et al., 2011), including Romania (Toma et al., 1998; Zagrai I. et al., 2010a), where PPV is endemic, and can drastically affects the yield and quality of susceptible plum cultivars (Minoiu, 1997; Macovei and Diaconu, 2001).

Described for the first time in 1917, in Bulgaria (Atanasoff, 1932), PPV has progressively spread around the world, excepted Australia, New Zeeland and South Africa (García and Cambra 2007; Barba et al., 2011; EPPO, 2019). A large-scale survey performed in Romania revealed that PPV is widespread in all plum orchards with an average rate of 69%, making the country as an endemic one where plum production is largely compromised (Zagrai I. et al., 2010b). Therefore, an adequate strategy for Sharka containment is strongly required.

PPV is naturally spread by aphids in a non-persistent manner (Labonne et al., 1995) making thus its containment extremely difficult in endemic countries. Although control of insect vectors is essential for control of some systemic pests, however controlling PPV by controlling its vectors is less feasible (Candresse, cited by Hadidi et al., 2011). Therefore, breeders had focused their efforts to increase resistance in plants as the most efficient strategy against PPV (Ravelonandro et al., 2011; Scorza et. al., 2013). Although a lot of works were done for a long term to identify resistance gene and their using in conventional breeding, the scarcity of such trait in *Prunus domestica* L. often led to get only tolerant cultivars, which rather decreased the economically impact of PPV infection, but allowed the virus to proliferates and spreads. It should be noted the breeding works directed towards hypersensitivity as a means of resistance that permitted the obtaining hypersensitive cultivars such as 'Jojo' (Hartmann and Neumüller, 2006) which reacts to PPV inoculation with a hypersensitive response. However, while such genotypes appeared to have exhibited a high level of resistance to PPV in the field there are reports showing that the resistance may be virus strain/isolate-dependent (Polák and Jarošová, 2011, 2012; Rodamilans et al., 2023). Nevertheless, hypersensitivity represents a mechanism that requires continued research attention (Scorza et al., 2013). However, progress in conventional breeding for resistance is slowed down by the long generation time and the polygenic nature of resistance in the most cases (Barba et al., 2011).

As an alternative or complementary approach to conventional breeding, pathogen derived resistance (PDR) approach (Sanford and Johnson, 1985) was used for obtaining virus-resistant plum. Thus, the first genetically engineered plum resistant to PPV, clone C5, subsequently named 'HoneySweet' was developed (Scorza et al., 1994) and presently is well known as an efficient tool to get high level resistance to *Plum pox* virus (PPV) (Ravelonandro et al., 1997; Malinowski et al., 2006; Zagrai et al., 2011a; Scorza et al., 2013).

As in any GMO cases, potential environmental safety issues have been expressed with the field release of 'HoneySweet'. The concerns include: i). Recombination events between transgene transcripts and infecting RNA that could led to the emergence of PPV recombinants. Two experiments performed in different environments (Mediterranean and continental) revealed that there is no detectable effect on the emergence of recombinant PPV species over ten years (Capote et al., 2008; Zagrai et al., 2011b); ii).Transgene flow by pollen from transgenic plum to wild relatives that could express undesirable traits such as weediness. An USDA assessment revealed that PPV resistance in 'HoneySweet' and its progeny will not impart weediness (USDA/APHIS - Federal Register Doc. E7-13649, July 12, 2007); iii). Potential impact on the non-target organism, such as its impact on indigenous aphid populations and bees. Experimental field trial performed in Spain, showed a neutral impact of 'HoneySweet' on aphid populations under Mediterranean conditions (Capote et al., 2008).

The objective of the present investigation was to assess the potential impact of 'HoneySweet' transgenic plum on the diversity and dynamics of indigenous aphid populations under temperate continental conditions, such as the Northern Romania.

2. Material and methods

Experimental orchard

A field trial (1200 m^2 size of the site plot) including 'HoneySweet' transgenic and two conventional plum cultivars ('Stanley' and 'Reine Claude d'Althan') grafted onto Myrobalan BN 4Kr seedling rootstocks was esthablished on the spring of 2013 at Fruit Research & Development Station Bistrița, Romania. The deliberate release into the environment of 'HoneySweet' transgenic plum was made under appropiate authorization provided by the Romanian Ministry of Environment (Authorisation no. 1/1032/GA/16.05.2012, European no. B/RO/11/01). The experimental plot design consisted in 12 blocks of 4 trees (two 'HoneySweet' + two conventional – one tree of two different cultivars) interspersed with *P. cerasifera* (4 plants) and *P. spinosa* (4 plants) for coexistence studies (not included in the current study). To minimize the pollen dispersal, the experimental plot was surrounded by non-transgenic border apple trees. The plot maintenance was similar as in conventional crops. The only difference was the reduced number of treatments with insecticides applied during the vegetative periods.

Winged aphids monitoring

Winged aphid populations visiting the experimental plum trees were monitored by using the sticky shoot method (Avinent et al., 1993) during the vegetative periods of 2016-2018. This method involves the spraying of selected shoots with an adhesive substance, and subsequently the counting the number of aphids captured in a time interval. Three shoots with similar length from one tree of each of three cultivars in the same block were sprayed with 'Soveurode' aerosol as glue. The blocks and the trees were changed every 10 days and new sticky shoots were initiated in these trees. Sticky shoots were collected at the end of each 10 days cycle. Traped winged aphids were removed by washing the collected shoots with turpentine. Aphids were preserved on 70% ethanol, and then counted and identified under a binocular microscope. The steps of "sticky shoot" method from orchard to laboratory are schematically shows in the figure 1.

The monitoring of the aphids landing on the plum trees was made starting from the first decade of May until the first decade of October, thus covering the entire vegetation period, three consecutive years. Data collected was used to draw up the dynamic and the flight curves of the aphids visiting the three plum tree cultivars, two conventional versus one transgenic respectively. Then a comparison of number and species of aphids landing on conventional versus transgenic trees was made.

Statistical analyses

The veracity of the results was statistically verified by using XLSTAT program (Addinsoft, New York, USA), ANOVA modelling data, Duncan multiple range test, 95% confidence interval, in order to evaluate differences in aphid's preference regarding to cultivar.

3. Results and discussions

Number of aphids collected from selected shoots

The results presented in the table 1 showed that the numbers of aphids captured from selected shoots vary mainly among years depending of specific climate conditions of each year. Thus, the number of winged aphids captured in 2016 was very similar on 'HoneySweet' (147 aphids) and 'Stanley' (145 aphids), and slightly higher on 'Reine Claude d'Althan' (162 aphids). In 2017, the number of winged aphids captured was very similar to all the three cultivars (144 aphids on 'HoneySweet', 138 on 'Reine Claude d'Althan', 149 aphids on 'Stanley'). A higher number of aphids were collected to all the three cultivars in 2018 (176 aphids on 'HoneySweet', 171 on 'Reine Claude d'Althan', 208 aphids on 'Stanley'). Thus, a very similar number of aphids landed on 'HoneySweet' and 'Reine Claude d'Althan', and slightly higher number on 'Stanley'.

Results revealed that there were three different situation when compared transgenic and conventional plum: i) transgenic and both conventinal plums were visited by a similar number of aphids in 2016; ii) transgenic and one conventional ('Stanley') were visited by a similar number of aphids in 2017; iii) transgenic and the other conventional ('Reine Claude d'Althan') were visited by a similar number of aphids in 2018.

Regarding the number of aphids collected during June in the period of study (2016-2018), when most of the catches were recorded, a similarity was found to all three studied cultivars. Consequently, there are no specific rule in which the number of aphids collected favoured or disfavoured one or other of the studied cultivars, transgenic or conventional respectively.

The statistical approach confirms that there is no significant difference between aphids captured on transgenic and those on each conventional plum cultivars (Table 2). Analysing the total number of aphids collected during 2016-2018, related to cultivars, revealed a similarity in 'HoneySweet' and 'Reine Claude d'Althan', and a slightly higher number in 'Stanley', but with no significant differences between variants.

Aphid's flight curves

The flight curves drawn up based on the total number of aphids collected at ten days along May - September in the three consecutive years of 2016-2018, on the three plum cultivars, were very similar. Thus, aphids flight started at the beginning of May with a continual increase until the mid of June, when a maximum peak was recorded. The flight curves then had a continual decrease until the end of July, when the flight ended. No aphids were captured during August along the three consecutive years. The flight slowly resumed throughout September and a smaller peak was noted in the middle of the month. Data collected along the three vegetative periods of 2016-2018 revealed that the three flight curves overlaps, almost perfectly (Fig. 2). Although the climate changes in the last decade have been modified the biology of some pests, the aphids flight landing on the plum trees in Bistrița area recorded a similar flight curve, as was previously reported (Zagrai L. et al., 2010). Unlike to our results, under a Mediterranean climate, in a similar experimental plot, the maximum flight of aphids was recorded earlier, respectively in May (Capote et. al., 2008), explainable by the warmer time during May in Spain.

Aphid's species visiting the experimental orchard

Different aphid species were landed on experimental orchard containing transgenic and conventional plums. The most of aphid species captured in all three plum cultivars belongs to *Aphis* spp. (40%), followed by *Hyalopterus pruni* (19%). Other important aphid species captured were represented by *Rhopalosiphym* spp., *Myzus* spp., *Brachycaudus* spp., *Phorodon humuli and Anoecia* spp. (Fig. 3).

Aphid's species landing on transgenic versus conventional plum

All aphid species captured were uniformly distributed among the three plum cultivars. Also, a similar frequency of aphid species visiting the three plum cultivars, respectively a similar presence on transgenic and conventional trees, was determined (fig. 4). Thus, *Aphis* spp. was the most prevalent visitors to both transgenic and conventional plum trees, followed by *Hyalopterus pruni*. The other aphid species represented by *Rhopalosiphum* spp., *Myzus* spp., *Brachycaudus* spp., *Phorodon humuli and Anoecia* spp. were also captured from all the three cultivars with similar frequency.

Moreover, ANOVA (analysis of variance) and Duncan's Multiple Range Test revealed no significant differences between the aphid's species landing on transgenic and conventional plum cultivars (Table 3). Thus, no significant difference between the three cultivars of plum, neither between transgenic and any the two conventional, nor between the two cultivars of conventional plum. This means that aphids, no matter of species, do not have preference for one or other cultivar, regardless if is transgenic or conventional. Overall, flight curves revealed a high similarity with an almost perfect overlapping between aphid flight curves, regardless by cultivar. Also, results showed that different aphid's species visited transgenic and conventional plums too, with similar frequency. Moreover, this similarity was supported by statistical analysis, meaning that there is no aphid preference for a particular plum cultivar, regardless of whether it is transgenic or conventional. Our results are consistent with those reported by Capote et al, (2008) under Mediterranean climate in Spain showing that the aphid species have no preference for transgenic or non-transgenic character of plum trees.

4. Conclusions

There is no aphid preference for a particular plum cultivar, regardless of whether it is transgenic or conventional.

'HoneySweet' transgenic plum does not affect the diversity and dynamics of native aphid populations.

References

1. Atanasoff D., 1932. Plum pox. A new virus disease. Yearbook University of Sofia, Faculty of Agriculture and Silviculture 11: 49-69.
2. Avinent L., Hermoso de Mendoza A., Llácer G., 1993. Comparision of sampling methods to evaluate aphid populations (Homoptera Aphidinae) alighting on apricot trees. Agronomie 13: 609-613.
3. Barba M., Hadidi A., Candresse T., Cambra M., 2011. Plum pox virus. In: Hadidi A., Barba M., Candresse T., Jelkmann W. (eds) Virus and Virus-like Disease of Pome and Stone Fruits. APS Press, pp. 185-197.
4. Cambra M., Capote N., Myrta A. and Llácer G., 2006. Plum pox virus and the estimated costs associated with sharka disease. Bull OEPP/EPPO Bull. 36: 202- 204.
5. Capote N., Pérez-Panádes J., Monzó C., Carbonell E.A., Urbaneja A., Scorza R., Ravelonandro M., Cambra M., 2008. Assessment of the diversity of Plum pox virus and aphid populations on transgenic European plums under Mediterranean conditions. Transgenic Res 17: 367-377.
6. Dunez J. and Sutic D., 1988. Plum pox virus. In: European handbook of plant disease, Eds. I.M.Smith, J. Dunez, R.A.Eliot, D.H.Phillips, S.A.Arches. Blackwell, London, UK: 44-46.
7. EPPO 2019. https://gd.eppo.int/taxon/PPV000/datasheet.
8. Food and Agriculture Organization of the United Nations (FAOSTAT, 2021). https://www.fao.org/faostat/en/#data.
9. García J.A. and Cambra M., 2007. Plum pox virus and sharka disease. Plant Viruses 1, 69–79.
10. Hadidi A., Pasquini G., Barba M., Spiegel S., Zaidi A., Crescenzi A., 2011. Strategies for control of systemic pathogens of fruit trees – an overview. In: Hadidi A., Barba M., Candresse T., Jelkmann W. (eds) Virus and Virus-like Disease of Pome and Stone Fruits. APS Press, pp. 373-375.
11. Hartmann W. and Neumuller M., 2006. Breeding for resistance: breeding for Plum pox virus resistant plums (*Prunus domestica* L) in Germany. OEPP/EPPO Bulletin 36: 332-336.
12. Kamenova I., Scorza R., Ravelonandro M., Callahan A., Paunovic S., Zagrai I., Dorokhov D., Blume Y., 2010. Case study: Reducing the harmful impacts of Plum pox virus through the use of biotechnology. In: Golikov A and Atanassov A (eds) Regional consensus documents on environmental risk and economic assessment of genetically modified crops. Black Sea Biotechnology Association, Infoprint, Pleven 2010: 127-151.
13. Labonne G., Yvon M., Quiot J., Avinent L., Llácer G., 1995. Aphids as potential vectors of Plum pox virus: comparison of methods of testing and epidemiological consequences. Acta Hortic. 386: 207-218.
14. Macovei A. and Diaconu M., 2001. Viro-geographical data concerning the actual spreading status of the plum pox potyvirus in Romania. Middle Eur. Meet. Plum Pox, Pitesti-Maracineni.
15. Malinovski T., Cambra M., Capote N., Zawadzka B., Gorris M.T., Scorza R., Ravelonandro M., 2006. Field trials of plum clones transformed with the Plum pox virus coat protein (PPV-CP) Gene. Plant Disease 90 (8): 1012-1018.
16. Minoiu N., 1997. Bolile și dăunătorii prunului (Plum Diseases and Pests). In: Prunul (The Plum). Cociu I., Botu I., Minoiu N., Modoran I. (eds.) Editure Conphys, Pitesti, Romania. pp. 343-374.
17. Polák J. and Jarošová J., 2011. Hypersensitivity of *Prunus domestica* L. "Jojo" was changed by PPV-D strain into very high sensitivity. Acta Hortic. 899: 87-93.
18. Polák J. and Jarošová J., 2012. Susceptibility of plum trees cv. 'Jojo' to a Czech isolate of Plum pox virus strain D. Canadian Journal of Plant Pathology. 34(2): 283-287.
19. Ravelonandro M., Briard P., Hily J.M., 2013. RNAi to silence the Plum pox virus genome. Acta Hortic. 974: 165-174.
20. Ravelonandro M., Scorza R., Bachelier J.C., Labonne G., Lery L., Damsteegt V., Callahan A.M., Dunez J., 1997. Resistance of transgenic plums (Prunus domestica L.) to Plum pox virus infection. Plant Disease 81: 1231-1235.
21. Ravelonandro M., Scorza R. and Hammond W., 2011. Biotechnological aproaches for resistance to viruses, viroids, and phytoplasmas. In: Hadidi A., Barba M., Candresse T., Jelkmann W. (eds) Virus and Virus-like Disease of Pome and Stone Fruits. APS Press: 395-400.
22. Rodamilans B., Hadersdorfer J., Berki Z., García B., Neumüller M. and García J.A., 2023. The mechanism of resistance of European plum to Plum pox virus mediated by hypersensitive response is linked to viral NIa and its protease activity. Plants, 12: 1-14.

https://doi.org/10.3390/plants12081609
23. Sanford J.C. and Johnston S.A., 1985. The concept of parasite-derived resistance-deriving resistance genes from the parasite’s own genome. J Theor Biol 113: 395-405.
24. Scorza R., Callahan A., Dardick C., Ravelonandro M., Polak J., Malinowski T., Zagrai I., Cambra M., Kamenova V., 2013. Genetic engineering of Plum pox virus resistance - ‘HoneySweet’ plum - from concept to product. Plant Cell, Tissue and Organ Culture. 115: 1-12.
25. Scorza R., Ravelonandro M., Callahan A.M., Cordts J.M., Fuchs M., Dunez J., Gonsalves D., 1994. Transgenic plums (*Prunus domestica*) express the Plum pox virus coat protein gene. Plant Cell Repts 14: 18-22.
26. Toma S., Isac M., Balan V. and Ivascu A. 1998. Detection of Plum pox virus by enzyme-linked immunosorbent assay in some apricot and peach varieties and hybrids in Romania. Acta Virologica 42(4): 276-277.
27. Zagrai I., Zagrai L., Kelemen B., Petricele I., Pamfil D., Popescu O., Preda S., Briciu A., 2010a. Typing and distribution of Plum pox virus isolates in Romania. Julius-Kuhn-Archiv 427: 342-346.
28. Zagrai I., Zagrai L., Preda S., Isac M., Cardei E., 2010b. Incidence of Plum pox virus in Romanian plum orchards. Bulletin UASVM, Horticulture 67(1-2): 488. ISSN 1843-5254.
29. Zagrai L., Zagrai I., Festila A., 2010. Monitoring of Aphid Species Landing in Prunus Nursery Plot from Bistrita Area. Bulletin UASVM, Horticulture 67(1-2): 489. ISSN 1843-5254.
30. Zagrai I., Ravelonandro M., Zagrai L., Scorza R., Minoiu N., 2011a. Overview of the investigations of transgenic plums in Romania. Acta Hortic. 899: 153-158.
31. Zagrai I., Ravelonandro M., Gaboreanu I., Ferencz B., Scorza R., Zagrai L., Kelemen B., Pamfil D., Popescu O., 2011b. Transgenic plums expressing the Plum pox virus (PPV) coat protein gene do not assist the development of PPV recombinants under field conditions. Journal of Plant Pathology 93 (1): 159-165.

Tables and Figures

Fig.1. The steps of "sticky shoot" method from orchard to laboratory

Table 1. The number of aphids captured on transgenic and conventional plum (2016-2018)

Decade- month/ cultivar	HoneySweet			Total	Reine Claude d'Althan			Total	Stanley			Total
	2016	2017	2018		2016	2017	2018		2016	2017	2018	
I May	3	3	0	**6**	0	6	1	**7**	0	0	0	**0**
II May	9	3	9	**21**	12	6	6	**24**	3	9	9	**21**
III May	15	10	33	**58**	9	9	15	**33**	15	6	39	**60**
I June	18	22	33	**73**	27	17	30	**74**	24	27	36	**87**
II June	48	44	24	**116**	48	39	18	**105**	39	42	28	**109**
III June	33	24	18	**75**	45	26	30	**101**	33	27	36	**96**
I July	3	14	39	**56**	6	10	33	**49**	9	15	33	**57**
II July	6	13	6	**25**	3	13	21	**37**	9	10	9	**28**
III July	0	3	6	**9**	0	3	3	**6**	0	3	9	**12**
I August	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
II August	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
III August	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
I Sept.	2	1	2	**5**	1	1	2	**4**	3	1	1	**5**
II Sept.	5	4	5	**14**	7	3	8	**18**	4	5	6	**15**
III Sept.	4	2	1	**7**	4	3	3	**10**	4	3	2	**9**
I October	1	1	0	**2**	0	2	1	**3**	2	1	0	**3**
Total	**147**	**144**	**176**	**467**	**162**	**138**	**171**	**471**	**145**	**149**	**208**	**502**

Table 2. Summary of all pairwise comparisons of total number of aphids on cultivar (Duncan) (2016-2018)

Plum cultivars	2016	2017	2018	Average of captured winged aphids
HoneySweet	147	144	176	155.667[a]
Reine Claude d'Althan	162	138	171	157.000[a]
Stanley	145	149	208	167.333[a]
Pr>F(model)				0.825
Significant				NO

**Different letters indicate differences at p < 0.0001 according to Duncan`s Multiple Range Test.*

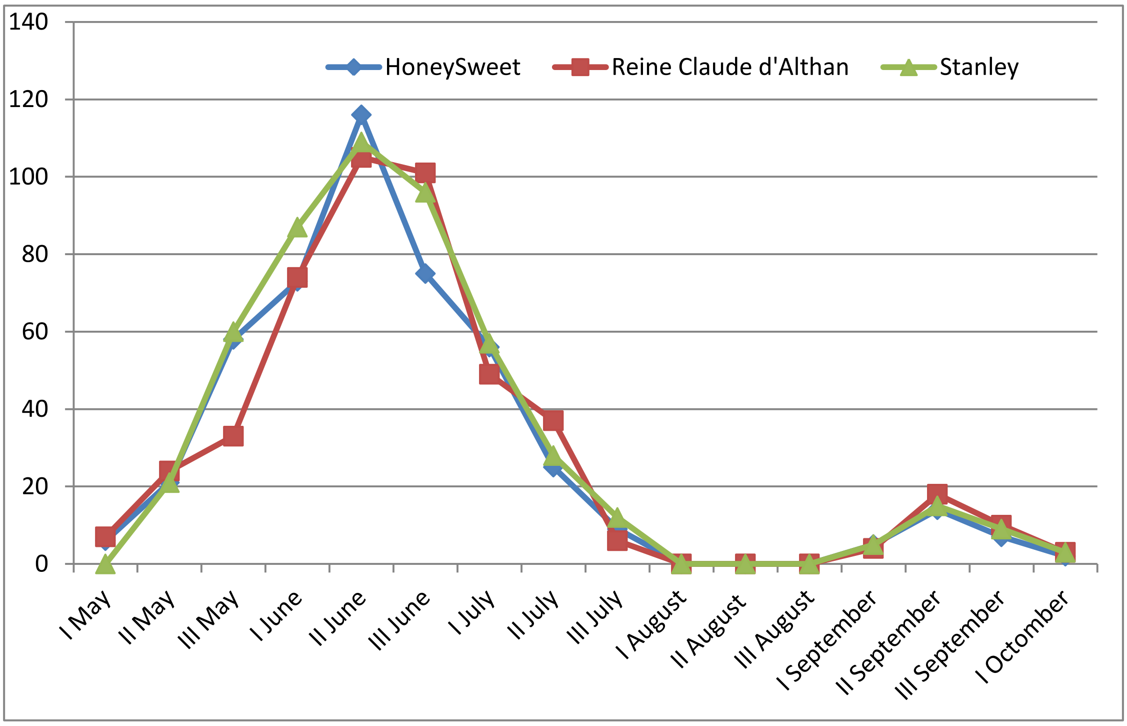

Fig. 2. Flight curves based on the number of aphids collected from control branches on transgenic and conventional plum cultivars (2016-2018)

DOI 10.33045/fgr.v39.2023.15

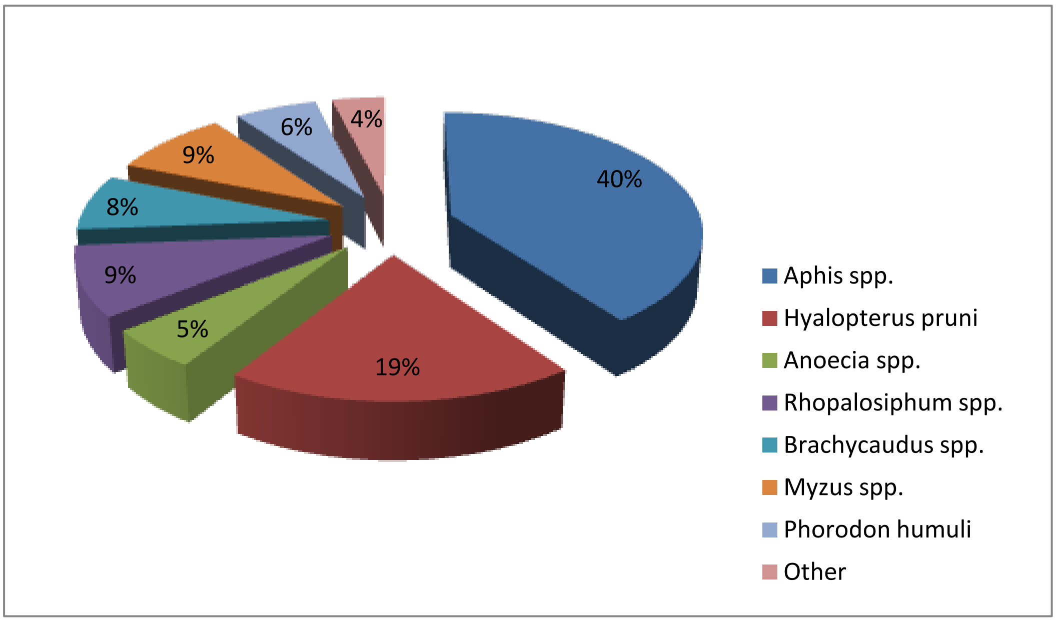

Fig. 3. The rate of aphid species captured in experimental plot containing transgenic and conventional plum cultivars during 2016-2018

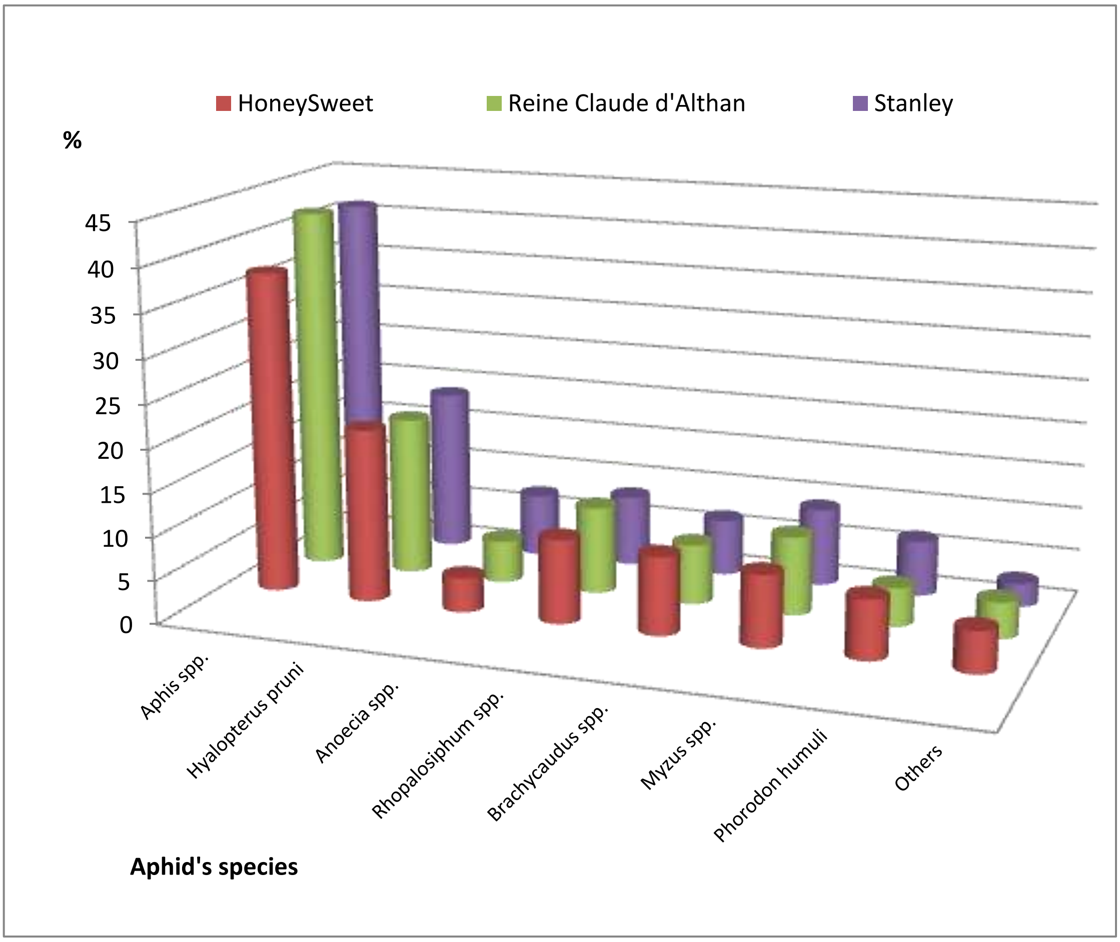

Fig. 4. The aphid species captured on transgenic and conventional plum cultivars (2016-2018)

Table 3. Summary of all pairwise comparisons for cultivar related to aphid's species captured on transgenic and conventional plum (Duncan) (2016-2018)

Aphid's species (%) / Plum cultivars	*Aphis* spp.	*Hyalopterus pruni*	*Anoecia* spp.	*Rhopalosiphum* spp.	*Brachycaudus* spp.	*Myzus* spp.	*Phorodon humuli*	Other aphids species
HoneySweet	37.133[a]	20.033[a]	4.000[a]	9.733[a]	9.033[a]	8.300[a]	6.933[a]	4.833[a]
Reine Claude d'Althan	41.967[a]	18.400[a]	4.867[a]	10.100[a]	6.900[a]	9.067[a]	4.533[a]	4.167[a]
Stanley	41.133[a]	18.700[a]	7.233[a]	8.233[a]	6.533[a]	9.000[a]	6.400[a]	2.767[a]
Pr>F(model)	0.632	0.920	0.593	0.497	0.591	0.978	0.795	0.660
Significant	NO	NO	NO	NO	NO	NO	NO	NO

**Different letters indicate differences at p < 0.0001 according to Duncan`s Multiple Range Test.*
The values in the table represent the percentage of winged aphid species captured on each plum cultivar