DIFFUSION AND HOST PREFERENCE OF *Orientus ishidae* (Hemiptera: Cicadellidae) IN NORTHER ITALY APPLE ORCHARDS

Giovanni DALMASO[1], Raffaele PIGNOLI[2], Gianfranco ANFORA[3], Claudio IORIATTI[4], Mario BALDESSARI[5]

[1, 4, 5] Technology Transfer Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
[2, 3]Center Agriculture Food Environment, University of Trento, San Michele all' Adige, Italy

ABSTRACT

Flavescence dorée (FD) is a serious disease affecting grapevines in Europe, primarily transmitted by the alien leafhopper *Scaphoideus titanus* Ball. Other potential vectors, including the non-European leafhopper *Orientus ishidae* (Matsumura) have been identified. Despite monitoring efforts in Trentino vineyards (North Italy) since 2015, populations of *O.ishidae* have remained scarce. However, abundant populations have been observed in Trentino apple orchards since 2019. Monitoring activities confirmed the widespread presence of *O.ishidae*, particulary in organic orchards compared to conventional ones. A laboratory experiment was conducted to assess insect fitness among different host plants. Mortality rates on apple trees were similar to wild hosts, but significantly higher on grapevines. Apple trees and hornbeam exhibited the shortest development time to the adult stage compared to other substrates.These findings suggest that *O.ishidae* is better adapted and more prevalent on apple trees and wild species than on grapevines in Italy. However, uncertainties remain regarding its role as a vector of phytoplasma in apple orchards. Further research is underway to clarify these aspects.

Key words: Flavescence doree, *Orientus ishidae*, alien species, vector, fitness

1 INTRODUCTION

Grapevine's Flavescence dorée (FD) is one of the most insidious and destructive phytoplasmas diseases, causing important economic losses to European viticulture (Chuche and Thiéry 2014, Tramontini et al. 2020). The phytoplasmas associated with FD (FDp) belong to the ribosomal subgroups 16SrV-C and D (Lee et al. 2004, Martini et al. 2007, Malembic-Maher et al. 2020) and the epidemic spread of FD is

[1] via E. Mach 1, 38010 San Michele all'Adige, Italy
[2] 38010, San Michele all' Adige, Italy
[3] Ibid.
[4] via E. Mach 1, 38010 San Michele all'Adige, Italy
[5] Ibid.

caused by the Nearctic leafhopper *Scaphoideus titanus* Ball, 1932 (Hemiptera, Cicadellidae, Deltocephalinae) (Schvester et al. 1961, Chuche and Thiéry 2014). However, certain FDp strain can be transmitted from additional plant hosts such as *Alnus glutinosa, Clematis vitalba* to grapevines by alternative vectors like *Dictyophara europaea, Orientus ishidae, Allygus mixtus* (Filippin et al. 2009, Malembic-Maher et al. 2020) or putative vectors such as *Phlogotettix cyclops, Hishimonus hamatus* which were capable of acquiring FDp under controlled conditions (Strauss and Reisenzein 2018, Malembic-Maher et al. 2020). Among these, the leafhopper *Orientus ishidae* Matsumura, 1902 (Hemiptera, Cicadellidae, Deltocephalinae) was identified as an alternative insect vector of FDp in different transmission experiments (Lessio, Icciau, et al. 2016, Malembic-Maher et al. 2020, Jarausch et al. 2023). Moreover, high population of *O. ishidae* has been reported in non-cultivated area suggesting that they may locally contribute to the maintenance of FD, particularly within the landscape (Parise 2017, Jermini et al. 2019, Malembic-Maher et al. 2020, Rizzoli et al. 2021).*Orientus ishidae* is indigenous to Asia and was initially documented in Europe in 1998, as reported by Guglielmino (Guglielmino 2005). Subsequently the leafhopper rapidly spread throughout the continent (EPPO 2023), probably helped by its wide host plant range, including *Corylus avellana, Acer spp., Alnus glutinosa, Salix spp.* and *Carpinus betulus* (Valley and Wheeler 1985, Lessio, et al. 2016, Parise 2017, Desqué et al. 2019). Some of these species, such as *A. glutinosa* and *C. avellana* could harbour different FDp genotypes (Malembic-Maher et al. 2020, Kogej Zwitter et al. 2023) and may thus be involved in alternative epidemiological cycles of FDp.

However, multiple studies have shown that *O. ishidae* populations are scarce within vineyards in contrast to wild areas, where they are found to be extremely abundant (Lessio, et al. 2016, Rizzoli et al. 2021, Guerrieri et al. 2024). It has been hypothesized that mandatory treatments against *S. titanus* may have depressed *O. ishidae* populations (Lessio et al. 2016), rendering cultivated environment, such as vineyard, hostile to that species. Unexpectedly, since 2019 abundant population of the mosaic leafhopper have been reported within apple orchards in Trentino (North East Italy) (Baldessari et al. 2020) in contrast to the findings in vineyards in the same region (Gelmetti et al. 2021). It remains unclear whether the low populations of *O. ishidae* within the vineyards are a result of the effects of pesticides or what factors contribute to the discrepancy between the populations recorded in Trentino apple orchards and vineyards. To address these hypotheses the main objects of the research were:

i. assess the presence of *O. ishidae* in apple orchards located in other regions of northern Italy regions where the leafhopper is only recorded in vineyards.
ii. investigate fitness parameters of *O. ishidae* to determine whether grapevines can serve as an optimal host plant for the mosaic leafhopper

2 MATERIAL AND METHODS

2.1 Study area and experimental design

During summer 2022 and 2023, a survey was conducted to monitor the presence of *O. ishidae* in apple orchards across northern Italian regions, including Trentino Alto Adige, Friuli Venezia Giulia, Lombardia, Piemonte, Veneto (only in 2022), Emilia Romagna (only in 2023) and Slovenia in 2023. In each region four organic and two IPM orchards were randomly selected. Three yellow sticky traps (YST, Glutor© Biogard) were positioned along the diagonal of each orchard, suspended at approximately 1.50 meters above the ground on the median wire of the training system. The YSTs were changed every two weeks, starting from mid-July and continuing until the end of September. The collected YSTs were stored at -20°C and sent to Fondazione Mach for leafhoppers determination using a stereo microscope.

2.2 Fitness test

The fitness parameters taken into account were nymphs/adult mortality and the 'nymph-adult' development time in apple (cv Golden Delicius), grapevine (cv Bronner) and two wild plants (hazelnut and hornbeam). *Orientus ishidae* was reared under greenhouse conditions (T = 22 ± 3°C, photoperiod 16:8 L:D), starting from eggs as described in previous work (Dalmaso et al. 2023). Dormant apple wood with eggs was collected in winter from apple orchards of the Trentino region, where a high number of adult *O. ishidae* were captured by YSTs during the previous summer and placed in a hatching box.

To test the nymphs' mortality rate, first instar nymphs were collected from the hatching box and enclose in group of three on leaves of potted plants in cylindrical cages covered with a nylon mesh (height 20 cm, diameter 13 cm). The leaves were checked daily, and the dead specimens were removed. Nymphs were randomly subdivided and equally assigned to each host. Nymphs were left growing undisturbed and checked daily for the presence of newly emerged adults. The day of emergence of each adult was recorded.

The newly emerged adults derived from nymphs developed on the four previously assigned plants. The adult survival status was recorded every day and dead insects were removed from the cage and discarded.

2.3 Data analysis

All the statistical analyses were conducted on R software v 4.1.2 (R Core Team 2024). A Wilcoxon rank sum test with continuity correction or a Kruskal–Wallis rank sum test were then used to determine differences among medians. If the Kruskal–Wallis rank sum test confirmed such differences, a Mann-Whitney pairwise test (confidence level = 95%, Bonferroni corrected) was used to rank differences (packages ggplot, ggstatsplot, rstatix). The effect of the host plant species on the survival of *O. ishidae* was assessed through survival curves, estimated by the Kaplan–Meier method for censored data. (Klein and Moeschberger 2003). For testing the statistical significance among groups, we used log-rank tests, an established non-parametric method for comparing survival distributions (packages survival, survminer).

3 RESULTS

3.1 Diffusion in apple orchards

Yellow sticky traps showed *O.ishidae* to be widespread in all monitored sites, but with higher captures in apple orchards located in the northeast and in Italian alpine regions. In the Po Valley (Italy), captures were relatively lower in both years considered. Extremely high populations of the mosaic leafhopper have been recorded in organic apple orchards when compared with integrated management in 2022 (average season catches in organic orchards = 82,1 vs 8,40 in IPM, Wilcoxon Test, W = 23, p-value = <0,0001) and 2023 (average season catches in organic orchards = 58,9 vs 11,9 in IPM Wilcoxon Test, W = 309, p-value = 0.0013), although in some regions this difference was less marked in both years.
Finally, a preliminary analysis has indicated that catches of *O. ishidae* did not vary between the centre and edges of apple orchards in 2022 (Kruskal-Wallis chi-squared=0,615 df = 2, p-value=0,735,) and 2023 (Kruskal-Wallis chi-squared= 0,450, df = 2, p-value= 0,798). A landscape analysis will provide a more comprehensive understanding of the data, revealing, for instance, whether there are any correlations between the recorded population levels and the complexity of the agroecosystem.

3.2 Fitness parameters in different host plant

No nymphs reared on vine did progress to the adult stage. In contrast, on hornbeam, apple, and hazel, adults consistently emerged in all replications, and no evident difference in mortality rates was observed among these host plants (Kruskal-Wallis chi-squared= 69,19 df= 3, p-value=<0.0001). In terms of development time, no significant differences were observed among hornbeam, apple and hazel, with the mean moulting time ranging from 28 to 31 days (Kruskal-Wallis chi-squared= 1,47 df= 2, p-value=0,49). However, for grapevine, this value could not be calculated because no nymphs reached the adult stage.
Adult longevity exhibited significant differences in survival probability among *O. ishidae* reared on the four host plants. Given the lack of adults on vines from the previous experiments, nymphs from a separate rearing were placed on vines as soon as they reached the adult stage. In particular, individuals reared on grapevine survived for a shorter time compared with all other groups (log rank test: chi-squared= 270; p <0,0001). After three days 50% of the tested specimen died and no adults survive more than 10 days. Hornbeam and Hazel specimens, along with those reared on apple, displayed similar survival rates. Half of the adults survived more than 26 days, and two individuals reared on hornbeam died after 60 days.

4 DISCUSSION AND CONCLUSION

Preliminary findings reveal widespread *O. ishidae* populations throughout northern Italy orchards, particularly in organic apple orchards in the Northeast. Further investigations into climate and landscape factors may shed light on the underlying reasons for this distribution. Moreover, unlike in vineyards, where *O. ishidae* populations are sparse and mainly confined to the edges (Lessio et al. 2016, Jermini et al. 2019, Gelmetti et al. 2021, Guerrieri et al. 2023), our observations indicate a broader colonization of apple orchards by the mosaic leafhopper, highlighting the suitability of apple trees as hosts for this species.

While it has been speculated that compulsory chemicals treatments targeting *S. titanus* might contribute to the suppression of *O. ishidae* population (Lessio et al. 2016), the possibility of grapevines serving as non-hosts for the mosaic leafhopper has never been explored. Our experiment revealed significantly lower fitness performances on grapevines compared to all other host, suggesting that grapevine is not a suitable host.

In conclusion, although grapevines may not be the preferred host, the significance of *O. ishidae* in vineyards ecosystems should not be underestimated. It is advisable to investigate its fitness across various grapevine varieties. Moreover, considering its established role as an FD vector among wild plants and the observed populations in apple orchards, it is worthwhile to explore the potential involvement of apple crops in FD epidemiology.

5 REFERENCE

Baldessari, M., G. Dalmaso, V. Mazzoni, and N. Mori. 2020. Infestazioni su melo in Trentino di Orientus ishidae. L'informatore Agrar. 60–61.

Chuche, J., and D. Thiéry. 2014. Biology and ecology of the Flavescence dorée vector Scaphoideus titanus: A review. Agron. Sustain. Dev. 34: 381–403.

Dalmaso, G., C. Ioriatti, V. Gualandri, L. Zapponi, V. Mazzoni, N. Mori, and M. Baldessari. 2023. Orientus ishidae (Hemiptera: Cicadellidae): Biology, Direct Damage and Preliminary Studies on Apple Proliferation Infection in Apple Orchard. Insects 2023, Vol. 14, Page 246. 14: 246.

Desqué, D., P. Salar, J. L. Danet, T. Lusseau, C. Garcion, E. Moreau, C. Dubus, J. Dureuil, L. Delbac, D. Binet, A. Auriol, D. Thiéry, X. Foissac, and S. Malembic-Maher. 2019. Impact of Orientus ishidae on "flavescence dorèe" emergence in the vineyards of riparian ecosystems. Phytopatogenic Mollicutes. 9: 69–70.

EPPO. 2023. Orientus ishidae (ORIEIS)[World distribution]| EPPO Global Database. (https://gd.eppo.int/taxon/ORIEIS/distribution).

Filippin, L., J. Jović, T. Cvrković, V. Forte, D. Clair, I. Toševski, E. Boudon-Padieu, M. Borgo, and E. Angelini. 2009. Molecular characteristics of phytoplasmas associated with Flavescence dorée in clematis and grapevine and preliminary results on the role of Dictyophara europaea as a vector. Plant Pathol. 58: 826–837.

Gelmetti, A., F. Ghidoni, L. Zapponi, V. Mazzoni, and M. Bottura. 2021. Three-year monitoring of potential insect vectors of "flavescence dorée" in Trentino vineyards through use of chromotropic traps, p. 33. *In* INFitoplasmi 2021 VIII Incontr. Naz. Sui Fitoplasmi e Mal. Da Fitoplasmi. Catania.

Guerrieri, E., V. Forte, E. Belgeri, M. Signorotto, L. Filippin, M. Burati, M. Pavasini, E. Angelini, and N. Mori. 2023. Presence and distribution of known, alternative and putative insect vectors of phytoplasmas associated with "flavescence dorée" disease in Northeast Italy. Phytopathogenic Mollicutes. 13: 29–30.

Guerrieri, E., V. Forte, M. Pavasini, E. Belgeri, L. Filippin, N. Mori, and E. Angelini. 2024. Does Orientus ishidae constitute a risk for "flavescence dorée" epidemics in Veneto region (Northeastern Italy) vineyards?, p. 51. *In* Bertaccini, A. (ed.), Eur. Work. "Flavescence Dorée." Elisa Angelini, Assunta Bertaccini, Nicola Mori, Annalisa Polverari, Verona.

Guglielmino, A. 2005. Observations on the genus *Orientus* (Rhynchota Cicadomorpha Cicadellidae) and description of a new species: *O. amurensis* n. sp. from Russia (Amur Region and Maritime Territory) and China (Liaoning Province). Marburg. Entomol. Publ. 3: 99–110.

Jarausch, B., A. Markheiser, W. Jarausch, S. Biancu, S. Kugler, M. Runne, and M. Maixner. 2023. Risk Assessment for the Spread of Flavescence Dorée-Related Phytoplasmas from Alder to Grapevine by Alternative Insect Vectors in Germany. Microorganisms. 11: 2766.

Jermini, M., M. Conedera, A. Rizzoli, E. Belgeri, L. Filippin, and E. Angelini. 2019. Potential role of *Orientus ishidae* in the "flavescence dorée" epidemics in Ticino, Switzerland. Phytopathogenic Mollicutes. 9: 67–68.

Klein, J. P., and M. L. Moeschberger. 2003. Survival Analysis. Springer New York, New York, NY.

Kogej Zwitter, Z., G. Seljak, T. Jakomin, J. Brodarič, A. Vučurović, S. Pedemay, P. Salar, S. Malembic-Maher, X. Foissac, and N. Mehle. 2023. Epidemiology of flavescence dorée and hazelnut decline in Slovenia: geographical distribution and genetic diversity of the associated 16SrV phytoplasmas. Front. Plant Sci. 14.

Lee, I. M., M. Martini, C. Marcone, and S. F. Zhu. 2004. Classification of phytoplasma strains in the elm yellows group (16SrV) and proposal of "Candidatus Phytoplasma ulmi" for the phytoplasma associated with elm yellows. Int. J. Syst. Evol. Microbiol. 54: 337–347.

Lessio, F., L. Icciau, E. Onella, M. Andrioli, F. Ota, and A. Lma. 2016. The mosaic leafhopper Orientus ishidae : host plants , spatial distribution , infectivity , and transmission of 16SrV phytoplasmas to vines. Bull. Insectology. 69: 277–289.

Lessio, F., L. Picciau, E. Gonella, M. Mandrioli, F. Tota, and A. Alma. 2016. The mosaic leafhopper *Orientus ishidae*: Host plants, spatial distribution, infectivity, and transmission of 16SrV phytoplasmas to vines. Bull. Insectology. 69: 277–289.

Malembic-Maher, S., D. Desqué, D. Khalil, P. Salar, B. Bergey, J. L. Danet, S. Duret, M. P. Dubrana-Ourabah, L. Beven, I. Ember, Z. Acs, M. Della Bartola, A. Materazzi, L. Filippin, S. Krnjajic, O. Krstić, I. Toševski, F. Lang, B. Jarausch, M. Kölber, J. Jović, E. Angelini, N. Arricau-Bouvery, M. Maixner, and X. Foissac. 2020. When a Palearctic bacterium meets a Nearctic insect vector: Genetic and ecological insights into the emergence of the grapevine Flavescence dorée epidemics in Europe. PLoS Pathog. 16: 1–28.

Martini, M., E. Murari, N. Mori, and A. Bertaccini. 2007. Identification and Epidemic Distribution of Two Flavescence Dorée—Related Phytoplasmas in Veneto (Italy). https://doi.org/10.1094/PDIS.1999.83.10.925. 83: 925–930.

Parise, G. 2017. Notes on the biology of Orientus ishidae (Matsumura , 1902) in Piedmont (Italy). Cicadina. 17: 19–28.

R Core Team. 2024. R: The R Project for Statistical Computing. (https://www.r-project.org/).

Rizzoli, A., E. Belgeri, M. Jermini, M. Conedera, L. Filippin, and E. Angelini. 2021. *Alnus glutinosa* and *Orientus ishidae* (Matsumura, 1902) share phytoplasma genotypes linked to the 'Flavescence dorée' epidemics. J. Appl. Entomol. 145: 1015–1028.

Schvester, D., P. Carle, and G. Moutous. 1961. Sur la transmission de la Fla- vescence dorée des vignes par une cicadelle. Comptes Rendus des Séances l'Académie d'Agriculture Fr. 47: 1021–1024.

Strauss, G., and H. Reisenzein. 2018. First detection of Flavescence dorée phytoplasma in *Phlogotettix cyclops* (Hemiptera, Cicadellidae) and considerations on its possible role as vector in Austrian vineyards. IOBC-WPRS Bull. 139: 12–21.

Tramontini, S., A. Delbianco, and S. Vos. 2020. Pest survey card on flavescence dorée phytoplasma and its vector *Scaphoideus titanus*. EFSA Support. Publ. 17.

Valley, K. R., and A. G. Wheeler. 1985. Leafhoppers (Hemiptera: Cicadellidae) Associated with Ornamental Honey Locust: Seasonal History, Habits, and Descriptions of Eggs and Fifth Instars. Ann. Entomol. Soc. Am. 78: 709–716.