LAURA ORZALI^a - FABIO MOSCONI^a - PIO FEDERICO ROVERSI^b
VALENTINO BERGAMASCHI^a - LUCA RICCIONI^a

FIRST REPORT OF THE INVASIVE PEST *DROSOPHILA SUZUKII* (MATSUMURA, 1931) (DIPTERA DROSOPHILIDAE) ON *OLEA EUROPAEA* L. IN ITALY

(a) CREA-DC, Research Centre for Plant Protection and Certification, Rome, Italy

(b) CREA-DC, Research Centre for Plant Protection and Certification, Florence, Italy

Corresponding Author: Laura Orzali, laura.orzali@crea.gov.it

Orzali L., Mosconi F., Roversi P.F., Bergamaschi V., Riccioni L. - First report of the invasive pest *Drosophila suzukii* (Matsumura, 1931) (Diptera Drosophilidae) on *Olea europaea* L. in Italy.

The fruit crop pest *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) is a highly polyphagous invasive pest listed in the EPPO A2 list. This species has been reported as host of a variety of cultivated plants and wild fruits. Herein, we report the record of some *D. suzukii* viable specimens that were found in olives directly collected from the tree branches in four different sites of Latium and Umbria (Italy). Adults emerging from these pupae were collected and morphological and molecular analysis were carried out to confirm the species identity. Olive tissues alterations were observed in association with the presence of the species. The analysis of the available literature showed us that this is the first record of *D. suzukii* as a phytophagous species on the olive tree in Italy and probably worldwide.

KEY WORDS: olive tree, olives, pest, spotted wing *Drosophila*.

INTRODUCTION

Drosophila suzukii is a globally widespread fruit crop pest, native to Western Asia (DOS SANTOS *et al.*, 2017). *D. suzukii* can damage a variety of economically important hosts, such as blackberries, blueberries, sweet cherries, table grapes, peaches, raspberries, strawberries and many others (LEE *et al.*, 2011; BELLAMY *et al.*, 2013) and, in general, it has been reported to cause devastating effects on soft-fruit production (CALABRIA *et al.*, 2012; CINI *et al.*, 2012; DE ROS *et al.*, 2013). Following the first records of *D. suzukii* in Spain in 2008 (CALABRIA *et al.*, 2012), the species has rapidly spread in Europe since it was added to the EPPO A2 List in 2011 (EPPO, 2020); in Italy, after the first appearance of the species in Tuscany in 2008, it was reported in several other regions, both on wild hosts and cultivated species (CINI *et al.*, 2012; TAIT *et al.*, 2017; ANTONACCI *et al.*, 2017).

Most species of Drosophilidae lay eggs in overripe or already damaged fruits and are therefore not considered as a threat for fruit crops; conversely, *D. suzukii* is able to lay eggs in healthy or unwounded ripening fruits, hanging on plants, thus causing economic damage to fruit production mainly due by larvae feeding on fruit flesh (KARAGEORGI *et al.*, 2017).

One of the most important features for *D. suzukii* is its high level of polyphagy. The species is in fact able to attack different types of fruit, both cultivated and spontaneous (WALSH *et al.*, 2011; KENIS *et al.*, 2016; ELSENSOHN and LOEB, 2018). The identification of alternative or new host plants (with special reference to crop plant species) on which *D. suzukii* can complete its life-cycle is to consider as a priority.

In this work we present a host plant never ascribed before to *D. suzukii*: the olive tree *Olea europaea* L. (EPPO, 2020). During the research activities concerning the study of new and emerging olive pests (SALVAOLIVI Project¹), we found several specimens of *D. suzukii* inside drupes still on the tree and we also observed many adults emerging from olives.

MATERIALS AND METHODS

During a monitoring activity in olive tree orchards between October and December 2019, ripening drupes were collected directly from trees from 23 different sites in Central and Southern Italy (Umbria, Latium and Calabria). A mix of 80 drupes showing symptoms of fungal rotting and insect damages and symptomless olives were chosen. The drupes were placed in sealed humid chambers (100% constant humidity at room temperature) to stimulate fungal development and after 3 days were observed and photographed under a stereomicroscope (Wild Heerbrugg with integrated LEICA IC80HD camera, up to 25x zoom lens). Following the observation of *Drosophila* pupae in some not rotting drupes, these were still kept in humid chamber for 3 more days to allow adults to emerge. Adults and non-hatched pupae were stored in 95% alcohol.

For species level identification we used both morphological and molecular approaches. Following the observation of some characters compatible with the *Drosophila suzukii* species (i.e., the dark spot at the apex of the wing in males and the morphology of the ovipositor of the females) the specimens were subjected to a molecular analysis for specific final recognition.

¹SALVAOLIVI Project: "Safeguard and valorization of Italian olive heritage with research actions in the field of plant protection" funded by the Italian Ministry of Agricultural, Food and Forestry Policies MIPAAF (D.M.33437 of the 21st/12/2017)

Molecular identification was carried out by amplification of a 687 bp fragment of the mitochondrial cytochrome c oxidase gene subunit 1 (COX1) with the primer pair LCO1494/HCO2198 (FOLMER *et al.*, 1994). DNA extraction was carried out as described in GLOOR *et al.* (1993), using liquid nitrogen for smashing the flies. Polymerase chain reaction (PCR) amplification mix was prepared in 30 μ l following CALABRIA *et al.* (2012) with some modifications: 0,4 μ M of each primer, 1x Bioline buffer with 1,5 mM MgCl₂ and 0.9 units of BIOTAQ DNA Polymerase. The thermocycling profile consisted in one cycle of 2 min 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 48 °C 1min 72 °C with a final elongation step of 7 min at 72 °C. Amplification product were sent to Eurofins Genomics (Ebersberg Germany) using both the forward and the reverse primers. The final sequences were analyzed using the Basic Local Alignment Search Tool Nucleotide (BLASTN) searches at GenBank (National Center for Biotechnology Information, <https://www.ncbi.nlm.nih.gov/>) in order to determine the closest matches and confirm the morphological identification.

RESULTS AND DISCUSSION

Drosophila specimens were found in olives sampled from 4 different localities: three from Latium, i.e. Rome (41.9335°, 12.55416°), Tor Mancina RM (42.09097°, 12.62068°), Grottaferrata RM (41.793846°, 12.664567°), and one from Umbria, i.e. Narni TR (42.48925°, 12.54585°). Out of a total of 80 drupes collected from the above-mentioned sample sites, 15 individuals were found emerging from intact drupes evenly distributed overall the four localities. Initially, the presence of the pupae inside olive drupes has been ascertained by observing the respiratory appendages protruding from the fruit surface, with the rest of the pupal body almost totally immersed inside the fruit tissue (1 or 2 pupae from each drupe, as shown in Figs I and II).

Fig. I - A *D. suzukii* pupa protruding from an olive fruit showing the typical respiratory appendages.

The specimens observed were viable, in fact they could end their biological cycle and emerge as adults from pupae after 3 days of incubation in humid chamber, as observed for 8 specimens. The morphological and molecular analysis allowed us to identify the species as *Drosophila suzukii* (WALSH *et al.*, 2011; COLOMAR, 2014). One haplotype (Genbank accession number: MT458049.1) was obtained from molecular analysis of individuals sequenced, and it showed 100% identity with several isolates of *D. suzukii* present in the GenBank database.

Fig. II - Direct damage caused by the development of *D. suzukii* larvae inside the olive tissue, visible as a circular depressed area close around a couple of pupae.

In the available scientific literature, *D. suzukii* is never reported as an olive phytophagous, despite its high level of polyphagia; in fact, the species has been sampled on the drupes of several cultivated and wild species (e.g., LEE *et al.*, 2011; MAIER, 2012; BELLAMY *et al.*, 2013; LUE *et al.*, 2017; CLOONAN *et al.*, 2018; EPPO, 2020), including a species belonging to the Oleaceae family (GRASSWITZ, 2015), but never on *O. europaea*. However, our finding seems to indicate that the species may attack undamaged olives not occasionally, in line with its feeding and reproductive habits on other fruits. In fact, the number and the distance of the sites where we found the species could suggest that this presence on the olive drupes could be not considered a sporadic or a fortuitous case.

After the emergence of the pupae, olive tissues alterations were observed in association with the presence of the species: a circular depression zone, probably due to the feeding action of the larvae, is clearly visible all around the pupae protruding from a hole on the surface of the drupes (Fig. II). In this regard, it would also be interesting to investigate the possibility of indirect damage caused by the fly in addition to the observed direct damage. This is in fact a well-known phenomenon for other fruits: the *D. suzukii* larvae feeding behavior create soft and sunken areas on the fruit surface (direct damage), also creating the opportunity for secondary fungal

or bacterial infection (indirect damage) compromising the commercial value of the production (WALSH *et al.*, 2011).

Finally, in addition to the possibility of losses, the olive tree could represent a "stepping stone" for the expansion and maintenance of populations of *D. suzukii* in new areas if used to complete the life cycle, and could therefore have important repercussions on the management of this pest. In fact, alternative hosts may allow the development of the species also where the preferred crop hosts are not available (LEE *et al.*, 2015).

Therefore, based on what is known about the biology of the species, more investigations are needed regarding the relationship between this species and the olive tree. In particular, it would be necessary to verify if the species regularly includes the olives in its diet and not in an occasional way, thus broadening the knowledge about its feeding habits and strengthening the prospect of adding *O. europaea* as suitable and not occasional host for *Drosophila suzukii*.

ACKNOWLEDGMENTS

The present work was carried out within the SALVAOLIVI Project funded by the Italian Ministry of Agricultural, Food, Forestry and Tourism Policies MIPAAF (D.M.33437 of the 21st/12/2017)

The authors would like to thank Elisabetta Gargani for the clarifications received on the biology of the species and Alberto Zilli, Ashley Kirk-Spriggs and Pierfilippo Cerretti for the initial advices on the specific identity of the specimens.

REFERENCES

- ANTONACCI R., TRITTO P., CAPPUCCI U., FANTI, L., PIACENTINI L., BERLOCO M., 2017 - *Drosophilidae monitoring in Apulia (Italy) reveals Drosophila suzukii as one of the four most abundant species*. - Bulletin of Insectology, 70: 139-146.
- BELLAMY D.E., SISTERTON M.S., WALSE S.S., 2013 - *Quantifying host potentials: indexing postharvest fresh fruits for spotted wing drosophila, Drosophila suzukii*. - Plos One, 8: e61227.
- CALABRIA G., MACA J., BACHLI G., SERRA L., PASCUAL M., 2012 - *First records of the potential pest species Drosophila suzukii (Diptera: Drosophilidae) in Europe*. - Journal of Applied Entomology, 136: 139-147.
- CINI A., IORIATTI C., ANFORA G., 2012 - *A review of the invasion of Drosophila suzukii in Europe and a draft research agenda for integrated pest management*. - Bulletin of Insectology, 65: 149-160.
- Cloonan K.R., Abraham J., Angeli S., Syed Z., Rodriguez-Saona C., 2018 - *Advances in the chemical ecology of the spotted wing Drosophila (Drosophila suzukii) and its applications*. - Journal of chemical ecology, 44 (10): 922-939.
- COLOMAR L. A.E., 2014 - *Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae) una nueva plaga de frutales que se está extendiendo mundialmente. Distribución, biología y ecología*. - Revista Agronómica del Noroeste Argentino, 34 (1): 13-19.
- DE ROS G., ANFORA G., GRASSI A., IORIATTI C., 2013 - *The potential economic impact of Drosophila suzukii on small fruits production in Trentino (Italy)*. - IOBC-WPRS Bulletin, 91: 317-321.
- DOS SANTOS L.A., MENDES M.F., KRÜGER A.P., BLAUTH M. L., GOTTSCHALK M.S., GARCIA F.R.M., 2017 - *Global potential distribution of Drosophila suzukii (Diptera, Drosophilidae)*. - Plos One, 12(3):e0174318.<https://doi.org/10.1371/journal.pone.0174318>
- ELSENJOHN J.E., LOEB G.M. 2018 - *Non-crop host sampling yields insights into small-scale population dynamics of Drosophila suzukii (Matsumura)*.- Insects, 9(1): 5.
- EPPO, 2020 - EPPO Global Database (available online). <https://gd.eppo.int>
- FOLMER O., BLACK M., HOEH W., LUTZ R., VRIJENHOEK R., 1994 - *DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates*. - Molecular marine biology and biotechnology, 3: 294-299.
- GLOOR G.B., PRESTON C.R., JOHNSON-SCHLITZ D.M., NASSIF N.A., PHILLIS R.W., BENZ W.K., ROBERTSON H.M., ENGEL W.R., 1993 - *Type I Repressors of P Element Mobility*. - Genetics, 135: 81-95.
- GRASSWITZ T.R., 2015 - *First Occurrence of Spotted Wing Drosophila, Drosophila suzukii, in New Mexico, with Notes on Its Host Plant Associations*. - Southwestern Entomologist, 40(4): 851-853.
- KARAGEORGI M., BRÄCKER L.B., LEBRETON S., MINERVINO C., CAVEY M., SIJU K.P., GRUNWALD KADOW I.C., GOMPEL N., PRUD'HOMME B., 2017 - *Evolution of multiple sensory systems drives novel egg-laying behavior in the fruit pest Drosophila suzukii*. - Current Biology, 27: 1-7. <https://doi.org/10.1016/j.cub.2017.01.055>
- KENIS M., TONINA L., ESCHEN R., VAN DER SLUIS B., SANCASSANI M., MORI N., HAYE T., HELSEN H., 2016 - *Non-crop plants used as hosts by Drosophila suzukii in Europe*. - Journal of Pest Science, 89: 735-748.
- LEE J.C., BRUCK D.J., CURRY H., EDWARDS D., HAVILAND D.R., VAN STEENWYK R.A., YORGEY B. M., 2011 - *The susceptibility of small fruits and cherries to the spotted-wing drosophila, Drosophila suzukii*. - Pest Management Science, 67: 1358-1367.
- LEE J.C., DREVES A.J., CAVE A.M., KAWAI S., ISAACS R., MILLER J.C., VAN TIMMEREN S., BRUCK D.J., 2015 - *Infestation of wild and ornamental noncrop fruits by Drosophila suzukii (Diptera: Drosophilidae)*. - Annals of the Entomological Society of America, 108(2):117-129.
- LUE C.H., MOTTERN J.L., WALSH G.C., BUFFINGTON M.L., 2017 - *New record for the invasive spotted wing drosophila, Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae) in Anillaco, western Argentina*. - Proceedings of the Entomological Society of Washington, 119(1): 146-150.
- MAIER C.T., 2012 - *First Detection and Widespread Distribution of the Spotted Wing Drosophila, Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), in Con-*

- necticut in 2011*. - Proceedings of Entomological Society of Washington, 114(3): 329-337.
- TAIT G., VEZZULLI S., SASSÙ F., ANTONINI G., BIONDI A., BASER N., SOLLAI G., CINI A., TONINA L., OMETTO L., ANFORA G., 2017 – *Genetic variability in Italian populations of Drosophila suzukii*. - BMC genetics, 18(1): 87.
- WALSH D.B., BOLDA M.P., GOODHUE R.E., DREVES A.J., LEE J.C., BRUCK D.J., WALTON V.M., O'NEAL S.D., ZALOM F.G., 2011 - *Drosophila suzukii (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential*. - Journal of Integrated Pest Management, 1: 1-7.