

**Diagnostics**  
**Diagnostic****PM 7/115 (1) *Drosophila suzukii*****Specific scope**

This standard describes a diagnostic protocol for *Drosophila suzukii*<sup>1</sup>.

**Specific approval and amendment**

Approved in 2013-09.

**Introduction**

*Drosophila suzukii* (spotted wing drosophila) is a polyphagous pest of many small fruits, especially from the genera *Vaccinium*, *Rubus*, *Prunus*, *Fragaria*, *Vitis*, *Ficus*, *Actinidia*, *Rhamnus*, *Lonicera*, *Sambucus* and also many others. Plant species from 15 families are recorded as host plants (Kanzawa, 1939; Grassi *et al.*, 2012; Seljak, 2011; Walsh *et al.*, 2011). *Drosophila suzukii* is a vinegar fly. Most species of vinegar flies are not pests because they infest overripe, fallen, rotting fruit. However, *D. suzukii* females lay eggs in ripening fruit and larvae develop in the fruit and cause it to become soft and unmarketable. It originates and is widely distributed in the temperate East Palaearctic zoogeographical region, but recently *D. suzukii* has been introduced and is now reported from North America as well as from Europe (Hauser, 2011; Cini *et al.*, 2012). The most recent and comprehensive overview on introduction, spread, distribution, host range, economic impact and control in Europe has been provided by Cini and collaborators (Cini *et al.*, 2012). The pest is currently spreading quickly in Europe. It can be found at different altitudes from coast to mountain. Because of the rapid spread, it is recommended to consult PQR (EPPO, 2013) for updated information on the geographical distribution.

**Identity**

**Name:** *Drosophila suzukii* (Matsumura 1931)

**Synonym:** *Leucophenga suzukii* Matsumura 1931.

**Taxonomic position:** Diptera, Brachycera: Drosophilidae; *Drosophila*, subgen.: *Sophophora*.

**EPPO code:** DROSSU.

**Phytosanitary categorization:** EPPO A2 List no. 363; EU not categorized.

**Detection**

*Drosophila suzukii* may be detected as larvae or pupae in fruits. Eggs may also be detected but are more difficult to see. Adults can be caught in traps.

**Detection of adults**

Different traps can be used: the most efficient method for first detection is by trapping. Any 250–750 mL plastic containers with closely fitting lids can be used as traps. Four to six holes should be made on the side to enable flies to enter through these holes. To make the trap more selective and to avoid the trapping of large insects the diameter of these holes should not be too large. The recommended diameter is about 3 mm. Various baits are used to attract the flies to the trap. Apple cider vinegar is considered to be highly effective, and the most practical bait to use (EPPO, 2010). In trials, a mixture of vinegar and wine trapped significantly more *D. suzukii* compared with vinegar alone or wine alone. These results indicate a synergy of acetic acid and ethanol as lures for *D. suzukii* (Landolt *et al.*, 2012). Several other feed attractants and lures are proposed to be effective in trapping *D. suzukii*.

**Identification**

Morphological identification to species level with a binocular microscope is the recommended diagnostic method. Magnification  $\times 10$  for males to  $\times 50$  for females,  $\times 200$  for male and female genitalia as well as for male sex combs (if needed, guidance for the preparation of genitalia and male sex combs is given in Appendix 1).

A reliable morphological identification can only be performed on adult specimens. It is not possible to identify with certainty eggs, larvae and pupae with this protocol (these should be reared to the adult stage). Immature stages can only be identified by molecular techniques (Hauser,

<sup>1</sup>Use of brand names of chemicals or equipment in these EPPO Standards implies no approval of them as others may also be suitable.

2011) however, there is currently little experience regarding molecular tests in the EPPO region. It should be noted that rearing adults from larvae is easy and rapid and morphological identification rather straightforward. An EPPO Standard PM 7/XX on *DNA barcoding as an identification tool for plant pests* is in preparation and COI sequences are currently available in Q-bank for this pest. However, sequence data for other members of the *suzukii*-subgroup are not yet available and therefore, it is unclear whether a barcode-gap exists for *D. suzukii*.

An excellent comparative diagnostic account on *D. suzukii* has been published by Hauser (Hauser, 2011). A good key for the identification has also been provided by Vlach (Vlach, 2012).

### Description of adults

#### General

Adults of *D. suzukii* are small flies approximately 2.25–4.0 mm long with a wing span of 6–8 mm. (Figs 1,2 and 3) Males are usually slightly smaller (2.0–3.5 mm) than females (2.5–4.0 mm) (Kanzawa, 1939).

#### Head

Head entirely yellow; compound eyes red to red orange in live specimens, covered with short and dense pilosity, gena about 0.3 times as high as eye, facial ridge with two subequal vibrissal setae (Fig. 4), palpi with only one prominent seta at tip; antennae 3-segmented all segments yellow, terminal segment (flagellum) with a dorsal plumose arista; arista typically forked at the tip through the main stem and the last lateral hair, which is one of the key characters in separating *Drosophilidae* from other Diptera families with plumose arista (Fig. 4); frons with 3 pairs of orbital setae (the most anterior one proclinate, the remaining two reclinate); ocellar setae present, inserted inside the ocellar triangle; a pair of postocellar setae well developed, convergent (Fig. 5).

#### Thorax

Dorsum yellowish brown, sometimes with two darker brown longitudinal stripes in the dorsocentral area that sometimes may be fused into one; 8 rows of acrostichal setulae between the anterior pair of dorsocentral setae, 2 subequal humeral setae (Fig. 5). Wings 2.3–3.3 mm, entirely hyaline in females, while in males a typical large black spot at the top of vein  $R_{2+3}$  is present (Figs 1,2 and 6) by which *D. suzukii* differs from all indigenous European *Drosophila* species (note, however, that many East-Palaearctic and Oriental *Drosophila* species belonging to the *suzukii* subgroup may exhibit similar feature); sometimes this spot may be fade or even absent in still teneral specimens. Very seldom mature “spotless” male individuals may also occur. In these cases, other morphological characters such as sex combs and genital segment have to be examined as well. Halteres are white to bright yellow. Sternum and legs yellow. Legs: preapical dorsal seta on all tibia



Fig. 1 *Drosophila suzukii* – male.



Fig. 2 *Drosophila suzukii* – male.



Fig. 3 *Drosophila suzukii* – female.

present; forelegs in males with two sex combs, one on the first and one on the second tarsomere, both in one row only and oriented parallel to the length of the tarsus, the first

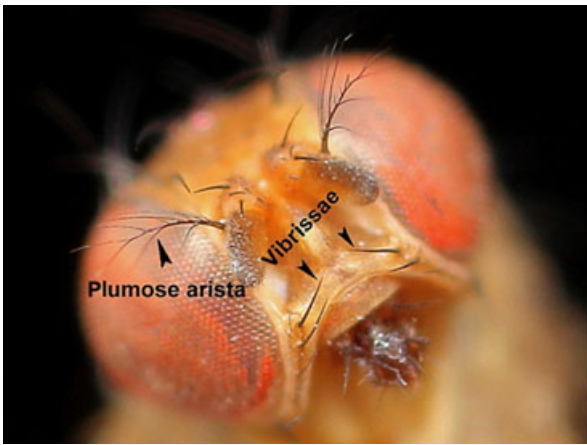


Fig. 4 *Drosophila suzukii* - head (frontal view).

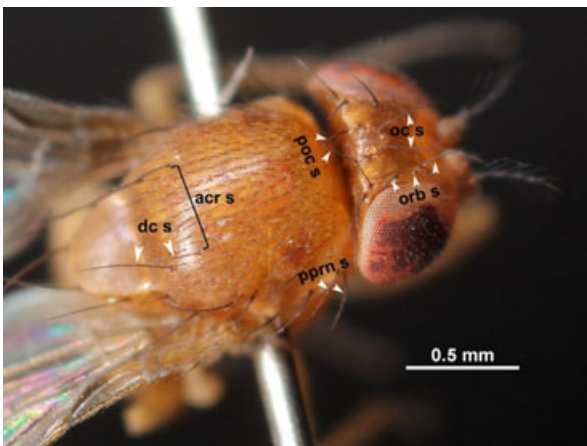


Fig. 5 *Drosophila suzukii* - the basic chaetotaxy of head and notum (*acr s* - acrostichal setae (8 rows), *dc s* - dorsocentral setae, *oc s* - ocellar setae, *orb s* - orbital setae, *poc s* - postocellar setae, *pprn s* - postpronotal setae).

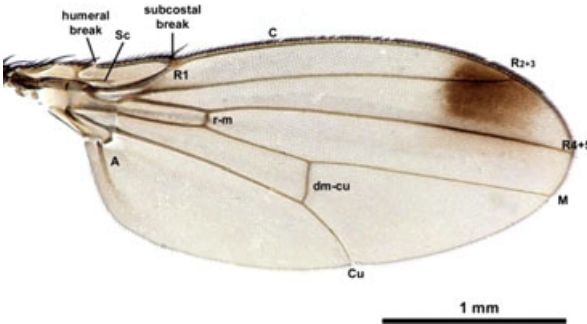


Fig. 6 *Drosophila suzukii* - right wing of a male with indicated veins.

one composed of 5–6 and the second one of 3–4 strong spines (Fig. 7); females lack such sex combs.

**Abdomen**

Abdominal segments with large dark brown to black transversal bands that are not broken or interrupted medially (Fig. 8; compare also to Fig. 9).

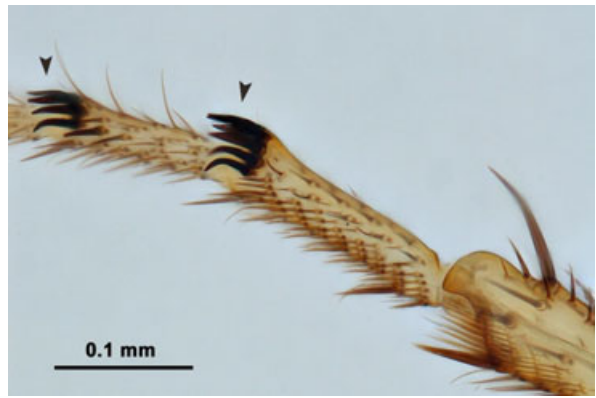


Fig. 7 *Drosophila suzukii* - sex combs on first and second tarsomere of male's forelegs.

**Male.** Male's genital segment see (Fig. 10); epandrium slightly constricted at middle; surstylus (clasper) large, with tapering tip, bearing 10–11 primary teeth (prensisetae) medially and 4–5 secondary teeth subapically; marginal bristles also in two sets, upper one 5–6, lower one 3–4 at the tip; anal plate oval, tapered ventrally (adopted after Hsu, 1949).

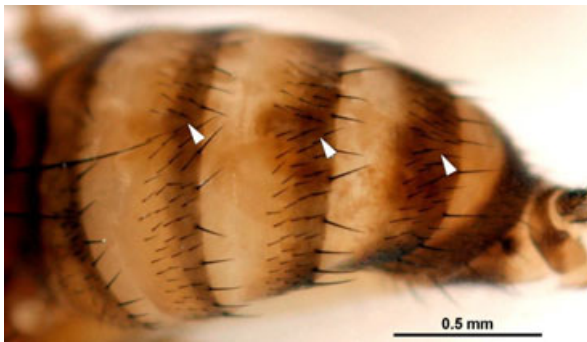
**Females.** Females have a strongly sclerotized ovipositor with strong, contrastingly black marginal teeth by which females of *D. suzukii* are easy distinguishable from all other European as well as North American *Drosophila* species; each ovipositor valve bears 30–36 teeth, whereby those in distal half of each valve are much stronger and darker than proximal ones (Fig. 11). The ovipositor is 6–7 times as long as the diameter of the spermatheca, while in most European species the ovipositor is only 2–4 times as long as the spermatheca (Hauser, 2011). Similar strong sclerotized ovipositors are exhibited by some other East-Palaearctic and Oriental species of the *suzukii* subgroup, such as *Drosophila subpulchrella* Takamori & Watabe, *Drosophila pulchrella* Tan, Hsu & Sheng, *Drosophila immacularis* Okada (Okada, 1966:81–89; Takamori *et al.*, 2006; Kimura & Anfora, 2011).

**Egg**

Eggs are milky white and glossy, semi-transparent, on average 0.62 × 0.18 mm wide (Fig. 12). The eggs have two subapical respiratory tubes (Kanzawa, 1939).

**Larva**

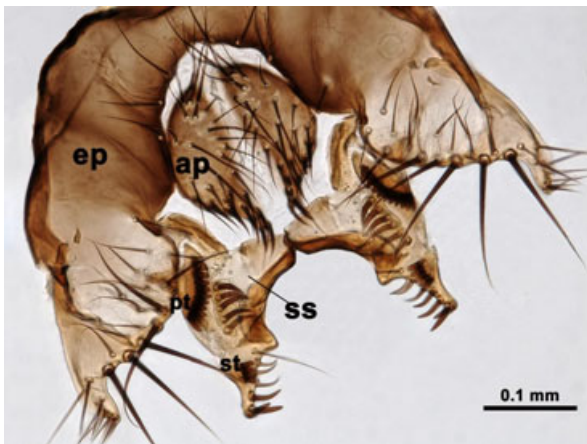
There are three larval instars that range in size (length × width) from 0.67 × 0.17 mm, 2.13 × 0.40 mm and 3.94 × 0.88 mm on average for first, second and third instars, respectively (Kanzawa, 1939) (Fig. 13). The larvae are white to cream in colour with visible internal organs and black cephalopharyngeal skeleton; mandibles (mouthhooks) of the third instar larva densely serrated ventrally; each anterior spiracle composed of 6 to 8 subparallel respiratory tubules; posterior spiracles horn-shaped, both tubes parallel, close to each other; sternites with transversal rows of brown rose-thorn-shaped sharp spinules.



**Fig. 8** *Drosophila suzukii* - abdominal tergites with transversal medially not interrupted dark bands (arrows).



**Fig. 9** *Drosophila immigrans* - abdominal tergites with transversal medially interrupted (arrows!) dark bands on segments 2–5.



**Fig. 10** *Drosophila suzukii* - genital segment of a male (flattened view): *ap* - anal plate, *ep* - epandrium, *pt* - primary teeth, *ss* - surstylus, *st* - secondary teeth.

#### Pupa

The pupae of *Drosophila suzukii* are creamy becoming tan-brown in colour and are about 3 mm long by 1 mm wide (Fig. 14). The horn-shaped respiratory tubes are about 0.3 mm long and are protrusions of the anterior spiracles



**Fig. 11** *Drosophila suzukii* - female with strongly sclerotized saw-like ovipositor.



**Fig. 12** *Drosophila suzukii* – eggs.

on both sides of the head. Each respiratory tube bears seven to eight radially arranged branches at the ends (Kanzawa, 1935).

#### Related and morphologically similar species that may be confused with *D. suzukii*

The conspicuous black spots on the wing top near the vein  $R_{2+3}$  (Fig. 6) and the two sets of tarsal sex combs in males, as well as strongly sclerotized and serrated ovipositor in females make the identification of *D. suzukii* in Europe fairly easy. No one indigenous *Drosophila* species exhibit similar characters. In the East-Palaeartic and Oriental zoogeographical region (India, South East Asia, Southern China), however, several close related species with similar appearance and behaviour occur (Kimura & Anfora, 2011). Nearly identical wing spotting in males are show by *D. subpulchrella*, recently described from Japan and China (Takamori *et al.*, 2006) and *Drosophila biarmipes* known from India (Malloch, 1924). Another similar species is *D. pulchrella*, but it has an additional much smaller black spot on the wing base as well (Okada, 1966). Males of all three species differ from *D. suzukii* by the shape of sex



Fig. 13 *Drosophila suzukii* - third instar larva.

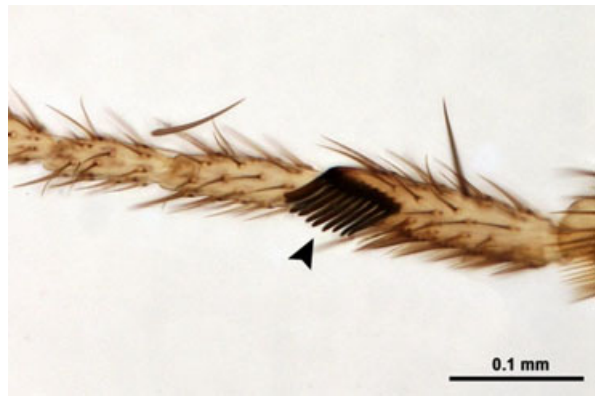


Fig. 16 *Drosophila melanogaster* - a sex comb on first tarsomere of male's forelegs.



Fig. 14 *Drosophila suzukii* - pupae.



Fig. 17 *Drosophila immigrans* - ovipositor moderately sclerotized with subequal coloured teeth on valve margins.



Fig. 15 *Drosophila suzukii* - ovipositor with strong black teeth on valve margins.



Fig. 18 *Drosophila melanogaster* - soft ovipositor with weak teeth; spermatheca (arrow).

combs on the first and the second tarsomere. In *D. suzukii* they are composed of a single row of spines on both the first and second tarsomere, while in the other three species sex comb spines are arranged in two separate rows (upper and lower) on each tarsomere or at least on the first one

(Okada, 1966; Bock & Wheeler, 1972). In *D. biarmipes*, the sex comb is only present on the first tarsomere and female's ovipositor is weakly sclerotized. As in *D. suzukii*,

strongly sclerotized ovipositors with dark stout teeth are also present in the females of *D. subpulchrella*, *D. pulchrella* and *D. immacularis* (Kimura & Anfora, 2011). The number of teeth on valvae margins, however, is lower (usually 20 to 25 in number) in these three species. Furthermore, upper marginal teeth (usually three in number) are as strong as lower marginal teeth, while they are markedly smaller in *D. suzukii* (Fig. 15). In *D. immacularis*, the black spot at the top of first vein is always absent in both sexes

and acrostichal hairs are arranged in six rows. Good keys for separation of *D. suzukii* from *D. pulchrella* and *D. pulchrella* from *D. immacularis* were provided by Okada (Okada, 1956, 1966). All species with long and strongly sclerotized ovipositors (*D. suzukii*, *D. subpulchrella*, *D. pulchrella* and *D. immacularis*) have putative similar ecology laying eggs in fresh fruits (Kimura & Anfora, 2011).

### Dichotomous key for identification of *Drosophila suzukii* (Matsumura)

This key only works properly when all characters are considered together step by step. Single characters cannot be taken from the key and used independently as this could lead to misidentification.

1	Small sized flies of <4 mm body length and golden yellow to yellowish brown colours (Figs 1,2 and 3); head with red or red orange, short pilose compound eyes (observe at slant or backlight), 3 segmented antennae, flagellum (3rd segment) with a plumose arista characteristically forked at the tip (Fig. 4), 3 pairs of orbital setae (one proclinate, 2 reclinate), a pair of convergent postocellar setae present (Fig. 5), vibrissae always present (Fig. 4); wing as in (Fig. 6), subcosta (Sc) incomplete, not reaching costal margin, humeral and subcostal break well developed ( <i>Drosophilidae</i> p. p.)	2
1*	Disagreeing with above in one or more of characters	Not <i>Drosophila</i>
2	Wings transparent (Fig. 3) or with a single dark spot near the end of wing on first vein $R_{2+3}$ (Figs 1, 2 and 6)	3
2*	Wings with bands, multiple spots, with a darkened crossvein, or a spot at the tip of the wing on the second vein ( $R_{4+5}$ )	Not <i>D. suzukii</i>
3	Abdominal tergites 2 to 5 with broad dark brown transversal bands NOT broken or interrupted in mid-dorsal line ( <i>Drosophila</i> subgenus <i>Sophophora</i> ) (Fig. 8, compare also Fig. 9)	4
3*	Abdominal tergites with stripes, spots, or patterns other than above or completely black; if transversal bands developed they are broken or interrupted medially by a longitudinal light coloured strip at least on tergites 2 to 5 (Fig. 9)	Not <i>D. suzukii</i>
4	Wing with a single black spot near wing tip at the junction of first vein ( $R_{2+3}$ ) with the costal margin (Fig. 6); forelegs with sex comb(s) on tarsomeres	5
4*	Without such a spot near wing tip (Fig. 3); with or without sex combs	6
5	Forelegs with two sex combs, one on 1st tarsomere composed of 5–6 spines and one on 2nd tarsomere composed of 3–4 spines respectively the spines of both oriented $\pm$ parallel to the length of the tarsus (Fig. 7); genital segment as in (Fig. 10)	<i>D. suzukii</i> (male)
5*	Forelegs with one (e.g. Fig. 16) or several sex combs on tarsomeres; if two sex combs then their spines directed more or less transversally or obliquely to the length of the tarsus and/or sex comb spines arranged in two or more transverse rows on each tarsomere (species not occurring yet in Europe)	Some other <i>Drosophila</i> species from the <i>suzukii</i> -subgroup
6	Females: ovipositor developed, forelegs without sex combs on tarsomeres	7
6*	Males: forelegs with one or several sex combs on tarsomeres (Figs 7 and 16)	10
7	Large and strong sclerotized ovipositor with strong teeth (Figs 11, 15 and 17)	8
7*	Ovipositor not sclerotized, soft with weak teeth (Fig. 18)	Other <i>Drosophila</i>
8	Marginal teeth on ovipositor strong, almost black, much darker than ovipositor itself (Figs 11 and 15)	9
8*	Teeth on ovipositor the same colour or only slightly darker as the rest of ovipositor (Fig. 17)	Other <i>Drosophila</i> , not <i>D. suzukii</i>
9	Ovipositor saw-like, with strong contrastingly black teeth in the distal half of each valve; 30–36 in number on each valve; teeth on upper margin (usually 3 in number) much weaker than distal lower teeth (Figs 11 and 15)	<i>D. suzukii</i> (female)
9*	Ovipositor arched; number of teeth lower, usually <25, teeth on upper margin as stout as that on lower margin (species not occurring yet in Europe)	<i>D. subpulchrella</i> , <i>D. pulchrella</i> , <i>D. immacularis</i>
10	Forelegs with two sex combs, one on 1st tarsomere composed of 5–6 spines and one on 2nd tarsomere composed of 3–4 spines respectively, the spines of both oriented $\pm$ parallel to the length of the tarsus (Fig. 7); genital segment as in (Fig. 10)	<i>D. suzukii</i> (“spotless” and teneral males)
10*	Forelegs with one or several sex combs on tarsomeres; if two then their spines directed more or less transversally or obliquely to the length of the tarsus or sex comb spines in more than one row on each tarsomere (e.g. Fig. 16)	Males of other <i>Drosophila</i> , but not <i>D. suzukii</i>

## Reference material

Material can be obtained from laboratories which have declared expertise on this pest in the EPPO Database on Diagnostic expertise.

## Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM7/77 (1) *Documentation and reporting on a diagnosis*.

## Further information

Further information on this organism can be obtained from:

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## Feedback on this diagnostic protocol

If you have any feedback concerning this Diagnostic Protocol, or any of the tests included, or if you can provide additional validation data for tests included in this protocol that you wish to share please contact diagnostics@epo.int.

## Protocol revision

An annual review process is in place to identify the need for revision of diagnostic protocols. Protocols identified as needing revision are marked as such on the EPPO website.

When errata and corrigenda are in press, this will also be marked on the website.

## Acknowledgements

This protocol was originally drafted by: G. Seljak, Agricultural and Forestry Chamber of Slovenia.

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**Appendix 1 – Examination of genitalia and male sex combs**

For more accurate examination of genitalia, preparation of microscope slides is recommended. First gently remove the abdomen and place it into absolute ethanol, to which an equal volume of approximately 10% KOH is added. Macerate the abdomen in this mixture at room temperature for about 12–24 h. This procedure can be accelerated by heating on a hot plate at about 90–95°C for 5–10 min. Gently squeeze abdomen with a small spatula or other tool to remove the macerated content. Transfer then the abdomen

in glacial acetic acid at room temperature for about 15 min and then to absolute ethanol for several minutes. Gently separate the genital segment or the last segments of abdomen. Further procedures depend on the type of preparation. For routine examination temporary slides are usually performed. For temporary mounts the simplest technique is to place the dissected parts in a drop of glycerol on a microscope slide and cover then carefully with a coverslip over it. For permanent slides Canada balsam, Berlese fluid, Euparal or some other mountants can be used.