

Threat of *Drosophila suzukii* as an Invasive Species and the Potential of Entomovectoring



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1 Introduction

Drosophila suzukii Matsumura (Diptera: Drosophilidae), also commonly referred to as spotted wing drosophila (SWD) and native to Southeast Asia (Kanzawa 1939; Tan et al. 1949), is a polyphagous invasive pest in America and Europe (Lee et al. 2011; Kinjo et al. 2014; Deprá et al. 2014). From its early detection in 2008, in California (USA), Spain and Italy (Europe), *D. suzukii* has rapidly spread through these two continents with the aid of global trading and absence of niche competitors (Hauser 2011; Calabria et al. 2012; Cini et al. 2012; Rota-Stabelli et al. 2013; Cini et al. 2014; Wiman et al. 2014; Asplen et al. 2015). Contrary to other closely related *Drosophila* species that would preferentially infest over-ripened and damaged fruits, and thus are not considered serious pests (Lee et al. 2011), *D. suzukii* has the ability to bore holes into the skin of maturing and undamaged healthy fruits using its serrated ovipositor and oviposits into them. The oviposition wounds caused by *D. suzukii* flies very often provide access points to other insects and undesirable secondary infections by pathogens, including fungi, yeasts and bacteria, hence, causing additional losses (Hamby et al. 2012; Ioriatti et al. 2015). All these together make *D. suzukii* a pest of great concern to maturing and ripening fruits (Mitsui et al. 2006; Calabria et al. 2012). A wide range of different soft and stone fruits including strawberry, raspberry, plums, blueberry and grapes are potential targets under *D. suzukii*'s damage range (Dreves et al. 2009; Cini et al. 2012; Bellamy et al. 2013). The damage caused by *D. suzukii* has been reported to reach up to 80% crop loss (Dreves et al. 2009; Walsh et al. 2011; Goodhue et al. 2011). Furthermore, the management of *D. suzukii* is primarily challenging because the fly can continuously

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infest various fruits available throughout the year (Lee et al. 2011), it can survive in a wide range of different climatic conditions in which their natural predators can sometimes not keep up (Chabert et al. 2012) and it also has a very short generation time (Kanzawa 1939; Lee et al. 2011; Wiman et al. 2014). Limited knowledge on how to effectively control this pest and the zero tolerance attitude for infested fruit bound for the fresh market or various export markets, has motivated the priority for more research into possible control options for this pest.

Entomovector technology, which utilizes insects as vectors of biological control agents for targeted precision biocontrol towards plant pests and diseases (Hokkanen and Menzler-Hokkanen 2007; Mommaerts and Smagghe 2011; Menzler-Hokkanen et al. 2013), presents an intriguing management option for the control of *D. suzukii* in an integrated pest management (IPM) system. Multiple studies have reported on the success of exploiting both honey bees and bumblebees to vector different entomopathogenic control agents into flowers to control pest insects which either feed on, or inhabit, the flowers (Gross et al. 1994; Butt et al. 1998; Carreck et al. 2007; Albano et al. 2009). However, the success of entomovectoring in the management of *D. suzukii* will be based on mutual and suited interactions between the appropriate components of vector, control agent, formulation and dispenser, and it needs to be safe for the environment and human health.

This chapter presents the threat of the occurrence of *D. suzukii* in Europe, and places this in context to the possible effects that it might have on entomovectoring. Insights into the possibility of exploiting entomovectoring as a management option for the biocontrol of *D. suzukii* are also discussed.

2 Threat of *Drosophila suzukii* to Fruit Production

Contrary to most other Drosophilidae, with the exemption of *D. subpulchrella*, *D. suzukii* is able to lay eggs in healthy, unwounded fruit and not only on damaged or overripe fruits, thanks to the serrated female ovipositor (Fig. 1) (Sasaki and Sato 1995; Cini et al. 2012; Bellamy et al. 2013). Hence, ripening fruits are preferred over overripe ones (Mitsui et al. 2006).

Although most of the damage caused by *D. suzukii* is largely due to the larvae feeding on fruit flesh, the insertion of its prominent ovipositor into the skin of the fruit can cause physical damage to the fruit. This in turn provides access to secondary infections of pathogens such as, yeasts, filamentous fungi and bacteria, which may cause faster deterioration and further losses (Hamby et al. 2012; Ioriatti et al. 2015) (Fig. 2).

Additional costs associated with the field management of *D. suzukii* are mostly related to increased production costs (monitoring and chemical input costs, increased labour and fruit selection, reduction of the fruit shelf life, storage costs) and to the decrease of foreign market appeal for fruit production from contaminated areas (Goodhue et al. 2011). Nevertheless, the oviposition habit itself is not enough to explain the dramatic impact of *D. suzukii* on fruit production. In the next sections

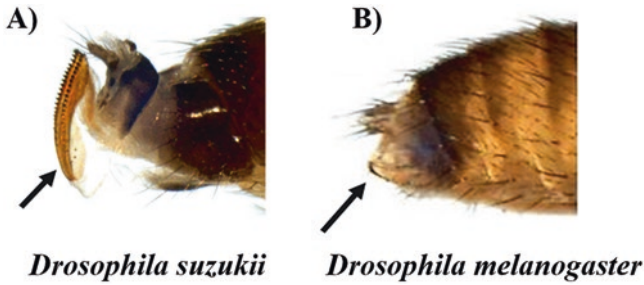


Fig. 1 Fly ovipositor. (A) Arrow indicates the serrated hook-like ovipositor of *D. suzukii* used in boring into unwounded ripening fruits on the fields (Photograph by Martin Hauser, California Department of Food and Agriculture). (B) Arrow indicates the shorter ovipositor of *D. melanogaster* used in boring into overripe and decaying fruits

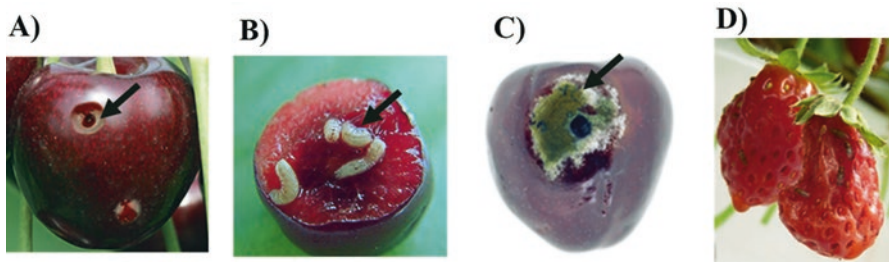


Fig. 2 Indirect and direct damages caused by *D. suzukii*. (A) Arrow indicates an oviposition spot created by the serrated hook-like ovipositor of *D. suzukii* on a healthy cherry. (B) Arrow indicates larvae feeding inside a cherry. (C) Arrow indicates fungi growing around an oviposition spot (Photograph by Martin Hauser, California Department of Food and Agriculture). (D) Deterioration and softening of strawberries following infestation with *D. suzukii*

the main characteristics making *D. suzukii* a threat of high concern for the European fruit production sector are discussed.

2.1 High Fecundity in *D. suzukii*

Mating in *D. suzukii* optimally occurs from the first days of life and females start to lay eggs already from the second day from emergence. Females are known to typically lay 1–3 eggs per fruit in up to 7–16 fruits per day, depending on the temperature (Kinjo et al. 2014). Since they are capable of ovipositing for 10–59 days, they can lay up to a total of 600 eggs during their lifetime (around 400 eggs on average). The eggs hatch within 2–72 h after being laid inside the fruits, and larvae mature (inside the fruit) in 3–13 days. *D. suzukii* pupae reside for 3–15 days either inside or less frequently outside the fruit. Depending on the temperature, a minimum of 10 days is required from the time the egg is oviposited to adult emergence. This very

short generation time exhibited by *D. suzukii* has a huge impact on fruit production. It implies that *D. suzukii* can complete several generations in a single cropping cycle and up to 7–15 generations in a year, depending on specific climatic conditions, thus allowing an explosive population growth [life-cycle details can be found in Kanzawa (1939); Mitsui et al. (2006); Walsh et al. (2011); Tochen et al. (2014); Wiman et al. (2014)].

2.2 *D. suzukii* is Tolerant to a Wide Range of Climatic Conditions

The ability to survive and reproduce in a wide range of different climatic conditions is obviously a relevant factor for pest insects. Limiting temperatures for *D. suzukii* reproduction have been reported to be between 10 and 32 °C for oviposition and up to 30 °C for male fertility (Sakai et al. 2005). Its development and peak activity is around 20–25 °C (Kanzawa 1939; Tochen et al. 2014). *D. suzukii* can also be described as being both heat tolerant (viable *D. suzukii* populations can resist hot summers in Spain) and cold tolerant (*D. suzukii* is present in cold areas, such as mountain regions in Japan and Alpine areas). Adult *D. suzukii* are particularly tolerant to cold compared to other drosophilids (Sasaki and Sato 1995; Mitsui et al. 2010) and mated females in reproductive diapause have been reported to be the *D. suzukii* stage that overwinters (Kanzawa 1939; Mitsui et al. 2010; Walsh et al. 2011). Whether the observed tolerance is physiological or mediated by behavioral adaptation is still unclear. However, several authors have suggested that *D. suzukii* survival under harsh conditions might be increased by acclimatization (Walsh et al. 2011), altitudinal migration (Mitsui et al. 2010), and/or overwintering in manmade habitats or other sheltered sites (Kimura 2004).

2.3 *D. suzukii* has a Broad Host Range

D. suzukii has a large host range, infesting both cultivated and wild soft-skinned fruits on host plants in both native and invaded areas, with berries being the preferred hosts (Table 1). Despite laboratory tests indicating that *D. suzukii* has a lower oviposition susceptibility and developmental rate on grapes compared to berries and cherry (Lee et al. 2011), reports from observations in vineyards in Northern Italy have clearly indicated that *V. vinifera* can become a field host (particularly with soft skinned varieties being more impacted) (Griffo et al. 2012). This could indicate that *D. suzukii* host preference is highly dependent upon the local abundance of hosts. *D. suzukii* can also be flexible with its host choice. This is demonstrated by its ability to develop on tomato under controlled laboratory conditions. However, tomato has not been so far recorded as its host in the field, even though *D. suzukii* adults have

Table 1 List of *D. suzukii* host plants grouped based on botanical family

Family name	Host plants ^a	References
Rosaceae	<i>Fragaria ananassa</i> (strawberry), <i>Rubus idaeus</i> (raspberry), <i>Rubus fruticosus</i> , <i>Rubus laciniatus</i> , <i>Rubus armeniacus</i> and other <i>Rubus</i> species and hybrids of the blackberry group, <i>Rubus ursinus</i> (marionberry), <i>Prunus avium</i> (sweet cherry), <i>Prunus armeniaca</i> (apricot), <i>Prunus persica</i> (peach), <i>Prunus domestica</i> (plum), <i>Eriobotrya japonica</i> (loquat)	Kanzawa (1939); Bolda et al. (2010); Grassi et al. (2011); Seljak (2011); Walsh et al. (2011); Klick et al. (2016); Kenis et al. (2016); Mazzi et al. (2017)
Ericaceae	<i>Vaccinium</i> species and hybrids of the blueberry group	Hampton et al. (2014)
Grossulariaceae	<i>Ribes</i> species including the cultivated currants	Cini et al. (2012)
Moraceae	<i>Ficus carica</i> (fig), <i>Morus</i> spp. (mulberry)	Lee et al. (2011); Cini et al. (2012)
Rhamnaceae	<i>Rhamnus alpina</i> ssp. fallax, <i>Rhamnus frangula</i> (buckthorn)	Asplen et al. (2015); Kenis et al. (2016)
Cornaceae	<i>Cornus</i> spp. (dogwood)	Kenis et al. (2016); Pelton et al. (2016)
Actinidiaceae	<i>Actinidia arguta</i> (hardy kiwi)	Kinjo et al. (2014)
Ebenaceae	<i>Diospyros kaki</i> (persimmon)	Kanzawa (1939)
Myrtaceae	<i>Eugenia uniflora</i> (Surinam cherry)	Cini et al. (2012); Lee et al. (2015)
Rutaceae	<i>Murraya paniculata</i> (orange jasmine)	Mann et al. (2011); Lee et al. (2015)
Myricaceae	<i>Myrica rubra</i> (Chinese bayberry)	Cini et al. (2012); Asplen et al. (2015)
Caprifoliaceae	<i>Lonicera</i> spp. (honeysuckle)	Lee et al. (2011); Cini et al. (2012)
Elaeagnaceae	<i>Elaeagnus</i> spp. (silverberry or oleaster)	Cini et al. (2012); Kinjo et al. (2013); Asplen et al. (2015),
Adoxaceae	<i>Sambucus nigra</i> (black elder)	Lee et al. (2011); Cini et al. (2012); Lee et al. (2015)
Vitaceae	<i>Vitis vinifera</i> (common grape vine), <i>Vitis labrusca</i> (fox grape)	Cini et al. (2012); Van Timmeren et al. (2013)

^aNon-exhaustive and tentative host list, since some information is not well documented

been trapped in France in tomato crop fields (EPPO website). In addition to cultivated fruits, many wild, ornamental, and uncultivated plants can serve as potentially important hosts (Lee et al. 2015; Klick et al. 2016).

Despite its relatively recent detection in Europe, *D. suzukii* has already caused severe yield losses in several small fruit crops grown across southern Europe, such as sweet cherries, strawberries, raspberries, blackberries, and blueberries. Extreme damage has been reported for locations in Northern Italy (Trentino) and in France, with up to 100% damage reported on cranberries, strawberries, and sweet cherries (Cini et al. 2012; Warlop et al. 2013). The first evaluation of the economic impact in

Europe was presented by De Ros et al. (2013), although the study only focused on Trento Province, Italy. It was estimated in the study that 400-ha of soft fruit production areas faced losses of around 500,000 € in 2010, and three million € in 2011. Although the level of these economic impacts recorded in Trentino can be ascribed to high levels of blueberry production, this estimate is also somewhat conservative in that it did not consider the costs of control strategies and other societal consequences resulting from increased chemical inputs. In France, *D. suzukii* has also been reported on apples and peaches, although without economically significant damage (Warlop et al. 2013).

The wide host range of *D. suzukii* represents a pest management constraint in many affected regions. This is not only because *D. suzukii* can cause damage to many species, but also because populations can survive almost everywhere, alternating hosts with different ripening times through the year, both cultivated and wild. Crop plants usually cultivated in high density monoculture, allow rapid and impressive population growth, while wild hosts and ornamental plants may serve as refuges from management treatments, and provide later re-infestation sources and overwintering habitats observed (Klick et al. 2016). The ability to damage thick ripening fruits and the wide host range, gives to *D. suzukii* a wide but at the same time specialized ecological niche. Nevertheless, the overlap of niches and the possibility of competition with other drosophilids needs to be investigated.

2.4 *D. suzukii* has a High Potential for Dispersal

The rapid spread of *D. suzukii* in invaded countries and its presence on several continents, as well as remote islands [e.g. Hawaii; Kaneshiro (1983)], confirms its high dispersal potential (Hauser 2011; Calabria et al. 2012). Similar to many other invasive species (Westphal et al. 2008), passive diffusion due to global trade is most likely the main cause of the spread of *D. suzukii*. Before larval activity, the intact and healthy appearance of fruits infested with *D. suzukii* is likely to mask the damage caused to the fruit. This will lead to the risk of infestation remaining undetected and thus an increase in the risk of passive dissemination of *D. suzukii* (Calabria et al. 2012).

3 Rapid Worldwide Spread of *D. suzukii*

D. suzukii was initially described for the first time in 1916, in Japan, where it was reported to attack cherries, however, it is still uncertain whether it is native to this region or was introduced (Kanzawa 1939). The presence of *D. suzukii* has also been reported in the eastern part of China (Peng 1937), Taiwan (Lin et al. 1977), North and South Korea (Chung 1955, Kang and Moon 1968), Pakistan (ud Din et al. 2005), Myanmar (Toda 1991), Thailand (Okada 1976), the Russian Far East

(Sidorenko 1992) and India (Kashmir region, (Parshad and Duggal 1965), where it was described as the *D. suzukii* subspecies *indicus* (Parshad and Paika 1964). *D. suzukii* is currently spreading in many areas, such as the USA (West and East coast), Canada, Brazil (Deprá et al. 2014), Mexico and Europe [a history of the introduction in North America is reviewed by Hauser (2011)]. A key feature of the rapid spread of *D. suzukii* was the initial lack of regulation over the spread of any *Drosophila* species.

D. suzukii is rapidly spreading across Europe (Fig. 3). First reports of its presence in Europe were in autumn 2008 in Spain (Rasquera Province) (Calabria et al. 2012), although a later proposal suggested that southern France was the first propagation center (Cini et al. 2014). Moreover, malaise traps deployed in Tuscany (San Giuliano Terme, Pisa, Italy) in 2008 caught *D. suzukii* adults simultaneously with those deployed in Spain (Raspi et al. 2011). By 2009, in other regions of Spain, (Bellaterra, near Barcelona), France (Montpellier and Maritimes Alpes) and Italy (Trentino) (Grassi et al. 2009; Calabria et al. 2012), *D. suzukii* adults were trapped and recorded. In Trentino, first oviposition on wild hosts (*Vaccinium*, *Fragaria* and *Rubus* spp.) and economically important damage on several cultivated berries species were reported (Grassi et al. 2009; Sarto and Royo 2011). By 2010–2011, the range of *D. suzukii* was further enlarged. In Italy it was reported in several other regions: Piedmont, Aosta Valley, Lombardy, Veneto, Emilia Romagna, Liguria, Marche and Campania (Franchi and Barani 2011; Pansa et al. 2011; Süß and Costanzi 2010; Griffo et al. 2012; Baser et al. 2015; Mazzetto et al. 2015) and in France it was found from Corsica up to Ile de France. Then, many other European countries made their first record: Switzerland (Baroffio and Fischer 2011; Baroffio et al. 2014), Slovenia (Seljak 2011), Croatia (Milek et al. 2011), Portugal (Rota-Stabelli et al. 2013), Austria (Lethmayer 2011), Germany (Vogt et al. 2012; Vogt



Fig. 3 Current worldwide *D. suzukii* distribution map (Asplen et al. 2015). It is worthwhile to note that the lack of reports from several areas is probably due to a lack of monitoring rather than to an actual absence of *D. suzukii*

2014; Briem et al. 2015), Belgium (Mortelmans et al. 2012; Belien et al. 2013), The Netherlands (Helsen et al. 2013), United Kingdom (EPPO 2012), Hungary (Kiss et al. 2014; Kiss et al. 2016), Poland (Łabanowska and Piotrowski 2015), Greece (Papachristos et al. 2013), Romania (Chireceanu et al. 2015), Bulgaria (EPPO 2015), Serbia (Toševski et al. 2014), Bosnia and Herzegovina (Zovko 2014) and Czech Republic (Breziková et al. 2014). This reflects the distribution of *D. suzukii* in Europe.

D. suzukii seems to be spreading rapidly and all of continental Europe is at risk for invasion (Fig. 3). It is important to note that the lack of reports from several areas is probably due to a lack of monitoring rather than to an actual absence of *D. suzukii*. Thus, the history of reports might reflect differences in the sampling effort and/or problems of awareness rather than the true distribution of *D. suzukii*. Considering the reports together with the outputs of available degree-day phenological models (Damus 2009; Coop 2010) and analysis of the distribution of *D. suzukii* host plants (EPPO website), it is very likely that *D. suzukii* will spread all over Europe. Ecological simulations have indicated that the northern humid areas are more suitable ecosystems for *D. suzukii* compared to the Mediterranean drier environments, especially because desiccation seems to be a limiting factor for drosophilids (Walsh et al. 2011). Taking the current climate changes into account, even Scandinavian countries cannot be considered out of reach from the risk of *D. suzukii* invasion. On a wider geographic perspective, according to the biology of *D. suzukii*, global expansion in regions with climatic conditions spanning from subtropical to continental is highly likely to happen (Walsh et al. 2011). Furthermore, the occurrence of niche shifts, as was observed for other pests (e.g. *Zaprionus indianus* Gupta, Da Mata et al. 2010), should not be excluded (Calabria et al. 2012), suggesting that *D. suzukii* could become a global problem for fruit production.

4 Potential of Entomovectoring in the Management of *D. suzukii*

The success of entomovectoring in the management of *D. suzukii* will depend on mutual and suited interactions between the appropriate components of vector, control agent, formulation and dispenser, and it needs to be safe for the environment and human health. A typical scenario will be the delivery of the microbial control agent (MCA) to the flowers by the vector (e.g. honey bee or bumble bee), which will in turn lead to the protection of the resulting fruit against *D. suzukii* coming to feed on the ripening fruits (Fig. 4). In this scenario, the MCA has to be able to survive long enough in the flower to the maturation of the fruit and subsequently to the ripening of the fruit. The choice for an MCA which can survive on flower dwelling insect pest prior to fruit maturation could be a good option.

The potential MCA of choice to be used in the control of *D. suzukii* will need to fulfil the criteria as defined for agents against postharvest diseases by Droby et al.

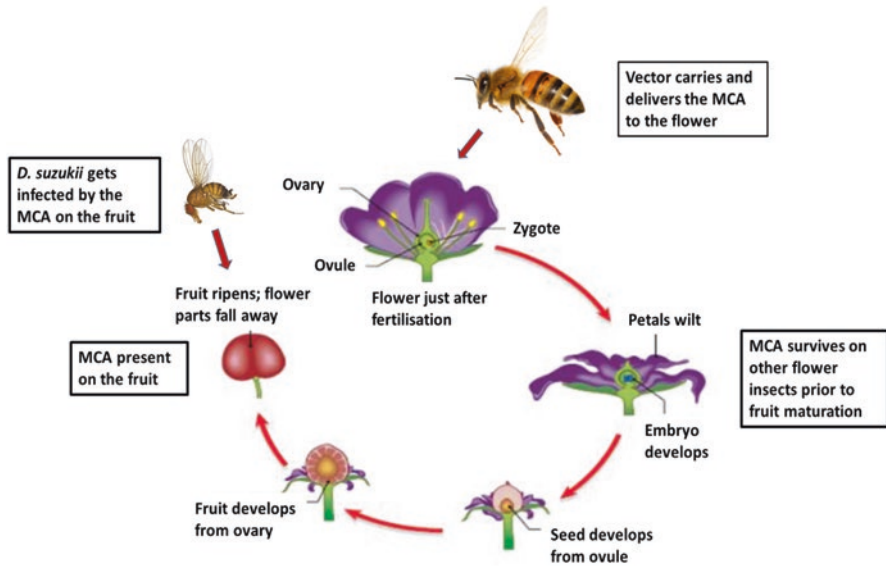


Fig. 4 Illustration of the management of *D. suzukii* through entomovectoring. In this scenario, the vector delivers the MCA to the flower during pollination. The MCA then survives by feeding on other flower dwelling insects until fruit maturation and ripening. *D. suzukii* attacking the fruits are exposed to the MCA, which subsequently leads to mortality in *D. suzukii*

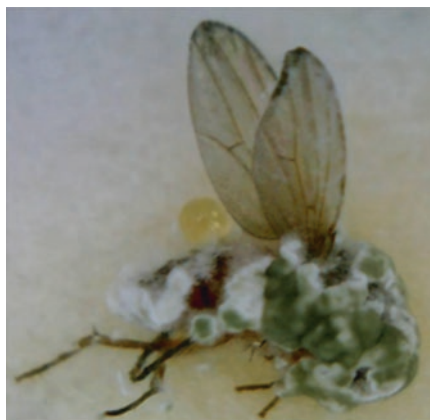
(2009) and Sharma et al. (2009): (a) effective at low concentrations, (b) not fastidious in its nutrient requirements, (c) genetically stable, (d) able to survive adverse environmental conditions, (e) non-pathogenic to the host, (f) resistant to pesticides, (g) preparable in a form that can effectively be stored and disseminated and (h) not detrimental to human health. In addition to these criteria three extra characteristics should be included for a suitable MCA, namely (i) safe for the vector and the crop, (j) effective against aerial and/or foliar plant insect pests, and (k) able to survive and grow under conditions present in the flower.

Metarhizium anisopliae, an entomopathogenic fungus has been observed to infect over 200 insect pest species (Cloyd 1999). *M. anisopliae* and its related species have been tested as biological insecticides against a number of pests such as termites, thrips, pollen beetle, cabbage seedpod weevil, sweet potato weevil and fruit flies (Butt et al. 1998; Carreck et al. 2007; Reddy et al. 2014; Quesada-Moraga et al. 2006; Yousef et al. 2015; Yousef et al. 2017). *M. anisopliae* could be exploited as a possible MCA for the management of *D. suzukii*. *M. anisopliae* does not infect humans or other animals and is therefore considered safe as an insecticide. Vectoring of *M. anisopliae* on oil seed rape and canola has been demonstrated to cause high mortality in some insect pests, including larvae/adults of *Meligethes aeneus* Fabricius (Coleoptera: Nitidulidae) and *Ceutorhynchus assimilis* Dejean (Coleoptera: Curculionidae) (Butt et al. 1998; Carreck et al. 2007). *M. anisopliae* typically causes the diseases known as ‘green muscardine disease’ (due to the green

color of its spores) in insects. When the mitotic (asexual) spores (called conidia) of the fungus come into contact with the body of an insect host, they germinate and the hyphae that emerge penetrate the cuticle. Then, the fungus develops inside the insect body eventually killing it only after a few days. It is very likely that the lethal effect is aided by the production of insecticidal cyclic peptides (destruxins). Most insect species living close to the soil have evolved natural defenses against entomopathogenic fungi such as, *M. anisopliae*. To overcome the insect host defenses, this fungus is locked in an evolutionary battle, which has resulted to a large number of different isolates (or strains) that are adapted to certain groups of insects (Freimoser et al. 2003). This implies that screenings will need to be performed to select isolates with insecticidal activities against *D. suzukii*, prior to any field trials. In a recent study, Yousef et al. (2016) reported on the effectiveness of *Metarhizium brunneum* Petch (Hypocreales: Clavicipitaceae) and its crude extract in the control of *D. suzukii*. The study evaluated the use of two *M. brunneum* strains, EAMa 01/58-Su and EAMb 09/01-Su, and their extracts for the respective development of lure-and-infect and lure-and-kill devices for the control of *D. suzukii* (Fig. 5). The EAMa 01/58-Su strain designed for a lure-and-infect strategy, caused 62.2% mortality in adult *D. suzukii* (survival time of 3.6 days). Furthermore, the evaluation of horizontal transmission and sublethal reproductive effects of the fungal strain showed 48.0% mortality in untreated males after mating with fungus-treated females, whereas only 24.0% of untreated females were killed after mating with treated males, thereby revealing the horizontal transmission potential of the strain. These results show the high potential of using *M. brunneum* as an MCA in entomovectoring, contributing to an IPM program for the control of *D. suzukii*.

Another MCA which could be used in the management of *D. suzukii* is the entomopathogenic fungus, *Beauveria bassiana*. It is known to attack a broad range of insects, acting as a parasite on various arthropod species (McNeil Jr. 2005; Barbarin et al. 2012). Studies with honey bees vectoring *B. bassiana* GHA in canola showed 22–56% mortality in *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) (Al Mazra'awi et al. 2006). *B. bassiana* causes white muscardine (due to the white

Fig. 5 *D. suzukii* adult with *M. brunneum* EAMa01/58-Su strain fungal outgrowth (from Yousef et al. 2016)



color of its spores) disease in insects, using a similar mechanism as describe for *M. anisopliae*. When the microscopic spores of the fungus come into contact with an insect host body, they germinate, penetrate the cuticle, and grow inside, killing the insect within a matter of days. New spores are then produce from a white mold which emerges from the cadaver. Since various isolates of *B. bassiana* differ in their host range and the factors responsible for host susceptibility are unknown, further research will have to be done to select an appropriate isolate to be used in the management of *D. suzukii*. A preliminary screening of some isolates of *B. bassiana* showed up to 44% mortality in *D. suzukii* (Cuthbertson et al. 2014). Another example of a possible MCA is *Isaria fumosorosea*. Cuthbertson and Audsley (2016) demonstrated the efficacy of *I. fumosorosea* against *D. suzukii* by immersing blueberries in suspensions of these fungi pre- and post-infestation. *I. fumosorosea* caused >40% mortality in adult flies within 7 days of fly contact with the fungi.

Once appropriate MCAs against *D. suzukii* are identified and tested, the next crucial step will be the development of appropriate carriers in which the MCA will be transported by the vector. An appropriate carrier will need to fulfil three criteria (Kevan et al. 2008): (a) No effect on the life span of the MCA. A good example is reported by Hjeljord et al. (2000), where the germination of *Trichoderma* spp. and *B. bassiana* spores were significantly slower when formulated with talc; (b) Safe for the vector. A good example is reported by Israel and Boland (1993), where talc irritated honey bees causing them to groom, whereas with flours as carrier, grooming decreased by 50% (Kevan et al. 2008). Similarly, Pettis et al. (2004) reported that minerals such as talc adversely affected the honey bee brood; (c) Enhance the transport capacity of the vector. In this context, Al-Mazra'awi et al. (2007) showed that direct honey bee load increased with decreasing carrier particle size and moisture content. A start point to the carriers for the management of *D. suzukii* could be adaptations from existing carriers. So far, known carrier substances are corn flour (Shipp et al. 2006), corn meal (Peng et al. 1992), bentonite (Kevan et al. 2008) and polystyrene beads (Butt et al. 1998). Despite the high efficiency of the latter carrier, these beads are prohibitively expensive for commercial formulations, whereas flours and meals have the advantage to be easily available and inexpensive, safe and food grade qualified. These carrier options could be used as basis for the evaluation of identified MCAs against *D. suzukii*, while research continues for the identification of better carriers.

It is evident that success in dissemination and deposition of the MCA is crucial in an entomovector strategy. Therefore it is of paramount importance that the most efficient vector should be selected, and this selection depends on the species, the crop visitation rate by the vector, and the deposition capacity of the MCA by the vector to the target. Honey bees and solitary mason bees are used to vector MCAs onto crops under field conditions. Besides the carrier substance and selection of an appropriate MCA against *D. suzukii*, all of the other components of an entomovectoring system (such as, the selection of the vector, vector safety, transport of MCA, dispenser design and safety of the control agent to the environment and human health) will probably be the same as reported in other cases (Kevan et al. 2008; Mommaerts and Smaghe 2011). These indicate the feasibility for the development

of an entomovectoring system, where bee-mediated dissemination of entomopathogenic MCAs could be exploited to target fruit pests, such as *D. suzukii*, within an IPM system that aims to enhance biological control and minimize insecticide use.

5 Effects of the Occurrence of *D. suzukii* on Entomovectoring

The control of *D. suzukii* populations in the field mainly relies on the use of chemical pesticides (Beers et al. 2011; Bruck et al. 2011; Whitener and Beers 2015; Andrezza et al. 2017), a practice with serious drawbacks such as indiscriminate killing of different insect species (including bees) and its use close to harvest which could lead to a risk of high residues left on fruits. The particular preference of *D. suzukii* for ripening fruit presents timing difficulties with respect to pollinator protection and pre-harvest intervals. This implies that the most effective time for applying chemical controls against *D. suzukii* is when the fruit is ripe or very nearly ripe, necessitating chemicals with a shorter pre-harvest interval. Therefore, growers of bee-pollinated crops may need to remove their bees slightly earlier than optimum to spray late-flowering fruit, before *D. suzukii* infestation, if bee kills are to be minimized.

The fast spread and establishment of *D. suzukii* in Europe will result to an increase in the use of chemical pesticides to manage this invasive pest. Certain pyrethrin and spinosad are among the authorized active materials for *D. suzukii* control (Diepenbrock et al. 2016). Increased pesticide usage to control *D. suzukii* will inevitably lead to an increase in bee mortality. Considering that bees are currently the only actively exploited vectors in the delivery of MCAs in entomovectoring, this will significantly impact efforts in promoting entomovectoring as an alternative to the use of chemical pesticides.

6 Conclusions

The rapid spread of *D. suzukii* poses a challenge to fruit production in Western countries. The biology of *D. suzukii* clearly indicates that an effective control effort requires an area wide IPM program. In order to accomplish this, research needs to address *D. suzukii* basic biology, the development of management tools, the transfer of knowledge and technology to users and, finally, the implementation of the IPM program also at a cultural and societal level. While short term solutions to limit the current dramatic damage are strongly needed, only long-term and environmentally friendly management approaches will allow a sustainable control of this pest. To this aim, research into entomovectoring as a possible biocontrol option, should be carried out to shed light on many knowledge gaps that are still present.

Acknowledgements The authors acknowledge support by the EU via Core-Organic II (Bicopoll, Targeted precision biocontrol and pollination enhancement in organic cropping systems), the Special Research Fund of the Ghent University, the Flemish **agency for Innovation by Science and Technology** (IWT-Flanders, Brussels), and the Research Foundation-Flanders (FWO-Vlaanderen, Brussels).

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