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Investigation on ecology and integrated pest management of *Drosophila suzukii* Matsumura

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Padova, 11 November 2016 Lorenzo Tonina

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Table of contents

Riassunto

Drosophila suzukii, carpofago di recente introduzione in Italia ed in Europa, sta causando ingenti danni alle coltivazioni di ciliegio e piccoli frutti. La sua gestione è particolarmente complessa a causa della rapidità del ciclo di sviluppo, della capacità di infestare la frutta in prossimità della raccolta e dell'ampia polifagia. Al fine di sviluppare strategie di gestione integrata (IPM) sostenibili ed efficaci, risulta essenziale caratterizzare la gamma di piante ospiti presenti negli habitat naturali, individuare la soglia termica minima di sviluppo e studiare gli spostamenti dell'insetto dalle aree naturali a quelle coltivate.

Per svolgere studi di ecologia e definire razionali strategie di controllo è necessario disporre di strumenti di monitoraggio (trappole ed esche) efficaci, caratterizzati da elevata selettività, praticità di utilizzo, economicità e basso impatto ambientale. Dalle prove svolte durante il dottorato in differenti areali e nel corso di tre anni, l'attrattivo Droskidrink si è dimostrato essere il più efficace, mentre Suzukii Trap il più selettivo. Attrattività e selettività variano durante la stagione in funzione delle condizioni climatiche, suggerendo la necessità di utilizzare esche diverse in funzione del periodo.

Nel Nord Italia, tra le oltre cento specie investigate, 34 piante ospiti non coltivate hanno permesso lo sviluppo di *D. suzukii*. La loro presenza favorisce l'incremento delle popolazioni che, successivamente, sono in grado di colonizzare le adiacenti aree coltivate.

Dai frutti selvatici, raccolti lungo due differenti gradienti altitudinali nelle zone di montagna, sono sfarfallati adulti quando la temperatura media giornaliera era di almeno 11,1°C. Risultati simili sono stati ottenuti allevando colonie di laboratorio in una grotta a cielo aperto caratterizzata da un gradiente naturale di temperatura. Il completamento del ciclo di sviluppo si è verificato con temperatura media giornaliera superiore a 11,6°C. I risultati ottenuti evidenziano la capacità di *D. suzukii* di svilupparsi anche a basse temperature.

Attraverso uno sistema di trappole disposte a differenti distanze dal margine delle aree selvatiche ed a diverse altezze da terra all'interno di frutteti è stato dimostrato come la presenza di *D. suzukii* negli impianti produttivi diminuisca fortemente all'aumentare sia della distanza dal margine che dell'altezza da terra. I differenti andamenti delle catture osservati durante il susseguirsi delle fasi fenologiche della coltura hanno permesso di evidenziare che *D. suzukii* utilizza diversi habitat nel corso delle stagioni. Il carpofago colonizza i frutteti verso l'interno e

le piante in altezza in presenza dei frutti maturi o in maturazione, mentre vola in prossimità dei margini e vicino al cotico erboso nei restanti periodi.

Dalle informazioni provenienti dai vari studi presenti in questa tesi emerge che per una efficace difesa contro questo nuovo insetto è necessaria la combinazione di tutti i mezzi di contenimento disponibili e la necessità di mettere a punto strategie di monitoraggio e contenimento a livello di agroecosistema. Inoltre è auspicabile l'instaurazione degli equilibri biologici, pertanto è necessario un maggiore approfondimento relativo all'efficacia nella limitazione da parte dei parassitoidi autoctoni.

Summary

The invasiveness of *Drosophila suzukii* in North America and Europe is causing several damage on soft fruits and cherry. The management of *D. suzukii* is particularly complex due to its rapid developmental cycle, its ability to infest fruit close to harvest and its polyphagy. Thus, characterizing the range of host plants in natural habitats, finding the lower thermal threshold for pest development and studying the spillover from natural areas to cultivated one become essential to develop sustainable integrated pest management (IPM) strategies.

A highly sensitive monitoring tools (traps and lures) characterized by high selectivity, practical in use, economic cost and low environmental impact, is essential to study the population dynamics and define rational strategies of *D. suzukii* control. From our studies, carried on multi-year and multi-regional comparison, the most attractive lure was Droskidrink, while Suzukii Trap was the most selective. Attractiveness and selectivity change during the season in dependence of climatic conditions, suggesting the need of implementing different lures in different periods.

Out of more than 100 investigated species in North Italy, 34 non-crop plants were found suitable for *D. suzukii* development, enhancing pest population in wild areas with subsequently invasion of fields.

From wild host fruits sampled along elevation gradients in mountain areas adults were obtained when the daily average temperature in the three weeks preceding the sampling was at least 11.1°C. Similar results were obtained with the laboratory colonies reared in a natural temperature gradient in an open-top cave, where oviposition and development from egg to adult occurred above 11.6°C. These findings indicate that *D. suzukii* performs well at low temperatures.

Using traps disposed at different distances from the forest margin and at different heights from ground, it emerged that *D. suzukii* abundance in the orchards declined strongly with increasing distances from border and heights from ground. The observed patterns varied across the crop phenological development stages, indicating that the pest used multiple habitats across the seasons. When the host plant was not suitable for reproduction, *D. suzukii* preferred to fly closer to the forest margin and near the grass. Differently, when the host plant was suitable, *D. suzukii* colonized further the orchards both horizontally and vertically exploring more in depth the canopy volume.

The knowledge provided by this thesis underline the importance to conjugate the monitoring and IPM strategies in order to control *D. suzukii* at agroecosystem level. In addition, an effective limitation by native parasitoids is desirable and needs further studies.

Chapter 1

Introduction

Biological invasions by non-native or 'alien' species are the greatest threats to the ecological and economic safety of the planet. Alien species can act as vectors for new diseases, alter ecosystem processes, change biodiversity, disrupt cultural landscapes, reduce the value of land and water for human activities and cause other socio-economic consequences for man (e.g.: migrations, cultural changes, etc; Kenis *et al.*, 2009; europe-aliens.org, 2016).

Globalization is a complex phenomenon, affecting cultural, social and political aspects with a considerable importance in biological terms. Indeed, the transport of goods and people involves also the movement of harmful organisms, whose establishment is often facilitated by climate change (Roques, 2010; Roques *et al.*, 2010; Marini *et al.*, 2011). Among animals, arthropods are the most commonly introduced alien organisms. During the period 2000-2008, in Europe, an average increase of 19.6 new exotic species of arthropods per year was observed. It is an almost twice value compared to that observed in the previous period 1950-1974 (10.9 new species/year; Roques, 2010; Roques *et al.,* 2010).

Among European countries, Italy is certainly one of the most exposed to accidental introductions due to its geographical position, crossroads of several international trade, and the variety of habitats and climatic conditions characterizing the territory. The heterogeneity of environments increases the risk of establishment of the introduced species into the new area. Moreover, the wide range of climatic conditions facilitates their acclimatization (Pellizzari e Dalla Montà, 1997; Roques, 2010; Roques *et al.*, 2010). The risk that introduced species can become invasive and potentially dangerous is related to their adaptive capacity, to their biological characteristics and to limiting factors present in the new environment. In recent years, many exotic pests have been introduced accidentally through trade, causing serious damage to the primary sector of many countries.

Delivering Alien Invasive Species In Europe (DAISIE) is a project funded by the European Commission that provides updated information on biological invasions in Europe. Recent examples of introduced species causing high damage in agricultural sectors are: *Diabrotica virgifera* LeConte (Coleoptera: Chrysomelidae), *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera; Cynipidae), *Popillia japonica* Newman (Coleoptera: Scarabeidae) and *Halyomorpha halys* Stål*,* (Rhynchota: Pentatomidae). Up to 2008, the most important alien carpophagous species was *Ceratitis capitata* Wiedemann (Diptera: Tephritidae; http://www.europe-aliens.org; 2016). The recent introduction of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) in Italian territory has alarmed the whole agricultural sector (Grassi *et al.*, 2012, Grassi and Pallaoro, 2012).

D. suzukii is also known as Spotted Wing Drosophila (SWD) in the US, Cherry drosophila in UK, Kirschessigfliege in German countries and Drosophile du cerisier in France. *D. suzukii* belongs to the vast order of Diptera, family Drosophilidae, genus *Drosophila,* subgenus *Sophophora* (Rondani, 1875; Sturtevant, 1939). A large number of species belongs to this family commonly known as fruit flies, including *Drosophila melanogaster*, also known as vinegar fly. In Italy, to date, it has been reported the presence of thirty species of Drosophilidae (O'Grady, 2002).

The interest for *D. suzukii* is related to the heavy damage caused to a wide range of healthy fruits, both cultivated (Sasaki and Sato, 1995, 1996). In fact, differently from other species of *Drosophilia* known in our areas, *D. suzukii* does not require rotten or over ripe tissues. In addition, the preference for unripe or overripe fruits is lower than for healthy and ripe fruit (Kanzawa, 1939; Mitsui *et al.*, 2006; Lee *et al.*, 2011a). The destructive potential is given by the strong and serrated ovipositor, able to tear the tissues of the fruit skin, during egg laying. The trophic activity of the larva causes a collapse of the pulp close to the oviposition site, the lesion caused from ovipositor also exposes the fruit to possible secondary attack by pathogens or other insects. The damage caused by the larvae of *D. suzukii* make the fruit unsuitable for marketing (Cini *et al.*, 2012, Hauser *et al.*, 2009). Due to the high potential for dissemination and economic damage at the global production, the European and mediterranean Plant Protecion Organization decided to include *Drosophila suzukii* in the alert list in 2010. Later it was moved to the A2 list (eppo.int, 2016).

Origin and diffusion

D. suzukii is a native species of Asia, it was discovered for the first time in 1916 on cherry in Japan (Kanzawa, 1936). At the time it was supposed that the pest was introduced in the country at the beginning of the 900th from countries such as China, India and Bangladesh (Kanzawa, 1936). Few years later the presence in China, India, Korea and other Asian Countries was documented (Kanzawa, 1936; eppo.int, 2016).

In 1980, the pest was found for the first time outside Asia, specifically in the Hawaiian Islands, though no damage were reported (O'Grady *et al.*, 2002).

The first detection in the United States is dated September 2008 in California (Hauser, 2011) and then spread in the following years throughout the country (Hauser *et al.*, 2009; Burrack, 2011; Isaac *et al.*, 2010; Hauser, 2011).

The year 2008 marks a change in the spread of *D. suzukii*. Once the pest arrived in the United States, the import-export trade related to the American food chain allowed to spread to all importing countries, and so also in Europe (Anfora *et al.*, 2012). The first catches in Europe were recorded in Catalonia in 2008 (Calabria *et al.*, 2012). Since 2011 *D. suzukii* has spread all over the other European countries (eppo.int, 2016).

For Italy, the first report was recorded in September 2009 in Trentino, on raspberry and blueberry (Grassi *et al.*, 2009). In Veneto the first official reporting was in 2010 on raspberry and cherry, with several damage in the following year (Griffo *et al.*, 2012). The pest reached quickly the whole Country (Franchi and Barani, 2011; Pansa *et al.*, 2011; Suss and Costanzi, 2011; Griffo *et al.*, 2012; Mazzetto *et al.*, 2015).

The current distribution is reported in Figure 1.1.

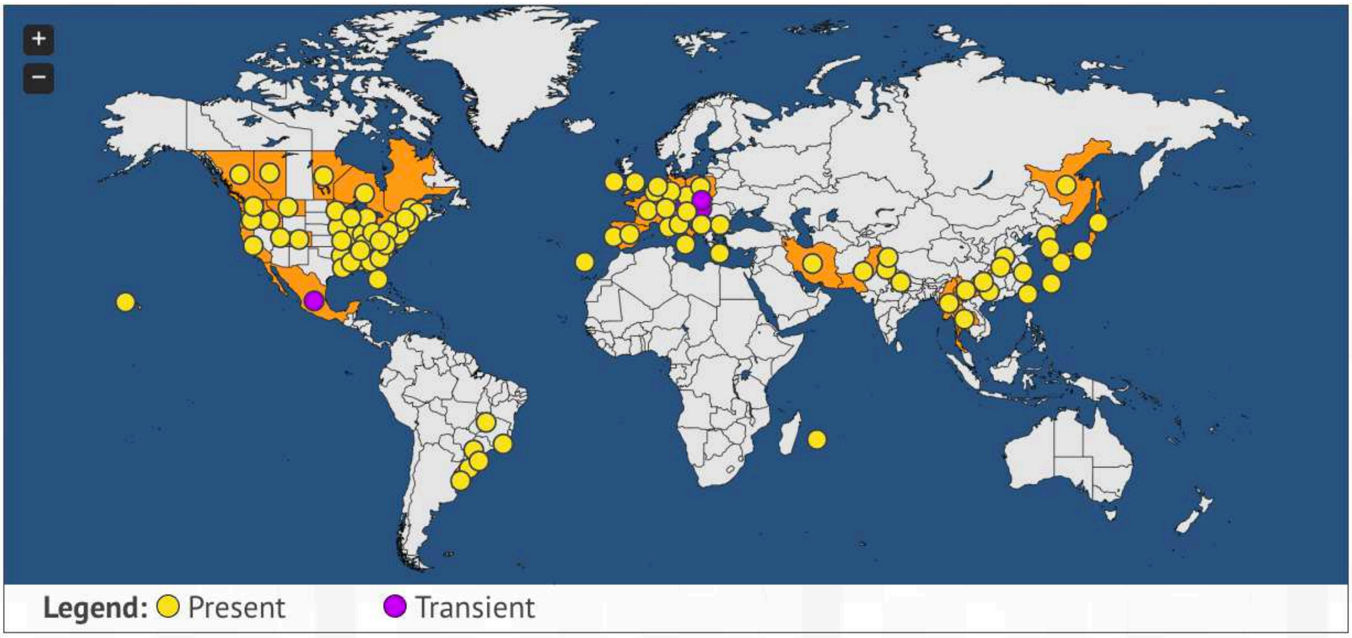

Figure 1.1 - Spread of *D. suzukii* around the world (eppo.int 2016).

This insect is characterized by high dispersion ability both active, with distances reaching 1400km/year, and passively through the commercialization of infested fruits and plants (Hauser, 2011; Calabria *et al.*, 2012; Westphal *et al.*, 2008). This last way of dissemination was the key factor for the first colonization of the western areas where, both in California and in Spain, the first findings occurred nearby of ports, suggesting that eggs or larvae arrived in infested fruits transported by ship from Asia (Rota-Stabelli *et al.*, 2012). The high distribution potential and the insect adaptation to sub-optimal environments further justify the speed of spatial diffusion (Cini *et al.*, 2014, Rota-Stabelli *et al.*, 2012). The ability to spread thanks to the infected material and the high adaptation characteristics to different conditions make the containing and the complete eradication unfeasible (Ioriatti *et al.*, 2011a, 2011b; eppo.int, 2016). Within a wider geographical perspective, taking account of the insect biology, it seems highly probable an imminent spread of *D. suzukii* in all the regions of the Earth with climatic conditions ranging from subtropical to continental (Walsh *et al.*, 2011). This because of three peculiar ecological factors, that make *D. suzukii* the subject of great concern for the world fruit production: adaptability to environmental conditions, high fertility and feeding habits (Cini *et al.*, 2012, Walsh *et al.*, 2011).

Ecology of the species

Morphology

The adult (Fig. 1.2) of *D. suzukii* has a length of 2-3 mm and a wingspan of 6-8 mm. Usually females are on average larger than males. The thorax is light yellow-brown in colour. It presents a pair of wings and a pair of halteres, like all the Diptera. The male has wide black spots at the tip of the wing on second vein. In males two dark coloured combs (sex combs) are present, on the first and second segments of the front legs (foretarsi). The abdomen is characterized by a light yellow colour and presents unbroken bands at the ends of abdominal segments (Kanzawa,1939; Kawase *et al.*, 2007).

Figure 1.2 – Adults of *D. suzukii*, female (left) and male (right).

The prerogative of *D. suzukii* females is a large sclerotized, hard, shiny and dark ovipositor with strong serrations of teeth, with saw-like edge when fully exposed (Vlach, 2010; Walsh *et al.*, 2011; Fig. 1.3).

Figure 1.3 – Relaxed ovipositor in *D. suzukii* female.

D. suzukii females lay between 7 and 16 eggs each day, for a total of 350-400 eggs during their life. On each fruit 1 to 3 eggs are laid on average (Kanzawa, 1939; Grassi *et al.*, 2009). Differently from other species of pests, several *D. suzukii* females may lay eggs in the same fruit, resulting in the emergence of many larvae (Smith and Saverimuttu, 2010).

The eggs are white and small, with an average size of 0.62 x 0.18 mm. They possess respiratory processes, starting from the egg surface and remaining outside of the fruit skin. Through these structures, called spiracles, the supply of oxygen to the embryo is guaranteed (Kanzawa, 1939; Walsh *et al.*, 2011; Fig. 1.4, 1.5).

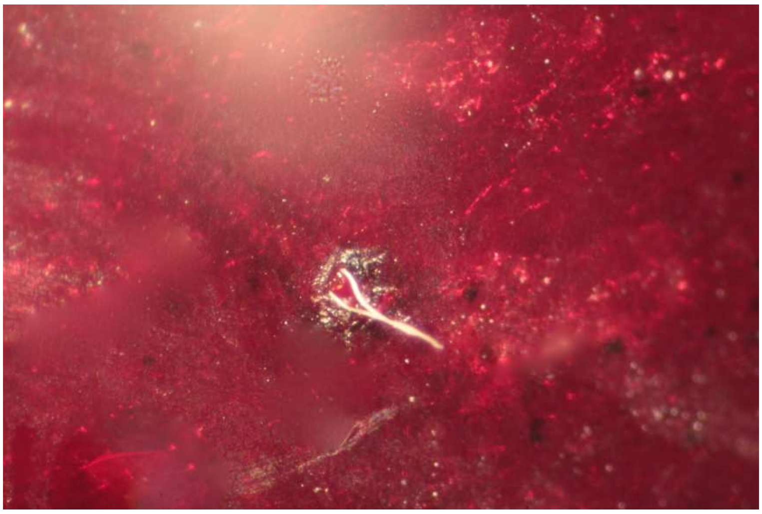

Figure 1.4 – Spiracles of *D. suzukii* in egg laid in ripe cherry.

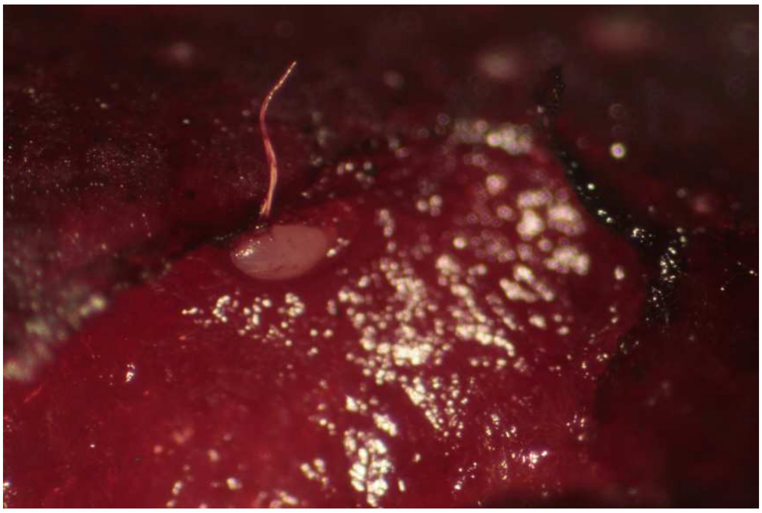

Figure 1.5 – Egg laid under cherry skin with the spiracles outside.

Larvae and pupae present characteristics common to all *Drosophila* flies. The larva is legless, cylindrical in shape and white-cream colour. The length is variable from 0.5 to 4 mm, depending on age. There are three larval instars. The mouthparts are black tearing sickle cell. Even the larval stages present spiracles in the end of the body, serving to breathe in a low oxygen environment, such as decaying tissues (Kanzawa, 1939; Walsh *et al.*, 2011; Fig. 1.6).

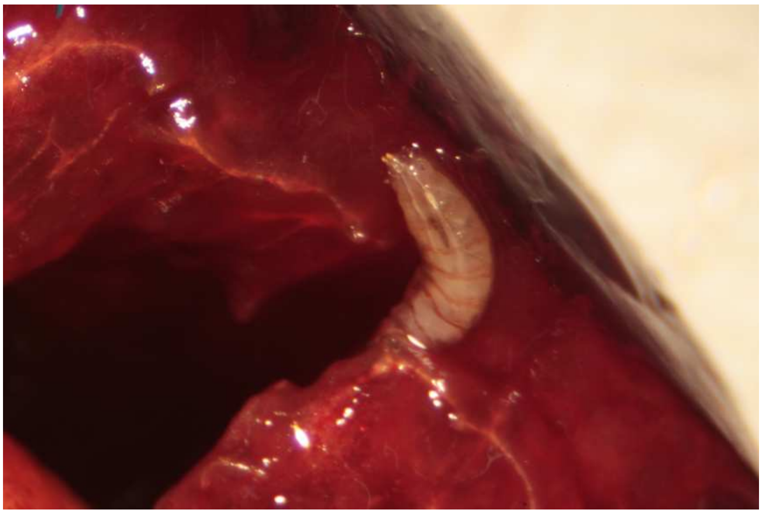

Figure 1.6 – Larvae of *D. suzukii* in cherry pulp.

The puparium of *D. suzukii* is thin and elongated, which is also very similar in the relevant species. The puparium is around 3 to 3.3 mm long and 1 mm wide. If still young, they have a yellow-golden colour, which gradually turns to opaque red and finally to dark red. Some adult structures are easily visible, including eyes and wings. Two breathing processes are present, which have a length of about 0.3 mm (Fig. 1.7). The pupation can take place directly in the fruit or external in the immediate vicinity (Kanzawa, 1935; Walsh *et al.*, 2011).

Figure 1.7 – Pupae of *D. suzukii*.

Biological cycle

The duration of the cycle, from egg laying to adult emergence is dependent on the ambient temperature, at a constant temperature of 15°C the cycle is completed in about 21-25 days in 9-

11 days at 25°C (Kanzawa, 1939; Walsh *et al.*, 2011). The short biological cycle allows to *D. suzukii* to complete many generations in a single harvesting period and complete up to 13 generations in a year, according to the zones. This permits an explosive population growth (Dreves *et al.*, 2009; Grassi *et al.*, 2009; Kanzawa, 1939).

After 12-72 hours from the hatching, the larvae start to feed voraciously the pulp. The adult requires 24-48 hours to reach the sexual maturity state (Dreves *et al.*, 2009).

Effect of temperature and humidity on life history

D. suzukii can be considered a specie very resilient at different temperatures, it is tolerant to heat and cold. Adults are particularly tolerant to cold, compared with other *Drosophila* species (Kimura, 1988). The temperature range that allows the reproduction is quite extensive and is between 10°C and 30°C. The higher fertility is registered at around 20-25°C. Above 29-30°C the adult male becomes sterile and ceases reproducing (Walsh *et al.*, 2011). *D. suzukii* is sensitive to drought: in the absence of water individuals die within 24 hours. Also high relative humidity and sultry climate allow the insect to live without dehydrate excessively. Often, the presence of small rivers promotes the development (Walsh *et al.*, 2011).

Overwintering

The adult is the most common durable stage of this species, indeed mated female in diapause are considered the overwintering stage of *D. suzukii* (Kanzawa, 1939; Mitsui *et al.*, 2010; Walsh *et al.*, 2011). The overwintering phase starts at temperature lower than 5°C. During the fall, with decreasing temperature, the neo-adults of *D. suzukii* do not sexually mature, but enter in winter diapause. Under these conditions even the sexually mature adults can get into this physiological state and resume the activities in spring or when temperatures return to be suitable for the biological cycle (Kanzawa, 1939; Mitsui *et al.*, 2006; Walsh *et al.*, 2011). During this period, overwintering adults can live an average of 200 days up to a maximum of 301 days (Dreves *et al.*, 2009).

Several places are suitable for overwintering, for example natural sites like forests and natural vegetation or anthropic one (Zerulla *et al.*, 2015).

Habitat and hosts

Temperature is the most influential factor on the development of the local populations of *D. suzukii*, that is closely affected by altitude, latitude and seasonal climate trends.

D. suzukii has been detected in different habitats, from sea level up to the 1550 m a.s.l. (Calabria *et al.*, 2012). Tolerance to harsh environment may depend on physiological causes or may be mediated by adaptations such as altitudinal migration (Mitsui *et al.*, 2010), acclimatization (Walsh *et al.*, 2011) and/or overwintering in anthropic habitats or other sites (Kimura, 2004).

An important characteristic of this species is the high polyphagy. *D. suzukii* is able to attack different kinds of fruitscultivated, wild or ornamental, mainly characterized by soft skin (Walsh *et al.*, 2011).

Several host plants were documented before the start of my PhD (Kanzawa, 1939; Grassi *et al.*, 2012, Grassi and Pallaoro 2012; Lee *et al.*, 2011b; Seljak, 2011; Walsh *et al.*, 2011), but a complete list of wild and ornamental host fruits is missing. The preference for a species rather than another is strongly influenced by the abundance of plant species present in the local area (Cini *et al.*, 2012).

Bellamy *et al.* (2013) tested the interaction of *D. suzukii* with host fruits, examining four aspects of interaction: attraction of the host plant on adults, egg-laying capacity at the population level, egg-laying capacity at individual level and key factors of development. It has been applied a Host Potential Index (HPI) methodology, resulting in higher susceptibility as a host for blueberry, strawberry and blackberry. This laboratory study revealed less attraction for peaches, cherries and blueberries, with grapes at the lowest potential. In choice conditions, it would seem that *D. suzukii* prefers fruits with peel thinnest as possible.

Without hosts fruits, adult of *D. suzukii* can feed and lay eggs also on some kinds of flower (Mitsui *et al.*, 2010).

The wide range of hosts makes difficult the management of this pest. First because the fly can cause damage at many species. In addition, the populations of *D. suzukii* can survive in many environmental contexts, both cultivated and wild, alternating on different hosts with different ripening periods during the year. The cultivated plants are usually managed in high-density orchards that enable to pest population rapid growth, while the wild and ornamental plants could serve as a refuge from the insecticide treatments, alternative source of breeding sites and refuge areas, with the subsequent re-infestation of the fields (Klick *et al.*, 2014).

Impact on crops production

D. suzukii is one of the few species of drosophilids able to infest healthy fruit (Fig. 1.8).

Figure 1.8 – Female of *D. suzukii* during oviposition on cherry in changing colour.

Females lay eggs on the fruits causing small injuries and lacerations of skin. The trophic action of larvae creates depressed areas and softening of its flesh. Very quickly, within a couple of days, the affected fruits collapse up to a complete disintegration of internal tissues.

In the few hours after the egg hatching, the damage is still limited and hardly noticeable, and this can prevent the farmer to notice the infestation at harvest, thus combining infested fruits with the healthy ones. This aspect can be a major problem during the marketing phase, as the affected fruit degenerate and cause the development of moulds, able to damage even the fruits not affected directly by *D. suzukii*. The commercial damage are considerable, due to appearance, the loss of texture and sour smell. The consequence of an important infestation is a total depreciation of the commercial value of the attacked fruits (Bolda *et al.*, 2010).

On the infested fruit, acetic fermentation, fungal and bacteria attacks could start entering through injuries and galleries created by the pest. These attacks therefore accelerate the rotting process of tissues. They are often associated with the presence of flies of others species of the genus *Drosophila* (Grassi *et al.*, 2012; Lee *et al.*, 2011b).

In addition to lower production, the economic losses are related to the increasing cost for the harvesting (fruit sorting time), for insect management (plant protection products, traps, nets, etc.) and for costs of marketing the product (possibility of a dispute about infested stocks; Grassi *et al.*, 2012).

In Japan, studies carried out during the '30s reported damage up to 100% on cherries and 80% on grapes on different orchards in a few years (Kanzawa, 1939). Damage comprised between 26% and 100% have been reported in Japan still more recently on some areas (Sasaki and Sato, 1995). In North America *D. suzukii* caused damage on blueberry, strawberry and raspberry. Gross revenues for farmers were assumed to decrease by 20 to 37%. Estimated annual costs to the US fruit production are more than US$ 500 million (Hauser *et al.*, 2009).

In Europe the documented damage concerns all berries, cherry in particular. Alerts were also made on some grape varieties (Mazzetto *et al.*, 2015). In the Trento district of Italy, annul losses in small fruit production were assumed €3.3 million per year (De Ros *et al.*, 2013). In Verona the harmfulness on cherries of this pest is very high. At consumptive of 2013 cherry harvest campaign, more than 30% of the hilly and mountainous production was damaged (data thanks to CCIAA Verona, 2013; Fig. 1.9).

Figure 1.9 – Cherries heavily infested by *D. suzukii*.

Management and control strategies

The research for population containment tools and pest control is extremely complicated, because of the high biological performance of this species (Griffo *et al.*, 2012). All of these components have obliged many researchers of the different countries to an intense work in an effort to provide to farmers increasingly effective protection methods (Grassi *et al.*, 2013). Due to the impossibility to remove the pest from areas where it is already present (eppo.int, 2016), the fruit protection must set up operations to depress the population of the pest continuously, in order to reduce the subsequently infestation.

Monitoring

In a perspective of increasing attention for integrated pest management (IPM) in fruit productions, characterized by low input of chemicals, the monitoring of target insects become fundamental. The need to detect the presence of *D. suzukii* and to collect data on the number of adults in the environment has prompted several research groups to start territorial monitoring activities (Ioriatti *et al.*, 2015; Fig 1.10).

Figure 1.10 – Monitoring trap (Droso-Trap) in strawberry orchard.

The aim of territorial monitoring is to collect information on the development of *D. suzukii* in relation to environmental and climatic conditions and to obtain technical details concerning the management of the protection from this pest (Ioriatti *et al.*, 2015).

In order to realize an effective control of the pest in the field it is essential to monitoring the presence of adults and oviposition on the fruits. To perform an efficient monitoring is necessary the availability of a technical device with a high selectivity; practical in use, economical and of low environmental impact (Ioriatti *et al.*, 2015).

The monitoring of *D. suzukii* adults is based on the use of traps of different colour, shape and structure baited with food lures in fermentation, pure or in mixture. The most currently used traps are bottles or other containers of the 500-1000 mL capacity, of different colours. Black and red traps were found to be the most attractive (Basoalto *et al.*, 2013; Mitsui *et al.*, 2006; Edwards *et al.*, 2012). Recent studies showed a high level of attraction from a mixture of vinegar and wine, probably due to a synergistic effect of acetic acid, ethanol and other volatile compounds present in wine and vinegar (Landolt *et al.*, 2012). Four chemical compounds resulted more attractive for *D. suzukii* adults; they are acetic acid, ethanol, acetoin and methionol (Cha *et al.*, 2013).

As previously reported, *D. suzukii* females usually lay their eggs in undamaged healthy fruit. Perhaps the odours produced by fermenting bait represent only a generic food signal, while females that need to lay eggs are more attracted to volatile compounds released from fresh fruit. Currently a species-specific trap-lure combination has not been developed yet, further research on traps, lures and monitoring protocols are therefore necessary (Cini *et al.,* 2012 Walsh *et al.*, 2011).

Chemical control

The insect biology and the phenological characteristics of susceptible crops greatly complicate the insecticide protection. The principal problems using plant protection products are the insect polyphagia, the attack close to the harvest, the length of the harvesting period and the contemporary presence of different crops and varieties (Ioriatti *et al.*, 2011a, 2011b). The sensitivity to the attack of the fruits is maximum close to harvesting and, especially for small fruit, the ripening period is long and the harvest is scalar and performed with very restricted turns (Ioriatti *et al.*, 2011a, 2011b).

These characteristics of the pest and host plants involve the need for repeated insecticide applications from the changing colour phase to the ripening period, in order to protect the most sensitive phenological stages. This clashes with the need to respect the Pre-Harvest Interval (PHI) and with the increasing risks of residues on fruits for the considerably tight collection intervals. In addition to these factors, the chemical control is further limited by the reduced availability of active substances registered for *D. suzukii* (Ioriatti *et al.*, 2015).

Regarding to this aspect, the new generation of insecticides characterized by selectivity of action against target organisms have proved relatively ineffective in containing the damage caused by *D. suzukii*, forcing farmers to use products with a wide spectrum of action. The use of such molecules, however, involves an increase in the risk of residues presence on fruits, the possibility of pest resistance development and the increase of the production costs (Grassi *et al.*, 2012, Grassi and Pallaoro, 2012).

Due to of the impossibility to make an effective control of larvae, an adulticide-ovicide and residual approach is needed. Studies in Europe and in the US shown good results for organo-phosphate products or continuous applications with pyrethrins and spinosyns (Beers *et al.*, 2011;

Bruck *et al.*, 2011; Profaizer *et al.*, 2012, 2015; Shawer *et al.*, 2015; Angeli *et al.*, 2012; Van Timmeren and Isaacs, 2013). Other tests indicate lambdacyhalothrin as a good product (Grassi *et al.*, 2012; Grassi and Pallaoro, 2012). Instead, the efficacy of neonicotinoids on adults was unsatisfactory (Bruck *et al.*, 2011). For organic production, at the moment, Spinosad seems to remain the insecticide providing the best results (Walsh *et al.*, 2011).

Mass trapping

Among the most widely studied control methods, searching for alternative to insecticides, there is mass trapping (Fig. 1.11). Objective of this method is to contribute at the containment of the *D. suzukii* damage reducing the pressure of adults using numerous traps. Tests were conducted to search for more attractive substances and to evaluate different distributions of traps inside and around the orchard to protect. Investigation in North Italy found this method suitable and cost-effective for growing situations less susceptible to strong attacks (isolated orchards of medium to large size, not surrounded by forest or hedgerow, in flat conditions, with hot and dry microclimate). Mass trapping showed disappointing results, because the traps are ignored when the fruits are ripe and the infestation equally occurs. The main problems for the effectiveness of this method is the unavailability of a highly specific and effective lure, able to competing with the fruits in the attraction of adults of *D. suzukii* (Ioriatti *et al.*, 2015).

Figure 1.11 – Row of traps used as mass trapping to protect cherry orchard.

Insect-proof nets

Among the sustainable protection techniques for the control of *D. suzukii*, the use of anti-insect nets was studied, obtaining encouraging results, reducing or avoiding completely the use of insecticides, and reaching high levels of control (Kawase *et al.*, 2007; Grassi and Maistri, 2013). The holes in the mesh size should not exceed 1mm^2. Different application methods were also evaluated, obtaining an almost total control on models "single plant" and "single row", while in the " whole orchard" model the efficacy was not total, due to the continuous lifting of the nets to allow the movement of farmers and machineries (Grassi and Maistri, 2013; Fig. 1.12, 1.13, 1.14). To obtain a good effectiveness of the nets, is necessary to apply it in advance, prior to changing colour of the fruits and keep them closed until the end of the harvest (Grassi *et al.*, 2013).

Further investigations will be useful to evaluate the economic sustainability of the investment, the possible side effects on fruit quality, microclimate, plant ecophysiology, fungal diseases and other insects (Ioriatti *et al.*, 2015).

Figure 1.12 – Insect-proof nets in cherry orchards: single tree system.

Figure 1.13 – Insect-proof nets in cherry orchards: single row system.

Figure 1.14 – Insect-proof nets in cherry orchards: whole orchard (Ph: Alberto Grassi).

Cultural practices

Pendant fruits are preferred than fruits fell to the ground (Walsh *et al.*, 2011; Dreves *et al.*, 2009), but fruits on the ground may constitute a dangerous inoculum. The whole collection and elimination of infested fruit through sunburn, disposal in closed containers, crushing, low temperature treatments, bagging and burying are essential IPM procedures to limit the infestation of healthy fruit, through the destruction of eggs and larvae in the orchard (Grassi *et al.*, 2009; Cini *et al.*, 2012).

In addition, a correct pruning and the harmonization of the canopy are useful to increase the light filtering, to decrease the relative humidity and to avoid favourable microclimate for the insect, to increase the penetration of the insecticide applications and to concentrate the ripening period of the whole plant and speed up the harvest (Fig 1.15).

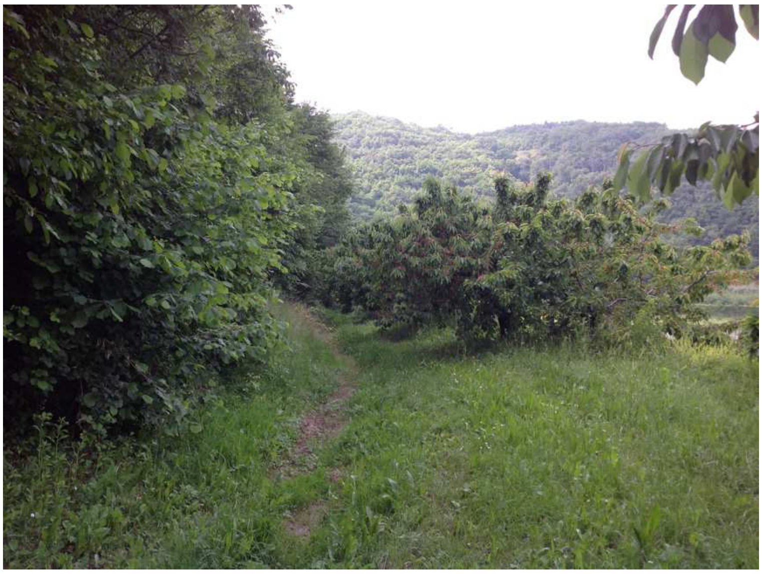

Figure 1.15 – Cherry orchard close to the forest, with tall grass, dense canopy and high humidity, all conditions favourable for pest development.

Biological control

Currently different biocontrol agents are under experimentation and development: viruses, bacteria, fungi and other natural enemies such as parasitoids and predators. Recently DNA viruses were isolated in *Drosophila* species resulted related to other viruses used for pest control (Unkless, 2011). A practical and effective control method, but often overlooked, profit by the close relationship between the pest species and their endosymbionts. These interactions can directly or indirectly influence the population dynamics of many pests and could become interesting for crop pest management (Zindel *et al.*, 2011). From preliminary results it seems that *Wolbachia* is able to infect *D. suzukii*, but further studies are needed (Cini *et al.*, 2012).

A valid way to restore favourable conditions for a sustainable and ecosystems respectful pest management, is represented by biological control. The parasitoids could have an important role in containing the populations of *D. suzukii* (Rossi-Stacconi *et al.*, 2015).

In Japan, numerous evaluation studies of management strategies for a long-term control were performed, based on the permanent introduction of natural enemies of *D. suzukii* from the origin area, where pest and antagonist are probably co-evolved (Cini *et al.*, 2012). The first experiments in the laboratory, have tested the effectiveness of *Phaenopria* spp. (Hymenoptera, Diapriidae) in the control of *D. suzukii* and the results were unsatisfactory (Kanzawa, 1939).

Some species of the genus *Ganaspis* (Hymenoptera: Figitidae) showed the best results with rates of parasitism of 2-7% (Ideo *et al.*, 2008; Mitsui *et al.*, 2007). They lay eggs in the larvae that feed on fruit and are characterized by a high specificity rate against *D. suzukii*. Two other species: *Leptopilina japonica* Novkovic et Kimura (Hymenoptera: Figitidae) and *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae) are able to attack larvae and pupae only in rotting fruit, attacking a wide range of drosophilids (Mitsui and Kimura, 2010).

In addition to the study of co-evolved organisms in the area of origin, recent surveys are aimed to improve the beneficial effects of existing parasitoids in the invaded territories. They are generalist and wide diffused species that have *D. suzukii* in their host range or that are able to adapt to it. In this regard *Pachycrepoideus vindemiae*, a common pupal parasitoid of many drosophilids, was found on *D. suzukii* (Ioriatti *et al.*, 2011a). The potential ability to adapt to *D. suzukii* was also demonstrated from *L. japonica*, *Trichopria drosophilae* Perkins (Hymenoptera:Diapriidae) and *A. japonica*. These three species are able to attack larvae and pupae in rotting fruit, having as host various species of drosophilids in Europe and showed their ability to develop on *D. suzukii* (Cini *et al.*, 2012; Rossi-Stacconi *et al.*, 2015, Mazzetto *et al.*, 2016).

Regarding predators, trophic activities were discovered by *Anthocoris nemoralis* Fabricius (Heteroptera:Anthocoridae), *Atheta coriaria* Kraatz (Coleoptera:Staphylinidae), *Cardiastethus nazarenus* Reuter (Heteroptera:Anthocoridae)*,* *Cardiastethus fasciventris* Garbiglietti (Heteroptera:Anthocoridae), *Dicyphus tamaninii* Wagner (Heteroptera: Miridae), *Hypoaspis miles* (Acari: Laelapidae), *Orius majusculus* Reuter (Heteroptera:Anthocoridae) and *Orius laevigatus* (Heteroptera:Anthocoridae) Fieber (Arnò *et al.*, 2012; Cuthbertson *et al.*, 2014).

In addition to the use of these biocontrol agents, it is possible to reduce the pest population through the realising of sterile individuals in the environment, in an inundative way. Integrated with other control activities, this technique could reach numerous benefits, by acting in a specific, non-dependent density mechanism (Vreysen *et al.*, 2006).

Aims

The arrival of *D. suzukii* in 2009 caused extensive damage and hardly changed the cultivation of cherry and soft fruit in Italy. Currently, to control the pest, there is an increase in the number of insecticide applications that are sprayed from fruit changing colour until complete harvest. This increasing use of chemicals produces a rise in production costs and could pose risks to consumers and to environment (Boselli *et al.*, 2012; Grassi *et al.*, 2012, Mori *et al.*, 2015). The control of *D. suzukii* damage is extremely complicated and is therefore essential to continue in intensive research studies. It is desirable and necessary to investigate deeper on effective methods and start experiments focused on still unclear aspects. For example, the identification of compounds able to increase the attractive potential of lures in the traps requires an articulated experimentation for longer times, to improve efficiency of the monitoring systems. As well as the development of strategies of biological control of the pest and the search for more efficient biocontrol agents. The knowledge of the biological aspect, is the prerequisites for research of environmentally friendly control methods (Griffo *et al.*, 2012; Ioriatti *et al.*, 2011a).

With the aim to apply integrated pest management (IPM) strategies and consequently to reduce the use of insecticides is necessary to study aspects of biology and ecology, to look for new methods for monitoring populations and to use new management strategies in the field.
The European DROPSA Project has started in 2014 with these purposes. The major objectives are to determine introduction and spread of *D. suzukii* and pathogens; to develop preventative strategies and recommendations against the introduction of invasive fruit pests and diseases; to identify the biology and ecology of *D. suzukii*, for the development of preventative and sustainable control methods; to develop practical, effective and innovative solutions to control *D. suzukii* and the pathogens, and transfer of best practices for inclusion in IPM strategies; to develop forecasting and decision support systems and risk management and, finally, to provide economic analyses to support decision making in the implementation of practical solutions to protect the EU fruit sector.

The aims of my PhD are focused on:

1) **Comparison of attractants for monitoring *Drosophila suzukii* in sweet cherry orchards**

   Developing monitoring techniques is of fundamental importance and it is the prerequisite to set up rational control strategies. For that reason, three tests were carried out between 2014-2016 to identify the best combination of effective trap and lure in monitoring adult populations. These tests were performed in collaboration with other research institutions of the North East of Italy (Fondazione Edmund Mach and Consorzio Fitosanitario di Modena), following same protocols. Furthermore, since 2013 a collaboration with some local cherry markets in Verona and Vicenza and with the Servizio fitosanitario della Regione Veneto has been activated in order to monitor the populations and the damage in the field.

2) Non-crop plants used as hosts by *Drosophila suzukii* in Europe

Due to the high polyphagy of *D. suzukii* for cultivated, wild and ornamental fruits in both native and new invaded areas (Walsh *et al.*, 2011), detailed studies on host plants present in our environment were necessary. As a consequence, the sampling of non-cultivated fruits were carried out in different landscapes in collaboration with two DROPSA partners (CABI Switzerland and Wageningen University Netherlands).

3) Development of *Drosophila suzukii* at low temperature in mountain areas

To assess the abundance of the fly in mountain areas in summer months, potential wild host fruits were sampled along two gradients of elevation to measure its emergence under different temperature conditions. In addition, a natural temperature gradient in an open-top cave, covering the lower range of temperatures known for *D. suzukii*, was exploited to deploy laboratory stock colonies. These experiments allow to find the minimum thermal threshold of development and the performance at low temperatures.

4) Spillover of *Drosophila suzukii* between non-crop and crop areas: implications for pest management

Considering the important role of semi-natural areas providing alternative host resources, overwintering habitats, or refuge areas when crops are sprayed with insecticides (Kenis *et al.*, 2016, Pelton *et al.*, 2016; Zerulla *et al.*, 2015), investigate the influence of forests in the distribution of catches inside the orchards should be necessary. Gradients in catches were studied placing traps at different distances from forest-margin and at different heights from the ground inside cherry orchards.

Chapter 2

A multi-regional comparison of attractants for *Drosophila suzukii* monitoring in sweet cherry orchards in Italy

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LT participated in conception and design of the research, conducted the experiments for Verona sites, analysed the data of all group and wrote the manuscript.

Abstract

The invasiveness of *Drosophila suzukii* Matsumura is causing sizable damage to soft fruits, cherry and grapevine. In order to define targeted strategy of *D. suzukii* control is essential to dispose of highly sensitive monitoring tools that presents high selectivity, practical in use, economical and with low environmental impact.

The aim of this study was to compare different combinations of traps and lures to define the best practice for the *D. suzukii* monitoring across different years and extended over a wide elevational gradient and landscape complexity.

The high attractiveness is not always combined with high selectivity, in some cases up to 95% of the *Drosophila* spp. captured were species different from the target *D. suzukii*. The most attractive lure was Droskidrink while Suzukii Trap was the most selective.

It is relevant to underline that the lure attractiveness and selectivity change during the season in dependence of climatic conditions suggesting the need of implementing different lures in different periods and for the different purposes (monitoring or mass trapping).

About the management of lures, Droskidrink showed the problem of bacterial-gel formation, Suzukii Trap and Trécé resulted more easy to manage since they do not need to be serviced at weekly interval.

Introduction

Drosophila suzukii has spread from its native distribution in Asia in recent years and is now a major pest in Europe and North America (Rota-Stabelli *et al.*, 2012; Asplen *et al.*, 2015). Due to the preference for the ripening fruits close to harvest, rapid developmental cycle and high polyphagy this invasive species is causing several damage to crop and non-crop species (Cini *et al.,* 2012, Poyet *et al.*, 2015; Kenis *et al.*, 2016).

The potential of *D. suzukii* to spread worldwide and its recognized detrimental impact have raised the need to specifically monitor its occurrence, promoting the development of effective traps and attractants and their practical application in monitoring actions (Grassi *et al.*, 2009; Lee *et al.*, 2012; Cini *et al.*, 2012; Ioriatti *et al.*, 2015; eppo.int, 2016). Monitoring the dynamic of *D. suzukii* populations both at individual field and at regional scale is crucial to understanding the spread into new areas and the potential impact and damage of the population to susceptible crops. Ultimately, effective monitoring tools constitute the essential prerequisite to better predicting pest outbreaks and implementing integrated pest management (IPM) strategies in order to reduce insecticides applications (Cini *et al.,* 2012).

However, to develop an effective monitoring method, it is necessary to determine the combination of trap and lure enabling high attractiveness in situations with low populations, high selectivity, ease of use, low-costs and low environmental impacts. Selectivity, the ratio between the pest and all other accidentally caught species, needs to be accurately assessed and integrated in the framework of an effective monitoring and pest management. In particular, the high selectivity minimizes detrimental effects on the biodiversity of non-target species and, at the same time, facilitates the screening operations during trap sorting.

To date, different types of baits and lures for *D. suzukii*, all based on food attractants, have been developed (Landolt *et al*., 2012; Lee *et al*., 2012, 2013; Basoalto *et al*., 2013; Cha *et al*., 2013, 2014, 2015a, 2015b; Grassi *et al.,* 2015; Kleiber, 2013; Kleiber *et al*., 2014; Burrack *et al*., 2015). Among the different baits the most widely used are based on fermenting substances (as apple cider vinegar (ACV) alone or in a mixture with wine) or in combination with peptides. The Droskidrink bait (75% of ACV, 25% red wine and 20 g/L of cane sugar) has been reported as an effective attractant in the early studies carried out in north Italy (Grassi *et al*., 2015). Synthetic lures are also available and are based on chemicals isolated from fermenting mixtures (Cha *et al*., 2013, 2014) released by specific commercial dispensers.

Comparisons among some baits and lures suitable for monitoring *D. suzukii* were carried out in USA and Europe (Cha *et al.* 2013; Grassi *et al.* 2015). However, these studies have the limit to analyse the solely attractiveness in narrows altitudinal gradients and over a short period of time.

From these premises emerge the need to better define the attractiveness of the different baits and synthetic lures, either alone or in combinations, investigating the seasonal dynamic of captures by performing continuous monitoring across different years. In addition, such monitoring needs be performed in different environments to cover the widest possible elevational gradient and landscape complexity. Finally, the analyses of attractiveness need to be paralleled by the accurate evaluation of lures selectivity in order to achieve the most targeted monitoring action.

The present work therefore aimed at comparing the attractiveness and selectivity of different baits and lures in time series of catches in order to evaluate the effect of environmental features and to provide reliable practice for the monitoring and control of *D. suzukii*.

All trap comparisons have been carried out in cherry orchards over three ripening seasons and in different locations in Northern Italy. Cherry is the most damaged fruit due to its ripening period, when few host fruits are available (Kenis *et al*., 2016), and due to its nutritive value and chemical-physical characteristics which are optimal for *D. suzukii* development (Lee *et al*., 2011a).

This long term approach performed over a wide geographical range provides large data sets needed to discriminate the variation in lure attractiveness due to unmanageable environmental causes and the effect of competition with the ripening fruits and it allows to determine the most suitable setup to efficiently monitor *D. suzukii* in cherry orchards.

Materials and methods

Sites and trap setting

The comparative surveys were conducted in four cherry orchards in the Provinces of Modena, Trento and Verona (North-East Italy) during 2014, 2015 and 2016 (Table 2.1).

Table 2.1 - Start, end, number of sampling periods undertaken and harvesting period in the different sites in the three years of survey.

Site	Start	End	n° of sampling	Harvesting period
2014				
Vignola (Modena)	Week 21	Week 30	10	Week 20 to 26
Pergine (Trento)	Week 21	Week 30	10	Week 23 to 28
Val d'Alpone (Verona)	Week 20	Week 29	10	Week 21 to 24
Valpolicella (Verona)	Week 21	Week 27	8	Week 22 to 26
2015				
Vignola (Modena)	Week 07	Week 29	23	Week 21 to 26
Pergine (Trento)	Week 07	Week 26	20	Week 24 to 25
Val d'Alpone (Verona)	Week 07	Week 26	20	Week 20 to 25
Valpolicella (Verona)	Week 07	Week 26	14	Week 22 to 25
2016				
Vignola (Modena)	Week 12	Week 16	5	Week 20 to 27
Pergine (Trento)	Week 12	Week 15	4	Week 23 to 28
Val d'Alpone (Verona)	Week 13	Week 16	4	Week 20 to 24

For this study commercial baits (Apple Cider Vinegar, Acentino, Modena Italy; Droskidrink®, Prantil, Trento, Italy; Biologische Essigfliegenfalle®, Riga-Gasser, Ellikon a. d. Thur, Switzerland; Suzukii Trap®, Bioiberica, Barcelona, Spain) were selected according to our preliminary results (unpublished). Droskidrink was added always with 5 g of brown sugar. ACV, Droskidrink and water were added with 1 drop of Triton® X-100 (Sigma-Aldrich Co. LLC.). In 2015, Droskidrink was added with a viable culture of selected lactic bacteria (*Oenococcus oeni*) produced by the Fondazione Edmund Mach laboratories and buffered the pH at 4.5 in order to evaluate their capacity as bio-catalyzers of the production of biologically active compounds to *D. suzukii* (Anfora *et al*., 2016). Comparison was performed using 250 mL of each bait. The synthetic lures were provided as numbered samples or commercial product (Pherocon SWD®)

by Tréce´ Inc. (Adair, OK, USA) and were used either in combination with 250 mL of the recommended water drowning solution. In 2016, Pherocon SWD was also tested in combination with some of the baits (Droskidrink, ACV, and Suzukii Trap) in order to verify potential synergistic effect. In each trial the lures were added with one drop of Triton surfactant, except Gasser-Riga and Suzukii Trap® ones.

A common protocol was followed at each site sampled. Four replications of all baits and lures under evaluation were deployed in Droso-Trap® (Biobest, Westerlo, Belgium) and installed within each orchard in a randomized complete block design. In 2014, a homemade trap commonly used in northern Italy for the monitoring of *D. suzukii* until 2014 (Grassi and Pallaoro 2012) baited with 200 mL of ACV and one drop of Triton was comparatively tested with Droso-Trap baited with the same attractant. From 2015 the Droso-Trap was modified adding a 3x3 mm net on the holes to avoid the entrance of bigger insects.

The lures tested, the amount inside a single trap, their replacement in the field and the year were reported in Table 2.2.

Table 2.2 - Composition, trap servicing interval of the baits and lures used in the field trials carried out during 2014-2016 for evaluation of attractiveness to *D. suzukii*

Lure	Bait/Lure change	Activity		
		ACV vs bait	Bait vs lure	Sinergidm
Apple Cider Vinegar 250 mL [1)]	weekly	2014		
Biologische Essigfliegenfalle 2 jars	weekly	2014		
Droskidrink 250 mL + brown sugar 5g	weekly	2014	2015	2016
Droskidrink 250 mL + brown sugar 5g + selected bacteria[1)]	weekly		2015	
Droskidrink 250 mL + brown sugar 5g + Pherocon SWD dispenser	4 weeks			2016
Pherocon SWD dispenser + Apple Cider Vinegar 250 mL	4-6 weeks [2)]	2014		
Pherocon SWD dispenser + water 250 mL	4-6 weeks		2015	2016
Suzukii Trap 250mL	4 weeks		2015	2016
Suzukii Trap 250mL + Pherocon SWD dispenser	4 weeks			2016
Trécé dispenser 1176 + water 250 mL	6 weeks		2015	
Trécé dispenser 1180 + water 250 mL	6 weeks		2015	
Trécé dispenser 1181 + water 250 mL	6 weeks		2015	

[1)] lactic bacteria (*O. oeni*) produced by FEM laboratories, pH buffered at 4.5
[2)] see supplementary trials

Each batch of traps was set within the orchard and spaced at least two rows apart the border and 20m the woods, in order to minimize possible gradients in population density. Each batch was set apart at least 20 m from another and the traps were hung on trees 1.5 m from the ground and 2.5 m apart from each other. The content of each trap (either bait or water drowning solution) was collected weekly and concurrently was renewed or refilled according to the type of attractant. At each sampling, traps were randomly reassigned to a new position. At each trap service the status of liquid and other management aspects such as bacterial gel, sludge and evaporative loss were recorded.

Data collection

The content of each trap was sorted under the stereo-microscope, recording the number of females and males of *D. suzukii*, the total number of other *Drosophila* species and the total number of insects other than *Drosophila* species (these last two counting were not performed in Pergine).

For each site in 2015 temperature data-loggers were deployed, at 2 m from the ground, to record hourly temperature (HOBO U23 ProV2 with RS1 solar radiation shield, Onset Computer Corporation, Bourne, MA, USA). Hourly temperature data were used to calculate thermal sums (over the 5°C minimum threshold) and to compute the number of hours in a week when temperature exceeded the 5°C minimum threshold.

Data analyses

Data on the weekly capture were used to calculate either the attractiveness as the number of *D. suzukii* caught or the selectivity as the percentage of *D. suzukii* within the total number of *Drosophila* species. Attractiveness and selectivity for each batch were analysed with traps-lure combination as fixed factors and site and data of collection as a random factor, by using a linear mixed-effect model (LME). Selectivity data since they are expressed as percentage, were arcsine square root transformed. The means were compared by using the Tukey-HSD test following ANOVA (The R Foundation for Statistical Computing http://www.R-project.org)

Results and discussion

These experimental monitoring trials, performed across different years and extended over a wide elevational gradient and landscape complexity, have provided clear indications for the best combinations of trap and attractant for monitoring *D. suzukii* in cherry-orchards in the North-East Italian regions.

Validation of the traps

The 2014 sampling campaign allowed us to compare the effectiveness of the two different trap types. Employing the same brand of apple cider vinegar, the commercial Droso-Trap recorded catches of *D. suzukii* four times higher than the homemade trap (averages: 3.13 vs 0.75 SWD/trap/week, LME $F_{1;194}$ 22.48, $p < 0.0001$). The average selectivity of both types of traps was similar and corresponding to about 15%; the differences were not statistically significant ($p=0.7895$).

Validation of the lures

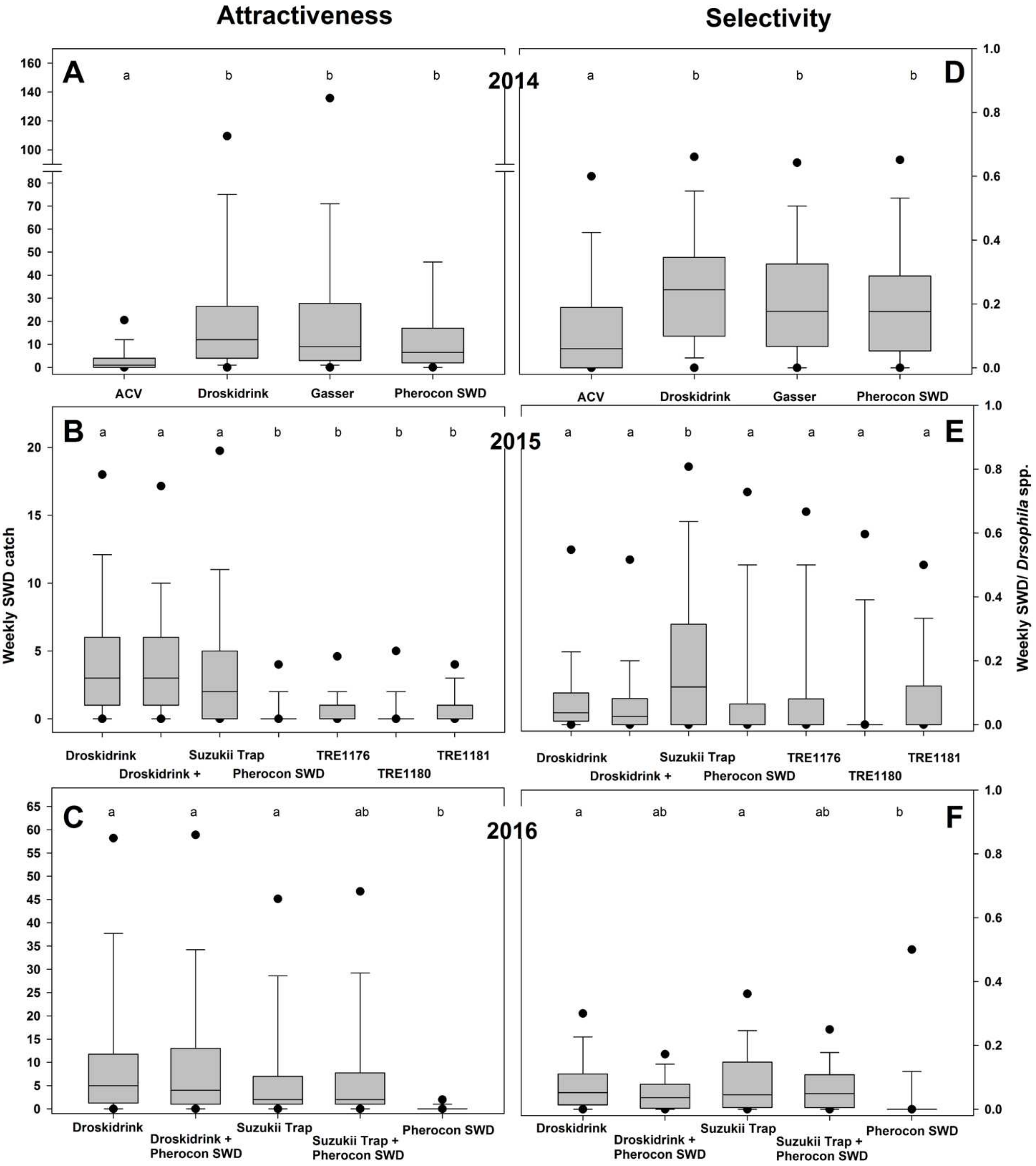

Figure 2.1 - Attractiveness and selectivity of the different *D. suzukii* baits and lures in three years of study. The boxes enclose the first and third quartiles; the ends of the whiskers represent the 5th/95th percentiles and the solid lines the median. Different letters indicate statistically significant differences between treatments (Tukey’s test following ANOVA, N=52-308, P<0.01). Droskidrink + indicates Droskidrink added with lactic bacteria bacteria, TRE indicates different dispensers of Pherocon SWD Trécé.

a. Attractiveness

Among the four lures tested during the 2014 cherry season only the pure apple cider vinegar showed a low capture efficiency. Droskidrink, Gasser and ACV+Trécé showed similar high catching capacity(Fig. 2.1.a; LME $F_{3;3411}$ 29.59, p<0.0001).

The attractiveness of Droskidrink, either used alone or with *O. oeni* culture, and Suzukii Trap differs from the Trecé lures tested in spring 2015, showing five times higher capture rates (Fig. 2.1.b; LME $F_{2;2067}$ 37.07, p<0.0001).

Synthetic lures over unscented drowning solution as tested in 2016 were significantly less attractive than baits. The use of synthetic lures in combination with baits did not show significant synergistic effect (Fig. 2.1.c; LME $F_{4;243}$ 8.10, p<0.0001).

Comparing the results among the three years the highest attractiveness was obtained with Droskidrink, Suzukii Trap and Gasser.

b. Selectivity

In 2014 ACV has shown the worst performance in selectivity among the tested lures and baits, with a value about 14%. Droskidrink, Pherocon SWD and Gasser captured on average 24% of the target species among the captures (Fig. 2.1.d; LME $F_{3;411}$ 31.22, p<0.0001).

Among the 7 lures and baits tested in 2015 (Fig. 2.1.e) Suzukii Trap was the most selective, with an average value of 20% SWD/all *Drosophila* species, statistically different from the other that are comparable between each other (LME $F_{6;1388}$ 19.59, p<0.0001). In two study sites, best selectivity of Suzukii Trap emerged also in relation at all other insects (not *Drosophila* species) captured from the trap (16% vs 5-11% of other baits; LME $F_{6;864}$ 9.55, p<0.0001).

The selectivity of Suzukii Trap in 2016 was comparable with Droskidrink, also when both were combined with Pherocon SWD dispenser, while Pherocon SWD dispenser alone showed a very low selectivity (Fig. 2.1.f; LME $F_{4;235}$ 5.82, p<0.0001).

These trials demonstrated that all the trap combinations tested are characterized by low selectivity, ranging on average from 6 to 23%. The mixture of peptides and organic acid (Suzukii Trap) increased the attractiveness to SWD with respect to other species. Compared with the fermenting substances and synthetic lures Droskidrink has shown low selectivity, higher than ACV, confirming that the addition of red wine and brown sugar is effective for SWD (Landolt *et al.*, 2012).

Table 2.3 - Overall captures of *Drosophila suzukii*, other *Drosophila* species and selectivity recorded in the different sites by the lures and baits tested during 2014-2015-2016 (note that not all lures were tested for the entire period), in bold the mean value for each lure, lures ranked by mean lure selectivity.

Lure and bait	Site	Sum *Drosophila suzukii*	Sum other *Drosophila* species	Mean selectivity
Suzukii Trap	Val d'Alpone	629	3314	21.3%
	Valpolicella	356	1649	24.4%
	Vignola	1226	6820	24.0%
				23.2%
Gasser	Val d'Alpone	253	1211	16.1%
	Valpolicella	688	2940	25.9%
	Vignola	1322	3843	26.0%
				22.7%
Pherocon SWD	Val d'Alpone	367	2532	7.2%
	Valpolicella	605	2706	22.1%
	Vignola	425	2012	13.7%
				14.4%
ACV	Val d'Alpone	58	981	6.3%
	Valpolicella	136	972	19.0%
	Vignola	154	688	16.8%
				14.0%
Droskidrink	Val d'Alpone	1317	14161	12.2%
	Valpolicella	898	4956	15.0%
	Vignola	1981	24232	14.5%
				13.9%
Trécé TRE1176	Val d'Alpone	14	917	1.7%
	Valpolicella	93	243	21.9%
	Vignola	163	403	13.1%
				12.3%
Trécé TRE1180	Val d'Alpone	3	672	0.3%
	Valpolicella	120	355	24.8%
	Vignola	89	699	6.9%
				10.7%
Trécé TRE1181	Val d'Alpone	75	960	5.5%
	Valpolicella	57	154	17.8%
	Vignola	133	466	8.5%
				10.6%
Droskidrink with selected bacteria	Val d'Alpone	198	6334	4.0%
	Valpolicella	190	2480	7.2%
	Vignola	1026	15428	11.2%
				7.5%
Suzukii Trap + Pherocon SWD	Val d'Alpone	296	4048	6.6%
	Vignola	112	2206	6.9%
				6.7%
Droskidrink + Pherocon SWD	Val d'Alpone	458	9060	5.6%
	Vignola	148	5768	6.2%
				5.9%

Overall, Suzukii-Trap and Gasser, consistently among sites, had the highest selectivity (Table 3). ACV, Pherocon SWD and Droskidrink had a lower selectivity, around 14%, but it is worth noting that the latter showed a highly constant selectivity across all the sites. All remaining lures provided lower selectivity and they are characterized by great variability across sites.

Altogether it is clear that attractiveness and selectivity are not correlated each other. Thus, an effective monitoring strategy should take into account the peculiar environmental conditions and balance between the options of high number of captures and high selectivity, in order to better approach the population density minimizing at the same time the detrimental effect on insects biodiversity. This aspect would become highly relevant when lures are used to reduce pest population size (e.g. mass trapping technique, attract & kill).

Temporal trends

c. Attractiveness

The greater efficiency of Droskidrink, Gasser and Pherocon SWD + ACV based on average weekly catches in 2014 is supported by data on constancy over time. These attractants showed the best performance for 42% of times for Droskidrink, 37% for Gasser and 18% for Pherocon SWD; the remaining 3% was for ACV.

In 2015 Droskidrink alone or with the addition of *O. oeni* were the most attractive during the period (39% and 36% of time respectively), followed by Suzukii-Trap (21%) and the other lures (4%).

An additional relevant parameter to consider is the early detection ability of a trap. Indeed, it is important to verify the presence of the pest earlier than the start of damage on fruits especially when the population density is low. In addition, a reliable early detection require lures able to attract adults even at temperature below the minimun threshold for larval development, 11°C (Tonina *el al.*, 2016). In 2014 Gasser and Pherocon SWD + ACV were able to record the first peak of capture before the fruit changing colour phase. Droskidrink alone or with selected bacteria and Suzukii-Trap were effective in 2015 during the period from blooming to beginning of ripening. In 2016 Droskidrink and Suzukii-Trap were efficient independently of the combination of dispenser Pherocon SWD.

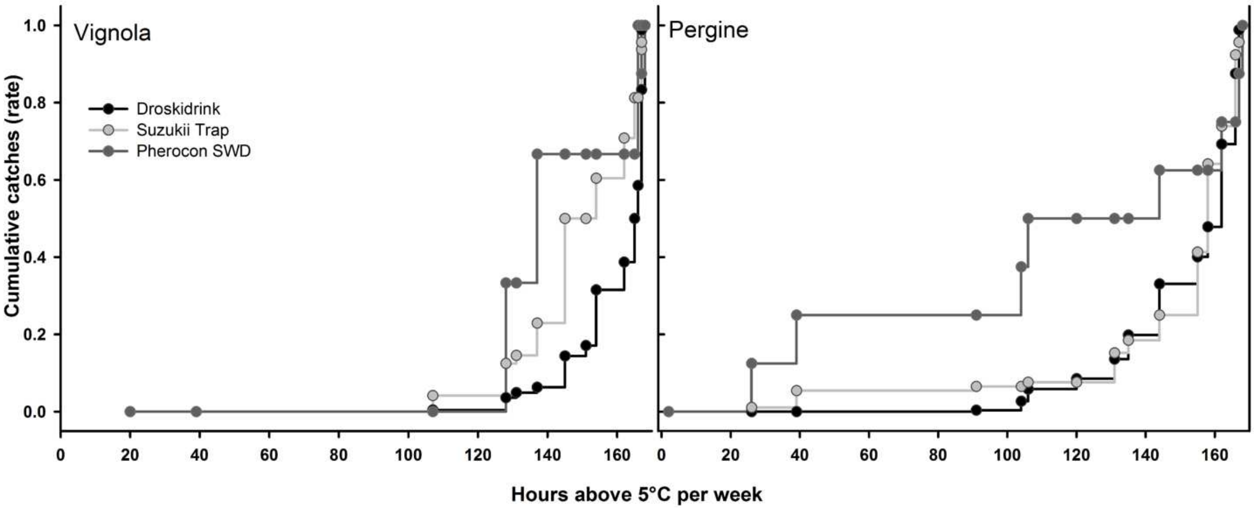

Figure 2.2 - cumulative catches of the three major lures according to the number of hours per week with temperature above 5°C in 2015.

The figure 2.2 shows the curves of cumulative catches recorded with the various lures and baits in 2015 according to the number of hours per week with temperature above 5°C. In Vignola, Suzukii Trap was able to start capturing earlier than other lures, being attractive already in cool periods. Pherocon SWD started capturing shortly after Suzukii Trap but the number of catches of Pherocon SWD increased more rapidly (50% of total captures reached in 135 hours) than those of Suzukii Trap (50% in 145 hours). Droskidrink instead needed hotter phases in order to increase the number of catches (50% in 160 hours). In Pergine site instead Pherocon SWD was performant with cool temperatures (50% in 105 hours). Suzukii Trap presented an early start compared to Droskidrink but then the two lures presented similar trend.

From these data we have the indication that below 60 hours at minumun 5°C Droskidrink was not able to work, insted of Pherocon SWD dispenser and Suzukii Trap. That because, in addition to smell, the effectiveness of Droskidrink is given by fermentation which need warm temperatures to increase its performance.

d. Selectivity

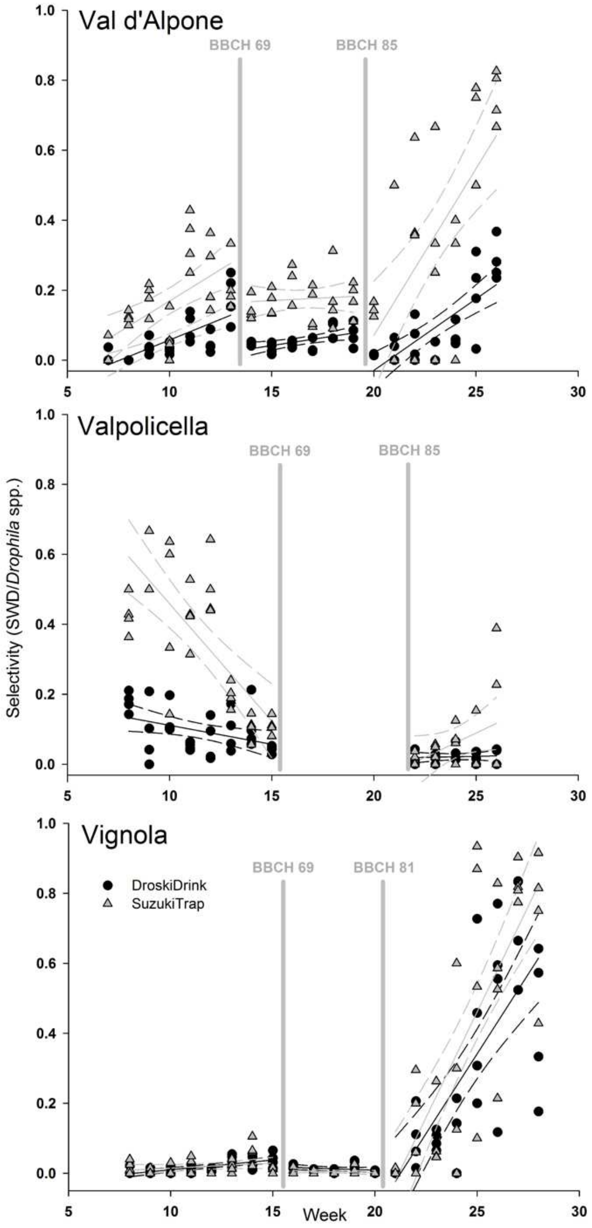

Figure 2.3 - Selectivity trends for Droskidrink and Suzukii Trap during the monitoring season 2015. Sampling periods were divided in 3 blocks, BBCH 69 and 81 (grey vertical lines) point the phenological phases of "End of flowering" and "Beginning of fruit colouring" respectively. The solid lines indicate the regression lines while the dashed lines indicate the confidence intervals at 95%.

The selectivity of the best attractants (Droskidrink and Suzukii Trap) is not constant over time and change greatly among sites (Fig. 2.3). In the three different stages for 2015 the Suzukii Trap was always more selective than Droskidrink, with value ranging from 5 to 80%. The selectivity over time and sites changes with the SWD population density. In the three sites where the selectivity was investigated there was a strong increase of this parameter after harvesting. This reflects the higher abundance of *D. suzukii* which emerged from the abandoned fruits on trees and the lack of competition during this phase between lures and ripening fruits.

In order to improve the lures, it is necessary to consider if it is better to have lures with a low but constant over time selectivity or with high but not constant selectivity. To improve the efficiency of monitoring programs it could be useful to change the lure types during the period to take advantage of higher selectivity or attractiveness in accordance with practical needs and different *D. suzukii* population structures.

These results and observations suggest new monitoring and control strategies that use combinations of different lures at the same time or in succession, taking climate and the dynamics of populations into consideration.

Operational and choosing aspects

In addition to the aspects of attractiveness and selectivity it appears also important to analyse additional parameters such as easiness to use and duration of attractiveness. Droskidrink presents problems related to the formation of bacterial gel, especially in periods with temperatures above 20°C. In 2014 in 65% of controls there was the formation of this gel. Gasser in 50% of controls had produced a black sludge at the bottom of the trap, making the replacing procedures more difficult. The dispenser Pherocon SWD instead are easy to use and they attraction is lasting for more prolonged periods, 4-6 weeks, replacing weekly only the drowning solution. Even Suzukii Trap can be replaced on a monthly basis, but still needs periodic refill, depending on temperature and relative humidity, to compensate for the evaporation loss. Droskidrink, ACV and Gasser require weekly replacement. Another very important aspect is related to the costs, that must be contained. Also the ease of purchase must be considered, attractants as ACV and Droskidrink have the advantage that can be easily procured. On this regard it should be remarked that homemade mixtures may not provide a comparable efficiency to the commercial lures.

Conclusion

Cherries are among the first fruits ripening in the year when the population density of *D. suzukii* is still relatively low (Asplen *et al.,* 2015) combining both adults exiting from overwintering diapause and those of the first spring generation. Thus a highly sensitive monitoring tool becomes a fundamental requirement to inform the timing of insecticides applications and set up selective and effective defences (Ioriatti *et al.,* 2015; Rossi Stacconi *et al*., 2016). In addition, an efficient combination of trap and lure/bait would be suitable also to perform mass trapping and attract and kill techniques.

From our multi-regional and multi-year comparison the best solutions for monitoring *D. suzukii* in cherry orchards is Droso-Trap® (Biobest®) baited with Droskidrink, Suzukii Trap or Gasser. In particular, Droskidrink shows the highest attractiveness while Suzukii Trap the highest selectivity. Suzukii Trap and Pherocon SWD when compared with Droskidrink, showed a better efficacy at the beginning of the growing season, when environmental temperatures are low.

In conclusion, Suzukii Trap is the most suitable option in consideration of the high selectivity, the early detection ability, the good attractiveness and the long persistence. While Droskidrink is the best solution in consideration of availability and high attractiveness.

Supplementary tests

During the season 2014 in the site of Modena also Suzukii Trap was tested, showing SWD capture similar to Pherocon SWD, lower than Droskidrink and Gasser, and higher than ACV ($F_{5:463}$ 72.68, $p<0.0001$). In addition, Suzukii Trap showed the highest selectivity (50% vs an average of 25%, LME $F_{5:224}$ 9.81, $p<0.0001$).

The dispenser of Pherocon SWD already exposed for 4 weeks were compared with the new ones, both combined with ACV, in 2014 in the site of Verona (Val d'Alpone), registering no differences in attractiveness ($F_{1;89}$ 0.03, $p=0.87$).

In the same site, 3 different brands of ACV were tested resulting in high differences among brands (Ponti and Prantil ACV catch 4 or 5 times Acentino; $F_{2;2}$ 5.60, $p=0.03$) Also the three different ACV brands show different ability to attract the target species, Acentino attracted only 9% of SWD, instead on 26 and 33% of Prantil and Ponti respectively ($F_{2;2}$ 14.17, $p=0.002$).

ShinEtsu baits (DSX 111, DSX 112 and DSX 113) were tested in 2014 in Pergine (Trento) showing attractiveness lower or comparable to ACV (Test Tuckey following one-way ANOVA $F_{7:623}$ 78.77, $p<0.0001$).

ACV and Droskidrink alone and added with Pherocon SWD were compared in two trials to check if the combination could increase the attractiveness and selectivity. No or low differences were emerged in both the experiments: $F_{1;92}$1.42, $p=0.24$ during 5 weeks in August 2014 in a cherry orchard in Canale di Pergine (Trento) and $F_{1;117}$5.57, $p=0.02$ during 8 weeks in September-October 2014 in a mixed berry orchard in Vigalzano di Pergine (Trento). These firsts outcomes suggested the use in a period characterized with lower temperature to explore better the synergic effects.

Gasser was tested for the second year (2015) in the site of Verona (Valpolicella) and it doesn't differ statistically from the best three lures (Test Tuckey following one-way ANOVA $F_{7;427}$ 17.24, $p<0.0001$), suggesting this is a good attractant.

Chapter 3

Non-crop plants used as hosts by Drosophila suzukii in Europe

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LT conducted the field sampling and laboratory rearing in Italy and participated in elaboration data and writing of the manuscript

Abstract

The invasive spotted wing drosophila *Drosophila suzukii*, a fruit fly of Asian origin, is a major pest of a wide variety of berry and stone fruits in Europe. One of the characteristics of this fly is its wide host range. A better knowledge of its host range outside cultivated areas is essential to develop sustainable integrated pest management strategies. Field surveys were carried out during two years in Italy, the Netherlands and Switzerland. Fruits of 165 potential host plant species were collected, including mostly wild and ornamental plants. Over 24,000 *D. suzukii* adults emerged from 84 plant species belonging to 19 families, 38 of which being non-native. Forty-two plants were reported for the first time as hosts of *D. suzukii*. The highest infestations were found in fruits of the genera *Cornus*, *Prunus*, *Rubus*, *Sambucus* and *Vaccinium* as well as in *Ficus carica, Frangula alnus, Phytolacca americana* and *Taxus baccata.* Based on these data, management methods are suggested. Ornamental and hedge plants in the vicinity of fruit crops and orchards can be selected according to their susceptibility to *D. suzukii*. However, the widespread availability and abundance of non-crop hosts and the lack of efficient native parasitoids suggest the need for an area-wide control approach.

Introduction

The spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a fruit fly of East-Asian origin that rapidly invaded other parts of the world in the late 2000s (Cini *et al.*, 2012; Deprá *et al.*, 2014; Asplen *et al.*, 2015). In contrast to most other *Drosophila* spp. that develop only on overripe or decaying fruits, *D. suzukii* is able to oviposit in ripe fruits due to the female's prominent serrated ovipositor (Lee *et al.*, 2011a; Walsh *et al.*, 2011). Since its first notification in 2008 in Europe, it has rapidly spread to most suitable areas of the continent (Cini *et al.*, 2014), becoming a major pest of a wide variety of berry and stone fruit crops (Asplen *et al.*, 2015).

Before becoming a worldwide invasive species, *D. suzukii* was considered a relatively minor pest in its area of origin (Asplen *et al.*, 2015; Haye *et al.*, 2016) and, therefore, efficient management techniques were not available. However, research on management methods has been carried out recently in various parts of the world (Haye *et al.*, 2016). *Drosophila suzukii* shows at least three important biological characteristics that may strongly influence the development of integrated management methods. Firstly, *D. suzukii* is poorly attacked by natural enemies in the invasion range (Haye *et al.*, 2016). In particular, larval parasitism is almost never observed while *Drosophila* spp. larvae are usually heavily parasitized by braconid and figitid wasps (Carton *et al.*, 1986). Laboratory assays suggest that European and American larval parasitoids are not able to develop on *D. suzukii*, apparently because of the strong host immune response of the invasive fly against these parasitoids (Chabert *et al.*, 2012; Kacsoh and Schlenke 2012; Poyet *et al.*, 2013). Secondly, its fast development, ca. two weeks to develop from egg to adult at 22°C (Tochen *et al.*, 2014) allows it to produce many generations per year between spring and autumn. In the temperate climate of Oregon, Western USA, Tochen *et al.* (2014) estimated that *D. suzukii* undergoes an average of 7.1 generations per year, but up to 13 generations per year have been cited for warmer climates (Asplen *et al.*, 2015); in temperate climates, the winter is spent as adults in reproductive diapause (Zerulla *et al.*, 2015). Thirdly, *D. suzukii* has a very broad host range, including fruits of many wild and ornamental host plants (Lee *et al.*, 2015; Poyet *et al.*, 2015), which allow it to move regularly from cultivated to wild and urban habitats. These characteristics imply that the potential development of the fly in wild and ornamental fruits in the vicinity of orchards and fruit fields has an important impact on the level of attack in cultivated fruits.

For obvious reasons, the host range of *D. suzukii* among cultivated fruits has been assessed extensively (e.g. Mitsui *et al.*, 2010; Lee *et al.*, 2011; Bellamy *et al.*, 2013; Burrack *et al.*, 2013) whereas less emphasis has been placed on non-cultivated hosts. Very recently, however, wild and ornamental non-crop hosts have been studied in Michigan and Oregon (USA) by Lee *et al.*, (2015) who found *D. suzukii* in 24 field-collected plant species belonging to 12 families. They also made additional assessments of host suitability in laboratory tests and provided a literature review on the host range of the fly worldwide. In Europe, the most extensive host range study is that of Poyet *et al.*, (2015). They tested, in the laboratory, *D. suzukii* on 67 fruit species collected in Northern France and found out that *D. suzukii* laid eggs on 50 of them and successfully developed in 33, belonging to 15 families. However, there have been discrepancies between host range data gathered from field surveys and laboratory tests in North America (Lee *et al.*, 2015) and, so far, no extensive field survey was carried out to assess the realised host range of *D. suzukii* in non-crop hosts in Europe.

The main objective of this study was to assess the host range realised by *D. suzukii* outside cultivated areas in Western and Central Europe. For this, surveys were carried out during two years in the Netherlands, Northern Italy and Switzerland to collect fruits in semi-natural and urban landscapes and rear out *D. suzukii*. Attempts were made to classify the host fruits according to the frequency and level of infestation.

Material and Methods

Potential host fruits, i.e. fruits that appeared sufficiently soft to allow the oviposition and development of *D. suzukii*, were collected through regular surveys in 2014 and 2015 at various sites in three countries: Italy, the Netherlands and Switzerland. In all three countries, sites were distant from each other's by at least one km. All sampling regions had been infested by *D. suzukii* at least since 2013, and the presence of *D. suzukii* in the areas during the sampling periods was confirmed by trapping campaigns for monitoring adult populations. The survey focused on ripe fruits of wild and ornamental non-crop hosts in various habitats, i.e. forests, forest edges, meadows, hedges in agricultural habitats, gardens and parks, etc. In a few cases, fruit trees planted as urban or garden trees at the surveyed sites were also sampled. Plants were identified using local reference guides (Pignatti, 1982; Meijden, 1996; Ferrari and Medici, 2008; Koning and Broek van den, 2012; Info Flora, 2015). Sampling techniques were rather similar in the three countries but with some differences. Therefore, they are described separately below.

Italy

Twenty-nine sites in semi-natural habitats located in seven different areas in North-eastern Italy (Veneto and Trentino Regions) were sampled every two weeks from March 2014 to October 2015. The fruits were collected when available from all potential host plants. Moreover, occasional collections were made in various landscapes in Liguria, Toscana and Veneto Region wherever new fruit species were found. Fruit were sampled from a total of 116 plant species. When possible, samples consisted in 2 dl of small fruits or 50 individuals of large fruits, but smaller amount of less abundant fruits were also collected. For each sample, the number of fruits was counted and their weight was measured. Fruits were then stored in containers, covered with fine mesh and kept at 23 °C. Emerging insects were collected three times a week and lasted three weeks after the last emergence of *D. suzukii*. Flies were stored in ethanol for later identification.

Netherlands

Three areas were selected in the centre of the Netherlands. The areas differed in respect to soil and vegetation type. The first was in the orchard dominated river clay area in Gelderland province. The second was in a semi-urban area in the Utrecht province, where river clay meets the sandy Pleistocene soils. The third was in forests and at forest edges on the sandy Pleistocene soils in Gelderland province. At each area, surveys were made at three sites of 0.5 ha each. The vegetation at each of the nine sites was sampled eight times from June to October 2014. Additionally, a large sampling effort was made on December 4, 2014, to determine whether *D. suzukii* could overwinter as a larva in fruits. In 2015, surveys were carried out between May and October at the same sites. At each sampling date, fruits were picked from all potential host plants. Occasional collections were also made in the region, wherever new potential host plant species were found. Fruits of 34 plant species were collected in 2014 and 68 in 2015. In total, 77 different plant species were sampled. When possible, samples consisted of ca. 50 fruits, but smaller numbers of less abundant fruits, or larger numbers of abundant but small fruits were also collected. After weighing, fruits were put in containers, covered with fine mesh and kept at 22 °C. Emerging insects were collected three times a week until three weeks after the last emergence of *D. suzukii* and stored in ethanol for later identification.

Switzerland

Collections were carried out only in 2014, mainly in the Canton Ticino, in the Southern Alps. Fruits of a variety of potential host species were collected at ten sites, once per month, from early May to early October 2014. Additional collections were made along elevation gradients in the Ticino, once in July and once in August 2014. Some collections were also made in the Jura Canton, Northwestern Switzerland. A total of 39 plant species were sampled. When possible, samples consisted of ca. 50 fruits, but smaller numbers of less abundant fruits, or larger numbers of abundant but small fruits were also collected. For each sample, the number of collected fruits was recorded. Fruits were then placed in photo-eclectors made of a cardboard cylinder surrounded by a funnel ending in a translucent plastic cup, in which the flies were collected daily until three weeks after the last emergence of *D. suzukii*. They were then killed in ethanol to allow a careful counting of the number of *D. suzukii* adults. After the emergence period, the cylinders were inspected to count the few flies that had died without reaching the cup.

Data analyses

Two parameters were calculated: the rate of occurrence and the infestation level. The rate of occurrence expressed the geographic frequency at which *D. suzukii* was found on a particular fruit species, without taking into account the level of attack at specific sites. It was calculated as the ratio between the number of sites x years (throughout all three countries) where a fruit was found attacked by *D. suzukii* divided by all sites x years combinations where the fruit was collected.

To allow a comparison of the infestation level among host species, the number of flies emerging per individual fruit is not a very good parameter because fruit size strongly varies among species. Instead, the number of flies should be expressed per fruit weight, volume or skin surface. In Italy and Switzerland, all fruits were counted but the size and weight of fruits could not be measured for all samples. Thus, for each fruit species collected in Italy and Switzerland, the average diameter was gathered in the literature, mainly in Info Flora (2015) and, if not indicated, an average of the average data found in various information sources (other books on regional flora and web sites from scientific societies and organisations) was calculated. In case of oval fruits, the length and the width were averaged. The fruit surface was estimated for each species (surface = $4\pi r^2$). Aggregate fruits composed of drupelets, e.g. *Rubus* spp., were treated in the same way, although we realise that, for these fruits, the surface was underestimated. Then, for each sample, a level of infestation was expressed as the number of *D. suzukii* adults emerged per dm^2 of fruit surface. In the Netherlands, the number of fruits was not counted but, instead, samples were weighed. Thus, for these samples, the level of infestation was expressed as the number of *D. suzukii* adults per kg of fruit. We realise that none of these two parameters are perfect. The fruit surface is probably a better expression of the potential of the fruit to attract *D. suzukii* and to support the development of a certain quantity of larvae than its weight or its volume. On the other hand, for some species, the size of the sampled fruits may be rather different from the average size found in the literature. Furthermore, if the fruit is very small in size individually, the fruit surface may not matter as much, and having other measures might be useful. But the aim of this parameter was not to finely compare fruit species but rather to broadly categorise the infestation levels of host fruits in the field. For a finer comparison of infestation levels, several confounding factors such as time of collection, habitat, fruit density and population size of the flies would have to be taken into account. For the same reasons, the infestation levels were not statistically tested. Only data from the years with the most abundant collections were considered for the calculation of the infestation levels, i.e. 2014 for Italy and Switzerland, and 2015 for the Netherlands.

Results

Fruits from a total of 165 plant species were collected in the three countries, providing 24,165 *D. suzukii* adults, 4,153 in Italy, 15,527 in the Netherlands and 4,485 in Switzerland. The list of the plant species from which *D. suzukii* emerged is listed in Table 1, with quantitative information on the sampling and emergence. The plant species from which no *D. suzukii* emerged are listed in Table 2. In total, 84 plant species from 19 families gave rise to adult emergence, 39 species in

Italy, 52 in the Netherlands and 24 in Switzerland. Forty-two of these are recorded for the first time as hosts of *D. suzukii* in the field, of which six had already been found to be suitable for larval development in laboratory studies (Baroffio *et al.*, 2014; Lee *et al.*, 2015; Poyet *et al.*, 2015) (Table 3.1). Thirty-eight host species are not native to any of the three investigated countries. Fifty are commonly found in the wild in at least one of the three regions, 53 are commonly planted as ornamental and 16 are commonly cultivated fruits.

Table 3.1 - Fruit species from which *D. suzukii* adults emerged, in Italy (IT), the Netherlands (NL) and Switzerland (CH: Ticino TI; Jura JU), in 2014 and 2015. a/b: a=number of sites where *D. suzukii* was obtained; b=number of sites were the fruit was collected.

Species (family)	New host record[1]	Main habitat/ purpose[2]	Native / Exotic[3]	IT 2014	IT 2015	NL 2014	NL 2015	CH-TI 2014	CH-JU 2014
Actinidia chinensis Planch. (Actinidiaceae)		F	E	1/1					
Amelanchier lamarckii F.G. Schr. (Rosaceae)	✓	O/W	E			0/2	1/2		
Amelanchier ovalis Medik. (Rosaceae)	✓	W/O	N	1/2					
Arbutus unedo L. (Ericaceae)		W/O	N	1/2	0/1				
Arum italicum Mill. (Araceae)	✓	O/W	N	0/1		0/2	1/1		
Cornus alba L. (Cornaceae)	✓	O	E				1/1		
Cornus kousa Hance (Cornaceae)		O	E				1/1		
Cornus mas L (Cornaceae)		W/O	N	1/3	1/1		1/1	1/1	
Cornus sanguinea L. (Cornaceae)		W/O	N	2/4	0/1	2/4	2/2	0/6	0/3
Cotoneaster franchetii Boiss. (Rosaceae)	✓	O	E				1/1		
Cotoneaster lacteus W.W. Smith (Rosaceae)		O	E	1/2	0/1				
Cotoneaster rehderi Pojark. (Rosaceae)	✓	O	E				1/1		
Crataegus chrysocarpa Ashe (Rosaceae)	✓	O	E				1/1		
Crataegus monogyna Jacq. (Rosaceae)	✓	W/O	N	0/4	0/1	2/6		0/1	0/2
Daphne mezereum L. (Thymelaeaceae)	✓	W	N	1/2	0/1				
Duchesnea indica (Andr.) Focke (Rosaceae)	Lab	O/W	E	1/2		0/1	3/3	2/5	
Eriobotrya japonica (Thunb.) Lindl. (Rosac.)		O	E	1/2					

Ficus carica (L.) (Moraceae)		F	N	1/3				3/3
Fragaria vesca L. (Rosaceae)		W	N	0/1	1/1		1/1	9/22
Frangula alnus Mill. (Rhamnaceae)		W	N	3/3		2/2	1/1	3/3
Gaultheria x wisleyensis M.&M. (Ericaceae)	✓	O	E				1/1	
Hippophae rhamnoides L. (Elaeagnaceae)		W/F	N	0/1				1/1
Lonicera alpigena L. (Caprifoliaceae)		W	N	2/3	2/3			
Lonicera caerulea L. (Caprifoliaceae)		W	N	1/1	1/1			
Lonicera caprifolium L. (Caprifoliaceae)	✓	W/O	N		0/1		2/2	
Lonicera ferdinandii Franch. (Caprifoliaceae)	✓	O	E				1/1	
Lonicera nigra L. (Caprifoliaceae)		W	N	1/2	2/2			
Lonicera nitida E. H. Wilson (Caprifoliaceae)	Lab	O	E				2/2	
Lonicera sp (Caprifoliaceae)								6/8
Lonicera xylosteum L. (Caprifoliaceae)		W	N	1/3	1/4			
Lycium barbarum L. (Solanaceae)	✓	O/F/W	N	1/2				
Mahonia aquifolium (Pursh) Nutt. (Berberid.)	Lab	O	E			0/1	1/5	
Mahonia sp. (Berberidaceae)		O	E	0/1	1/1			
Malus baccata Borkh. (Rosaceae)	✓	O	E				1/1	
Paris quadrifolia L. (Melanthiaceae)	✓	O	N	0/3	0/1			1/1
Parthenocissus quinquefolia (L.) (Vitaceae)		O	E	0/2				2/2
Photinia beauverdiana C. K. Schn. (Rosaceae)	✓	O	E				1/1	
Photinia villosa (Thunb.) DC. (Rosaceae)	✓	O	E				1/1	
Photinia prunifolia Lindl. (Rosaceae)	✓	O	E				1/1	
Phytolacca americana L. (Phytolaccaceae)		O/W	E	4/4	0/1	0/1		5/5
Phytolacca esculenta Van Houtte (Phytolacc.)	✓	O/W	E				1/1	
Polygonatum multiflorum (L.) All. (Liliaceae)	✓	W	N				1/1	0/1

Prunus armeniaca L. (Rosaceae)		F	E	1/1					
Prunus avium (L.) (Rosaceae)		W/F/O	N			0/2	1/2	5/10	
Prunus cerasifera Ehrh. (Rosaceae)		O/W	E	1/1					
Prunus cerasus L. (Rosaceae)		F/W	N	1/1	0/1				
Prunus domestica L. (Rosaceae)		F	E					2/2	0/1
Prunus laurocerasus L. (Rosaceae)		O/W	E	1/1	0/1	1/1	1/1	2/2	
Prunus lusitanica L. (Rosaceae)		O	E	1/1					
Prunus mahaleb L. (Rosaceae)		W/O	N	2/3	1/3				
Prunus padus L. (Rosaceae)	Lab	W/O	N			0/1	1/1	1/1	
Prunus serotina Ehrhart (Rosaceae)		W	E			1/2	1/1		
Prunus spinosa L. (Rosaceae)	Lab	W/O	N	1/5	0/2	2/5	2/3		0/1
Pyracantha sp. (Rosaceae)		O	E				1/1		
Rhamnus cathartica L. (Rhamnaceae)		W	N				2/2		
Ribes rubrum L. (Rosaceae)	Lab	F	N			0/1	2/3	0/1	
Rosa acicularis Lindl. (Rosaceae)	✓	O	E				1/1		
Rosa canina L. (Rosaceae)	✓	W/O	N	0/7	0/5	3/5	0/2		
Rosa glauca Pourr. (Rosaceae)	✓	O/W	N				1/1		
Rosa pimpinellifolia L. (Rosaceae)	✓	O/W	N				1/1		
Rosa rugosa Thunb. (Rosaceae)	✓	W/O	E			0/1	3/3		
Rubus caesius L. (Rosaceae)	✓	W	N		1/2		3/3		
Rubus fruticosus aggr. (Rosaceae)		W/F	N	4/5		8/9	6/6	29/324	5/7
Rubus idaeus L. (Rosaceae)		W/F	N	2/2		0/3	1/2	12/16	
Rubus phoenicolasius Maxim. (Rosaceae)	✓	F	E					2/2	
Rubus saxatilis L. (Rosaceae)	✓	W	N		2/2			0/1	
Sambucus ebulus L. (Adoxaceae)		W	N	1/2					
Sambucus nigra L. (Adoxaceae)		W	N	2/3	0/2	33/34	4/4	5/8	2/3
Sambucus racemosa L. (Adoxaceae)		W/O	N	1/3	5/6	0/3	1/1	4/5	
Solanum dulcamara L. (Solanaceae)		W	N	0/3	0/1	0/2	3/4	1/6	
Solanum nigrum L. (Solanaceae)		W	N	0/4			1/4	1/1	

Sorbus aria (L.) (Rosaceae)	✓	W/O	N	1/3	0/1			
Sorbus aucuparia L. (Rosaceae)	✓	W/O	N	0/4	0/1	1/4	0/1	
Symphoricarpos albus (L.) (Caprifoliaceae)		O/W	E	0/1		2/3	0/1	0/1
Tamus communis L. (Dioscoreaceae)	✓	W	N	2/4				2/3
Taxus baccata L. (Taxaceae)		O/W	N	2/3		0/1	1/1	1/1
Vaccinium myrtilloides Michx. (Ericaceae)	✓	F/O	E				1/1	
Vaccinium myrtillus L. (Ericaceae)		W/F	N	1/1				1/1
Vaccinium oldhamii Miquel. (Ericaceae)	✓	O/F	E				1/1	
Vaccinium praestans Lamb. (Ericaceae)	✓	O	E				1/1	
Vaccinium vitis-idea L. (Ericaceae)		O	E	0/1			1/1	
Viburnum lantana L. (Adoxaceae)		W/O	N	0/1	1/3	0/1		
Viburnum rhytidophyllum Hemsl. (Adoxaceae)	✓	O	E				1/1	
Vitis vinifera L. (Vitaceae)		F	N	1/1				0/3

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[1] New host record: ✓ = species not yet reported in the literature as host in the field, based on Cini et al. (2012), Baroffio et al. (2014), Asplen et al. (2015) and Lee et al. 2015; Lab = species not yet found as host in the field but suitable host in laboratory tests in Baroffio et al. (2014), Lee et al. (2015) or Poyet et al. (2015)

[2] Main habitat / purpose: W: commonly found in the wild in at least one of the three regions; O = commonly planted as ornamental; F = commonly planted as fruit crop; minor habitats / purposes are not indicated

[3] Native (N) = native in at least one of the investigated regions; Exotic (E) = exotic in the three regions.

[4] In Ticino, *Rubus fruticosus* aggr. may have also included *Rubus caesius*

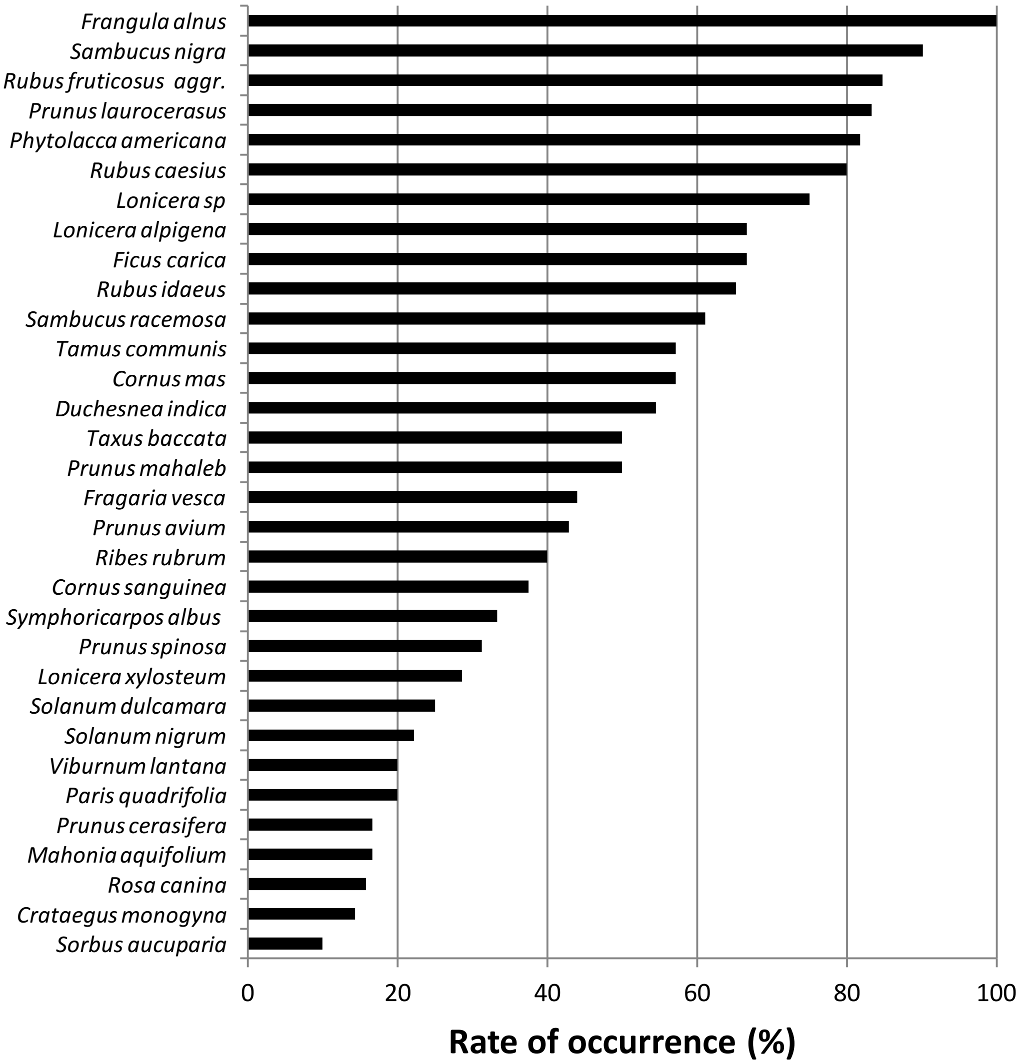

Figure 3.1 - Rate of occurrence of *D. suzukii* in the host plants in which it emerged, expressed as the % of sites x years in which *D. suzukii* was found. Only the fruits found in at least 5 sites x years are presented.

The rate of occurrence is presented for all fruit species collected in at least five different sites x year (Fig. 3.1). *Drosophila suzukii* emerged from fruit of *Frangula alnus*, *Sambucus nigra, Rubus fruticosus* aggr., *Rubus caesius*, *Prunus laurocerasus* and *Phytolacca americana* in at least 80% of the sites x years. In contrast, *Sorbus aucuparia, Crataegus monogyna, Rosa canina, Mahonia aquifolium, Prunus cerasifera, Paris quadrifolia,* and *Viburnum lantana* were only occasional hosts, with *D. suzukii* emerging at maximum 20% of the sites x years. Among the 81 plant species that did not provide *D. suzukii*, only six were frequently collected (at least five sites x year) and 10 of them had been found as field or laboratory hosts in previous studies (Table 3.2).

Table 3.2 - Fruit species from which no *D. suzukii* adults emerged, in Italy (IT), the Netherlands (NL) and Switzerland (CH: Ticino TI; Jura JU), in 2014 and 2015, with the number of sites sampled. In bold: six species collected in at least five combinations of sites x years

	Known host of *D. suzukii*[1]	**Number of sites where fruits were sampled**					
Species (Family)		**IT 2014**	**IT 2015**	**NL 2014**	**NL 2015**	**CH-TI 2014**	**CH-JU 2014**
***Actaea spicata* L.** *(*Ranunculaceae)		2	1			3	
Ampelopsis brevipedunculata (Max.) Tr. (Vitaceae)			1				
Aronia x prunifolia (Rosaceae)		1					
Asparagus acutifolius L. *(*Asparagaceae)		1					
Asparagus officinalis L. (Asparagaceae)					1		
Atropa bella-donna L. (Solanaceae)	Lab						1
Aucuba japonica Thunberg (Garryaceae)	Field	1	2				
Berberis x media (Berberidaceae)		1					
Berberis vulgaris L. (Berberidaceae)		3				3	
Berberis sp. (Berberidaceae)					2		
Bryonia dioica Jacq. (Cucurbitaceae)		1	1				
Callicarpa bodinieri H. Lév. (Lamiaceae)		1			1		
Cephalotaxus harringtonia (K. ex F.) Koch (Cephalotaxaceae)		1					
Chamaerops sp. (Arecaceae)		1					
Convallaria majalis L. (Nolinoideae)		2					
Cotoneaster acutifolius Turcz. (Rosaceae)		1					
Cotoneaster dammeri C. K. Schneid. (Rosaceae)		1					
Cotoneaster horizontalis Decne. (Rosaceae)	Field	1			1	1	
Cotoneaster microphyllus Wall. ex Lindl. (Rosaceae)		2					
Cotoneaster salicifolius Franch. (Rosaceae)		1	2				
Cotoneaster suecicus G.Klotz (Rosaceae)					1		
Cotoneaster × watereri Exell (Rosaceae)				1			
Crataegus azarolus L. (Rosaceae)			1				
Crataegus coccinea L. (Rosaceae)		1					
Crataegus crus-galli L. (Rosaceae)		1					
Crataegus kansuensis E. H. Wilson (Rosaceae)					1		
Crataegus laevigata (Poir.) DC. (Rosaceae)		1	1				
Crataegus sp. (Rosaceae)		2					
Diospyros kaki Thunberg (Ebenaceae)	Field	1					
Euonymus europaeus L. (Celastraceae)		1		3			
Gaultheria shallon Pursh (Ericaceae)					1		
Gaultheria sp. 1 (Ericaceae)		1					
Gaultheria sp. 2 (Ericaceae)		1					
***Hedera helix* L.** (Araliaceae)		1	3	1	3		
Hypericum sp. (Hypericaceae)					1		
Hypericum androsaemum L. (Hypericaceae)		1	1				
Ilex aquifolium L. (Aquifoliaceae)		1		1	1		
Ilex sp. (Aquifoliaceae)		1					

Juniperus sp. (Cupressaceae)		1					
Laurus nobilis L. (Lauraceae)		1	1				
Ligustrum lucidum W. T. Aiton (Oleaceae)		1					
***Ligustrum vulgare* L.** (Oleaceae)		4	1	2	1	2	
Lonicera etrusca Santi (Caprifoliaceae)		1					
Lonicera henryi Hemsl. (Caprifoliaceae)		1			1		
Lonicera periclymenum L. (Caprifoliaceae)	Field			1			
Lonicera pileata Oliv. (Caprifoliaceae)		1					
Malus floribunda Siebold ex Van Houtte (Rosaceae)		1	1				
Malus x Red Sentinel (Rosaceae)					1		
Mespilus germanica L. (Rosaceae)		1				1	
Morus alba L. (Moraceae)	Field	1					
Myrteola sp.(Myrtaceae)		1					
Myrtus communis L. (Myrtaceae)			2				
Nandina domestica Thunb. (Berberidaceae)		2	2				
Olea europaea L. cv. Leccino (Oleaceae)		1					
Parthenocissus tricuspidata (Sieb. & Zucc.) Planch. (Vitaceae)		1					
Phillyrea angustifollia L. (Oleaceae)			2				
Phillyrea latifolia L. (Oleaceae)			3				
Prunus persica (L.) var. florepleno (Rosaceae)	Field		1				
Punica granatum L. (Lythraceae)		2					
Pyracantha coccinea M. Roem. (Rosaceae)		1			1		
Pyracantha 'navaho' (Rosaceae)		1	1				
Rhamnus pumila Turra (Rhamnaceae)		1	1				
Rhodotypos scandens (Thunb.) Makino (Rosaceae)		1					
Ribes alpinum L. (Rosaceae)	Field			1			
Rosa pendulina L. (Rosaceae)		2					
Rubus ulmifolius Schott. (Rosaceae)			4				
***Ruscus aculeatus* L.** (Asparagaceae)		3	5				
Skimmia japonica Thunberg (Rutaceae)					1		
Smilax aspera L. (Smilacaceae)			1				
Solanum pseudocapsicum L. (Solanaceae)		1					
Solanum sisymbrifolium Lam. (Solanaceae)		1					
Sorbus chamaemespilus (L.) Crantz (Rosaceae)		2	1				
Sorbus intermedia (Ehrh.) Pers. (Rosaceae)				1			
Symphoricarpos x chenaultii Hanc. (Caprifoliaceae)					1		
Symphoricarpos orbiculatus Moen. (Caprifoliaceae)					1		
Vaccinium uliginosum L. (Ericaceae)		1					
***Viburnum opulus* L.** (Adoxaceae)	Field	2		4		2	2
Viburnum tinus L. (Adoxaceae)		1					
Viscum album L. (Santalaceae)	Lab	1					
Vitis labrusca L. (Vitaceae)		2					
Withania somnifera (L.) Dunal, (Solanaceae)			1				

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[1] Known host of *D. suzukii*: Field = plant already recorded as host in the field, based on the reviews of Cini et al. (2012), Baroffio et al. (2014) and Lee et al. 2015; Lab = species not yet found as host in the field but suitable host in laboratory tests in Baroffio et al. (2014), Lee et al. (2015) or Poyet et al. (2015)

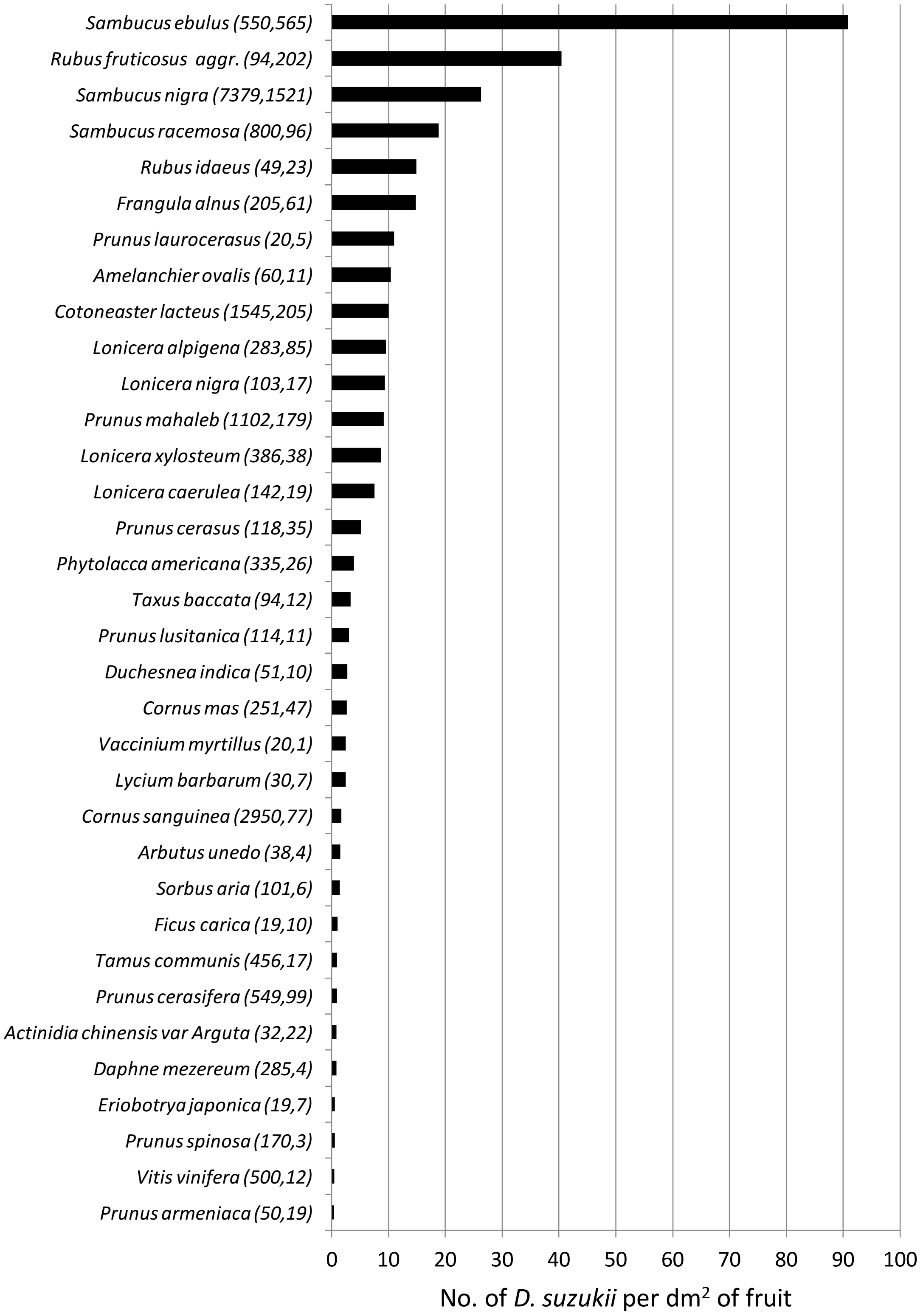

Figure 3.2 -No. of *D. suzukii* emerged per dm^2 of fruit collected in 2014 in Italy. Numbers in parentheses after the fruits' names indicate the total number of fruits collected and the total number of *D. suzukii* adults emerged from these fruits, respectively. Only fruits from which *D. suzukii* emerged are shown.

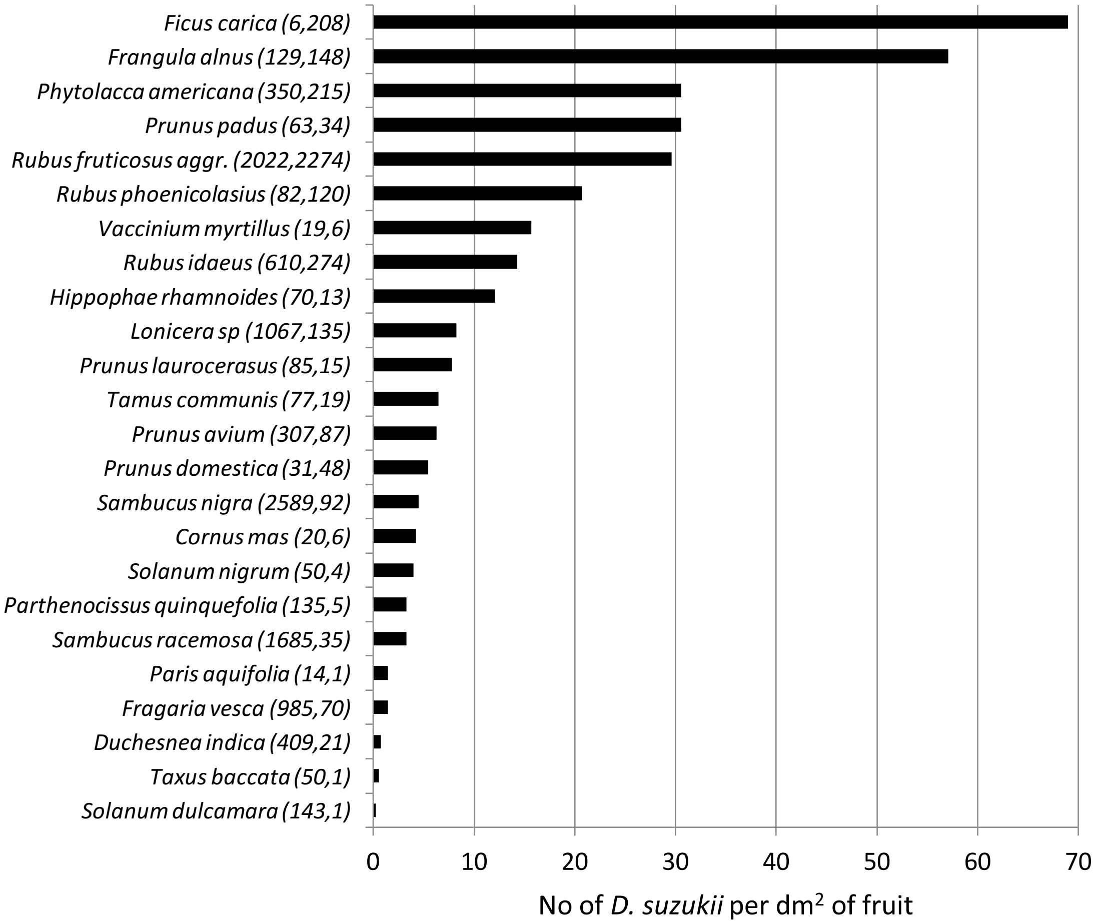

Figure 3.3 - No. of *D. suzukii* emerged per dm^2 of fruit collected in 2014 in Ticino, Switzerland. Numbers in parentheses after the fruits' names indicate the total number of fruits collected and the total number of *D. suzukii* adults emerged from these fruits, respectively. Only fruits from which *D. suzukii* emerged are shown.

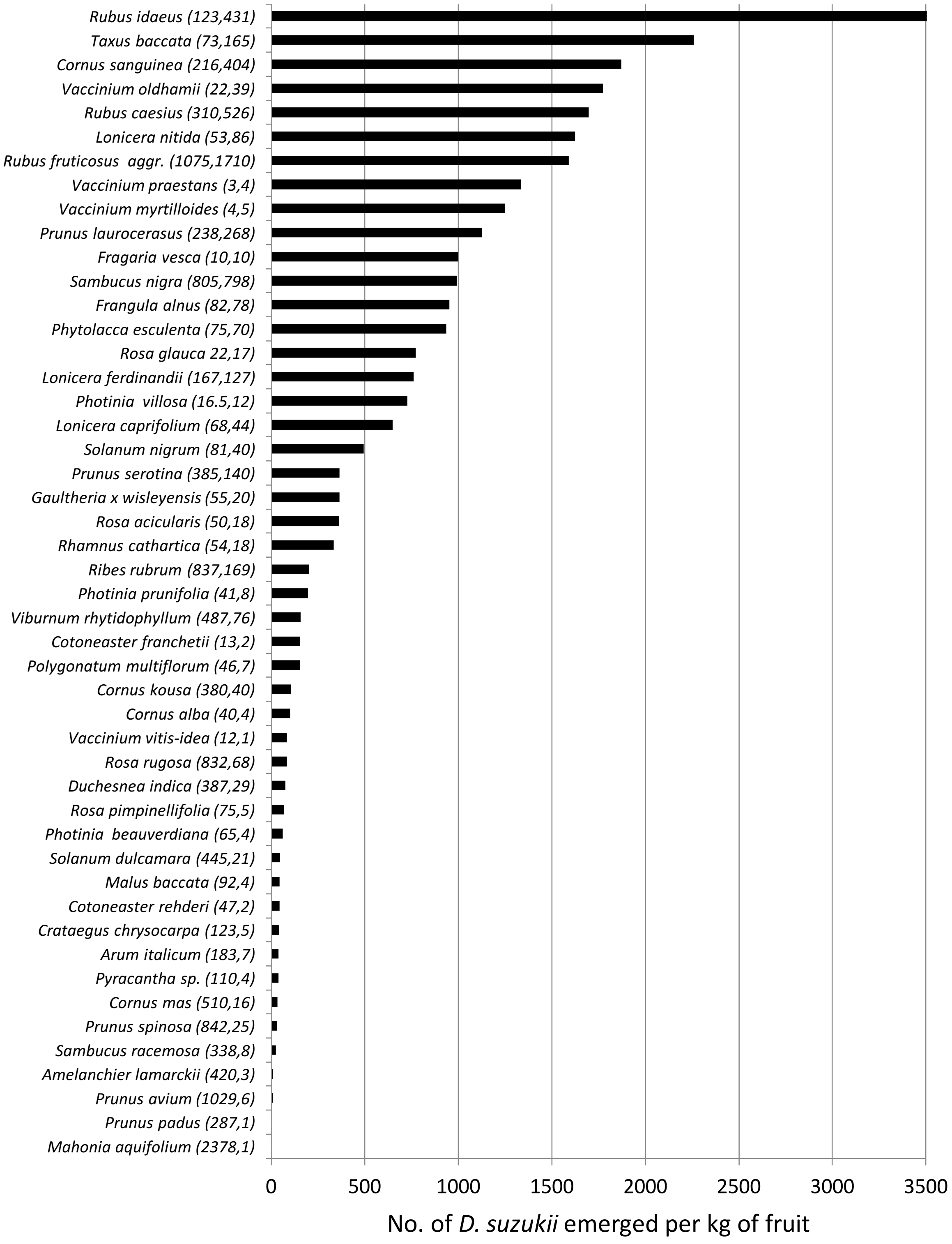

Figure 3.4 - No. of *D. suzukii* emerged per kg of fruit collected in 2015 in the Netherlands. Numbers in parentheses after the fruits' names indicate the total weight of fruits collected and the total number of *D. suzukii* adults emerged from these fruits, respectively. Only fruits from which *D. suzukii* emerged are shown.

The infestation levels are presented for Italy 2014, Switzerland (Ticino only) 2014 and the Netherlands 2015 in Figs 3.2, 3.3 and 3.4. In Italy, the highest levels, measured as the number of flies emerging per dm^2 of fruit, were found in plants of the genera *Sambucus* and *Rubus* followed by *Frangula alnus* (Fig. 3.2). The high score in *Sambucus ebulus*, a species sampled only in Italy, was obtained on the basis of a single site providing an enormous amount of flies emerging from its small fruits. In Switzerland, the same parameter provided slightly different results, with the highest scores obtained from a few figs (*Ficus carica*), followed by *Frangula alnus, Phytolacca americana* and *Prunus padus. Rubus* spp. showed a similar infestation level in Italy and Switzerland, while fewer flies emerged from *Sambucus* spp. in Switzerland. In the Netherlands, where the infestation level was measured as the number of *D. suzukii* adults per kg of fruit, the highest scores were also obtained by *Rubus* spp. but, more surprisingly, also by *Cornus sanguinea*, a species that was not or only poorly attacked in Switzerland and Italy. Similarly, *Taxus baccata* was heavily infested in the Netherlands and much less so in the two other countries. Other heavily attacked species in the Netherlands included three *Vaccinium* species and several species that also scored high in the other countries such as *Prunus laurocerasus, Sambucus nigra* and *Frangula alnus* (Fig. 3.4).

The fruits that were found early in the season, i.e. before June 1 in Italy, before July 1 in the Netherlands and on June 6 in Ticino (no fruits were found in Ticino at the first survey on May 9) are listed in Table 3.3. In general, fruits found in spring fall into two categories: those that are produced in spring and those that are produced in late summer and autumn but that last until spring of the following year. Fruit species of the first category were all infested by *D. suzukii*. In contrast, most fruit species that are able to last over winter were not infested and, from the six species that were, five of them contained *D. suzukii* only in autumn (Table 3.3). Only *Cotoneaster lacteus* fruits were found infested in November and again throughout April. A large collection of fruits was carried out in the Netherlands on 4 December 2014 to assess whether some could potentially host overwintering larvae. However, no fly emerged, even from fruits that had provided numerous flies until October. Plant species that were sampled that date included *Crataegus monogyna, Prunus spinosa, Rosa canina, Rosa rugosa, Rubus fruticosus* aggr.*, Symphoricarpos albus* and *Taxus baccata.*

Table 3.3 - Host suitability of fruits found early in the year: before June 1st in Italy, before July 1st in the Netherlands and on June 6 in Switzerland.

Fruits formed in spring		**Fruits formed in summer or autumn and lasting until the following year**		
Suitable hosts	**Host suitability not proved in this study**	**Suitable hosts in autumn and spring**	**Suitable host only in autumn**	**Host suitability not proved in this study**
Amelanchier lamarckii		*Cotoneaster lacteus*	*Crataegus monogyna*[1]	*Aucuba japonica*[2]
Eriobotrya japonica			*Duchesnea indica*[1]	*Cotoneaster microphyllus*
Fragaria vesca			*Prunus laurocerasus*[1]	*Cotoneaster salicifolia*
Lonicera xylosteum			*Pyracantha sp.*	*Hedera helix*
Mahonia aquifolium			*Rosa canina*	*Ilex aquifolium*
Prunus avium				*Laurus nobilis*
Prunus mahaleb				*Ligustrum vulgare*
Prunus padus				*Malus floribunda*
Ribes rubrum				*Mespilus germanica*
Rubus sp				*Morus alba*[2]
Sambucus racemosa				*Nandina domestica*
				Pyracantha 'navaho'
				Ruscus aculeatus
				Viburnum opulus

[1] For these species, only few fruits pass the winter
[2] Plants already recorded as suitable hosts in the field in Japan (Lee et al. 2015)

Discussion

This survey confirmed that *D. suzukii* is highly polyphagous and can attack and develop in a wide range of fruits of wild and ornamental plants as well as cultivated fruits. Forty-one plant species, both indigenous and exotic, have been added to the list of suitable hosts. Several hosts such as *Rubus* spp., *Sambucus* spp., *Prunus* spp., *Lonicera* spp. and *Frangula alnus* were consistently found throughout the sites and years as being heavily infested, confirming similar observations made in other studies (Mitsui *et al.*, 2010; Baroffio *et al.*, 2014; Lee *et al.*, 2015; Poyet *et al.*, 2015). Other results were more surprising. In particular, we did not expect so many adults emerging from species such as *Rosa* spp. and *Malus baccata*, which tend to have a rather tough skin. Similarly, flies were obtained from many other plant species considered to be unsuitable in laboratory tests carried out by Poyet *et al.* (2015), such as *Sorbus aria, Sorbus aucuparia*, *Polygonatum multiflorum*, *Paris quadrifolia* and *Crataegus monogyna*. These unexpected infestations can be partly explained by the very high population levels of *D. suzukii* in the second half of 2014 (Italy and Switzerland) and 2015 (the Netherlands). Moreover, although surveys focused on ripe, undamaged fruits, it is likely that some adults emerged from “hard” fruits, such as *Malus baccata*, resulted from eggs laid in unnoticed damaged fruits. Lee *et al.*, (2015) also obtained *D. suzukii* from field-collected fruits that appeared unsuitable in laboratory tests. They attributed these discrepancies to differences in fruit suitability among picked (laboratory) versus hanging (field) fruit and the timing of sampling.

Eighty-one plant species did not give rise to *D. suzukii* adults, among which ten have been reported as hosts in other field surveys or laboratory tests (Baroffio *et al.*, 2014; Lee *et al.*, 2015; Poyet *et al.*, 2015). These negative results must be considered with great caution. Most of the fruit species from which nothing emerged were collected in low numbers or at few sites. Laboratory tests could be carried out to confirm the unsuitability of these fruits, taking into account that discrepancies between laboratory tests and field surveys may occur (Lee *et al.*, 2015). Only the six fruit species collected in high numbers in at least five combinations of sites x years can be regarded as “unsuitable”: *Actaea spicata*, *Berberis vulgaris*, *Hedera helix*, *Ligustrum vulgare*, *Ruscus aculeatus* and *Viburnum opulus,* even though, for the latter species, some larval development but no adult emergence had been observed in laboratory tests (Poyet *et al.*, 2015).

This survey also illustrated the close association between *D. suzukii* and invasive plants. Forty plants identified as hosts are exotic to the survey areas and many of them are considered as invasive species. The interaction between *D. suzukii* and the invasive American black cherry *Prunus serotina* has been studied in France by Poyet *et al.*, (2014), who suggest that the heavy infestation of *Prunus serotina* fruits by *D. suzukii* could reduce the life span of fruits and their attractiveness to seed consumers and dispersers. In contrast, *Prunus serotina* could represent a suitable plant reservoir enhancing *D. suzukii* invasion. A similar scenario was proposed by Asplen *et al.*, (2015) regarding the invasion of the European buckthorn, *Rhamnus cathartica,* in North America, which was found to be a suitable host of *D. suzukii* both in North America and in our study. The invasive ‘Himalayan’ blackberry, *Rubus armeniacus,* is also known to favour the spread and abundance of *D. suzukii* in berry production systems in Western North America (Klick *et al.*, 2016). Besides *Prunus serotina*, other important invasive plants infested by *D. suzukii* in our samples include, e.g., *Duchesnea indica*, *Phytolacca americana* and *Phytolacca esculenta*, *Prunus laurocerasus*, *Rosa rugosa* and *Symphoricarpos albus.* The interactions between the invasion processes of *D. suzukii* and these invasive plants should be further investigated.

Implications for sustainable *Drosophila suzukii* management

Knowing the realised host range and the preferred host plants outside cultivated habitats is essential for the development of sustainable IPM strategies against *D. suzukii* (Klick *et al.*, 2016; Lee *et al.*, 2015). Pelton *et al.*, (2016) showed that the amount of woodland in the landscape positively affects early season crop risk and the high numbers of *D. suzukii* in the woods have implications for understanding overwintering. Non-crop hosts in the vicinity of susceptible fruit crops may also enhance *D. suzukii* populations before or during the crop season, as shown by Klick *et al.*, (2016) in a raspberry crop system in Western North America. These alternative hosts may also be used as refuges for *D. suzukii* when crops are sprayed with insecticides. Therefore the management of these non-crop hosts should be integrated in control strategies. For example, our results and other host range studies (Lee *et al.*, 2015, Poyet *et al.*, 2015) now allow us to advise on suitable and unsuitable ornamental plants to be used in the vicinity of susceptible crops. Species such as *Cornus* spp., *Lonicera* spp., *Prunus* spp., *Sambucus* spp. and *Taxus baccata,* which are abundantly used as hedge plants in Europe, should be avoided. In contrast, there is now sufficient evidence showing that, e.g. *Ligustrum* spp., *Viburnum* spp., *Crataegus* spp. or *Pyracantha* spp. do not increase populations of *D. suzukii* on site. Similarly, field margins could be cleared of susceptible wild plants (Klick *et al.*, 2016). However, the management of wild hosts in the surroundings of crops is often more problematic than ornamental hosts because of the high number of highly susceptible species and the difficulty in managing them in areas that do not always belong to the fruit producer. Furthermore, more should be known on the natural dispersal capacities of *D. suzukii* to determine the areas requiring management. If dispersal studies show that *D. suzukii* can be attracted over long distances, removing native wild host plants from a large area may become unpractical and have a negative effect on the functioning of local ecosystems.

The fruiting period of host plants is also an essential consideration when developing management strategies. In Europe, populations of *D. suzukii* often dramatically increase from spring to autumn, due to the high number of generations (Asplen *et al.*, 2015). Only a few plants produce fruits in spring, suggesting that the availability of suitable fruits in spring is a key element in the population dynamics of *D. suzukii.* Therefore*,* efforts should be made to control the presence of these early fruits in the surroundings of fruit crops and orchards (Asplen *et al.*, 2015; Poyet *et al.*, 2015). Not only non-crop hosts should be controlled. In the surveyed area in Northern Italy and the Netherlands, the first heavy infestations occur on abandoned or untreated cherry tree, which probably play an important role in the local increase of *D. suzukii* populations in summer (Ioriatti *et al.*, 2015; H. Helsen and B. van der Sluis, unpublished data). This survey showed that all plants fruiting in spring were attacked by *D. suzukii*. In contrast, most fruits that are formed in autumn and overwinter until spring were used neither as overwintering hosts nor as early hosts in spring, with the possible exception of fruits of *C. lacteus* that were found attacked in April. We did not find any evidence that *D. suzukii* larvae may overwinter in fruits. In Northern Italy, the monitoring and dissection of female flies throughout the winter showed that *D. suzukii* overwinters as adults in reproductive diapause from November to April (Zerulla *et al.*, 2015).

More generally, the ability of *D. suzukii* to attack such a large number of widely distributed ornamental and wild host plants strongly suggests the need for an area wide control approach. Since insecticides are often not effective (e.g. Rogers *et al.*, 2016) and cannot be used in many of the non-crop habitats, in particular forests, and since sanitation is impossible on a large scale, classical biological control through the introduction of specific parasitoids from the region of origin of the fly could be a long term solution (Haye *et al.*, 2016; Daane *et al.*, 2016). Preliminary studies in Japan have suggested that some larval parasitoids are specific to *D. suzukii* (Kasuya *et al.*, 2013; Nomano *et al.*, 2015) and recent surveys in East Asia have shown that larval parasitism rates are not negligible, e.g. less than 10% in the Tokyo region (Kasyua *et al.*, 2013), up to 16% in South Korea (Daane *et al.*, 2016) and over 50% in Yunnan, China (M. Kenis, unpublished data). It would be worth assessing the suitability of these parasitoids for introduction into invaded areas, including the evaluation of potential non-target effects of such introductions on the community of native Drosophilidae.

Chapter 4

Development of *Drosophila suzukii* at low temperature in mountain areas

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LT conceived and designed the research, conducted the experiments, analysed the data and wrote the manuscript.

Abstract

As a fly tracking the availability of fruits along climatic gradients, *Drosophila suzukii* is deemed to be rather flexible in relation to environmental factors, among which, temperature is a major player. We sampled potential wild host fruits of *D. suzukii* along two elevational gradients in mountain areas of north-eastern Italy, in order to measure fly performance in relation to temperature. In addition, we used a strong natural temperature gradient in an open-top cave, covering the lower range of temperatures known for *D. suzukii*, to deploy laboratory stock colonies to mimic conditions existing along elevational gradients. At least nine wild host species yielded adults of *D. suzukii* in the mountain area (*Daphne mezereum, Lonicera alpigena, Lonicera caerulea, Lonicera nigra, Lonicera xylosteum, Rubus caesius, Rubus saxatilis, Sambucus nigra,* and *Sambucus racemosa*) when the daily average temperature in the three preceding weeks was at least 11.1°C. Similar results were obtained with the laboratory colonies reared on an artificial medium in the cave, where oviposition and development from egg to adult occurred above 11.6°C. Both values are lower than previously recorded lower thresholds for development at both constant and fluctuating temperatures. These findings indicate that *D. suzukii* performs well at low temperatures, suggesting that population build up may occur even under these conditions, with likely consequences on crops and wild host reproduction.

Introduction

Temperature is one of the most important abiotic factors affecting the activity, performance, and geographic distribution of insects (Chown and Nicholson 2004; Angilletta 2009; Doucet *et al.*, 2009). In temperate climates, therefore, temperatures seasonally drop below 0°C, causing overwintering mortality (Williams *et al.*, 2015), determining the life history and limiting the performance and establishment of an invasive pest species (Doucet *et al.*, 2009). In response, insects have evolved physiological and behavioural strategies to avoid extreme temperatures, such as diapause or migratory flights (Doucet *et al.*, 2009).

Climate change is dramatically affecting the temperature regime in the world (Field *et al.*, 2014). The projected changes of air temperature for the entire Alpine region are positive in both time horizons and seasons. According to Gobiet *et al.* (2014), warming will be stronger in winter than in the rest of the year, with an increase of 1.6°C in air temperature by 2050, with even higher rates of warming at higher elevations. Patterns indicate less precipitation and a decrease of relative humidity in summer, particularly south of the Alps, and potentially more precipitation in winter at the end of the 21st century. These increases of temperature, within the vital limits of a species, would allow the spreading of insects northward and upward. Faster development may cause an increase in population density, promoting a further expansion (Battisti 2008). In the Alps mean air temperature decreases regularly as elevation increases, at a lapse rate of 0.56°C every 100 m (Theurillat and Guisan 2001); this implies a shift of 200-250 m in elevation.

The Spotted Wing Drosophila *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae), which has recently invaded Europe from Asia (Cini *et al.*, 2014), tracks the availability of wild fruits along latitudinal and elevational gradients, and it is expected to be rather flexible in relation to environmental factors, among which temperature is a major player. In its native range of Japan, *D. suzukii* moves from low to high elevation during summer and then returns to favourable overwintering conditions in the autumn (Mitsui *et al.*, 2010). *D. suzukii* is a relatively cold-intolerant and chill-susceptible species as 50% of adult flies are killed following a 24 h exposure to temperatures just below 0°C (Kimura 2004). Adults can also be killed by chronic exposure to 0°C or by short exposure to chilling temperatures (Jakobs *et al.*, 2015). The migration along elevational gradients has been explained as a mechanism to escape summer heat and to track available host fruits (Kimura 2004; Kinjo *et al.*, 2014). In late summer or autumn the flies return to low elevation where overwintering conditions are milder (Mitsui *et al.*, 2010). Based on the first studies of Kanzawa (1939), the developmental periods from egg to adult varies between 9 and 25 days at the constant temperature of 25°C and 15°C, respectively. Recent studies confirmed these results and indicated that development was also possible at temperatures as low as 10°C on cherry under laboratory conditions (79.4 days), although oviposition was not observed at this temperature (Tochen *et al.*, 2014). Asplen *et al.*, (2015) estimated the lower threshold of development at 6.0°C and created a temperature-dependent model where the development rate from egg to adult ranged from about 0.02 day^{-1} at 10°C to 0.1 day^{-1} at 30°C. All these studies provide laboratory-based indications or model estimation of the lower temperature threshold for development of *D. suzukii*, however an empirical study under natural conditions is still missing.

As *D. suzukii* has recently invaded the Alps where it has reached pest status (Grassi *et al.*, 2011), we were interested to explore how the insect responds to low temperatures that can be observed in the upper mountains during summer, where adult flies are commonly caught in traps. Thus, we sampled wild potential host fruits of *D. suzukii* along elevational gradients in a mountain area of northern Italy. We also tracked temperature conditions in the weeks before the sampling, in order to verify if data concerning temperature-related development available in the literature match with the exploitation of the hosts, and if there are hosts which cannot be exploited because of limiting temperature. In addition, we used a strong natural temperature gradient, in the lower range known for *D. suzukii*, to study fly performance at low temperatures on an artificial medium, by mimicking the conditions existing along the elevational gradients. We expected to find a temperature threshold for oviposition and development of *D. suzukii* under natural conditions and to assess the insect performance at low temperature. In particular, we wanted to find the lowest temperature allowing development and measure the related offspring yield. The results would help to explain the success of the species on natural hosts and to clarify the adaptation potential of the species to host availability in a new invasion area, taking into account the possible effects of climate change.

Materials and methods

Insect collection

Two mountain elevational gradients where *D. suzukii* was known to occur, at least in the lower part, were selected in Trento (TN) and Verona (VR) districts, respectively, in north-eastern Italy. The gradients consisted of three sites for TN (elevation span 1640-1940 m) and of six sites for VR (elevation span 1100-1820 m) (Table 4.1 and 4.2) and were selected in order to mimic the average temperatures tested in the natural gradient experiment, located along the VR gradient (see below). The presence of the fly was assessed with a specific red trap (Droso-Trap® Biobest) baited with the food lure Droskidrink (Prantil, 75% apple cider vinegar, 25% red vine, 20 g/l sugar) (Grassi *et al.*, 2015; Ioriatti *et al.*, 2015). Along these gradients, fruits of potential host plants were collected whenever available during the summers of 2014 and 2015. For each plant species, fruits were picked as soon as they were ripe, avoiding rotten or damaged fruits. In the laboratory, the precise number and the total weight were measured (Table 4.1 for 2014 and 4.2 for 2015). The samples were put in emergence containers (jars with 0.1 mm mesh net) and checked daily. The emerged insects were stored in small tubes with 95% ethanol. The insects in each vial were counted; dividing *D. suzukii* males and females, other *Drosophila spp.*, and parasitoids. For each site along a gradient, a weather station (www.meteotrentino.it) was identified nearby or data-loggers were deployed on-site, at 2 m from the ground, to record hourly temperature (HOBO U23 ProV2 with RS1 solar radiation shield, Onset Computer Corporation, Bourne, MA, USA). The average daily temperature of the three weeks before the collection was calculated (Table 4.1.a and 4.1.b).

Insect rearing

The *D. suzukii* population used for the experiment was obtained from the laboratory of the author's department of the University of Padua and originated from adults collected from cherry, blueberry and grape in the Verona district. The flies were reared in plastic vials (Falcon-type with 50 ml capacity, diameter 30 mm, length 115 mm) with specific medium for *D. suzukii* rearing (modified by Fondazione Edmund Mach from Bloomington Drosophila Stock Center at Indiana University, personal communication). The medium contained raw cornmeal 75 g, dry-yeast 17 g, sucrose 15 g, soybean meal 12 g, agar 5.6 g, propionic acid 5 ml, and water to 1000 ml. All components were mixed and heated for 20 minutes at about 100°C, with the exception of propionic acid that was added at a temperature lower than 50°C, just before pouring 15 ml of medium inside the plastic vials. The insect rearing, and the laboratory reference trials, were conducted at the constant temperature of 23.3°C, 70-80% relative humidity and under a photoperiod of 16L:8D in a climatic room. The same type of vials and medium were used to perform the natural gradient experiments.

Cave description

The experiment was conducted in a large, open-top cave well known for its strong temperature gradient (Benetti and Cristoferi, 1968), called Covolo di Camposilvano (Velo Veronese, 1220 m, 45°37'34" N, 11°05'34" E) during August and September 2014. The cave had previously been used to study the relationship between temperature and development in the sawfly *Cephalcia arvensis* (Battisti, 1994). The choice of the site was motivated by the occurrence of a strong gradient of naturally fluctuating temperatures. Seven sites were chosen, from the bottom of the cave (site 1) to the top (site 7) (Fig. 4.1 and Fig. 4.2).

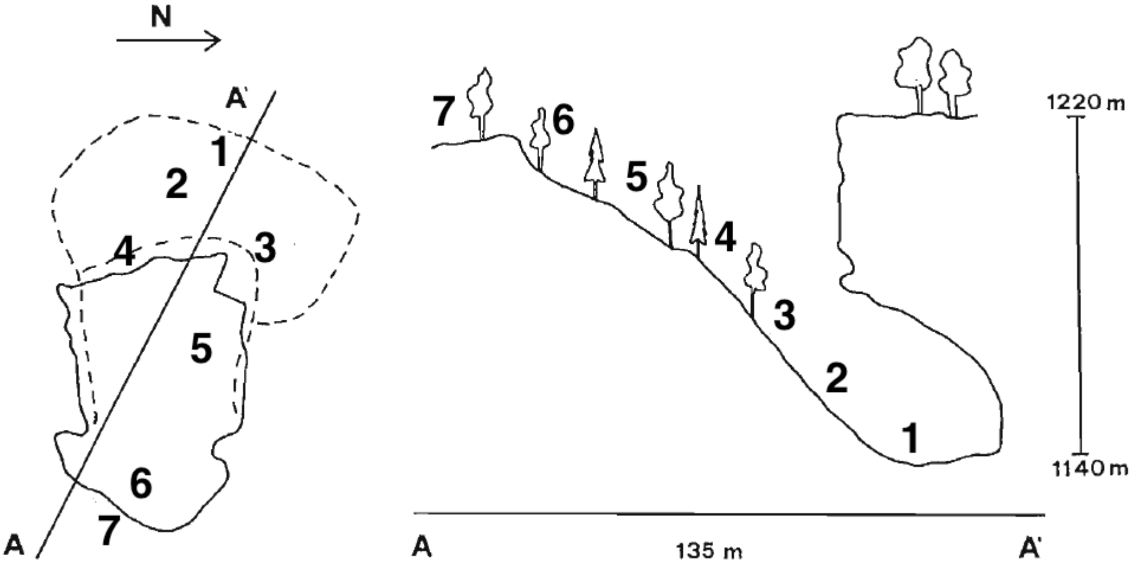


Figure 4.1 - Cross-section of the open-top cave used for the experiment, the numbers mark the position of the 7 different sites, with values of the average daily temperature and the average daily temperature variation (maximum – minimum) throughout the period of the trials (August 4-September 20, 2014). The numbers on the right indicate the elevation in meters above sea level.

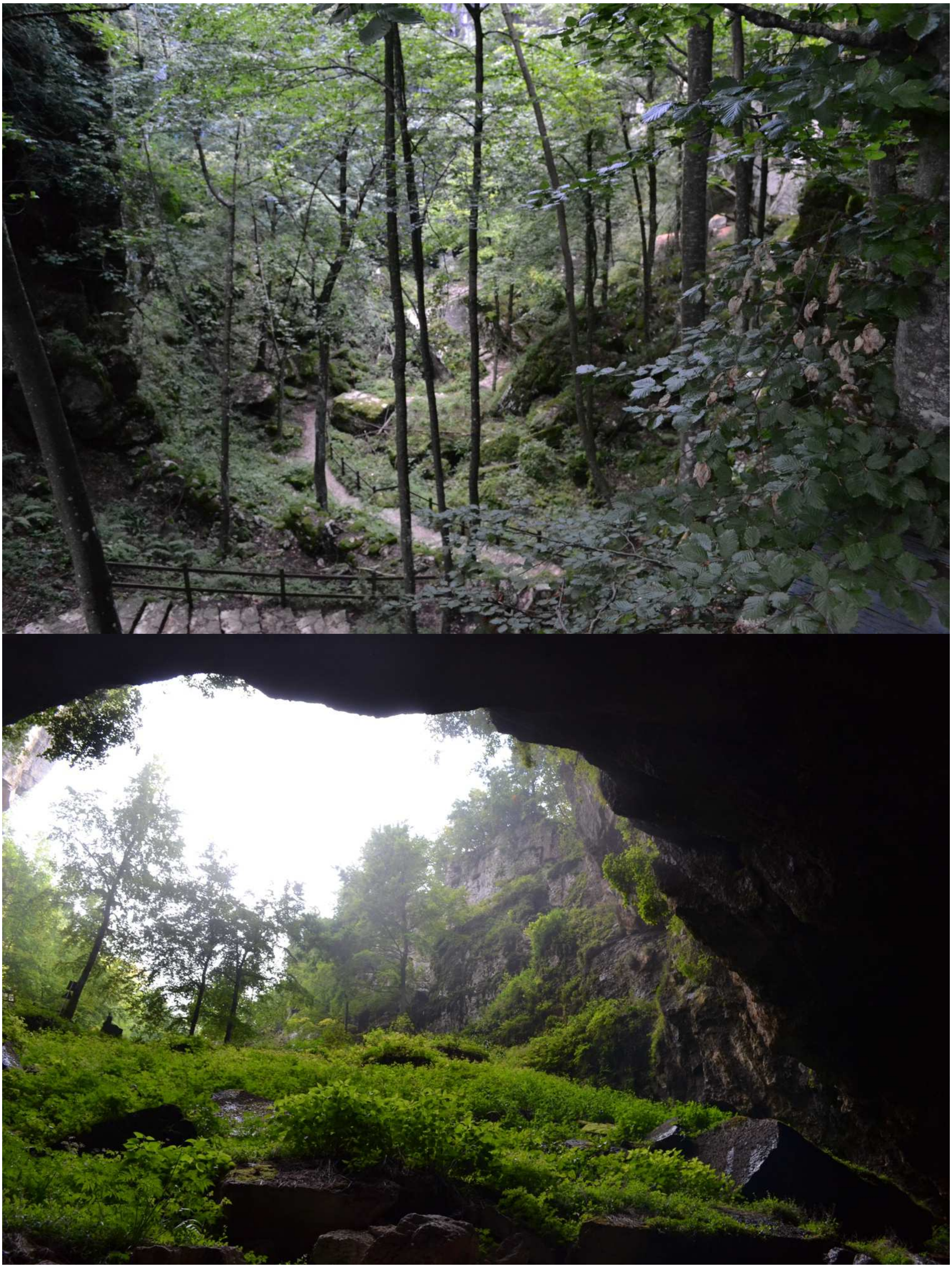


Figure 4.2 - Views of the cave used for the experiments, above from the top and below from the bottom.

During the study period, sites 1 to 4 were characterized by differences among sites of 2-3°C in the average daily temperature, while sites 5, 6 and 7 presented similar average daily temperature but differed in daily temperature variation (Fig. 4.3). All sites had the same natural photoperiod of the season (12-13 hours of light). The rearing vials were located close to the ground and protected by a shelter, ensuring the same light intensity but offering rainfall and animal exclusion. The shelter was made of a vertical cylinder of plastic net (diameter 25 cm, square mesh 5 mm x 5 mm) covered by a plastic plate as a roof. Each shelter was sitting on the ground and protected on the sides by stones. The vials were hung on the shelter roof and thus about 10 cm from the ground (Fig. 4.4). The temperature inside each shelter was measured with HOBO data-loggers (previously described) hung close to the vials. Seven data-loggers were used during the experiment in the cave and another one was placed in the laboratory (23.3 ± 1.4°C). Data were recorded every hour and downloaded twice during the experiment.

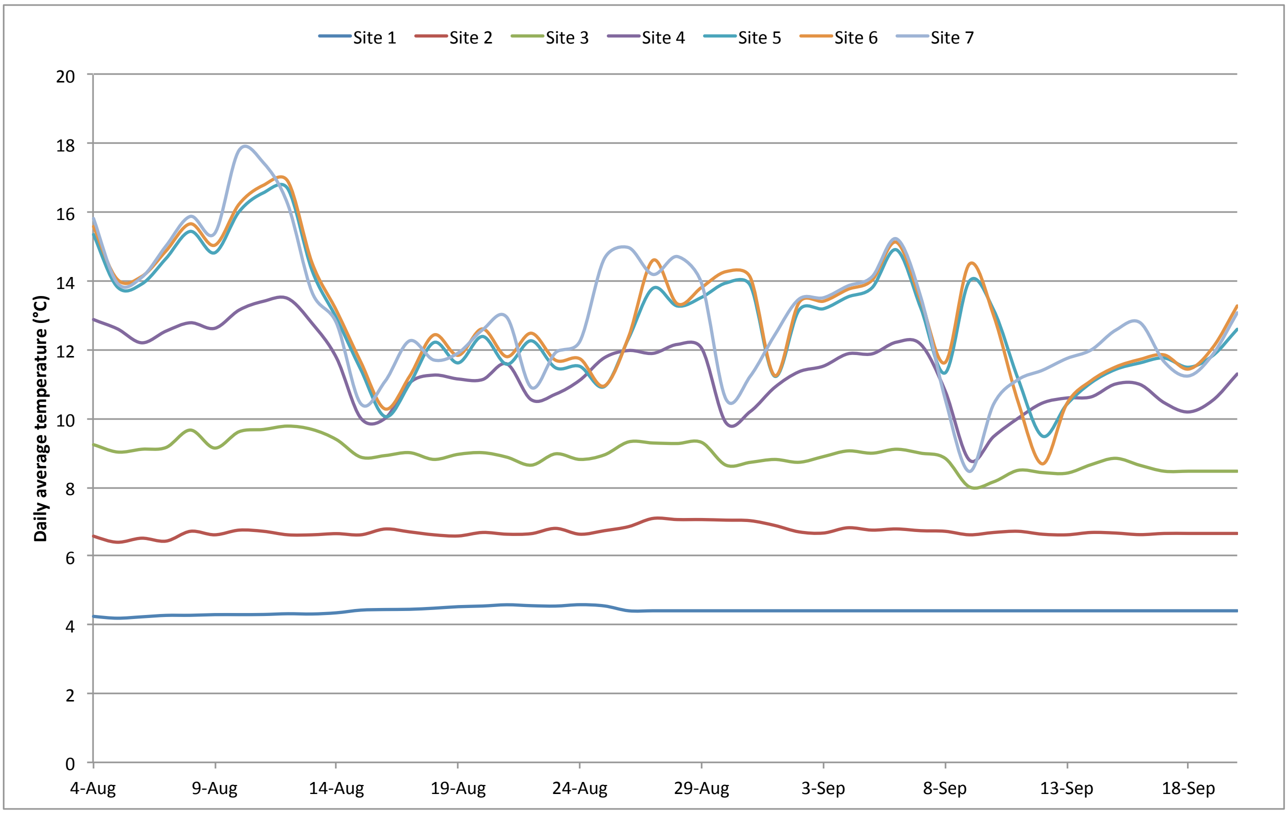


Figure 4.3 - Daily average temperatures at the seven sites of the cave during the period of the trials, from August 4 to September 20, 2014.

Figure 4.4 - Shelter used to protect the test tubes and the data-logger in the cave.

Natural gradient experiment

Four trials were implemented to test the effect of temperature on both oviposition and development of *D. suzukii* in the cave. Together, these trials aimed to assess a lower temperature threshold for fly development and to test for the capacity of acclimatization within and among generations. Different developmental stages and durations of acclimatization (permanence in the cave sites) were used to quantify possible variations in the response to the lower thermal threshold. In three out of four trials, individuals from constant laboratory conditions (23.3 ± 1.4°C) were moved to each of the seven sites in the cave. Specifically, larvae were used in Trial 1 while adults were used in Trial 2 for the first 2 days, and in Trial 3 for the remaining 3-14 days of their permanence in the site. The 4th Trial was intended to test the effect of temperature on the individuals originating in the cave sites, using the adults developed from the other Trials.

The four trials were implemented as follows:

Trial 1: A total of 5 females and 5 males (both 7-day-old) from the laboratory rearing culturing (23.3°C) were placed in individual vials with medium, allowed to mate and oviposit for 5 days in the laboratory before being removed. No mortality was observed during this period. These vials were then checked for the presence of the larvae and brought to the cave on August 6th 2014. Two vials were placed at each of the seven sites, while three vials were kept in the laboratory as a control. The vials were left on site for 32 days and checked biweekly until September 8th, when the insects were either emerged or dead, while the laboratory vials were recorded for 12 days (until August 18th).

Trials 2 and 3: A total of 8 flies (4 female and 4 male) from the laboratory rearing culture were placed in each vial in the laboratory and immediately transferred in a cold box (about 5°C) to the cave, where three vials were deployed at each site. For these Trials, 6-day-old flies were used, in order to have a constant egg-laying rate (Kinjo *et al.*, 2014). For Trial 2, insects were left in the vials for two days, from August 4th to August 6th, then removed and checked biweekly until September 8th (32 days). The same design was used with three additional vials kept in the laboratory for 15 days (until August 21th). For Trial 3, insects that survived the first 48 hours in Trial 2 were transferred to new vials, in the number of three vials per site. Insects were left in the vials for 12 days from August 6th to August 18th, when the adults were removed and the vials were checked biweekly until September 8th (21 days). For each Trial, the adult mortality was assessed when they were removed from the vials.

Trial 4: New adults emerging from Trials 1, 2 and 3 were removed in two dates (September 1st and 8th) and inserted into new vials with fresh medium. The number of flies and vials used differed among sites. At sites 1 to 3, no adults were produced, and therefore Trial 4 was not carried out. From sites 4 to 7, a number from 2 to 5 females and from 1 to 5 males for each vial were used, depending on the number of flies emerged at each site/date. In order to assess the effect of the adult age on the offspring yield, when possible adults were removed from the vials after 7 days and transferred into new vials for other 12 days. Due to a sudden temperature drop on September 20th, the vials were taken to the laboratory under constant temperature of 23.3°C to follow the development into pupae and adults, with regular checks until October 9th. This Trial was also used to verify a possible effect of the time from emergence on the capacity of the flies to generate offspring at low temperatures.

The presence and the number of pupae and adults were assessed in general twice a week. Unfortunately, it was not possible to detect precisely the number of eggs and larvae, although it was possible to detect whether they were present. Dead adults were always left in the vials. The presence of moulds was also annotated at each check and the relevant vials discarded from further analysis. At the end of each experiment (September 8th for Trials 1, 2 and 3, and October 9th for Trial 4) the content of each vial was checked, counting adults (male and female), pupae and larvae present in the medium. Moreover, the quality of the medium was visually checked, based on its colour/texture, in order to assess whether development could have been negatively affected.

Data analyses and statistics

All temperature data were referred to as the average daily temperature of each site, calculated using hourly temperature data. The standard deviation (SD) of average daily temperature was also calculated for the period of interest in each site. In addition, degree-days were calculated on an hourly basis using the lower thermal threshold of 5.975°C as suggested by Asplen *et al.*, (2015). The biweekly checks allowed the calculation of the development rate for pupae and adults. The development rates (r_p for pupae and r_a for adults) were calculated as the reciprocal of days elapsed since the beginning of oviposition to the detection of the first pupa or adult.

development rates (r_p or r_a) = (days from oviposition to first pupa or adult)$^{-1}$

The counts of all stages of development of insects in the vials at the end of the trials were necessary to assess the yield. The yield was related to the individual female fly per day of oviposition. The number of female flies was considered as the average between the number of females at the beginning of the trial and the number of females alive at the end of the trial, assuming the mortality rate was constant over time.

We considered the following indices:

- Immature yield, as the number of larvae and pupae developed per female per day of oviposition (only for Trial 4);

- Pupal yield, as the number of new pupae developed per female per day of oviposition;
- Adult yield, as the number of new adults developed per female per day of oviposition.

The data obtained for each vial were then used for calculating the average and the standard error (SE) at each site.

Statistical analyses were carried out using SPSS version 21.0 for Windows. In consideration of the low number of replicates, the results of Trial 1 were represented with a descriptive statistical only. Conversely, to find the lower temperature threshold for the development for Trial 1, 2, and 3, averages were compared using ANOVA and Dunnet's post-hoc test was applied to identify differences from the stock maintained under laboratory conditions (significance level set at $P<0.05$). Since this test sizably reduces the number of multiple comparisons, it provides a reliable estimation of the difference among means also in the cases of low number of replicates and an unbalanced dataset. To test for the effect of time from the emergence on the capacity to generate offspring at low temperatures (Trial 4) the number of larvae and pupae developed at sites 5 to 7 were pooled in two separate groups in accordance with the first and for the second week of oviposition. Differences between averages of these two groups were tested using the t-test for paired samples.

Results

Development in wild host fruits

Among 25 potential host fruits encountered along the gradients, a total of 9 (Tables 4.1a and 4.1.b) resulted in being suitable for the development of *D. suzukii*. The successful development of the fly depended on the site and the date of collection, and was mainly related to temperatures in the period prior to sampling.

In 2014 (Table 4.1.a), *Daphne mezereum* yielded adults at 1420 m (11.1°C) but not at 1940 m (10.7°C). *Lonicera alpigena* yielded adults at 1230 m (two collection dates) and 1420 m, with temperatures higher than 13°C, but not at 1820 m (about 10°C). The number of flies emerged from 1 g of fruit was higher at 14°C than at 13°C (0.5 and 0.14-0.20 adults/g, respectively). *Lonicera nigra* yielded adults at 1660 m (12.9°C) but not at 1820 (10.0°C). *Lonicera xylosteum* did not yield adults in early August (August 4th) but it did later in the month (August 18th), despite temperatures being higher at the beginning of the month (above 14°C). *Sambucus nigra* collected at 1420 m in early September (11.1°C) yielded adults as well as *Sambucus racemosa* collected earlier at the same site (13.2°C). The latter, however, did not yield adults at 1820 m (10.0°C).

In 2015 (Table 4.1.b), *Daphne mezereum* did not yield adults at low elevation (1230 m, 18.8°C). Also *Lonicera alpigena* did not yield adults at 1230 m (July 20th) while adults were obtained at the same site from *Rubus caesius*. At 1420 m, adults emerged only from samples collected at the end of August (14.9°C). At 1600 m, adults emerged during mid August (16.3°C). From the sample of *Lonicera caerulea* collected at high elevation (1880 m, 13.8°C), adults were obtained in abundance (2 adults/g of fruits). *Lonicera nigra* yielded adults at both 1600 m (16.3°C) and 1880 m (13.8°C), with higher yield at higher elevation (0.67 *vs* 1.83 adults/g of fruits). *Rubus saxatilis* also yielded adults at 1600 m (16.3°C). *Sambucus racemosa* yielded adults at all collection sites (ranging from 1100 to 1580 m) and dates (July 12th to August 4th). The yield ranged from 0.21 adults/g at lower temperature to 1.23 adults/g at higher temperature.

Table 4.1.a - Wild fruits collected along elevational gradients in mountain areas, during the summer season 2014, which yielded adults of *D. suzukii* in at least one sample. Plant species are listed alphabetically and according to increasing elevation within each species. Samples that yielded *D. suzukii* are reported in bold. Plant species that never produced adults are reported in the supplementary material (Table 4.2). The weather stations are indicated with the site: [1] Pian delle Fugazze 1170 m; [2]Barricata di Grigno 1345 m; [3] Malga Casapinello 1710 m; [4] Viotte, Monte Bondone 1552 m; [5]Malga Prato di sopra, Val d'Ambiez 1880 m. If not specified, on-site data logger were used.

Species	Family	Site	Coordinates	Elevation (m)	Dominant landscape	Date	Average daily temperature 3 weeks before collection ± SD	Adults *D. suzukii*	Number of fruits	Fruit fresh weight (g)
Daphne mezereum	***Thymelaeaceae***	**Malga Porcarina (VR)** [2]	**45°40'18.84"N 11°5'19.96"E**	**1420**	**Pasture and hedges**	**1 September 2014**	**11.14 ± 2.05**	**4**	**91**	**32**
Daphne mezereum	*Thymelaeaceae*	Passo Rolle (TN) [5]	46°17'50.77"N 11°46'47.68"E	1940	Pasture and hedges	16 August 2014	10.72 ± 1.88	0	57	20
Lonicera alpigena	***Caprifoliaceae***	**Camposilvano (VR)**	**45°37'52.29"N 11°5'19.89"E**	**1230**	**Wood (beech, shrubs, undergrowth)**	**18 August 2014**	**14.13 ± 2.30**	**60**	**120**	**109**
Lonicera alpigena	***Caprifoliaceae***	**Camposilvano (VR)**	**45°37'52.29"N 11°5'19.89"E**	**1230**	**Wood (beech, shrubs, undergrowth)**	**8 September 2014**	**13.03 ± 1.45**	**10**	**50**	**45**
Lonicera alpigena	***Caprifoliaceae***	**Malga Porcarina (VR)** [2]	**45°40'18.84"N 11°5'19.96"E**	**1420**	**Pasture and hedges**	**18 August 2014**	**13.18 ± 2.62**	**15**	**108**	**84**
Lonicera alpigena	*Caprifoliaceae*	Passo Malera (VR) [3]	45°41'31.25"N 11°6'30.15"E	1820	Pasture and hedges	1 September 2014	10.04 ± 1.83	0	40	23
Lonicera nigra	***Caprifoliaceae***	**Malga Prato di sotto (TN)** [4]	**46°7'20.89"N 10°52'49.90"E**	**1660**	**Pasture and hedges**	**19 August 2014**	**12.93 ± 2.43**	**17**	**93**	**38**
Lonicera nigra	*Caprifoliaceae*	Passo Malera (VR) [3]	45°41'31.25"N 11°6'30.15"E	1820	Pasture and hedges	1 September 2014	10.04 ± 1.83	0	10	2
Lonicera xylosteum	*Caprifoliaceae*	Camposilvano (VR) [1]	45°37'52.29"N 11°5'19.89"E	1230	Wood (beech, shrubs, undergrowth)	4 August 2014	15.46 ± 1.65	0	212	62
Lonicera xylosteum	***Caprifoliaceae***	**Camposilvano (VR)**	**45°37'52.29"N 11°5'19.89"E**	**1230**	**Wood (beech, shrubs, undergrowth)**	**18 August 2014**	**14.13 ± 2.30**	**38**	**160**	**37**
Sambucus nigra	***Adoxaceae***	**Malga Porcarina (VR)** [2]	**45°40'18.84"N 11°5'19.96"E**	**1420**	**Pasture and hedges**	**1 September 2014**	**11.14 ± 2.05**	**8**	**250**	**44**
Sambucus racemosa	***Adoxaceae***	**Malga Porcarina (VR)** [2]	**45°40'18.84"N 11°5'19.96"E**	**1420**	**Pasture and hedges**	**18 August 2014**	**13.18 ± 2.62**	**96**	**600**	**101**
Sambucus racemosa	*Adoxaceae*	Passo Malera (VR) [3]	45°41'31.25"N 11°6'30.15"E	1820	Pasture and hedges	1 September 2014	10.04 ± 1.83	0	40	3

Table 4.1.b - Wild fruits collected along elevational gradients in mountain areas, during the summer season 2015, which yielded adults of *D. suzukii* in at least one sample. Plant species are listed alphabetically and according to increasing elevation within each species. Samples that yielded *D. suzukii* are reported in bold. Plant species that never produced adults are reported in the supplementary material (Table 4.2). The weather stations are indicated with the site: ^{1} Pian delle Fugazze 1170 m; ^{2}Barricata di Grigno 1345 m; ^{3} Malga Casapinello 1710 m; ^{4} Viotte, Monte Bondone 1552 m; ^{5}Malga Prato di sopra, Val d'Ambiez 1880 m. If not specified, on-site data logger were used.

Species	Family	Site	Coordinates	Elevation (m)	Dominant landscape	Date	Average daily temperature 3 weeks before collection ± SD	Adults *D. suzukii*	Number of fruits	Fruit fresh weight (g)
Daphne mezereum	*Thymelaeaceae*	Camposilvano (VR) ^{1}	45°37'52.29"N 11°5'19.89"E	1200	Wood (beech, shrubs, undergrowth)	20 July 2015	18.78 ± 1.84	0	140	40
Lonicera alpigena	*Caprifoliaceae*	Camposilvano (VR) ^{1}	45°37'52.29"N 11°5'19.89"E	1200	Wood (beech, shrubs, undergrowth)	20 July 2015	18.78 ± 1.84	0	25	9
Lonicera alpigena	*Caprifoliaceae*	Camposilvano (VR) ^{1}	45°37'52.29"N 11°5'19.89"E	1200	Wood (beech, shrubs, undergrowth)	4 August 2015	18.03 ± 2.59	0	12	10
Lonicera alpigena	*Caprifoliaceae*	Malga Porcarina (VR)	45°40'18.84"N 11°5'19.96"E	1420	Pasture and hedges	4 August 2015	15.55 ± 1.97	0	59	31
Lonicera alpigena	***Caprifoliaceae***	**Malga Porcarina (VR)**	**45°40'18.84"N** **11°5'19.96"E**	**1420**	**Pasture and hedges**	**31 August 2015**	**14.92 ± 2.86**	**6**	**70**	**49**
Lonicera alpigena	***Caprifoliaceae***	**Malga Prato di sotto (TN)** ^{4}	**46°7'20.89"N** **10°52'49.90"E**	**1600**	**Pasture and hedges**	**15 August 2015**	**16.26 ± 2.16**	**22**	**37**	**21**
Lonicera caerulea	***Caprifoliaceae***	**Malga Ben (TN)** ^{5}	**46°07'39.4"N** **10°53'20.3"E**	**1880**	**Pasture and hedges**	**15 August 2015**	**13.79 ± 2.16**	**4**	**34**	**2**
Lonicera nigra	***Caprifoliaceae***	**Malga Prato di sotto (TN)** ^{4}	**46°7'20.89"N** **10°52'49.90"E**	**1600**	**Pasture and hedges**	**15 August 2015**	**16.26 ± 2.16**	**4**	**28**	**6**
Lonicera nigra	***Caprifoliaceae***	**Malga Ben (TN)** ^{5}	**46°07'39.4"N** **10°53'20.3"E**	**1880**	**Pasture and hedges**	**15 August 2015**	**13.79 ± 2.16**	**22**	**42**	**12**
Rubus caesius	***Rosacee***	**Camposilvano (VR)** ^{1}	**45°37'52.29"N** **11°5'19.89"E**	**1200**	**Wood (beech, shrubs, undergrowth)**	**06 July 2015**	**14.64 ± 3.24**	**8**	**30**	**15**
Rubus saxsatilis	***Rosacee***	**Malga Prato di sotto (TN)** ^{4}	**46°7'20.89"N** **10°52'49.90"E**	**1600**	**Pasture and hedges**	**15 August 2015**	**16.26 ± 2.16**	**16**	**150**	**17**
Sambucus racemosa	***Adoxaceae***	**Bosco Chiesanuova (VR)**	**45°38'01.6"N** **11°01'48.6"E**	**1100**	**Pasture and hedges**	**12 July 2015**	**16.33 ± 2.96**	**62**	**400**	**62**
Sambucus racemosa	***Adoxaceae***	**Malga S. Giorgio (VR)** ^{2}	**45°41'04.5"N** **11°05'02.9"E**	**1400**	**Pasture and hedges**	**25 July 2015**	**17.80 ± 1.78**	**340**	**2000**	**276**
Sambucus racemosa	***Adoxaceae***	**Malga Porcarina (VR)** ^{2}	**45°40'18.84"N** **11°5'19.96"E**	**1420**	**Pasture and hedges**	**20 July 2015**	**17.48 ± 1.87**	**76**	**2000**	**204**
Sambucus racemosa	***Adoxaceae***	**Malga Porcarina (VR)** ^{2}	**45°40'18.84"N** **11°5'19.96"E**	**1420**	**Pasture and hedges**	**27 July 2015**	**17.38 ± 1.84**	**37**	**500**	**84**
Sambucus racemosa	***Adoxaceae***	**Passo del Branchetto (VR)** ^{3}	**45°40'54.3"N** **11°04'10.8"E**	**1580**	**Pasture and hedges**	**4 August 2015**	15.51 ± 2.65	**56**	**1300**	**260**

Table 4.2 - Wild fruits collected along elevational gradients in mountain areas, during the summer 2014 and 2015. In bold are reported species that yielded adults of *D. suzukii* in at least one sample

Species	Family
Actaea spicata	*Ranunculaceae*
Arum italicum	*Araceae*
Convallaria majalis	*Convallariaceae*
Daphne mezereum	***Thymelaeaceae***
Juniperus sp.	*Cupressaceae*
Lonicera alpigena	***Caprifoliaceae***
Lonicera caerulea	***Caprifoliaceae***
Lonicera nigra	***Caprifoliaceae***
Lonicera xylosteum	***Caprifoliaceae***
Paris quadrifolia	*Melanthiaceae*
Rhamnus pumila	*Rhamnaceae*
Rosa canina	*Rosacee*
Rosa pendulina	*Rosacee*
Rubus caesius	***Rosacee***
Rubus fruticosus	***Rosacee***
Rubus saxatilis	***Rosacee***
Sambucus nigra	***Adoxaceae***
Sambucus racemosa	***Adoxaceae***
Solanum dulcamara	*Solanaceae*
Sorbus aria	*Rosacee*
Sorbus aucuparia	*Rosacee*
Sorbus chamaemespilus	*Rosacee*
Vaccinium myrtillus	*Ericaceae*
Vaccinium uliginosum	*Ericaceae*
Vaccinium vitis-idaea	*Ericaceae*

Natural gradient experiment

The results of Trial 1 (Table 4.3) show that the larvae produced in the laboratory developed into pupae at all temperature conditions, even at the lowest site with an average daily temperature of only 4.4°C. Below 9°C, however, the yield of pupae was very low, with less than 0.3 pupae/female/day, the remaining larvae were dead. Above 9°C, the yield increased to values higher than 0.5, although with high variability in individual response (as standard error). With temperatures above 13.3°C, the yield reached values higher than 1 pupae/female/day, with low variability (1.25 and 1.13 for sites 6 and 7, respectively). In the laboratory (23.3°C) the yield was 0.8 pupae/female/day. Adults were obtained only at site 4 and above, characterized by temperatures higher than 10.5°C. The yields were around 0.5 adults/female/day of oviposition, with the exception of site 5 which yielded less (0.2 adults/female/day). In the laboratory (23.3°C) the adult yield was lower than at the most productive site of the cave, amounting to 0.3 adults/female/day.

Table 4.3 - Number of pupae and adults (± SD) developed in the trial 1 per female and per day of the oviposition period. Hatching and first larval development were in the laboratory, larval development, pupae and adult emergence were observed in the cave. The last column indicates the number of vials used for the experiment and the total number of starting egg laying females.

Site	Temperature	Pupal yield	Adults yield	No. vials, No. females
1	4.42 ± 0.09	0.14 ± 0.20	0.00 ± 0.00	2, 10
2	6.75 ± 0.16	0.22 ± 0.12	0.00 ± 0.00	2, 10
3	9.09 ± 0.31	0.67 ± 0.28	0.00 ± 0.00	2, 10
4	11.65 ± 0.95	0.54 ± 0.67	0.48 ± 0.59	2, 10
5	13.15 ± 1.67	0.53 ± 0.18	0.20 ± 0.00	2, 10
6	13.37 ± 1.70	1.25 ± 0.07	0.57 ± 0.07	2, 10
7	13.45 ± 1.91	1.13 ± 0.05	0.52 ± 0.05	2, 10
Laboratory	23.28 ± 0.41	0.83 ± 0.21	0.30 ± 0.04	3, 15

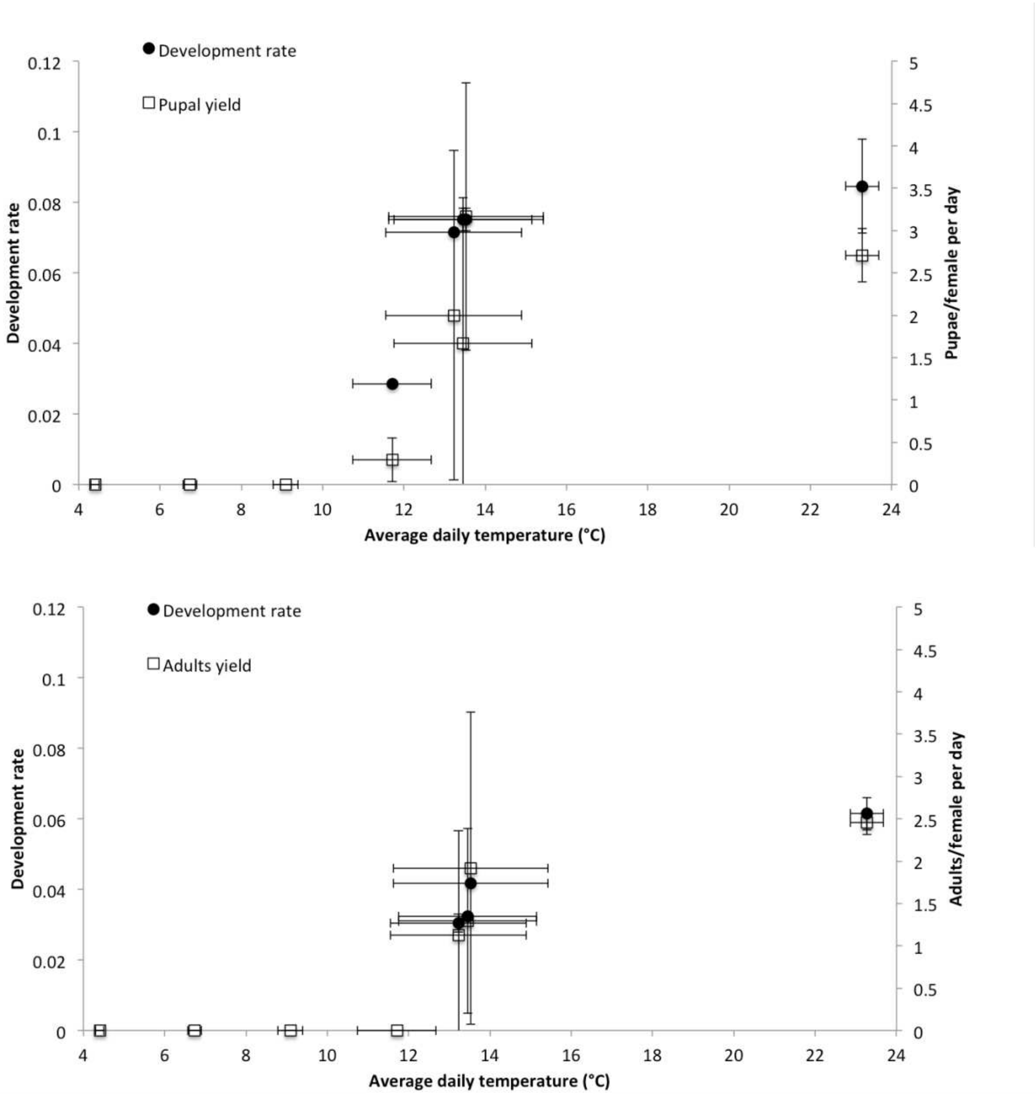
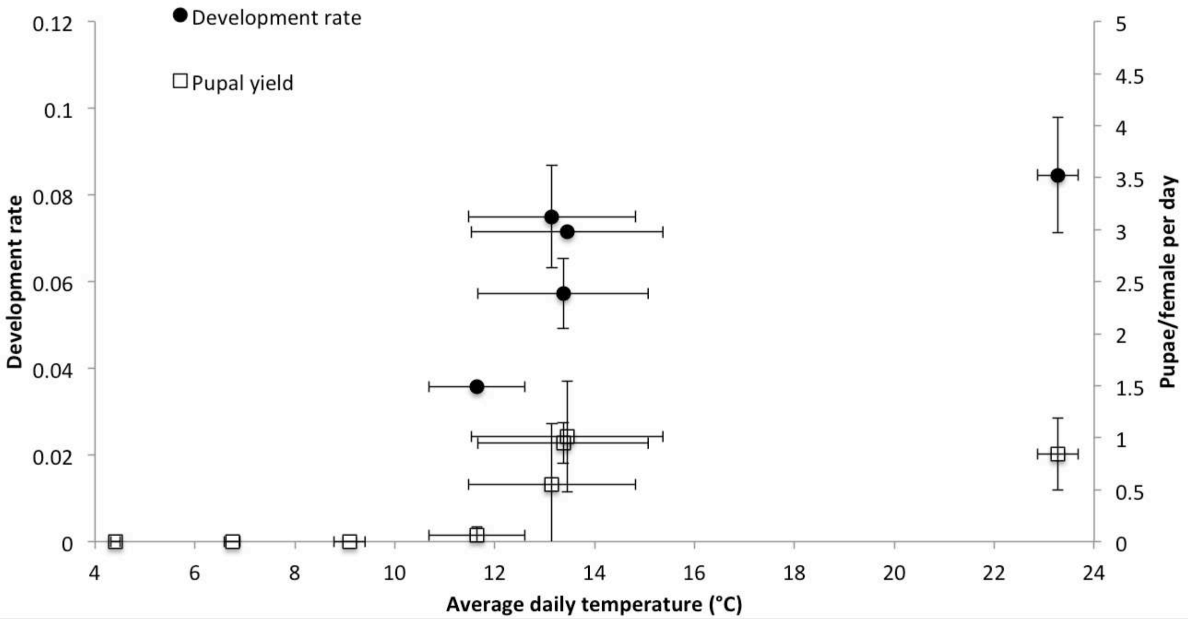


Figure 4.5 - Development rate from egg to pupa (above) or from egg to adults (below) and yield of pupae (above) or yield of adults (below) in trial 1 in the vials in the cave (T<16°C) and in the laboratory. Vertical bars indicate standard deviation of three replicates and horizontal bars indicate standard deviation of the average daily temperature.

The results of Trials 2 and 3 indicate that there was no development of pupae from eggs laid on site when the temperature was below 10°C. The first pupae appeared at 11.7±1.0°C in Trial 2 (0.29 pupae/female/day; Fig. 4.5 panel A) and at 11.6±0.9°C in Trial 3 with a very low yield (0.06 pupae/female/day; Fig. 4.2 panel B). Both rate of development and yield increased with temperature, with yields of 2.00, 1.67 and 3.17 pupae/female/day for Trial 2 (Fig. 4.5 panel A) and 0.55, 0.95 and 1.01 pupae/female/day for Trial 3 (Fig. 4.5 panel B) for the average temperature of 13.2, 13.4 and 13.5°C, respectively. Pupal development rate (r_p) increased to 0.071-0.075 days^{-1} at 13.2 and 13.5°C, respectively, for Trial 2 (Fig. 4.5 panel A) and 0.057-0.075 days^{-1} at 13.2-13.5°C for Trial 3 (Fig. 4.3 panel B). In the laboratory, however, an increase was observed for both yield

(2.7 and 0.8 pupae/female/day for Trial 2 and Trial 3 respectively; Fig. 4.5 panels A and B) and growth rate (r_p = 0.097 days^{-1}; Fig. 4.6 panels A and B). The value for pupal yield reached in the laboratory statistically deviated from the site 4 (11.6°C) for both Trials (Dunnett's test following one-way ANOVA; $F_{7,22}$=5.1 for Trial 2 and $F_{7,23}$=7.4 for Trial 3, $P < 0.05$). The laboratory pupal development rate for the Trial 2 was significantly higher only in comparison to that observed at the lowest temperature, while for Trial 3 it was always higher, with the exception of the one observed at 13.15°C (Dunnett's test following one-way ANOVA; $F_{4,12}$=21.4 for Trial 2 and 10.7 for Trial 3). Adults emerged at temperatures higher than 12°C and 11.6°C for Trial 2 and 3, respectively. In Trial 2, at temperatures around 13°C the yield in adults increased at values higher than 1 adult/female/day (Fig. 4.5 panel C) and the development rate reached values of 0.03-0.04 days^{-1} (Fig. 3 panel C). In Trial 3, the yield in adults slightly increased (0.23, 0.13 and 0.73 for temperatures of 13.2, 13.4 and 13.5°C, respectively; Fig. 4.5 panel D) while the speed of development increased greatly (r_a=0.06 days^{-1} for the three sites; Fig. 4.6 panel D). In the laboratory, the development rate was much higher compared to the cave for Trials 2 and 3 (r_a=0.079 days^{-1}; Fig. 4.6 panel C) with statistical differences with all sites (Dunnett's test following one-way ANOVA; $F_{3,10}$=246.0 for Trial 2 and $F_{4,12}$=323.0 for Trial 3). For Trial 2 the yield in the laboratory setting was significantly higher only in site 4 at 11.6°C (Dunnett's test following one-way ANOVA; $F_{7,22}$=4.1) while for Trial 3 the yield in the laboratory was similar only to that of site 5 at 13.1°C (Dunnett's test following one-way ANOVA; $F_{7,23}$=27.6). The number of degree-days required for the development from egg to adult was between 185 and 237 for Trial 2 and between 125 and 187 for Trial 3, while in the laboratory it was 220.



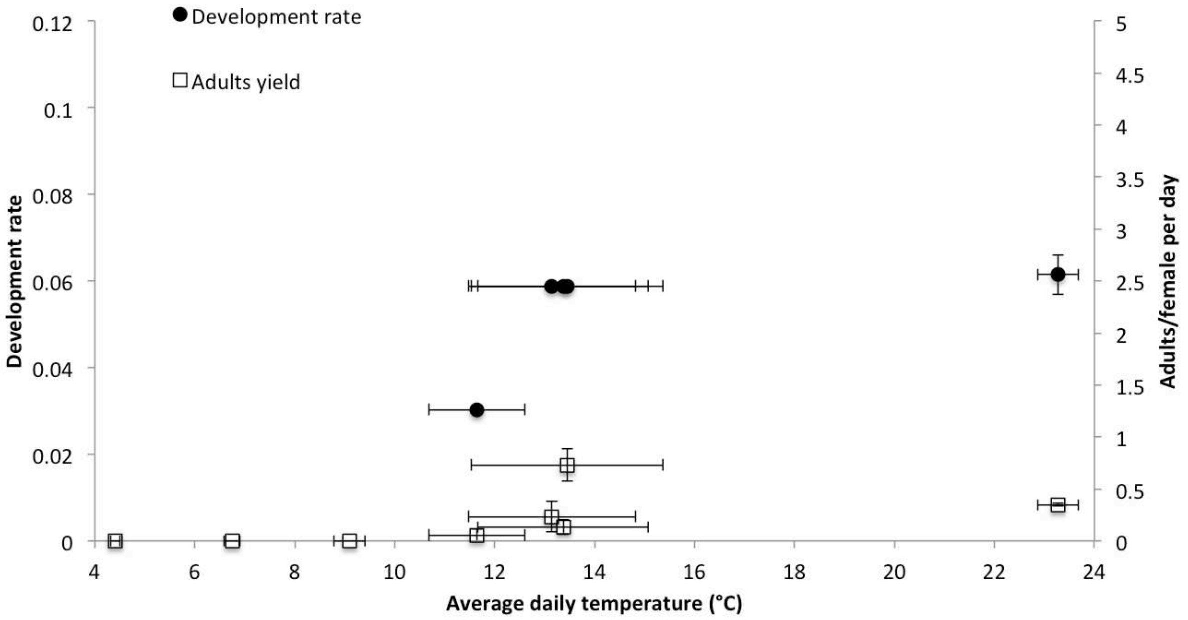


Figure 4.6 - Development rate from egg to pupa (above) or from egg to adults (below) and yield of pupae (above) or yield of adults (below) in trial 2 in the vials in the cave (T<16°C) and in the laboratory. Vertical bars indicate standard deviation of three replicates and horizontal bars indicate standard deviation of the average daily temperature.

The results of Trial 4 indicated that at the coldest site where adults were produced from the other Trials, they did not generate any progeny (larvae and pupae) both in the first 7 days (average daily temperature 11.6°C) and in the next 12 days (average daily temperature 10.4°C). Adults from Trial 1 laid eggs which later developed into new adults both in the first 7 days (average daily temperature 13.0°C) and in the next 12 days (average daily temperature 11.6°C) in the sites 5, 6 and 7, with a yield ranging from 0.35 to 0.70 young/female/day. Although in site 4 oviposition did not occur during the first week at temperatures lower than 13°C, in the upper sites the capacity of developing offspring was maintained after the drop of temperature from 13.2 (adults from Trial 1 at the first week from the emergence) to 11.6°C (adults from Trial 1 at the second week from the emergence), with no significant differences in the yield (T-test, $p > 0.05$). Adults from Trials 2 and 3 were tested only at sites 5, 6 and 7 and they were not able to generate progeny, with the exception of 0.02 immature/female/day at site 5 (average temperature of 11.6°C) and 0.29 immature/female/day at site 7 (second period, average temperature of 11.5°C).

Table 4.4 - Number of larvae and pupae (± SD) developed in the laboratory per female and per day of the oviposition period in the cave. The figures within brackets indicate the number of vials used for the experiment and the total number of starting egg laying females.

Site	Temperature at oviposition	Adults from trial 0 – first week	Adults from trial 0 – second week	Adults from trial 1 – first week	Adults from trial 1 – second week	Adults from trial 2 – first week
4	10.41 ± 0.87	-	0.00 (1, 4)	-	-	-
7	11.46 ± 1.54	-	0.00 (1, 5)	0.00 ± 0.00 (2, 8)	0.29 (1, 5)	0.00 ± 0.00 (3, 15)
4	11.60 ± 0.54	0.00 ± 0.00 (3, 7)	-	-	-	-
5	11.64 ± 1.36	-	0.44 ± 0.42 (3, 6)	-	-	0.02 (1, 7)
6	11.67 ± 1.59	-	0.35 ± 0.31 (3, 7)	0.00 ± 0.00 (2, 6)	-	0.00 (1, 1)
5	13.05 ± 1.23	0.43 ± 0.38 (3, 6)	-	-	-	-
6	13.25 ± 1.26	0.64 ± 0.50 (3, 7)	-	-	-	-
7	13.34 ± 1.36	0.70 ± 0.97 (3, 6)	-	0.00 ± 0.00 (3, 6)	-	-

Discussion

Both experimental results and field surveys conducted in the invasion range of *D. suzukii* in the Alps indicate that the Spotted Wing Drosophila is a species well adapted to cold conditions and ample fluctuating temperatures typical of mountains, where it presented relatively short development cycles and good reproductive performance. In addition, it invaded progressively the upper elevations during the summer, as shown in its native range in Japan (Mitsui *et al.* 2010). In Japan, a number of *Drosophila* species specializing on flowers and fruits move from low to higher elevations during summer, some as early as June (*D. unipectinata*) while others (*D. oshimai*, *D. suzukii* and *D. subpulchrella*) in July. Migration of *D. unipectinata* is considered as a means for avoiding summer heat or exploiting early-summer resources at a higher elevation. On the other hand, *D. oshimai*, *D. suzukii* and *D. subpulchrella* have the capacity to spend the summer at low elevation, and therefore their migration is assumed to be a means to escape from resource-poor conditions in summer at low elevations or simply to exploit resources at a higher elevation. Other generalist species of *Drosophila*, i.e. those associated with accidentally fallen immature fruits and/or decayed leaves, however, would not perform such extensive movements between low and higher elevations (Mitsui *et al.*, 2010).

The uncertainty associated with the migration behaviour of *D. suzukii* seems to be rather high, as in mountains areas the temperature often drops below 0°C even during the favourable season, and in general during winter (Williams *et al.*, 2015). Although insects are able to adapt their behaviour, drops in temperature cause first the suspension of reproduction and development, followed by a chilling coma and death (Chown and Nicholson 2004). The escape from adverse conditions through behavioural strategies such as migratory flights (Doucet *et al.*, 2009) is a risky process, which may have important consequences for the population growth as the insects have to find hosts in a stage suitable for oviposition. In turn, it may expose invaded ecosystems as well as crop systems to a sudden herbivore pressure, implying both economic and ecological costs as long as fruit harvest and regeneration potential of wild plants are affected, respectively.

Nine plant species were suitable for the development of *D. suzukii* in the mountain areas (*Daphne mezereum, Lonicera alpigena, Lonicera caerulea, Lonicera nigra, Lonicera xylosteum, Rubus caesius, Rubus saxatilis, Sambucus nigra,* and *Sambucus racemosa*). With the exception of *Daphne mezereum, Rubus caesius* and *Rubus saxatilis*, these species were already known as hosts of *D. suzukii*, although not in situations of limiting temperature (Grassi *et al.*, 2011; Lee *et al.*, 2015). The successful development of the fly was obtained only when the average temperature in the three-week period before the collection was higher than 11.1°C, as observed in 2014, which was a summer with temperatures much lower than average (ARPAV 2014). On that year it was also possible to show that performance on the same host (*Lonicera alpigena*) and site increased with temperature, as more flies per unit sample were obtained at 14°C than at 13°C. In 2015, temperatures in the three-week period preceding the collection were higher than those observed in 2014, nevertheless performance on *Sambucus racemosa* also increased from 0.21 adults/g at 15.5°C to 1.23 adults/g at 17.8°C. The higher temperatures recorded in 2015 caused an increase in the elevation where successful development of *D. suzukii* was observed; a shift from 1660 to 1880 m. This can be taken as an indicator of how quickly the insect may respond to a temperature shift, likely based on the intense migratory activity, suggesting that the response of *D. suzukii* to climate change (Field *et al.*, 2014) will likely be positive, although strongly dependent on the annual variation of weather (Battisti and Larsson, 2015).

The natural gradient experiment in the cave generally supported the field observations by using a laboratory population reared on an artificial medium. There were, however, a number of issues that deserve a thorough analysis. First, the larvae carried from the laboratory developed into pupae at all temperature conditions tested. The formation of pupae even at the lower temperature was likely influenced by the advanced stage of the larvae (third instar) taken to the cave. This concerned few individuals at 4.4°C and progressively more at 9°C, while above 13.3°C the number reached values comparable to those obtained in the laboratory (23.3°C). Adults, however, were obtained only at temperatures above 10.5°C. The adult yield was similar among temperatures from 10.5 to 13.4°C, and did not differ from values observed in the laboratory. The ample daily temperature fluctuation observed at the upper sites is likely responsible for the better performance, indicating that *Drosophila* flies are responding to the thermoperiod (Schou *et al.*, 2015) as well as other insect species (the sawfly *Cephalcia arvensis*) tested in the same experimental set-up (Battisti and Cescatti, 1994). Adult females taken from the laboratory and allowed to oviposit during the first 48 hours at different sites did not produce pupae when the temperature was lower than 10°C. Pupae, however, appeared at temperatures just below 12°C and adults above 13°C. It is likely that adults could have been obtained at lower temperatures, as observed in Trial 3, if the experiment had not stopped because of a sharp drop of temperature. At 10°C the duration of development from egg to adult stage has been shown to take more than 70 days, although eggs were laid at 22°C, since oviposition is not possible at that low temperature (Tochen *et al.*, 2014; Asplen *et al.*, 2015). In addition, it seems very difficult to keep either artificial medium or natural fruits suitable for larval development through that period of time. Anyhow, the development rate and yield increased with temperature up to 13.5°C for both pupae and adults, when it was comparable to the values observed in the laboratory at 23.3°C.

The development rate observed for the second oviposition period (starting from the 49th hour of adult female life at different temperatures inside the cave) was significantly higher than that of the first oviposition period. This may be interpreted as an adaptation of the females to the thermal conditions to which they were exposed during the first two days of their life. The observed trade-off with yield was predicted by the phenotypic plasticity theory, where mothers invest larger quantities of nutrients in fewer eggs, which in turn develop faster (Chown and Nicholson, 2004). However, yields of pupae and adults obtained from the second oviposition period were significantly lower than those of the first oviposition period. This is probably due to both competition caused by a higher number of larvae and the aging of the females (8 day-old at the beginning of the test, 20 day-old at the end). The yield values obtained in the laboratory at 23.3°C were similar to those obtained in the field (0.35 adults/female/day); perhaps due to the competition-related mortality within the test tubes.

By comparing all the data from the natural gradient experiment, it is possible to derive the minimum threshold of average daily temperature under natural conditions for the egg-to-adult development of *D. suzukii*, which corresponds to 11.6°C. This value is slightly higher than that obtained by weather stations close to the collection site of wild fruits which yielded adults along the elevational gradient (11.1°C). Both are lower than the lower threshold (13.4°C) for intrinsic rate of population increase assessed by Tochen *et al.* (2014) using nonlinear estimation, indicating that the species is particularly adapted to exploit at best the naturally fluctuating temperatures typical of mountain weather, for both development rate and yield. At the most favourable conditions tested in this study, an egg needed 24 and 17 days to develop into an adult in the first and second oviposition period, respectively. These development times are similar or shorter than those observed by Kanzawa (1939) and Asplen *et al.*, (2015), at the constant temperature of 15°C (21-25 days), or by Tochen *et al.* (2014) at 14°C (28.8 days), possibly because of the effect of the fluctuating thermal condition. They are, however, in contrast with other data obtained by Kanzawa (1939) under fluctuating low temperatures (10-14°C, 37 days) but experimental details are not available. The number of degree-days required to complete the development from egg to adult in the laboratory was similar to that observed by other authors under similar conditions, whereas it was considerably lower in the natural gradient experiment likely because fluctuating temperatures, as hypothesised by Tochen *et al.* (2014).

This study has shown that *D. suzukii* may quickly adapt to changing conditions, and that the temperature experienced by individuals in a given phase of their life may affect the performance of the subsequent stages. These findings open the way to better understanding the possibility of the fly to colonise mountain habitats, although in a temporary way because of the limiting conditions for overwintering. With the increases in temperature due to climate change the insect will expand to higher elevations, where it will be able to reproduce depending on the availability of host plants, which in turn will be affected in their reproductive potential. Results also indicate that *D. suzukii* performs well at rather low temperatures, suggesting that population build up may occur even under those conditions. If high density populations occur in the mountains at the end of the summer, then the flies may move to low elevation habitats when the cold arrives, threatening crops in early spring. This pattern is indirectly shown by the high number of flies caught in traps at low elevation during winter (unpublished results). Comparing the elevational distribution of the insect with the temperature-dependent performance over a wider range of temperatures and environmental conditions could contribute to modelling the species performance in the invasion range, as well as to predicting the impact of climate change.

Chapter 5

Spillover of *Drosophila suzukii* between non-crop and crop areas: implications for pest management

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LT conceived and designed the research, conducted the experiments, analysed the data and wrote the manuscript.

Abstract

Semi-natural areas may affect *D. suzukii* population dynamics by providing alternative host resources, overwintering habitats, or refuge areas when crops are sprayed with insecticides. Here, we investigated the spillover of *D. suzukii* between non-crop and crop areas using sweet cherry as a model crop. We sampled the pest at different heights and at different distances from the forest from the stage of vegetative rest to the stage after crop harvest. We showed that *D. suzukii* abundance in the orchards declined strongly with increasing distance from the forest margin and with increasing height from ground. The observed patterns varied across the crop phenological development stages indicating that the pest used multiple habitats across the seasons. When the host plant was not suitable for reproduction, *D. suzukii* preferred to fly closer to the forest margin and at lower heights. Differently, when the host plant was suitable (i.e. ripe cherries), *D. suzukii* colonized further the orchards both horizontally and vertically exploring more in depth the canopy volume. Our results can contribute to help developing physical or mechanical control measures such as the modification of the spatial configuration of the orchards and surrounding native vegetation or the possibility of application of insect-proof nets.

Introduction

Since its arrival in Europe *D. suzukii* Matsumura has become one of the most damaging pests attacking a large variety of crops. Severe yield losses have been reported for cherry, grape, and several other crops with soft-skin fruits (Mitsui *et al.*, 2010; Lee *et al.*, 2011a; Bellamy *et al.*, 2013; Burrack *et al.*, 2013). The damage is primarily caused by the feeding activity of the larvae inside the fruit tissues, followed by secondary rots (Lee *et al.*, 2011a; Cini *et al.*, 2012). The high polyphagia of the fly allows *D. suzukii* to infest different species among cultivated, wild and ornamental species (Walsh, 2011), making pest control particularly challenging.

Along with crops, many wild plant species are suitable for the development of *D. suzukii* and most of these species usually inhabit woody habitats such as forests or hedgerows (Kenis *et al.*, 2016; Tonina *et al.*, 2016). Therefore, these semi-natural areas may affect pest population dynamics by providing alternative host resources, overwintering habitats, or refuge areas when crops are sprayed with insecticides (Kenis *et al.*, 2016, Pelton *et al.*, 2016; Zerulla *et al.*, 2015). Previous research has indicated that proximity to semi-natural habitats can anticipate crop colonization early in the season, and also enhance *D. suzukii* density during the crop ripening (Asplen *et al.*, 2015; Klick *et al.*, 2016; Pelton *et al.*, 2016). Due to the potential role of semi-natural habitats in driving pest population and attack dynamics, it is crucial to investigate how and when the pest can move between crop and non-crop areas. Knowing dispersal parameters such as average flight height or dispersal distance over the season can contribute to understand the pest spatio-temporal dynamics and develop more effective control strategies.

In the native range, early studies on vertical flight in semi-natural and natural habitats found that *D. suzukii* mainly flies above plant tops while few individuals are usually found close to the ground (Beppu, 1980; Toda, 1987, 1992). *D. suzukii* presents a strong and constant preference for the canopy layer, variable in height as a function of vertical foliage complexity of the trees (Tanabe, 2002). However, pest movement can drastically change passing from a close forest to more open agricultural landscapes, and potential movement of *D. suzukii* from field margins to commercial crops is largely unknown (Klick *et al.*, 2016).

Here, we investigated the spillover of *D. suzukii* between non-crop and crop areas using sweet cherry as a model crop. Among the sensitive crops, cherry is one of the earliest species to ripe in temperate regions. As in early spring *D. suzukii* populations are usually low in the orchards (Asplen *et al.*, 2015), the positive effect of forest habitats as refuge and overwintering habitat is expected to be relevant for this crop. In this study, we investigated the behaviour of *D. suzukii* in the transition zone between semi-natural forest and cherry orchards. Specifically, we first aimed to understand the spillover distance of pest populations from the forest margin into the crop also evaluating the flight height. Second, the observations were made from the stage of vegetative rest to the stage after crop harvest in order to test if the pest movement between crop and non-crop areas changed over time. Third, observations were also made within forest, to understand pest behaviour in semi-natural habitat. Our results can contribute to help developing physical or mechanical control measures such as the modification of the spatial configuration of the orchards and surrounding native vegetation or the possibility of application of insect-proof nets.

Materials and methods

Study area

We selected seven cherry orchards located in the hills of Verona (Veneto region, Northern Italy, south of the eastern pre-Alps), four sites in Valpolicella and three in Soave area. The cherry orchards had a surface area ranging from 0.5 to 1 ha. Each orchard contained different varieties (e.g. Adriana, Mora di Verona, Ferrovia, Lapins, Kordia) covering a harvesting period of c. 1 month. Trees were grown with free form and were 3-4 m high. The orchards were located in mixed landscapes with relatively large cover of forest (on average 25%, ranging from 15 to 40% within a 500m buffer) and cherry orchards (30% average, ranging from 10 to 50%). The altitude varied from 200 m to 730 m a.s.l. The surrounding forests were mostly coppice with trees 10-15 m high. The most common tree species were *Carpinus betulus, Quercus* spp. *Fraxinus ornus, Robinia* *pseudoacacia,* while the understorey vegetation included several host plants such as *Sambucus nigra, Rubus* spp. *Cornus mas, Cornus sanguinea,* and *Lonicera* spp.

Sampling design

In each orchard, a single transect was positioned. The transect consisted of 4 poles placed at four distances from the margin into the orchard (0, 10, 25 and 50 m; Fig. 5.1). The poles allowed to place the traps up to a height of 6 m from the ground with a vertical distance of 1 m between the traps (1, 2, 3, 4, 5, and 6 m). To observe the height of the flight within the core forest habitat, a supplementary pole was placed inside the forest at 10 m distance from the margin. This pole was placed only in two sites.

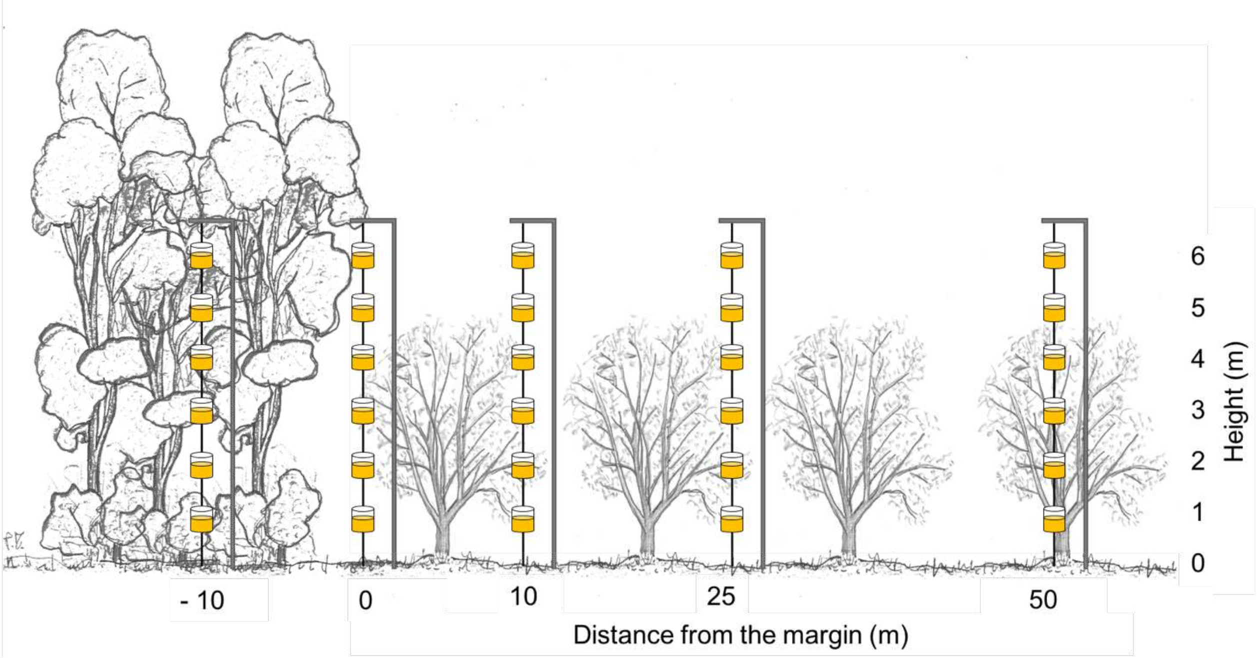

Figure 5.1 - Scheme of sampling design to collect *D. suzukii* in the 7 cherry orchards.

The traps were transparent white Kartell 250 mL bottles with 6 holes of 4 mm diameter, baited with 100 ml of apple cider vinegar (Prantil® Trento, Italy) plus a drop of surfactant Triton® X-100 (Sigma-Aldrich Co. LLC.). The trap and lure were chosen for their low attractiveness capacity in order to obtain a limited volume of attraction around the trap, thereby reducing reciprocal interferences between the nearby traps. Other solutions were excluded as transparent plastic adhesive panels, both perpendicular or circular, for the difficulty of use and the possible interference with the flight of insects or red traps baited with Droskidrink due to their higher attractiveness. The absence of individuals at the higher traps confirmed the validity of our system (Tanabe, 2002; Toda, 1992).

Insect sampling

The insect sampling took place during the cherry season of 2015. The surveys started in the early vegetative stage (6th of March), and continued until the pause after harvest (8th of August). The sampling was carried out weekly or every two weeks depending on the phenological stage. The content of each trap was sorted under a stereo-microscope, recording the number of females and males of *D. suzukii* and the total number of other *Drosophila* species abundance.

Phenological development stage

To test for a seasonal effect on pest spillover, a phenological development stage was assigned to each sampling date. Six stages were identified: before-flowering (BBCH<59), flowering (60<BBCH<69), fruit enlargement (71<BBCH<79), fruit colouring (81<BBCH<83), ripening (85<BBCH<87), and after harvest (BBCH>89).

Statistical analysis

To test the effects of trap distance, trap height and phenological development stage on SWD activity-density, a general linear mixed-effects models (GLMMs) was used. As the duration of the trapping periods changed during the season the total abundance was standardized by dividing number of individuals by the number of days between top consecutive sampling events. The log-transformed standardized abundance (+0.01) was our response variable. Trap distance (+1 m) was also log-transformed. In each model, phenological development stage was entered as categorical fixed factor, while trap distance and trap height were included as continuous fixed factors. Along with the main effects all possible interactions were also tested. To account for the nested design and the repeated measures, site identity (n=7), pole identity (n=28) and trap position (n=168) were included as random factors. The analyses were performed using the packages for generalized and "nlme" (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team 2015) for general mixed-effects models, implemented in R 3.0.2 (R Development Core Team 2013). In two sites, the difference in *D. suzukii* between forest, margin and cherry orchards were further tested using the same mixed model described above but including trap position (forest vs. margin vs. cherry-orchards) instead of trap distance.

Results

General results

D. suzukii was collected in all survey sites and during the whole study period. The catches were higher at the end of overwintering period and during the flowering stage. Subsequently, a sharp decline was recorded when fruits were present on plants. After harvest, a strong increase in catches was observed.

Effects of distance and height in the different phenological development stage

We found a marginal significant three-way interaction between distance, height and stage (Tab 5.1).

Table 5.1 - Results of the mixed-effect model testing the effect of height, distance from margin, and phenological development stage on *D. suzukii* abundance. Distance and abundance was log-transformed prior the analyses.

	df	F	p-value
Distance	1, 20	69.23	<0.0001
Height	1, 38	196.46	<0.0001
Stage	5, 1753	149.46	<0.0001
Distance x Height	1, 138	3.67	0.0573
Distance x Stage	5, 1753	7.30	<0.0001
Height x Stage	5, 1753	9.47	<0.0001
Distance x Height x Stage	5, 1753	1.99	0.0769

The interaction indicated that *D. suzukii* abundance declined with increasing height but in different ways according to distance from the margin and phenological development stage. *D. suzukii* abundance generally tended to decline after flowering and to increase after the end of crop harvest (Fig. 5.2). Generally, *D. suzukii* abundance decreased progressively with the distance increasing from the margin. The distance effect tended to weaken during the period with the fruits and to increase again after harvest. With distance above 25 m from the margin, SWD abundance showed a more stable trend. The height effect was weaker close to the margin and tended to disappear during the stage of fruit enlargement. The height effect also changed between the phenological stages, i.e. the differences tended to decline during the period with fruits and to increase after harvest.

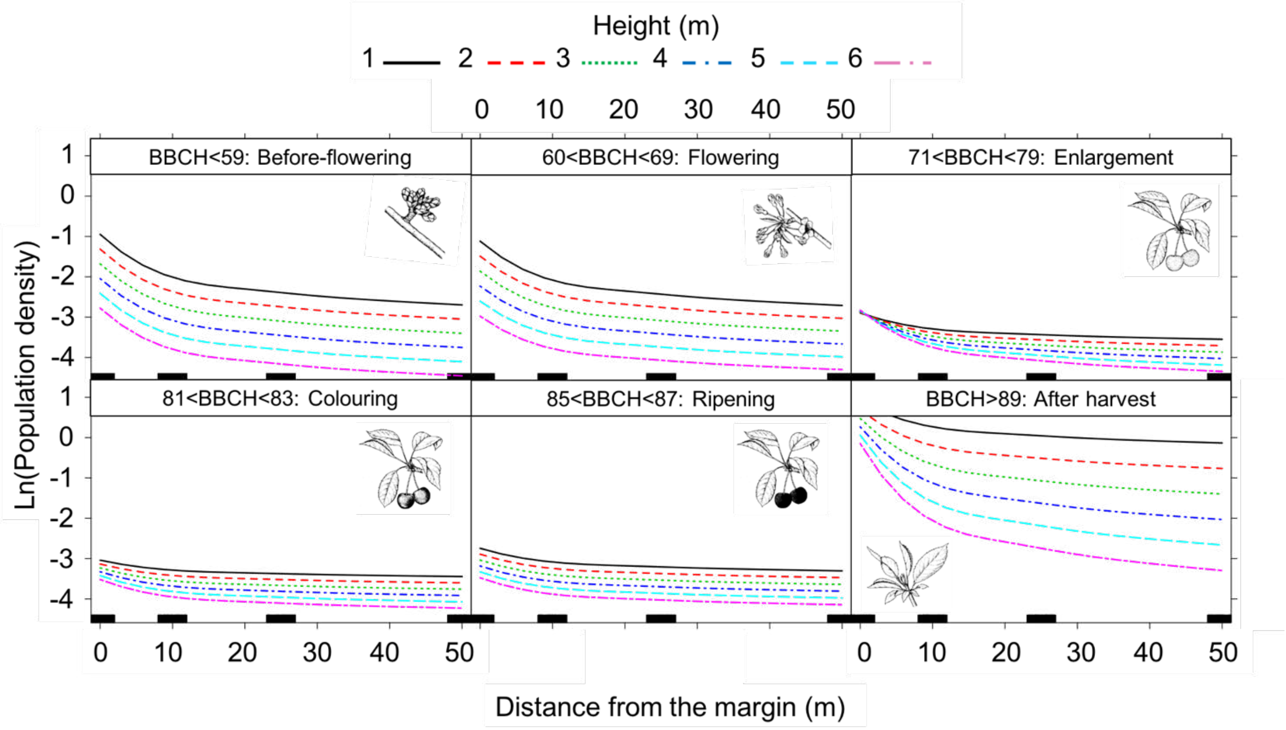

Figure 5.2 - Relationship between *D. suzukii* abundance and the distance from the margin (0, 10, 25 and 50 m) at the six different heights, subdivided into the six investigated phenological development stages.

Effects of woodland and height

We found a significant interaction between position and height (Table 5.2) indicating that the decline in *D. suzukii* abundance with increasing height observed within the orchard weakened at the margin and disappeared within the forest habitat (Fig. 5.3).

Table 5.2 - Results of the mixed-effect model testing the effect of height, position (forest, margin, orchard), and phenological development stage on *D. suzukii* abundance. Abundance was log-transformed prior the analyses.

	df	F-value	p-value
Position	2, 2	5.160	0.1623
Stage	5, 568	71.74	<.0001
Height	1, 27	23.5	<.0001
Position x Stage	10, 568	2.966	0.0012
Position x Height	2, 27	18.176	<.0001
Height x Stage	5, 568	2.48	0.0306

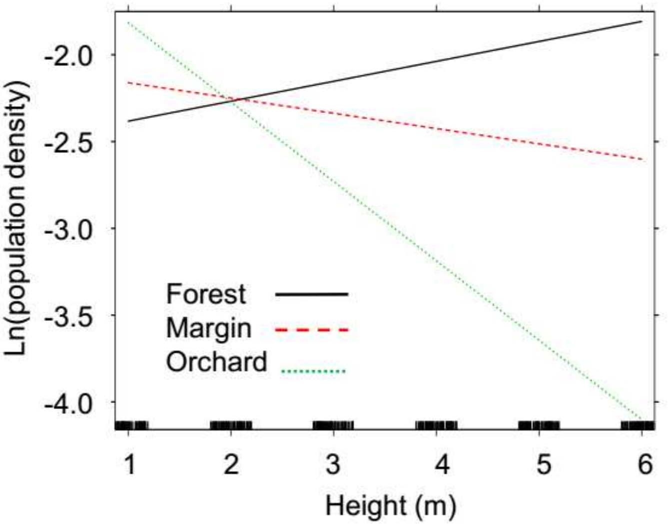

Figure 5.3 - Relationship between *D. suzukii* abundance and height from the ground (from 1 to 6 m) in the cherry orchard, in the forest and at the margin between the two habitats.

Inside the forest, *D. suzukii* abundance was generally higher than in the other two habitats and the density did not depend on height. At the margin *D. suzukii* abundance was intermediate and mainly distributed at lower heights. 10 m inside the cherry orchard *D. suzukii* abundance was lower and concentrated close to the ground, tending to zero at the maximum height of investigation (Fig. 5.3).

We also found an interaction between phenological development stage and position indicating that, during the vegetative rest, catches inside the forest were higher than the margin and the cherry orchard. During the flowering period catches at the margin and into the forest were similar and higher than in the orchard. During fruit enlargement and ripening, catches were similar in all three habitats, while during fruit changing colour, catches were higher in the margin than in the cultivated and wild areas. After harvesting catches had increased restoring the original gradient among the three habitats (Fig. 5.4).

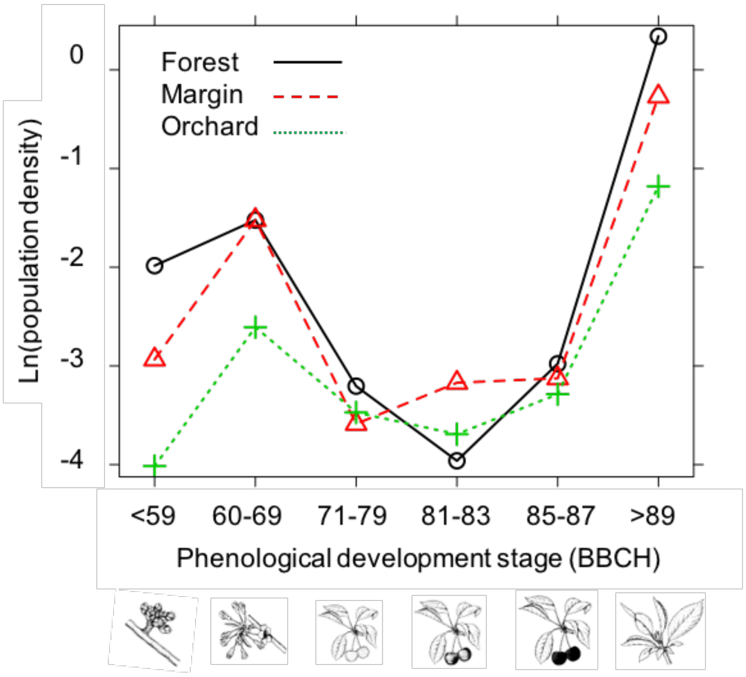

Figure 5.4 - Relationship between *D. suzukii* density and phenological development stage in the cherry orchard, in the forest and at the margin between the two habitats.

We found a significant interaction between height and phenological development stage within the forest habitat indicating a different trend in catches with increasing height during the stages. Before flowering (March-April) catches were concentrated close to the ground. Subsequently, *D. suzukii* explored mostly the higher areas of the forest canopy. When the cherries were present on trees (June and July), the pest was detected at all heights inside the forest.

Discussion

Understanding the movement of insects between crop and non-crop areas is extremely important for designing effective landscape management for enhancing pest control and increasing the populations of beneficial arthropods (Dyer & Landis, 1997; Gladbach *et al.*, 2011; Rand *et al.*, 2006; Tscharntke *et al.*, 2005). Here, we showed that *D. suzukii* abundance in the crop varied strongly depending on the distance from the forest margin and on the height from ground. The observed patterns varied across the crop phenological development stages indicating that the pest used multiple habitats across the seasons. When the host plant was not suitable for reproduction, *D. suzukii* preferred to fly close to the forest margin and at lower heights. Differently, when the host plant was suitable (i.e. ripe cherries), *D. suzukii* colonized the orchards both horizontally and vertically exploring more in depth the canopy volume.

Concerning the pest spillover, *D. suzukii* density generally decreased with increasing distance from the forest margin. The differences between the margin and the core crop area were stronger at the beginning of the growing season but they were less marked in the stages when the fruits were present, indicating an increased horizontal exploration of *D. suzukii* inside the orchard when the host plant was suitable for reproduction. The large availability of host plants probably attracted individuals from the forests into the orchards (Asplen *et al.*, 2015). This was confirmed by the fact that, after the crop harvest, a higher abundance in forests than in orchards was observed, indicating a return of the pest population into the refuge areas.

Overall a gradient of decreasing density was observed with increasing height from the ground. As for the pest spillover, the differences among heights changed with the phenological development stages. The differences between low and high traps were more evident in the early stages and tended to decrease in the stages where fruits were present, indicating an increased vertical exploration of the tree canopy. The strong gradient was reinstated after crop harvest, indicating a higher insect density at lower heights where more humid and cool conditions are present (shading by foliage and grass; Hamby *et al.*, 2016; Tochen *et al.*, 2016b).

D. suzukii density in forest was generally higher than both forest margin and cherry orchards, indicating that the species prefers forest habitats also in the newly invaded regions. However, these differences became lower when cherries were present. During the fruit changing colour stage, pest density was higher at the margin possibly due to the frequent passage of adults between the two habitats. Within forest *D. suzukii* catches were more localized to higher heights, with the exception of the early stages in which catches were concentrated on the ground probably due to the overwintering in the forest litter (Zerulla *et al.*, 2015). These data are supported by studies performed in the native range (Beppu, 1980; Toda, 1987, 1992; Tanabe, 2002). Studying the flying height inside the forest is very important to better understand the potential of insect in infesting wild fruits present on the top of native trees (e.g. *Viscum album*; Poyet *et al.*, 2015). In addition, it is useful to understand the colonization dynamics of canopy of higher cultivated plants and the height from which *D. suzukii* can invade neighbouring cultivated orchards.

Concerning seasonality, *D. suzukii* abundance was relatively high before and during cherry flowering. This is probably due to the attractiveness of the cherry flowers that can provide food for the adults after the overwintering period (Tochen *et al.*, 2016a). Abundance, then, tended to decrease after flowering probably due to climatic factors affecting the mortality of overwintering individuals (Hamby *et al.*, 2016; Zerulla *et al.*, 2015) and also for the competition between our low attractive traps and the fruits (Asplen *et al.*, 2015). After crop harvest, abundance increased strongly (Wang *et al.*, 2016) due to the new adults emerging from the infested fruits and the end of the fruit-trap competition. The results obtained allow to draw some conclusions and practical implications for the management of cherry cultivation in orchards close to forests or to other refuge/overwintering areas for *D. suzukii*. Concerning orchard plantation design, we suggest to plant cherry trees along rows parallel to the source of the infestation, in order to intercept the individuals dispersing from the forest in the first rows. The less sensitive or early ripening variety should be also planted close to the forest. Our results may also be useful for the application of mass trapping (Hampton *et al.*, 2014). For this purpose, the traps should be placed at the margin and should be already deployed during the flowering period, and before the colonization of the whole orchard. The ability to fly higher than 6 m close to the margin does not permit the application of anti-insect nets only at the sides of the orchards, as instead it is possible for other pests but it implies the need to cover the whole rows or orchard. Our study focused on the pest spillover at the interface between crop and non-crop areas but more research is needed to understand the role of forests on the population dynamics in the long run and the scale at which the pest can disperse and move across the agricultural landscapes.

Conclusions

The arrival in Italy of *Drosophila suzukii* Matsumura in 2009 caused extensive damage to cherry and soft fruit affecting their cultivation. Currently, to control the pest, there is an increase in the number of insecticide applications that are sprayed from fruit changing colour until harvest period. This increasing in use of chemicals produces a rise in production costs, could pose risks to occurrence of resistances, to consumers and to environment (Boselli *et al*., 2012; Grassi *et al*., 2012, Mori *et al*., 2015).

With the aim to apply integrated pest management (IPM) and consequently to reduce the use of insecticides is it necessary to study the biology and the ecology of the new invasive pest, such us the ability to infest the cultivated plant, the host range, the role of environmental parameters (temperatures, elevation, etc.) on the development.

To study the *D. suzukii* presence and diffusion, it is necessary to have an effective tool for monitoring the population. In addition, a proper monitoring is the prerequisite to set up rational control strategies. From our survey (see chapter 2) Droso-Trap (Biobest) baited with Droskidrink, Suzukii Trap or Gasser showed the best result for monitoring *D. suzukii*. In particular, Droskidrink had the highest attractiveness for SWD, while Suzukii Trap showed the highest selectivity in the captures. Selectivity (percentage of *D. suzukii* within the total number of *Drosophila* species) is an important factor related to ecological aspects and to the easiness of control in the laboratory.

According to the thermal threshold for insect development (see chapter 4), and the level of attractiveness of different lures at lower temperatures (see chapter 2), Droskidrink is the best lure for monitoring *D. suzukii* and to manage its control. This lure is also easy to manage in the field, cheaper and available at the market.

The amount of catches and damage found on the different crops were highly variable among areas and changeable with the location of the orchards and their surrounding environment. The environmental factors as temperature, landscape composition and hostplant abundance play an important role on pest abundance and population dynamics. Preliminary studies performed in Trentino show that catches and damage on the berries (blueberry and strawberry) are higher at the margins of the orchards, close to hedges and forest (Grassi *et al*., 2013), due to the presence of numerous host plants that provide food and refuge areas (more favourable temperature and humidity) (Cini *et al*., 2012; Walsh *at al*., 2011).

Out of 112 collected species, 34 of them resulted suitable for the development of *D. suzukii* in no crop areas (see chapter 3). Fruits founded infested at high altitudes allowed to establish that *D. suzukii* is able to colonize and develop in the mountain environment at 1900m a.s.l. Catches were recorded also at higher altitudes (2000 m a.s.l.) probably due to the migration of adults during hot summer weeks. In addition to infestations in the mountain, the minimum thermal threshold of *D. suzukii* development and the high developmental rates at low temperatures (see chapter 4) influence the infestations of orchard situated at higher elevations or crops ripening in early or late seasons.

Semi-natural areas have an important role providing alternative host resources, overwintering habitats, or refuge areas when crops are sprayed with insecticides (Kenis *et al.*, 2016, Pelton *et al.*, 2016; Zerulla *et al.*, 2015), with implication on distribution and population dynamics inside the orchards. Using traps disposed at different distances from the forest margin and at different heights from ground, it emerged that *D. suzukii* abundance in the orchards declined strongly with increasing distances from border and heights from ground (see chapter 5). The observed patterns varied across the crop phenological development stages, indicating that the pest used multiple habitats across the seasons. When the host plant was not suitable for reproduction, *D. suzukii* preferred to fly closer to the forest margin and near the grass. Differently, when the host plant was suitable, *D. suzukii* colonized further the orchards both horizontally and vertically exploring more in depth the canopy volume. These gradients must be considered in the design of new orchards and in insecticide applications. This information may also be useful for the application of mass trapping. Therefore, traps should be applied when a strong gradient in catches is still present, during the flowering period, and before colonization of the whole orchard. The ability to fly higher than 6 m close to the margin does not permit the application of anti-insect nets only at the sides of the orchards.

The catches in forest are more abundant at canopy level, except in the early season when are concentrated close to the ground confirming the overwintering preference of *D. suzukii* for the forest litter (Zerulla *et al.*, 2015).

Considering the insect ecological aspects and the location of the orchards often situated in hill conditions, close to the forest are more at risk due to the favourable environmental conditions and the large supply of susceptible fruits. Therefore, it is necessary to pay more attention in the control of this new pest in these situations (see chapter 3, 4 and 5). In addition, catches and damage of *D. suzukii* are not uniform but vary widely depending on the years. Years characterized by mild and humid winters and summers are favourable for the development of pest, in contrast with cold, dry winters and hot summers (Sancassani *et al.*, 2016).

These considerations indicate the need for a management at agroecosystem level and not just limited to the individual orchard.

Due to the biology (see chapter 4) and ecology (see chapter 3 and 5) of *D. suzukii,* the integration of all the defence strategies for an effective pest control is required, in combination with a proper monitoring (see chapter 2). Inside the filed, to define a rational control strategy the monitoring of population, of female fertility and of oviposition from the end fruit enlargement is essential (Sancassani *et al.*, 2016). From our data emerged the necessity to place traps also in the neighbouring wild areas to obtain information of the population abundance in the area useful to apply IPM strategies. This is important considering the high capacity of active insect flight and the important role of wild areas in growing population ready to invade the nearby fields (see chapter 5; Kenis *et al*. 2016, Pelton *et al*., 2016; Zerulla *et al*. 2015).

First of all, all cultural and agronomical practices in order to disadvantage the development of the pest should be adopted in the orchard as pruning, mowing, borders management and ripe abandoned fruit destruction. From fruit changing colour, the use of mass trapping can help in some situations characterized by lower pest pressure. Problems related with residues and side effects on the environment require careful management of chemical treatments, to be limited to the most sensitive fruit stages. Is of crucial importance a rapid and timely harvesting of all fruits and proper management of discarded fruits (Cini *et al.*, 2012; Dreves *et al.*, 2009; Grassi *et al.*, 2009; Walsh *et al.*, 2011).

In the near future, it is hoped that infestations of *D. suzukii* can find an effective limitation with biological control, as present in origin areas. Great hopes are placed on the autochthonous parasitoid *Trichopria drosophilae* Perkins (Hymenoptera: Diapriidae) which potential adaptability to *D. suzukii* has already been demonstrated (Rossi-Stacconi *et al*., 2015). Their activity could be positively influenced by abundance of forest and wild host species in the landscape around cultivated fields.

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Annexes

Information of the experiments performed during my PhD were also disseminated at the national level to technicians and farmers through two famous specialized magazines.

"Approccio integrato per la difesa dalla *Drosophila suzukii*"- in English: "Integrated approach for the control of *Drosophila suzukii*" was published on: "Frutticoltura", an important specialized magazine for fruit grower and technicians. This article contains the results of the pest management trials conducted by the inter-regional technical committee, in which UNIPD actively participates.

Ioriatti C., Boselli M., Caruso S., Galassi T., Gottardello A., Grassi A., **Tonina L**., Vaccari G., Mori N. (2015) Approccio integrato per la difesa dalla *Drosophila suzukii*. Frutticoltura 4:32-36

LT participated in conception and design of the researches, conducted the experiments for Verona sites, analysed the data of traps trials and wrote the part on traps comparison.

"*Drosophila suzukii* su ciliegio, esperienze di lotta integrata"- in English: "*Drosophila suzukii* on cherry, IPM experiences" was published on: "L'Informatore Agrario", an important specialized magazine for grower and technicians. This article contains the results of the monitoring actions and pest management trials conducted by UNIPD group in cherry orchards.

Sancassani M., **Tonina L**., Tirello P., Giomi F., Marchesini E., Zanini G., Mori N. (2016) *Drosophila suzukii* su ciliegio, esperienze di lotta integrata. L'Informatore Agrario 15:59-63

LT conceived and designed the researches, conducted the experiments, analysed the data and wrote the manuscript.

Approccio integrato per la difesa dalla *Drosophila suzukii*

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Nelle difficili condizioni climatiche del 2014 solo l'applicazione di reti anti-insetto ha consentito una soddisfacente protezione della produzione. Gli altri mezzi di controllo, da soli, non hanno contrastato efficacemente l'infestazione e richiedono di essere opportunamente integrati fra loro. Auspicabile che *D. suzukii* possa trovare un'efficace limitazione da parte di insetti benefici (parassitoidi autoctoni).

▲ ***Fig. 1 - Danni da* D. suzukii *su diverse varietà di ciliegio (foto Mori, sinistra, e Boselli, destra).***

Dal 2009, da quando cioè *Drosophila suzukii* Matsumura è *stata riscontrata per la prima volta in Italia, la difesa fitosanitaria di molte colture* da frutto si è drasticamente modificata, con un generale incremento del numero di interventi insetticidi nel tentativo di controllare questo pericoloso dittero. Una delle caratteristiche che fanno di questo insetto una vera e propria calamità per la frutticoltura risiede nella sua elevatissima polifagia (CABI, 2014; Lee *et al.*, 2015), essendo esso in grado di ovideporre e svilupparsi su una vasta gamma di frutti coltivati e selvatici, a cominciare dalle prime specie che vanno a frutto nella tarda primavera fino alle ultime fruttificazioni autunnali, in un crescendo demografico di difficile contenimento con le tradizionali armi in mano al frutticoltore. Questa peculiarità è dovuta alla presenza di un ovopositore particolarmente robusto, provvisto di una struttura dentellata molto caratteristica ed adatta ad incidere l'epicarpo di frutti perfettamente sani (Ioriatti *et al.*, 2012).

Nella ampia varietà di ospiti di *D. suzukii*, il ciliegio (Fig. 1) è *segnalato fra quelli più suscettibili* (Lee *et al.*, 2011) sia perché fruttifica in un periodo nel quale non sono presenti ospiti alternativi, sia in quanto per le sue caratteristiche chimico-fisiche risulta un substrato ideale per le larve che riescono a svilupparsi senza incorrere in significativi tassi di mortalità (Ballamy *et al.*, 2013). Le infestazioni su ciliegio sono state segnalate per la prima volta già nel 1916 in Giappone; queste infestazioni si sono in seguito intensificate, fino a compromettere la totalità della produzione e a comportare il rigetto delle partite sul mercato (Kanzawa, 1935). Più recentemente, in questo Paese si sono registrati danni su ciliegio variabili dal 26 al 100% in funzione dell'andamento climatico e della località (Sasaki e Sato, 1995).

Il ciliegio è stata una delle colture a frutto fra le prime a soffrire dell'attacco di *D. suzukii* allorché l'insetto ha fatto la sua comparsa in Nord America e in Europa sul finire del decennio scorso (Beers *et. al.*, 2011; Cini *et al.*, 2012). Nel 2008 l'impatto economico dell'attacco di *D. suzukii* è stato stimato pari all'84% del valore commerciale della produzione di ciliegie di California, Oregon e Washington (Walsh *et al.*, 2011). In Italia, nei primi due anni di infestazione sono stati registrati danni significativi fino al 90% di frutti attaccati in ceraseti di varietà tardive e in aziende collinari sia in Trentino che in Emilia-Romagna (Boselli *et al.,* 2012; Grassi *et al.,* 2012).

A cinque anni dalle prime segnalazioni di infestazione su ciliegio la comprensione della biologia dell'insetto, dei fattori che determinano il rischio per la coltura e dei mezzi di controllo da mettere in atto è *significativamente* aumentata, anche se rimangono aspetti che necessitano di approfondimenti. Al fine di ottimizzare le risorse investite nella ricerca e nel contempo per avere un quadro complessivo delle diverse situazioni in cui ci si trova ad operare, dallo scorso anno è stato istituito un tavolo tecnico fra Università, istituti di ricerca e centri di consulenza operanti in Trentino, Veneto ed Emilia-Romagna. L'obbiettivo è quello di concertare protocolli sperimentali e condividere i risultati dei principali progetti di ricerca attivati a livello regionale.

Di seguito si darà conto dei primi risultati che sono emersi da questa iniziativa con particolare riferimento a quanto traducibile fin da subito in consiglio tecnico per il cerasicoltore.

▲ *Fig. 2 - Diversi tipi di trappole ed attrattivi per* D. suzukii *impiegati per il monitoraggio territoriale (foto Tonina).*

Trappole ed attrattivi

Ad inizio di stagione, caratterizzata da una presenza di adulti dell'insetto ancora piuttosto contenuta, poter disporre di strumenti di monitoraggio altamente sensibili diventa un'esigenza fondamentale per cerasicoltori e tecnici, al fine di giustificare gli interventi insetticidi e impostare una difesa il più possibile mirata ed efficace. Inoltre, per svolgere un efficiente monitoraggio occorre avere a disposizione un mezzo tecnico dotato di un'elevata selettività; pratico nell'impiego, economico e di basso impatto ambientale.

A tal fine, in quattro aree d'indagine situate in tre regioni sono state messe a confronto la trappola Drosotrap® (Biobest), che ha fornito i migliori risultati in precedenti indagini (Grassi *et al.*, 2015) e che è stata innescata con differenti attrattivi come aceto di mele Acentino, Prantil (Vervò TN) e Ponti, Droskidrink® (Prantil-TN), Pherocon_SWD® (Trecè), Biologische Essigfliegenfalle® (Riga) e SuzukiiTrap® (Bioiberica), ed una trappola artigianale rossa attivata con aceto di mele (Fig. 2). La trappola Drosotrap® è stata modificata apponendo una retina con maglia di 3mm dietro ai fori di ingresso per migliorarne la selettività. Ove non presente, negli attrattivi è stato aggiunto un tensioattivo inodore (Triton®) per migliorarne la capacità umettante. Le trappole sono state appese alle piante a circa 1,5 m dal suolo. I risultati evidenziano che, indipendentemente dall'esca alimentare, la trappola Drosotrap® ha presentato efficacia e selettività superiori a quella artigianale, probabilmente grazie alle sue caratteristiche costruttive.

Tra i differenti attrattivi, Droskidrink® e Riga hanno mostrato una capacità significativamente superiore a Trecè e SuzukiiTrap®. I tre tipi di aceto di mele hanno evidenziato la minore efficacia. Gli aceti pastorizzati hanno sortito attrattività nettamente inferiori rispetto a quello crudo. Oltre all'insetto "target", sono state catturate numerose specie appartenenti al genere *Drosophila* (in alcuni casi anche il 75% delle catture).

Al fine di fornire utili indicazioni per il loro impiego, nella tabella 1 sono state riassunte le informazioni riguardanti l'efficacia, la selettività, la formazione di gel batterici (madre) e la frequenza di sostituzione dell'attrattivo per le differenti tipologie di esca testate.

Monitoraggio territoriale

Nel corso del 2014 il gruppo di lavoro interregionale ha condotto

TAB. 1 - EFFICACIA, SELETTIVITÀ DI CATTURA, FORMAZIONE DI GEL BATTERICI (MADRE) E FREQUENZA DI SOSTITUZIONE DELL'ATTRATTIVO PER LE DIFFERENTI TIPOLOGIE DI ESCA TESTATE

Attrattivo	Efficacia	Selettività	Formazione di madre	Sostituzione attrattivo
Droskidrink®	Buona	Media	SI	Settimanale
Trecè®	Buona	Buona	NO	Mensile
Gasser®	Buona	Buona	SI	Settimanale
Suzukiitrap®	Buona	Ottima	NO	Mensile
Aceto di mele crudo	Scarsa	Scarsa	SI	Settimanale
Aceto di mele pastorizzato	Scarsa	Scarsa	NO	Settimanale

▲ *Fig. 3 - Copertura con reti anti-insetto nel modello monoblocco, sinistra, e nel modello monofila, a destra (foto Caruso).*

una specifica indagine, con un protocollo sperimentale comune, in alcuni impianti di ciliegio sui rispettivi territori, con l'obiettivo di ricostruire e comparare le dinamiche di popolazione del carpofago (volo degli adulti, ovOdeposizione e danno sui frutti) e di valutare l'efficacia su larga scala di alcuni strumenti e metodi di monitoraggio tra quelli più largamente applicati.

Dai rilievi in Emilia-Romagna è *emersa* una maggior gravità degli attacchi sulle prime produzioni (maggio), grazie alla più precoce e consistente disponibilità di frutti e ad un maggior quantitativo di adulti sopravvissuti all'inverno rispetto alle altre regioni. In generale, sono state registrate importanti ovodeposizioni anche su frutti appena invaiati, a conferma di una attività più anticipata dell'insetto nella stagione scorsa. Inoltre, danni più rilevanti sono stati documentati da metà giugno in poi, specialmente sulle varietà più tardive delle aree produttive collinari e di montagna. L'incremento demografico che interessa abitualmente questo periodo, la forte pressione della stagione scorsa e la breve persistenza degli insetticidi autorizzati hanno reso in molti casi insoddisfacenti i risultati del controllo chimico.

Per quanto riguarda invece i mezzi e metodi di monitoraggio saggiati (Tab. 2), le trappole hanno segnalato con precisione l'inizio delle infestazioni. In virtù di una maggiore attrattività espressa durante tutto il periodo di prova, la trappola Droso Trap® attivata con Droskidrink si è dimostrata comunque più affidabile e quindi più valido strumento di monitoraggio, confermando quanto osservato nell'indagine sul confronto trappole. Tuttavia, anche questa sperimentazione, come altre condotte negli anni scorsi in Trentino, ha confermato l'impossibilità di dedurre dal dato di cattura informazioni circa il grado di attacco sui frutti. Occorre però associare al rilievo delle catture quello delle uova sui frutti, possibilmente verificando anche la successiva schiusa delle larve. Non sempre, infatti, le uova si sviluppano, specialmente se deposte su frutti immaturi.

Cattura massale

Obiettivo della cattura massale, in una strategia combinata di più tecniche di controllo, è di contribuire al contenimento del danno da *D. suzukii* riducendo la pressione di adulti immigranti negli impianti tramite l'impiego di numerose trappole. Le principali criticità nell'applicazione del metodo contro *D. suzukii* risiedono nella indisponibilità di attrattivi e trappole altamente specifici ed efficaci, capaci di competere con i frutti nel richiamo degli adulti, e nelle elevate capacità biologiche dell'insetto. Un aspetto tuttavia che può giustificare l'applicazione della cattura massale su ciliegio è *la bassa concentrazione di adulti che caratterizza il periodo della sua maturazione rispetto alla seconda parte della stagione*. Per verificare l'efficacia della cattura massale combinata con la difesa chimica, sono state condotte prove sperimentali nelle tre regioni, adottando un protocollo comune (Tab. 3).

In tutte le prove è stato dimostrato un contenimento delle catture di adulti nella parte interna della parcella gestita con l'integrazione della cattura massale. Relativamente all'infestazione sui frutti, in Emilia-Romagna è stata ridotta del 75 e 40 % rispettivamente su cv Lapins e Sweet Heart (più tardiva). In Trentino l'attacco su varietà Regina è stato contenuto in maniera decrescente passando dall'85 al 37% rispettivamente a tre e una settimana dalla raccolta. In Veneto, in un ceraseto multivarietale, l'efficacia è variata dal 65 al 56% durante il periodo di raccolta. Sembra

TAB. 2 - MEZZI E METODI DI MONITORAGGIO SAGGIATI. DROSKIDRINK (ACETIFICIO PRANTIL, PRIÒ - TN) È COMPOSTO PER IL 75% DA ACETO DI MELA E PER IL 25% DA VINO ROSSO

Regione	N° siti	Tipo trappola	Tipo esca	Gestione	Valutaz. infestazione sui frutti
Trentino	4	flacone PE Kartell® 1000 ml rosso, con 6 fori da 5 mm Ø	200 ml aceto mela	ogni 7 giorni, sostituz.esca e conteggio adulti (maschi e femmine)	ogni 7 giorni tra inizio invaiatura e fine raccolta, verifica della % di drupe con uova e larve su camp. 50 frutti da raggio di 30 m attorno alla trappola
E. Romagna	8				
Veneto	3	flacone PE Kartell® 1000 ml bianco, con 6 fori da 5 mm Ø			
Trentino	10	Droso Trap® di Biobest, con 21 fori da 8 mm Ø coperti con rete maglia 6 mm^2	200 ml Droskidrink + 4 gr. zucchero canna grezzo		
Veneto	3				
E. Romagna	4	flacone PE Kartell®1000 ml rosso, con 6 fori da 5 mm Ø			

TAB. 3 - PROTOCOLLO DI CATTURA MASSALE APPLICATA IN INTEGRAZIONE ALLA DIFESA CHIMICA ADOTTATO NELLE TRE REGIONI

Regione	Superficie (mq) tesi a confronto (blocchi non ripetuti)		Tipo trappola	Tipo esca	N° trappole	Modalità impiego trappole	Valutazione efficiacia		
	cattura massale + insetticidi	insetticidi					presenza adulti		infestazione
Trentino	2000	1500	Droso Trap® di Biobest, con 21 fori da 8 mm Ø coperti con rete maglia 6 mm^2	200 ml Droskidrink + 4 gr. zucchero canna grezzo attivato con preparato di batteri lattici appartenenti alla specie Oenococcus oeni	52	da inizio invaiatura, sulle piante di bordo a 2-2,5 m una dall'altra - sost. esca ogni 7 gg	barriera singola	Trappole posionate al centro e ai bordi di ogni parcella	Numero uova e di larve su 100 frutti raccolti sia al centro che sui bordi di ciascuna parcella
E. Romagna	9000	11000		200 ml Droskidrink + 4 gr. zucchero canna grezzo	180				
Veneto	2400	3600			140		barriera doppia		

evidente, pertanto, che l'effetto protettivo della barriera tende a calare progressivamente con il passare del tempo.

Livelli di infestazione superiori a quelli dell'appezzamento di riferimento trattato solo con insetticidi sono stati invece registrati sulle piante di bordo. Questo fenomeno, già documentato in prove condotte in Trentino su piccoli frutti (Grassi, *com. pers.)*, conferma la necessità di posizionare queste trappole sufficientemente distanti dalla coltura da proteggere. Da queste esperienze emerge che l'integrazione con la cattura massale si presta per quelle situazioni di coltivazione meno suscettibili di forti attacchi (impianti isolati, di dimensioni medio-grandi, non circondati da bosco, in fondovalle, con microclima caldo e secco).

Le reti antinsetto

Fra le tecniche di difesa sostenibili per il controllo di *D. suzukii* si è recentemente sperimentato l'uso delle reti anti-insetto. Le sperimentazioni hanno riguardato, in una prima fase, la dimensione della maglia della rete, dalle quali è emerso che il foro non dovrebbe superare 1 mm^2, e, successivamente, l'applicabilità in pieno campo su piccoli frutti (Grassi e Maistri, 2013).

Su ciliegio, date le più ampie superfici degli appezzamenti e l'altezza delle piante, l'uso delle reti richiede diversi metodi di applicazione. Sono stati valutati tre diversi approcci; nei moderni impianti già dotati di copertura anti-pioggia si è operato tramite la chiusura del perimetro e lasciando le aperture tra i filari per la circolazione dell'aria (modello monoblocco) (Fig. 3a). In quelli non dotati di teli antipioggia sono state coperte le singole file con reti dotate di doppia trama nella parte superiore (modello monofila) (Fig. 3b), mentre negli impianti collinari a vaso si è testata la copertura delle singole piante (modello monopianta) (Fig. 3c).

Le prime osservazioni realizzate nei diversi areali evidenziano un controllo pressoché totale di *D. suzukii* sui modelli "monopianta" e "monofila", mentre nel modello "monoblocco" l'efficacia è buona, ma non totale a causa del continuo sollevamento dei teli per consentire l'entrata e uscita dei mezzi agricoli. Inoltre, la sperimentazione condotta ha messo in luce un leggero incremento della temperatura e dell'umidità relativa, ma nessuna influenza significativa

TAB. 4 - CARATTERISTICHE ED EFFICACIA SU D. SUZUKII DEGLI INSETTICIDI TESTATI

Sostanza attiva	Classe chimica	Class. IRAC	Posizionamento	Tempo di carenza (gg)	Efficacia	Note
Fosmet	Fosorganico	1B	Adulticida/Ovo-larvicida	10	Buona	Fitotossico su alcune cultivar
Dimetoato	Fosorganico	1B	Adulticida/Ovo-larvicida	14	Buona	Uso straordinario di 120 giorni nel 2014
Lambda-cialotrina	Piretroide	3A	Adulticida	7	Buona	
Deltametrina	Piretroide	3A	Adulticida	3/7	Buona	Formulati commerciali con tempo di carenza diverso
Acetamiprid	Neonicotinoide	4A	Ovo-larvicida	14	Media-scarsa	
Thiametoxam	Neonicotinoide	4A	Ovo-larvicida	7	Medio-scarsa	
Thiacloprid	Neonicotinoide	4A	Ovo-larvicida	14	Medio-scarsa	
Imidacloprid	Neonicotinoide	4A	Ovo-larvicida	21	Medio-scarsa	
Spinosad	Spinosine	5	Adulticida	7	Buona	Ammesso in agricoltura biologica
Spinetoram	Spinosine	5	Adulticida	7	Buona	Uso straordinario di 120 giorni nel 2014
Cyantraniliprole	Diamidi	28	Adulticida	?	Buona	In attesa di registrazione

sulle caratteristiche qualitative della produzione (zuccheri, acidità, ecc.). Infine, non sono stati osservati effetti collaterali sulle altre avversità biotiche (afide nero, acari e monilia).

I primi risultati sono quindi incoraggianti, ma saranno necessarie ulteriori indagini per verificare gli aspetti legati alla produttività degli impianti e alla sostenibilità economica.

Controllo chimico

Il ricorso al controllo chimico è limitato dalla ridotta disponibilità di sostanze attive registrate per il contenimento di *D. suzukii,* dall'elevata intensità di popolazione, dalla mobilità degli adulti, dalla scalarità di maturazione dei frutti e, di conseguenza, dal rischio di superare i limiti dei residui ammessi. Sono state pertanto eseguite diverse prove sperimentali sia di laboratorio, sia di pieno campo per caratterizzare l'efficacia e le modalità di applicazione di insetticidi registrati e in via di registrazione (Tab. 4).

Conclusioni

L'inverno mite e il clima fresco e umido che ha caratterizzato l'estate 2014 sono state condizioni estremamente favorevoli per lo sviluppo importante delle popolazioni di *D.suzukii* che hanno cagionato danni significativi a tutte le colture ospite. In tali condizioni solo l'applicazione delle reti anti-insetto ha consentito una soddisfacente protezione della produzione di ciliegie. Gli altri mezzi di controllo, da soli, non hanno dimostrato di controllare efficacemente l'infestazione e richiedono di essere opportunamente integrati fra di loro. Nel prossimo futuro è auspicabile che le infestazioni di *D. suzukii* possano trovare un'efficace limitazione da parte dei parassitoidi larvali e pupali auctotoni, in particolare *Leptopilina heterotoma* Thomson (Hymenoptera: Figitidae), *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) e *Trichopria drosophilae* Perkins (Hymenoptera: Diapriidae), dei quali è già stata dimostrata la potenziale capacità di adattamento al nuovo fitofago (Rossi Stacconi *et al.,* 2015).

SUMMARY

Among the huge number of plant hosts, cherry is considered one of the most susceptible to the *Drosophila suzukii* infestation and it is suffering of considerable economic losses. Despite the knowledge of biology and control tool efficacy have improved considerably since the first reports of infestations in cherries, a number of aspects remain to unravel.

In 2014, concerted research actions were carried out by Universities, Research Institutes and extension agencies operating in Trentino, Veneto and Emilia Romagna in order to foster the advance in knowledge about the epidemiology and control of *D. suzukii*. The present paper is reporting the first year results that could help the growers to cope with this devastating pest.

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▲ ***Fig. 4 - Copertura con reti anti-insetto nel modello mono-pianta (foto Tonina).***

ATTIVITÀ CONDOTTA IN VENETO DAL 2013

Drosophila suzukii su ciliegio, esperienze di lotta integrata

La biologia e l'ecologia del moscerino *Drosophila suzukii* impone di integrare tutti i mezzi di contenimento, da quelli agronomici (potature, sfalci, gestione delle bordure e della frutta matura abbandonata) alla cattura massale e all'utilizzo delle reti antinsetto, ai trattamenti chimici. Fondamentale risulta la programmazione di una raccolta rapida e tempestiva dei frutti

di **M. Sancassani, L. Tonina, P. Tirello, F. Giomi, E. Marchesini, G. Zanini, N. Mori**

L'arrivo di *Drosophila suzukii* Matsumura nel 2009 ha profondamente cambiato la coltivazione del ciliegio in Italia. Prima della sua comparsa i principali fitofagi dannosi a questa coltura erano l'afide *Myzus persicae*, alcuni lepidotteri tortricidi (*Archips* spp.) e il dittero tefritide *Rhagoletis cerasi* per i quali erano sufficienti 1-2 trattamenti insetticidi.

Attualmente nel tentativo di controllare *D. suzukii*, che sta causando ingenti danni in ceraseti di varietà tardive e in aziende collinari in Emilia-Romagna, Trentino e Veneto (Boselli *et al.*, 2012; Grassi *et al.*, 2012, Mori *et al.*, 2015), c'è un **generale incremento del numero di interventi insetticidi che vengono applicati dall'invaiatura fino alla raccolta. Questo aumento di impiego di sostanze chimiche, oltre a un innalzamento dei costi di produzione, può comportare dei rischi per i consumatori e l'ambiente.** Dal 2013 il Dipartimento Dafnae dell'Università di Padova in collaborazione con i mercati cerasicoli di Verona e Vicenza sta effettuando dei progetti di ricerca, finanziati dal Settore fitosanitario della Regione Veneto, sulle possibilità di contenimento di *D. suzukii* da mettere in atto su ciliegio, con particolare attenzione ai mezzi di lotta ecosostenibili. Nel presente lavoro vengono esposti i risultati di queste attività.

Foto 1 Trappola Drosotrap innescata con Droskidrink impiegata per il monitoraggio. *Foto M. Sancassani*

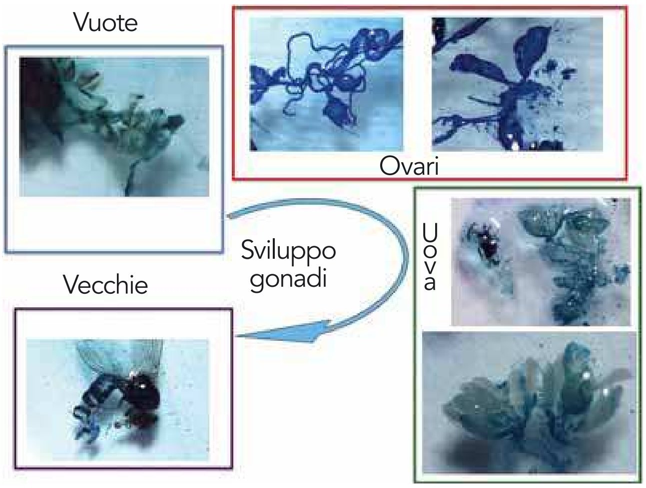


Foto 2 Sviluppo delle gonadi in *Drosophila suzukii*. *Foto L. Tonina*

Monitoraggio

Un corretto monitoraggio delle popolazioni e delle infestazioni *D. suzukii* è il prerequisito essenziale per impostare una razionale strategia di controllo. Le trappole Drosotrap (Biobest) (*foto 1*), innescate con l'attrattivo Droskidrink (75% aceto di mele, 25% vino rosso e 20 g/L zucchero, sostituito settimanalmente) applicate dalle prime giornate tiepide di aprile fino alla completa raccolta delle ciliegie, sono risultate il mezzo tecnico più efficace per il monitoraggio degli adulti, confermando quanto osservato in studi condotti in altre regioni del Nord-Est (Grassi e Maistri, 2013; Ioriatti *et al.* 2015).

La semplice osser-

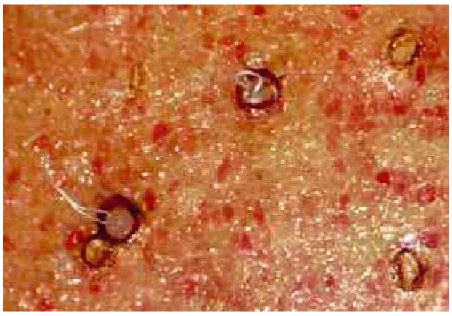


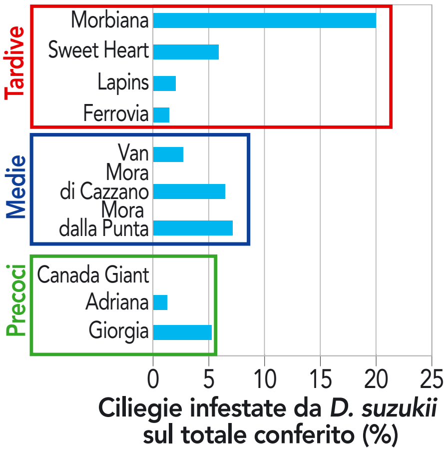
Foto 3 Stadio di maturazione delle prime ciliegie infestate dalle femmine svernanti. *Foto L. Tonina*

vazione delle catture, però, non è uno strumento sufficiente per definire un'adeguata strategia di difesa, sia perché in aree dove l'insetto è presente durante tutto l'anno o con densità di popolazione basse è difficile individuare l'epoca di inizio dei trattamenti (Harris *et al.*, 2014), sia perché non sono ancora emerse relazioni tra il numero degli adulti catturati e la entità del danno (Burrack *et al.*, 2012, Ioriatti *et al.* 2015).

Per rilevare l'inizio e l'entità delle infestazioni è necessario osservare la fertilità delle femmine catturate nelle trappole (valutando lo sviluppo delle gonadi) (*foto* 2) dalla fase di ingrossamento del frutto **e la presenza di ovideposizioni sulle drupe a partire dall'invaiatura**.

Dalle osservazioni effettuate in campo è emerso che le femmine svernanti depongono sulle prime drupe in invaiatura delle varietà precoci (*foto* 3), mentre quelle delle generazioni successive prediligono le ciliegie mature rispetto a quelle in maturazione. Tale capacità di scelta è in relazione alla densità di popolazione. Da prove di laboratorio è infatti emerso che solo dove sono presenti molte femmine queste ovidepongono anche su drupe non mature (*grafico* 1). Inoltre, per quanto riguarda le infestazioni, a parità di condizioni bisogna sempre considerare l'influenza della cultivar (*grafico* 2).

GRAFICO 2 - Infestazioni registrate presso un mercato cerasicolo suddivise per varietà e gruppo di maturazione



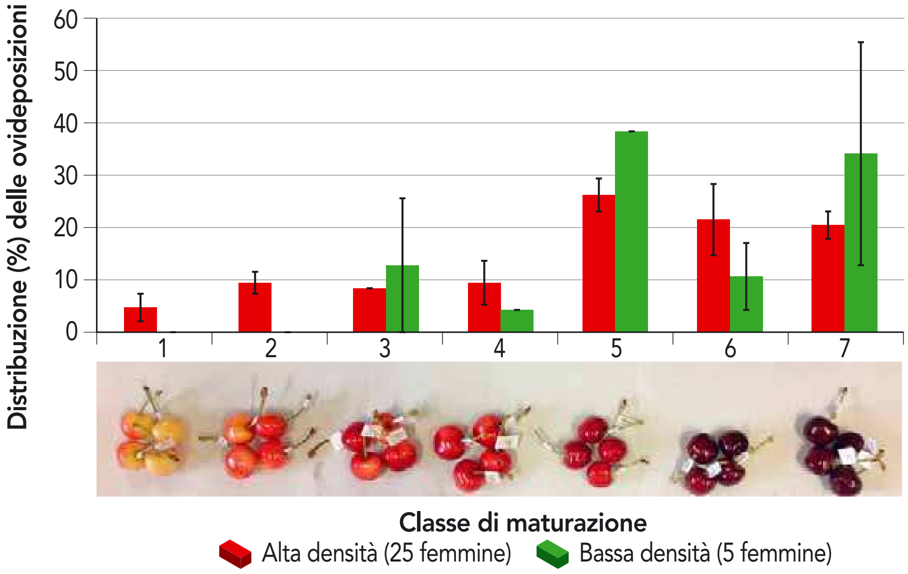
Influenza del clima su catture e infestazioni

Il monitoraggio territoriale, iniziato nel 2013 in 22 aree della provincia di Verona, ha permesso di evidenziare che le catture differiscono significativamente in base all'altimetria. **Nei siti in alta collina sono stati catturati più adulti rispetto a quelli localizzati in media e bassa collina/pianura** (*grafico* 3) confermando quanto già riportato in precedenti indagini svolte su vite (Marchesini e Mori, 2014).

Questo può essere imputabile all'abbondanza di piante ospiti e luoghi rifugio nei boschi in collina o alle condizioni ambientali maggiormente favorevoli alle quote superiori ai 400 m s.l.m. Infatti, dall'analisi delle catture e dei parametri ambientali registrati nei diversi siti indagati (*grafico* 4), si è rilevato che **le temperature miti verificatesi nell'inverno 2013-2014 in zone di media e alta collina hanno costituito condizioni ideali per lo svernamento del dittero, facendo registrare elevate catture nella primavera successiva.** Questo andamento non si è verificato in pianura a causa delle temperature più rigide e la carenza di luoghi rifugio.

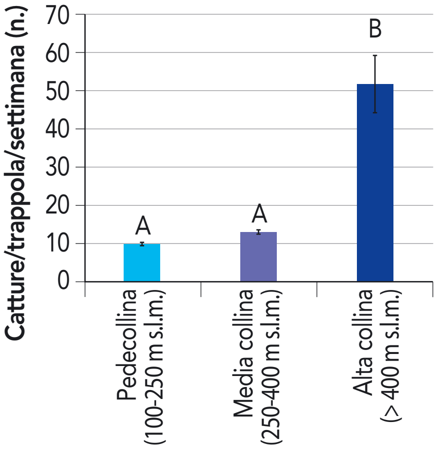
Nel 2014 temperature miti e l'elevata umidità relativa dei mesi primaverili hanno consentito un precoce e frenetico sviluppo delle popolazioni già da inizio maggio, facendo registrare ingenti danni anche alle ciliegie precoci di pianura e rendendo non com-

GRAFICO 1 - Distribuzione delle ovideposizioni su ciliegie a differenti gradi di maturazione (1)



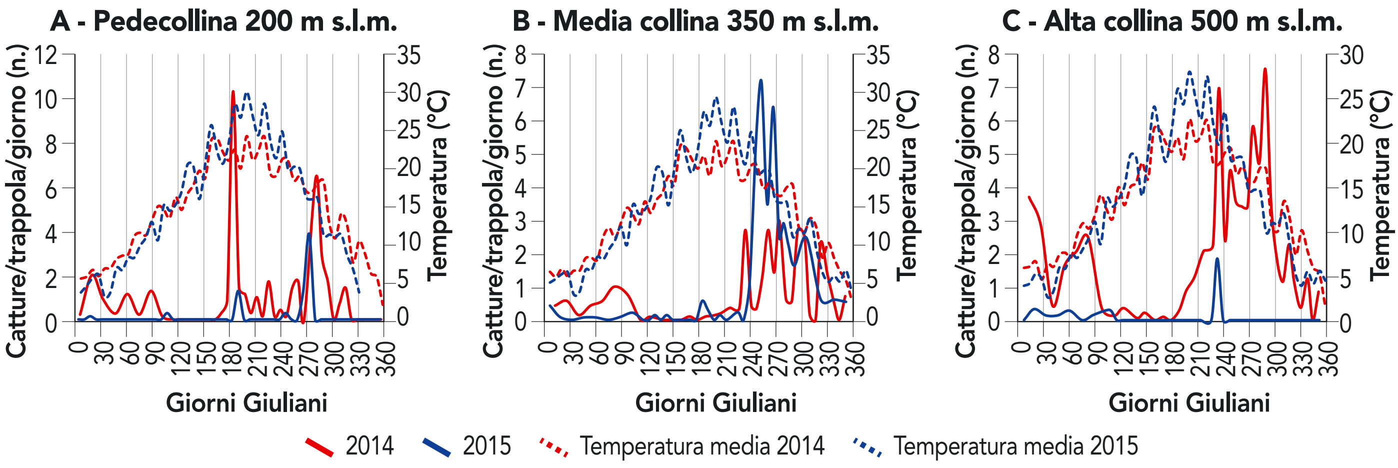
(1) Poste contemporaneamente in gabbie con diverso numero di femmine di *D. suzukii*. Le barre indicano la variazione tra il valore minimo e massimo riscontrato.

GRAFICO 3 - Catture di *D. suzukii* nel periodo 2013-2015 in siti collocati a differenti quote (1)



(1) Lettere maiuscole differenti indicano differenze significative per P≤ 0,01 al Anova e test di Tukey, le barre di errore indicano l'errore standard della media.

GRAFICO 4 - Andamento delle catture di *D. suzukii* e delle temperature in 3 ambienti



merciabili le produzioni dalla seconda decade di giugno in media ed alta collina.

Nel 2015 le temperature rigide dell'inverno, il prolungato periodo secco (piovosità inferiore a 150 mm) nel primo quadrimestre e soprattutto le elevate temperature estive (media giugno-luglio 25-28 °C) hanno fortemente ridotto la presenza degli adulti. In pianura non sono stati rilevati danni, mentre un aumento delle infestazioni è stato registrato solo su varietà a media maturazione in media (prima decade di giugno) e in alta collina (seconda decade di giugno).

Successivamente l'aumento delle densità delle popolazioni ha reso difficile il contenimento delle infestazioni sulle varietà tardive in alta collina (inizio luglio).

Distribuzione spaziale di catture e infestazioni

Da studi realizzati in Trentino emerge che **le catture e i danni sui frutti sono superiori ai margini degli appezzamenti in prossimità di siepi e boschi** (Grassi e Maistri, 2013), **per presenza di numerose specie ospiti e la disponibilità di zone di rifugio (temperature e umidità più favorevoli) negli ambienti boschivi** (Cini *et al*., 2012). Nei boschi adiacenti ai frutteti nel Nord-Est Italia sono state ritrovate più di 30 specie di piante selvatiche adatte allo sviluppo del dittero (Kenis *et al*. 2016).

Al fine di verificare la disposizione spaziale delle catture all'interno dei frutteti, in 7 impianti sono state installate trappole a differenti distanze dai margini boschivi e ad altezze progressive dal suolo fino a 6 m. Dai dati raccolti è emerso che la dispersione di *D. suzukii* presenta un significativo gradiente dai margini e dal suolo (*figura 1*). In prossimità del bosco il dittero è stato catturato fino a 6 m di altezza, con una distribuzione uniforme degli adulti, mentre all'interno del frutteto le basse catture erano concentrate a 2 m di altezza (*figura 1*).

Come per le catture, anche le infestazioni non sono distribuite uniformemente all'interno degli appezzamenti, differenziandosi in funzione della presenza di boscaglia, siepi o zone umide, con la formazione di un gradiente di danno dal bosco al centro del frutteto (*figura 2*). Questi aspetti ecologici devono essere considerati nella gestione della potatura, dei trattamenti insetticidi e nella progettazione di nuovi impianti, preferendo varietà precoci ai margini.

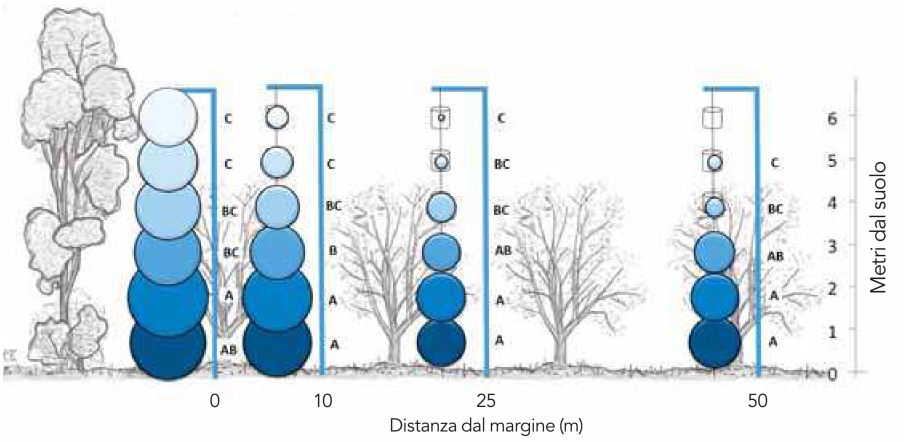
Strategie di difesa

Allo stato attuale, la lotta contro *D. suzukii* è basata principalmente sull'impiego di sostanze chimiche che vengono applicate dall'invaiatura alla raccolta, in base ai tempi di carenza dei formulati commerciali impiegati. Le elevate densità di popolazione, la mobilità degli adulti e la scalarità di maturazione dei frutti, e di conseguenza il rischio di superare i limiti di residui ammessi, rendono il ricorso al controllo chimico limitato (Ioriatti *et al*., 2015). Per questo, per un efficace contenimento del carpofago è necessaria l'integrazione di più tecniche di controllo.

Pratiche culturali e agronomiche

D. suzukii predilige microclimi freschi e umidi. Al fine di sfavorire lo sviluppo del carpofago **è necessario adottare all'interno della pianta o del frutteto tutte quelle pratiche agronomico-culturali che evitano la formazione di ristagni di umidità e zone ombrose quali: corretta gestione della**

FIGURA 1 - Distribuzione della popolazione di *D. suzukii* in funzione della distanza dal margine boschivo o dal suolo



Il diametro dei cerchi indica l'abbondanza di catture. Lettere differenti indicano differenze statisticamente significative all'interno della singola distanza dal bosco all'ANOVA e test di Tukey per P < 0,01.

FIGURA 2 - Distribuzione dell'infestazione di *D. suzukii* a differenti distanze dal margine boschivo

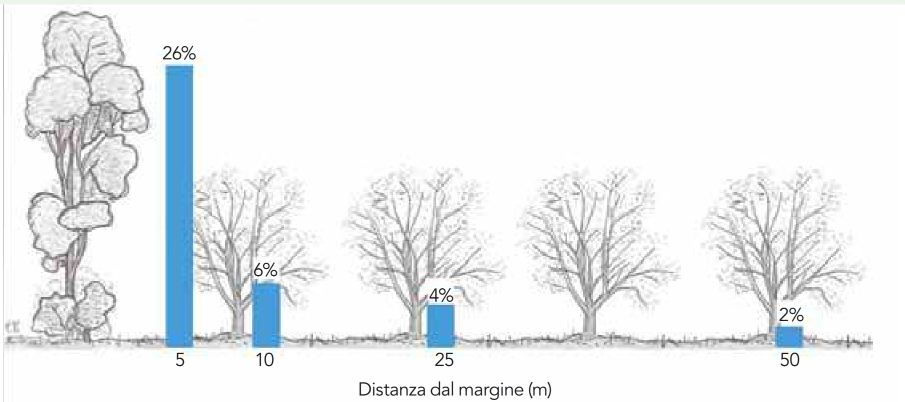


Foto 4 Con una corretta gestione del ciliegeto (***destra***) è stato possibile ridurre del 75% sia le catture (735 *vs* 104) sia le infestazioni (26% *vs* 4%) di *D. suzukii*.
Foto N. Mori

Foto 5 Frutta di scarto eliminata in modo non opportuno.
Foto M. Sancassani

chioma (che oltre a migliorare l'aerazione della chioma ottimizza anche la penetrazione della soluzione insetticida), sfalcio frequente del cotico erboso, gestione delle bordure. Con queste semplici pratiche (*foto* 4) è stato possibile ottenere una significativa riduzione sia delle catture sia delle infestazioni di *D. suzukii*.

La frutta matura non raccolta e abbandonata sulla pianta risulta adatta alle infestazioni e al conseguente aumento della densità di popolazione nel ceraseto. Attraverso uno studio effettuato in 18 ciliegeti veneti è emerso come la percentuale di infestazione, al termine dell'efficacia dei trattamenti insetticidi, aumenti in maniera significativamente superiore negli appezzamenti in cui le ciliegie vengono abbandonate rispetto a quelli nei quali vengono completamente raccolte (*grafico* 5).

Oltre alla frutta abbandonata, l'incremento degli adulti e del danno può essere provocato anche dallo scorretto smaltimento della frutta di scarto in campo o in zone prossime alle piante ancora da raccogliere (*foto* 5). Queste ciliegie andrebbero correttamente eliminate attraverso solarizzazione o interramento.

Cattura massale

La tecnica della cattura massale consiste nel collocare un numero elevato di trappole sul bordo degli appezzamenti al fine di contenere il danno da *D. suzukii* attraverso la riduzione del numero di adulti immigranti. Dalle prove effettuate in provincia di Verona e da analoghe indagini condotte in provincia di Trento e Modena (Ioriatti *et al*., 2015) emerge che i migliori risultati sono stati ottenuti dal posizionamento di una trappola attivata con un'esca alimentare, Droskidrink (o con la corrispondente miscela casalinga) ogni 2 m (ad un'altezza di 1,5 m circa dal suolo) lungo il bordo, alla distanza di 4-5 m dalle piante da proteggere dall'inizio della fase di allegagione (*foto* 6). La sostituzione dell'attrattivo deve avvenire a cadenza di una o due settimanale e il contenuto deve essere correttamente eliminato al fine di non incorrere negli stessi problemi della frutta di scarto. **Generalmente con questa tecnica è stato ottenuto un contenimento delle catture di adulti nella parte interna degli appezzamenti, ma una ridotta capacità nel contenimento del danno.** In alcune situazioni la cattura massale, abbinata a una corretta strategia insetticida, ha permesso un ritardo nella comparsa delle infestazioni con un prolungamento del periodo di raccolta di frutti non danneggiati.

Le principali criticità nell'applicazione del metodo contro *D. suzukii* risiedono nella indisponibilità di attrattivi e trappole altamente specifici ed efficaci, capaci di competere con i frutti nel richiamo degli adulti (Ioriatti *et al*., 2015).

Tuttavia tale tecnica può risultare idonea ed economicamente vantaggiosa, in combinazione alla lotta chimica, per situazioni di coltivazione meno suscettibili ai forti attacchi, quali impianti isolati di dimensioni medio-grandi, in fondovalle e con microclima caldo-secco.

Reti antinsetto

Con l'impiego delle reti antinsetto si realizza una barriera fisica che impedisce l'incontro tra il fitofago e il frutto. Il foro della rete non dovrebbe superare 1 mm^2 (Grassi e Maistri, 2013) e sul mercato sono presenti diverse tipologie che coprono la singola pianta (monopianta) il singolo filare (monofila a tunnel o a capannina) o l'intero

Foto 6 Disposizione delle trappole per la cattura massale lungo il perimetro dell'appezzamento.
Foto L. Tonina

frutteto (monoblocco). Dalle indagini effettuate in provincia di Verona con sistema monopianta e studi condotti nelle provincie di Trento e Modena con sistemi monofila e su monoblocco emerge che **l'utilizzo delle coperture con reti chiuse in fase di pre-invaiatura consente un'ottima efficacia, con livelli di controllo prossimi al 100% permettendo l'eliminazione o la forte limitazione dei trattamenti insetticidi** (Ioriatti *et al.*, 2015). Le reti non influiscono sulla qualità delle ciliegie alla raccolta (colore, peso, dimensioni, durezza, gradazione zuccherina, acidità e pH) e solo con il sistema monoblocco l'innalzamento della temperatura all'interno degli appezzamenti può rappresentare un problema soprattutto nelle annate calde.

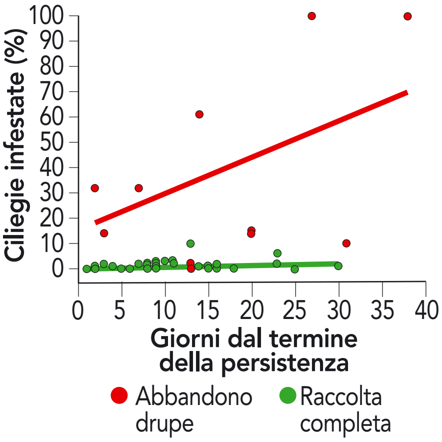
Considerata l'ottima efficacia nel contenimento di *D. suzukii* e i trascurabili effetti collaterali, l'impiego delle reti rimane sottoposto solo ad attente valutazioni economiche.

Controllo chimico

L'attività insetticida sui diversi stadi di sviluppo di *D. suzukii* su ciliegio è riportata in diversi articoli bibliografici (Beers *et al.*, 2011, Profaizer *et al.*, 2015, Shawer *et al.*, 2015).

In considerazione che le infestazioni aumentano con il progredire della maturazione, **è molto importante intervenire in prossimità della raccolta nel rispetto dei tempi di carenza dei singoli formulati commerciali e organizzare il cantiere di raccolta in modo da completare lo stacco completo dei frutti entro pochi giorni.** Negli impianti estesi o multivarietali sarà necessario trattare diversamente il frutteto, considerando oltre che l'epoca di maturazione anche i tempi di carenza e la velocità di raccolta delle ciliegie.

GRAFICO 5 - Effetto dell'abbandono delle ciliegie precoci sulla percentuale di infestazione di quelle medie e tardive, al termine della persistenza degli insetticidi



Integrare tutti i mezzi disponibili

Il ciliegio è la pianta più suscettibile agli attacchi di *D. suzukii*, sia perché fruttifica in un periodo nel quale non sono presenti altri ospiti alternativi, sia per le caratteristiche chimico-fisiche della polpa, (Lee *et al.*, 2011, Bellamy *et al.*, 2013).

Per la definizione di una razionale strategia di controllo è essenziale il monitoraggio della fertilità delle femmine e delle ovideposizioni sin dalla fase di fine ingrossamento del frutto, in quanto la percentuale di infestazione non è in stretta relazione con il numero delle catture nelle trappole alimentari.

La biologia e l'ecologia del carpofago impongono, per una difesa efficace della coltura, la combinazione di tutti i mezzi di contenimento. Anzitutto è necessario adottare nel frutteto tutte quelle pratiche colturali e agronomiche atte a sfavorire lo sviluppo del moscerino, quali potature, sfalci, gestione bordure e frutta matura abbandonata. Dall'invaiatura l'uso della cattura massale aiuta ad abbassare le densità delle popolazioni presenti. L'impiego delle reti che garantirebbe un efficace controllo deve essere sottoposto a una preventiva valutazione economica. I problemi legati ai residui e agli effetti collaterali sull'ambiente impongono un'attenta gestione dei trattamenti chimici, che dovranno essere limitati alle fasi di maggiore infestazione. Di fondamentale importanza è la programmazione di una raccolta rapida e tempestiva di tutti i frutti.

Nel prossimo futuro è auspicabile che le infestazioni di *D. suzukii* possano trovare un'efficace limitazione con altri mezzi biotecnologici e biologici. Molte speranze sono poste sul parassitoide auctotono larvo-pupale *Trichopria drosophilae* Perkins (Hymenoptera: *Diapriidae*) del quale è già stata dimostrata la potenziale capacità di adattamento a *D. suzukii* (Rossi Stacconi *et al.*, 2015).

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Drosophila suzukii su ciliegio, esperienze di lotta integrata

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RIASSUNTO

L'arrivo nel 2009 di *Drosophila suzukii* Matsumura 1931 ha profondamente cambiato la coltivazione del ciliegio in Italia. Attualmente nel tentativo di controllare questo dittero, che sta causando ingenti danni nelle varietà tardive e in aziende collinari in Emilia-Romagna, Trentino e Veneto, c'è un generale incremento del numero di interventi insetticidi che vengono applicati dall'invaiatura fino alla raccolta. Questo aumento di impiego di sostanze chimiche, oltre che a un innalzamento dei costi di produzione, può comportare dei rischi per i consumatori e l'ambiente.
Dal 2013 il Dipartimento Dafnae dell'Università di Padova in collaborazione con i mercati Cerasicoli di Verona e Vicenza sta effettuando dei progetti di ricerca, finanziati dal Settore fitosanitario della Regione Veneto, sulle possibilità di contenimento di *D. suzukii* da mettere in atto su ciliegio con particolare attenzione ai mezzi di lotta ecosostenibili.
Dai risultati è emerso che per la definizione di una razionale strategia di controllo è essenziale svolgere il monitoraggio della fertilità delle femmine e delle ovideposizioni sin dalla fase di fine ingrossamento del frutto, in quanto la percentuale di infestazione non è in stretta relazione con il numero delle catture nelle trappole alimentari. Data la biologia e l'ecologia del carpofago, per una difesa efficace è necessaria la combinazione di tutti i mezzi di contenimento. Anzitutto è importante adottare nel frutteto quelle pratiche colturali e agronomiche atte a sfavorire lo sviluppo del moscerino, quali potature, sfalci, gestione delle bordure e della frutta matura abbandonata. Dall'invaiatura, l'uso della cattura massale aiuta ad abbassare le densità delle popolazioni presenti; mentre l'impiego delle reti, che garantirebbe un efficace controllo, deve essere sottoposto a una preventiva valutazione economica. I problemi legati ai residui e agli effetti collaterali sull'ambiente impongono un'attenta gestione dei trattamenti chimici che dovranno essere limitati alle fasi di maggiore infestazione. Di fondamentale importanza inoltre è la programmazione di una raccolta rapida e tempestiva di tutti i frutti. Nel prossimo futuro è auspicabile che le infestazioni di *D. suzukii* possano trovare un'efficace limitazione con altri mezzi biotecnologici e biologici; molte speranze sono poste sul parassitoide auctotono larvo-pupale *Trichopria drosophilae* Perkins (Hymenoptera: *Diapriidae*) del quale è già stata dimostrata la potenziale capacità di adattamento a *D. suzukii*.

SUMMARY

The introduction, from 2009, of *Drosophila suzukii* Matsumura has deeply affected the cherry production in Italy. This diptera is actually causing high damages to the late ripening varieties, especially in hilly farms of Emilia-Romagna, Trentino and Veneto. Today, the attempts to limit the impact of this species produce increasing insecticide spraying efforts occurring from colour changing to the harvest. The increased employment of chemicals not only rises the production costs but could determine side-effects for the consumers and for the environment.
From the 2013, the Dafnae department of the University of Padova is collaborating with cherry market of Verona and Vicenza, on projects granted by Settore fitosanitario della Regione Veneto. This projects aims to develop reliable strategies to restrain the impact of *D. suzukii* on cherry trees, particularly through the adoption of eco-friendly systems.
The results demonstrate that the accurate monitoring of female fertility and ovideposition is of primary importance to address subsequently and effective control strategies. This become evident from the initial phases of fruit enlargement, when the level of infestation is not reflected in the number of animals attracted by the feeding traps.
In consideration of the ecology and life history of *D. suzukii* an effective control strategy require the integrated adoption of all restraining approaches. First of all, orchards should be managed with cultural and agronomic practices that restrain the development of the fly such as: pruning, frequent grass cuttings, management of the borders and, especially, avoiding the abandon of ripe fruit. From the beginning of fruit colour changing, the use of mass trapping helps in controlling the size of the populations. The use of net, which would ensure the most effective control, must be subjected to an economic evaluation. Concerns for the residuals and for the secondary environmental impact should drive an accurate use of chemical treatments that require to be limited to the major infestation events. In addition, the rapid and timely harvest of all fruits is revealed of outmost importance.
Hopefully, the introduction of additional biotechnological and biological approaches would further implement the control strategies for the future infestation of *D. suzukii*. Especially the implementation of the autochthonous parasitoid *Trichopria drosophilae* Perkins (Hymenoptera: *Diapriidae*) is highly awaited. Indeed, this species has been demonstrated that successfully parasitize larval and pupal stages of the no native *D. suzukii*.

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