Plant Health AUSTRALIA

Literature review on alternative control options to chemical control

Improving national biosecurity outcomes through partnerships

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SWD Management Approaches

The emergence of *Drosophila suzukii* as a new pest of horticultural production in the US and Europe has challenged established production systems. As is often case in early invasions of new pests, a well integrated pest management strategy is not available and growers can be reliant on chemical control programs to produce crops. This has been the case for early control efforts for *Drosophila suzukii* where management relied on repeated applications of broad-spectrum insecticides (Hamby and Becher, 2016). However in order to develop sustainable management practices, alternative control measures will be required.

Current SWD management approaches include:

1. Make fields less favourable for SWD
    - Cultivar selection
    - Weed fabric
    - Pruning
    - Netting
2. Monitor SWD flies in spring to detect first activity
3. As fruit begin to ripen, sample for larvae
4. Protect ripening and ripe susceptible fruit
    - Weekly application
    - Good coverage
    - Reapplication after rain
    - Rotate chemical classes
    - Consider adult and larval control
5. Post-harvest methods

The cost of management is less than the cost of doing nothing. Doing nothing can result in up to 100% crop loss. In the UK the cost of managing SWD is estimated to be $36-54 million per annum. No single control method will work to reduce SWD populations. Rather multiple methods used as part of an integrated pest and disease management plan is recommended. This review will focus on chemical-free pest control.

Biocontrols

Given legitimate concerns over the risks and limitations of using a chemical control method, research efforts have focused on the development of environmentally sound and sustainable methods. There is a wide variety of biocontrol agents including fungi, bacteria, viruses and natural enemies of the pest that could be employed in the control programs for *D. suzukii*.

Natural enemies of insect pests are endemic species that occur abundantly in agricultural fields. Natural enemies including pathogens, predators and parasitoids can be specialists or generalists, and they can induce a high level of mortality in their hosts (Flint and Dreistadt, 1998). Biological control approaches based on arthropod natural enemies are currently studied and developed worldwide. The pathogens and insects discussed below are some of the more promising biocontrols that might be applicable in an Australian setting for use when *D. suzukii* establishes in Australia. More research is required and a government process would have to be followed before the biocontrols are actively used in Australia. This could be done as part of preparedness activities for *D. suzukii*.

Bacteria

Photorhabdus luminescens, a member of the Gammaproteobacteria, is a Gram-negative and mutualistic bacterium that lives in the gut of entomopathogenic nematodes belonging to the Heterorhabditidae family (Shawer et al., 2018). Both *P. luminescens* alone and its symbiotic *Heterorhabditis spp*. nematode are known to be highly pathogenic to insects. Once the nematode infects an insect, *P. luminescens* is rapidly released into the haemocoel, where it secretes enzymes and high-molecular-weight toxin complexes (Tc) that disintegrate and bioconvert the body of the infected insect into nutrients, which can be consumed by both the nematode and bacterium. Shawer and colleagues (2018) investigated the possible use of *P. luminescens* to control *D. suzukii* larvae and pupae. The bacterium caused a high mortality of pre-immaginal stages (mortality ranging between 86.7 % - 100 % in larvae and 43.3 % - 63.3 % in pupae) through both oral and contact toxicity. A single bacterial application may maintain a sufficiently high population on fruit for at least 5 days making it an economic control method.

Entomopathogenic bacteria can be used as stand-alone products for pest management in organic farming, their use in rotation or combination with chemicals is strongly encouraged to achieve full efficacy and eco-sustainability. This work shows that *P. luminescens* is a promising tool for the containment of *D. suzukii* population. However, for its technological application in open field conditions, further studies are needed to assess the efficacy and formulation stability of products based on bacterial suspensions in different crops and environmental conditions.

Nematodes and predators

Some reports of *Drosophila suzukii* within the UK indicated that population levels had remained low in the UK with no widespread reports of damage (Cuthbertson and Audsley, 2016). This paper investigated several fungi and nematode biological agents to assess their ability to reduce population numbers of *D. suzukii.* Both the fungus *Isaria fumosorosea* and the entomopathogenic nematode *Heterorhabditis bacteriophora* offer much potential to be incorporated into control strategies to be employed against *D. suzukii* following the laboratory study that found they significantly reduced *D. suzukii* levels (Cuthbertson and Audsley, 2016).

A subsequent study by Hubner and colleages (2017) was performed on entomopathogenic nematodes examining their ability to infect larvae and pupae of *D. suzukii* within directly sprayed fruit, fruit placed on soil, and soil. *Steinernema feltiae* and *Steinernema carpocapsae* were more efficient at infecting soil-pupating host larvae than *H. bacteriophora*. Applied as a soil drench, *S. feltiae* and *S. carpocapsae* were able to infect *D. suzukii* larvae in the soil as well as hidden inside fruit. Direct application of entomopathogenic nematodes on the fruit was less successful, although emergence of flies was significantly reduced.

Another recent study found, *Orius insidiosus* plus *Heterorhabditis bacteriophora,* resulted in an 81 % reduction in blueberries and a 60 % reduction in strawberries (Renkema and Cuthbertson, 2018). It was not as effective in strawberry, likely due to drier substrate conditions. These results were not consistent with the study of Woltz and colleagues (2014) which found that *H. bacteriophora* had low infection rates while the predator *O. insidiosus* decreased *D. suzukii* survival in simple laboratory arenas but not on potted blueberries or bagged blueberry branches outdoors. The use of *O. insidiosus* and *H. bacteriophora* as natural enemies may therefore have a limited success rate.

Although entomopathogenic nematodes should be easily incorporated into existing invertebrate control programmes individually, they are unlikely to control/eradicate populations. Multiple combinations of *O. insidiosus* with other agents (parasitoids, fungal entomopathogens) should be tested.

Parasitoids

Parasitoid species are insects attacking other arthropods in the egg, larval or pupal development stages. Various Drosophila species are subjected to strong selective pressures by egg, larval and pupal parasitoids which play a key role in their population suppression. Most studies agree that Drosophila parasitoids induce a high rate of mortality on their host populations although the level of parasitism varies with breeding sites, local conditions and seasons (Nikolouli et. al., 2017). Studies on natural parasitoid enemies of *D. suzukii* in its invaded regions have shown that parasitism rates are limited, and thus their use is nonefficient for population suppression. This is attributed to the fact that *D. suzukii* exhibits a high level of resistance to the majority of the larval parasitoids tested, associated to a highly efficient cellular immune system and production of a constitutively high hemocyte level.

Two main native parasitic wasp species are known to attack *D. suzukii* pupae in the USA; *Pachycrepoideus vindemiae* and *Trichopria drosophilae* (Rufus Isaacs, personal communication). They were found in laboratory and field studies to successfully reproduce on *D. suzukii* pupae (Gabarra et al., 2015, Rossi Stacconi et al., 2015). In California, the highest parasitism was found in non-crop plants that are refuges for *D. suzukii* e.g. cactus fruits, blackberry in riparian zones and figs and loquat. Release of these parasitic wasps in commercial cropping situations may help manage *D. suzukii.*

Optimized timing of parasitoid release is essential for biological control of any parasitoid. Using a mathematical model Pfab and colleagues (2018) found that based on the climate of the province of Trento (northern Italy) the optimal time of *Trichopria drosophilae* release is estimated to lie between late spring and early summer. These timings would also be consistent in Australia with *D. suzukii* infestation predicted to peak in summer (dos Santos et al., 2017). Using a mathematical model it is predicted that a single parasitoid release event can be more effective than multiple releases over a prolonged period, but multiple releases are more robust to suboptimal timing choices (Pfab et al., 2018).

Progressively, government regulations require the development of host-specialised biological control agents. Extensive field studies and detailed evaluations are required to identify a novel strategy based on introduction and establishment of natural enemies of *D. suzukii* from its native range for a long-term control and determine their effectiveness and safety with regard to nontarget species. A petition is currently in revision to release SWD parasitoid wasps from China into the USA.

In Europe testing on larval parasitoids from *D. suzukii's* native Asia occurred on three Asian larval parasitoids and *Asobara japonica*, *Leptopilina japonica*, and *Ganaspis cf. brasiliensis*, and one European species, *Leptopilina heterotoma* (Girod et al., 2018). *Ganaspis cf. brasiliensis* had the highest level of specificity but variations occurred between two geographical populations tested. A Japanese population was strictly specific to *D. suzukii*, whereas another population from China parasitized *D. suzukii, D. melanogaster* and sporadically *D. subobscura*. These results show that more studies are needed on *G. cf. brasiliensis's* taxonomic status and the existence of biotypes or cryptic species varying in their specificity before field releases can be conducted in Europe and by extension, Australia.

Cultural Control Measures

Exclusion netting

Exclusion netting has been shown to be effective at reducing and delaying *D. suzukii* infection (Leach et al., 2016, Rogers et al., 2016). Nets need to be installed before the fruits begin to ripen to prevent any *D. suzukii* being trapped inside the nets. Cormier and colleagues (2015) found nets over blueberry fields had no significant effect on sugar content, yield and damage from other pests. Blueberries harvested inside the nets were significantly larger than blueberries from control plots which had no treatments applied. A larger study in raspberries investigated research plantings with insecticide and exclusion treatments (Leach et al., 2016). Each of the two control approaches provided significant reduction of infestation in raspberry fruit, but the combination treatment had the lowest overall abundance of larvae in fruit. The combination treatment also delayed the first detected larval infestation by 10 d compared to the untreated plots. Exclusion netting applied to commercial size high tunnels resulted in a significant reduction in overall *D. suzukii* infestation in raspberries, as well as a 3-wk delay in the average first detectable fruit infestation. Importantly raspberry size and quality were not affected by the exclusion treatments, indicating that this approach can be an important component of growers' response to invasion by *D. suzukii* in temperate climates.

While the fine mesh netting would block air flow, it also provides shading, which may be responsible for the similarity in temperature between the high net tunnels and no tunnels (Leach et al., 2016). However, the presence of the netting has the potential to increase the ambient temperature, especially in the later parts of the growing season or in warmer production regions. Extreme temperatures in netted high tunnels is a concern that should be kept in mind for fruit production in regions with different climates. However, there are fan systems and venting options that can be used to minimize the risk of extreme temperatures in high tunnels. Exclusion netting and screening can have additional pest management benefits by acting as a barrier against other pests including insects and birds. Not all pests can be managed by netting for example raspberry aphids and raspberry beetles were relatively unaffected by netting, perhaps because they were already established in plantings (Leach et al., 2016). The cost and potential for intensive labour for installation and maintenance are concerns for growers (Rogers et al., 2016). It is therefore likely that high netted tunnels are a suitable option for small-acerage and organic production systems but not necessarily for large scale set ups.

Cultivar selection

D. suzukii populations are lower early in the growing season. Planting regionally appropriate, early-ripening varieties can therefore help decrease the chances of heavy infestations (Sial et al., 2018). Fruit varieties with thicker skins may also be beneficial when selecting fruit cultivars.

Harvest frequency

Harvesting is a powerful tool for disrupting the SWD life cycle (Rufus Isaacs, personal communication). Increasing the harvest frequency reduces detectable larvae, particularly in the first and second instars. It is recommended to harvest soft fruit every 2-3 days (Sial et al., 2018).

Humidity control

As viability of *D. suzukii* eggs is lower under dry, warm conditions (Burrack et al. 2014), cool humid microhabitats should be avoided by pruning to open up the canopy and using wider tree spacing to increase airflow to the canopy and reduce shading (Sial et al., 2018). Thinning the canopy will enhance spray coverage of insecticides when they are applied (McGinnis et al., 2018). Heavier pruning may even result in larger berries that ripen earlier in the season (Sial et al., 2018).

D. suzukii larvae often emerge from fruit to pupate in a suitably protected place. Some pupating larvae drop to the ground to pupate below the soil surface. Studies suggest that using black plastic weed barrier as a mulch on the ground provides an effective barrier that prevents larvae from pupating underneath the soil surface, reducing *D. suzukii* survival (Sial et al., 2018). The plastic barrier also helps with weed management and water retention. The use of mulches reducing standing water can further contribute to the reduction of humidity in fruit orchards (Hoashi-Erhardt and Bixby-Brosi 2014).

Sanitation

It is important that waste or unmarketable fruit is disposed of correctly. Many farms have their pickers use two buckets, one for marketable fruit and another for waste fruit that are disposed of to reduce the population (Sial et al., 2018). Bagging is often the best method as flies can emerge from unbagged infested fruit. An effective disposal method is to put infested fruit in clear bags sealed and left in the sun for more than 32 hours (Rufus Isaacs, personal communication). This will ensure the larvae are exposed for long enough to the lethal temperate (30 ^{O}C).

Alternative plant hosts present on the edge of the field should be removed to decrease the onset and severity of *D. suzukii* in your crop (Sial et al., 2018).

Control Measures

Incompatible Insect Technique (IIT)

Wolbachia bacteria are naturally present in many insects and often induce a form of conditional sterility called cytoplasmic incompatibility (CI): the offspring of infected males die, unless the eggs are rescued by the compatible infection, inherited from the mother that protects the embryo (Cattel et. al., 2017, Nikolouli et. al., 2017). A long-recognized strategy called the incompatible insect technique (IIT) makes use of the CI phenotype to control insect populations through the mass release of infected males. One of the main points of IIT is that, contrary to SIT that allows both sexes to be released as long as they are sterile, this is not possible for IIT which requires strict male release (Nikoloui et. al., 2017). Indeed, the accidental release of females infected by Wolbachia may result in the replacement of the targeted population by a population carrying the Wolbachia infection. Providing that IIT produced females are compatible with the wild males, the success of IIT could be compromised, since the Wolbachia-infected females would be compatible with either the wild or the released males.

To implement IIT in *D. suzukii*, back and forth *Wolbachia* transfers between *D. suzukii* and *Drosophila simulans* were used to identify *Wolbachia* strains that sterilize *D. suzukii* females (Cattel et.al., 2017). Two *Wolbachia* strains were identified as potential candidates for developing IIT in *D. suzukii*. Importantly the fitness or the mating competitiveness of the sterilized males was not compromised in this study. While a promising control option for SWD several critical steps still need to be tested and developed outside the laboratory before the incompatible insect technique can be used to control *Drosophila suzukii* in a large scale operational program.

Sterile Insect Technique (SIT)

The sterile insect technique (SIT) is a species-specific and environment-friendly method of pest population suppression or eradication. The method is based on the sterilization of males (although releases of both sterile males and females have been successfully used), mainly using ionizing radiation which causes dominant lethal mutations in the sperm. A sufficient number of sterile males to create an overflow ratio over a period of time are released, and they are expected to compete with wild males and mate with wild females (Dyck et al. 2005). Mating results in infertile eggs and the developing zygotes die during early embryogenesis, thus inducing sterility in the wild females. Therefore, over time, the target population declines or it is potentially eradicated.

Apart from being an environmentally sound biological control approach, SIT can be easily integrated with other biological control strategies (parasitoids, predators and pathogens). It is a species-specific method, and the release can be performed from the air thus overcoming any topography limitations. Successful development and application of an SIT operational program depends on: (a) the target population being at low levels; (b) extensive knowledge on the genetics, biology and ecology of the target pest being available before the application; (c) mass-rearing facilities being available and capable of providing large numbers of high-quality sterile insects; (d) a release technology having been developed, and the sterile individuals being efficiently monitored; (e) the releases being applied on an area-wide basis covering the whole pest population and (f) the released sterile individuals not causing any side effects on humans or the environment. The majority of the SIT programs have been applied for the control of fruit fly species as they represent one of the major insect groups of economic importance (FAO/IAEA 2013, https://nucleus.iaea.org/sites/naipc/dirsit/)

First results show X-ray radiation can inhibit the development of all stages (egg, larva, pupa and adult) of *D. suzukii* and induce adult sterility (Follett et. al., 2014, Kim et. al., 2016). Nevertheless, there are some reasonable concerns about the feasibility of SIT for this pest considering its high fecundity and the recurrent immigration of flies into the crop that are not completely confined. The short generation time of *D. suzukii* indicates that SIT management should be intensive, otherwise there is a risk that the population will recover rapidly. In addition, control of large field populations of *D. suzukii* poses an extra challenge for SIT. Nikolouli and colleagues (2017) recommend greenhouses and other confined locations, e.g. exclusion netting high tunnels, as the ideal environment for the biocontrol of *D. suzukii* by using the SIT. Recent studies on plastic- and mesh-covered tunnels have shown that *D. suzukii* populations are significantly decreased in these confined areas, not only due to their physical exclusion, but also because of the unfavorable microclimate that is created in these locations (Rogers et al. 2016). Although complete exclusion is not achievable solely by this technique, its combination with SIT could increase the biocontrol levels of *D. suzukii*, thus limiting the use of insecticides. An additional challenge is that an adequate sexing system is not available for *D. suzukii*, and this means that both males and females will be included in the mass-reared and released flies. Bisexual SIT has been successfully used in the past; however, male only releases have been shown to be by far more cost effective and efficient (Rendon et al. 2000).

Combination SIT/IIT

A promising alternative approach for the biological control of *D. suzukii* is coupling SIT with IIT. In general, female insects are more sensitive to radiation than male insects in terms of the induction of sterility. The minimum dose of irradiation to induce full female sterility can be achieved at 75 Gy while an adequate level of male sterility (99.67%) was obtained at 200 Gy (Krüger et al., 2018). As a result, any accidentally released Wolbachia-infected females will be sterile and the risk of population replacement is reduced. In such a system, the released cytoplasmically incompatible males could also receive a low dose of radiation to ensure complete sterility of females that were not removed (Nikolouli et al., 2018). In this case, the sterility of released males would be due to both Wolbachia and irradiation, while the female sterility would only be caused by irradiation. This combined strategy could in principle be applied to any targeted species for which an adequate sexing system is not available. Integration of such a protocol combining low irradiation dose with CI has proved to be an efficient strategy in programs targeting the population suppression of *Aedes albopictus* (Nikolouli et al., 2017).

Before the application of a SIT and/or IIT program against *D. suzukii,* it is, nevertheless important to consider potential limiting factors that may render the program ineffective. An artificial larval and adult diet along with the factors affecting mass-rearing, like ensuring biological quality and consistency in captive populations, are considerations that need to be developed. SIT and IIT are therefore not ready for use in Australia as a control method if *D. suzukii* was to enter Australia today. SIT and/or IIT may however be a viable control method in the future pending successful outcomes to the hurdles listed above.

Conclusions

Although agrochemicals are a convenient method for controlling pests, the use of alternative methods is increasing due to the negative side effects of pesticides. Even if a certain degree of prudence is recommended in the use of these biological pest control agents, entomopathogenic bacteria or their by-products, SIT and/or IIT could be a valid alternative or combined method to reduce the intensive use of xenobiotic chemicals, resulting in a significant environmental benefit.

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