Native parasitic wasps: a new biological tool for fruit fly incursion management in Australia

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Reynolds *et al.*

EH Graham Centre for Agricultural Innovation (a collaborative alliance between NSW Department of Primary Industries and Charles Sturt University)

HAL Project CT07049

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Purpose of report: This project has identified key fruit fly parasitoids present in our major horticultural production areas and advanced our knowledge of the biology and pre-release feeding requirements of *Diachasmimorpha tryoni*, for the augmentative biological control of the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae).

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30 August 2012

Table of Contents

Appendix 1 – Gurr GM and Kvedaras OL. (2009). Synergizing biological control: Scope for sterile insect technique, induced plant defences and cultural techniques to enhance natural enemy impact? *Biological Control* 52 (3): 198-207. (Abstract).

Appendix 2 - Spinner, JE, Cowling, AM, Gurr, GM, Jessup, AJ and Reynolds, OL (2011). Parasitoid fauna of Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera: Tephritidae) in inland New South Wales, Australia and its potential for use in augmentative biological control. *Australian Journal of Entomology* 50, 445-452. (Abstract).

Media Summary

Fruit flies are considered the world's worst pests of edible fruit. Australia's $7 billion-plus per annum horticultural industry is threatened by the Queensland fruit fly 'Qfly'. In addition to Qfly's direct damage to horticultural crops, its presence leads to restrictions on the access of Australian fruit and vegetables to domestic and international markets. In Australia from 2002-2006, the total average export value of our top 25 commodities that are host to fruit fly was $432 million. Interstate trade of all host commodities subject to fruit fly quarantine is worth $1 billion.

Growers and consumers alike are increasingly aware of the hazards associated with heavy reliance on pesticides to control such pests. In the case of Qfly, two of the main chemicals currently used, dimethoate and fenthion have recently undergone a review, with the former now facing severe restrictions on its use and the latter soon likely to follow suit. More than ever before, Australia needs effective, non-chemical methods for the management of Qfly and against related species that threaten to invade from overseas. Non-chemical alternatives would enhance the safety and sustainability of fruit fly control as well as allow an expansion of organic fruit production.

This project examined scope to control Qfly using native Australian wasps that parasitise the larvae as they develop in fruit.. These wasps do not attack people. They are environmentally friendly, target specific and they are self-dispersing, so give wide coverage including areas where other techniques, such as spraying, cannot readily be applied.

When released in large numbers as part of an integrated pest management (IPM) program, such parasitoid wasps have given improved management of fruit flies in several regions of the world, including Hawaii and Guatemala.

This project has resulted in the identification of two species of fruit fly parasitoid not previously known to occur in inland NSW, making them prime candidates for biological control of fruit fly pests in this important fruit growing region. Further, studies have identified factors that will provide the parasitoids with the best possible opportunity to survive, locate their host and reproduce; key factors in the success of biological control. In Australia, this technique is likely to provide more economic and effective management of fruit fly populations as part of an integrated pest management program in addition to providing a sound option for controlling exotic fruit fly incursions. This project has considerbale practical relevance for management of a major Australian horticultural pest and offers a new fruit fly management tool for Australia, based on inundative parasitoid releases as successfully used in several overseas countries. This approach offers scope to markedly increase the efficacy of emergency plant pest incidents including the control of *B. tryoni* and incursion management of exotic fruit fly species.

Technical Summary

The horticulture industry in Australia is worth over $7 billion. The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) is probably the most economically important pest of edible fruit in Australia. Citrus alone, a favoured host of *B. tryoni*, produces close to 700,000 tonnes each year (75% oranges, 20% mandarins and 5% lemons/limes/grapefruit) and is Australia's largest fresh fruit export, with annual exports worth $200 million. In addition to causing serious damage to numerous horticultural crops, *B. tryoni* causes significant restrictions on the access of Australian fruit and vegetables to many domestic and international markets.

Growers and consumers alike are increasingly aware of the hazards associated with heavy reliance on pesticides. The development of more effective non-chemical alternatives for fruit fly management will help reduce such reliance, and may even allow an expansion of organic fruit production. Biological control using inundative or augmentative releases of opiine braconid wasps has resulted in effective suppression of target fruit flies species in several regions of the world, including Latin America and parts of the United States. Key to the success of an augmentative release program is that a parasitoid is provided with the best possible opportunity to survive, locate its host and reproduce. The pre-release environment provides opportunities for interventions including pre-release feeding to maximise longevity and fecundity and the exploitation of a parasitoids learning ability to maximise the chance of locating and parasitising a host. The aim of this study was to identify parasitoid species that are able to survive and persist in inland eastern Australia, and to identify those interventions in the pre-release environment that would maximise a parasitoids performance once it is released.

Initially, we conducted a field survey to determine the *B. tryoni* parasitoid species present and their existing levels of parasitism in fruit fly populations in inland eastern Australia, where populations of wild fruit flies are present. Fruit fly-infested fruits were collected from October 2008 to April 2009 to detect the presence of parasitoids of fruit fly in Wagga Wagga, Cootamundra, Ganmain, Gundagai, Lockhart and Lake Cargelligo on the south-west slopes and plains of NSW, and in Albury-Wodonga on the NSW-Victorian border. Based on the results of the survey, laboratory studies then determined the mating status, fecundity and size of female *Diachasmimorpha tryoni* (Cameron). The effect of a range of pre-release diets (10% concentrations of honey, white sugar and golden syrup) on the longevity of *D. tryoni* was also assessed in the laboratory. A further laboratory bioassay compared six pre-release diets i)quartered orange (two quarters/cage), ii) orange juice (of half an orange), iii) whole apricot (halved, seed removed), iv) whole macerated apricot (halved, seed removed and the flesh macerated), v) water only (control) and vi) honey (which is commonly provided in mass-rearing programs) on the longevity of *D. tryoni*.

In the survey, two species of opiine parasitoids were detected, *Diachasmimorpha kraussii* (Fullaway) and *D. tryoni*, with nine per cent of fruit samples yielding parasitoids. *Bactrocera tryoni* and island fruit fly, *Dirioxa pornia* (Walker) were also detected from the same fruits. There were significant differences between fruit type, fruit species, sampling events and towns. Fruit fly parasitoids were most commonly detected in fig (27.2% of samples), followed by stone fruit (11.5%), pome fruit (6.1%), loquat (4.3%) and citrus (2.1%). Parasitoid occurrence varied throughout the fruit fly season, peaking in February–March 2009 (17.4%). Of the towns surveyed, Cootamundra had the highest occurrence of parasitoids (28.8%), followed by Wagga Wagga (9.5%), Gundagai (10.2%) and Lockhart (1.2%), with no parasitoids detected in Albury-Wodonga, Ganmain or Lake Cargelligo. *Diachasmimorpha tryoni* was detected in all surveys except January–February 2009, during a heatwave. *Diachasmimorpha tryoni* was most common in November–December 2008 (5.2%), while *D. kraussii* was most common in February–March 2009 (14.5%), but was not

detected in October 2008 or April 2009. *Diachasmimorpha tryoni* was detected in Wagga Wagga (6.1%) and Cootamundra (1.9%), with *D. kraussii* detected in Wagga Wagga (9.5%), Cootamundra (26.9%), Gundagai (10.2%) and Lockhart (1.2%).

The egg load of mature wasps and progeny yields of mated and unmated parasitoid females were statistically similar, demonstrating that mating status is not a determinant of parasitoid performance. Female lifespan was not negatively impacted by the act of oviposition though larger females carried more eggs than smaller individuals, indicating a need to produce large females in mass-rearing facilities to maintain this trait. Adult females had the highest lifespan when fed on white sugar, whilst honey and golden syrup shared similar survivorship curves; all were significantly greater than water-only control females. Pre-release feeding of *D. tryoni*, particularly with white sugar, may enhance the impact of released parasitoids on *B. tryoni.* These results are important because honey is currently the standard diet for mass-reared braconids, yet white sugar is less than one third the cost of other foods; however, further work is required to assess diets at different concentrations in the laboratory and the post-release performance of the parasitoid.

The carbohydrate honey maximised the survival of female *D. tryoni* under laboratory conditions when compared with other diet treatments (water only and fruit). Parasitoids fed honey had at least 10% survival beyond 24 days, compared to the other diet treatments with 90% of parasitoids surviving a maximum of 11 days. Daily mortality rates of the parasitoids fed with either cut apricot or orange juice did not differ significantly from those fed with water only. Given so little is known about the dietary requirements or indeed potential food sources in the field of braconids parasitoids, an assessment of their natural foods could lead to diet improvements. Such dietary enhancements could produce a healthier mass-reared parasitoid with greater fecundity and survival and ultimately a more effective biological control agent.

This project has identified the parasitoid species present in our major horticultural production areas and which are prime candidates for augmentative parasitoid release programs for the control of *B. tryoni* in inland NSW, based on their presence and ability to persist in this area. In addition, this project has advanced our knowledge of the biology and pre-release feeding requirements of *D. tryoni*, which will aid augmentative release programs. Two of the three studies have been published in peer-reviewed journals, in addition to two review papers. Future work should include: i) determining the dietary requirements/potential food sources of native and naturalised parasitoids in the field, ii) bioassays and field cage pre-release feeding studies to confirm the food source that will maximise a parasitoids performance while minimising rearing costs, iii) parasitoid learning studies to maximise a parasitoids chance of locating their host and reproducing, iv) continued development of mass rearing techniques of selected parasitoids, v) identification of ways in which we can manipulate landscapes (i.e. composition and connectivity of landscapes) to ensure that parasitoids can readily find and exploit ‘islands’ of fruit fly habitat and vi) determine the effectiveness of the combined use of parasitoids and the sterile insect technique in controlling *B. tryoni* populations, compared with either technique alone.

Introduction

The horticulture industry in Australia is worth over $7 billion dollars. The Queensland fruit fly, *Bactrocera tryoni* is probably the most economically important pest of edible fruit in Australia. Citrus alone, a favoured host of *B. tryoni*, produces close to 700,000 tonnes each year (75% oranges, 20% mandarins and 5% lemon/limes/grapefruit) and is Australia's largest fresh fruit export, with annual exports worth $200 million. In addition to causing serious damage to numerous horticultural crops, its presence in Australia causes significant restrictions on the access of Australian fruit and vegetables to many domestic and international markets.

Growers and consumers alike are increasingly aware of the hazards associated with heavy reliance on pesticides. The development of more effective non-chemical alternatives for fruit fly management will help reduce such reliance and even allow an expansion of organic fruit production. Sterile insect technique (SIT) is an environmentally friendly option to control or suppress fruit fly populations or outbreaks. It uses mass-reared fruit flies that are irradiated before release to render them infertile. The wild female flies with which released males mate produce only non-viable eggs. This biologically-based approach has enjoyed significant success both overseas and in Australia, including treating outbreaks in the FFEZ (e.g. Fisher 1996; Jackman *et al.,* 1996; Perepelicia *et al.,* 1997). The success of SIT relies on sterile releases 'flooding' the wild population, minimising the possibility of wild males and wild females mating to produce viable eggs. However, SIT can be expensive when used against dense or widely dispersed pest populations (Parker & Mehta 2007). An alternative, or indeed synergistic non-chemical approach is biological control using parasitic wasps. Releases of these parasitoids (opiine members of the braconid family) have resulted in effective suppression of fruit flies in several regions of the world, most notably Hawaii (Haramoto & Bess 1970; Wong *et al.,* 1984, 1991). Unlike SIT which is most economical for small pest populations (that are easily 'flooded' by released steriles), parasitic wasp releases work best against a high pest population where wasps can readily locate pests. Simple logic suggests that combining these two methods together could avoid the limitations of each individual method: parasitoids used to bring a high pest population down to a level where SIT becomes effective. This logic is supported by theory. Population modelling has demonstrated that the combined use of SIT and parasitoids would be much more efficient than either method alone for suppressing or eradicating a host species (Barclay, 1987). This paper proposed that the greater combined efficiency of SIT and parasitoid release, as opposed to use of either singularly, was an example of a broader principle: that two pest control methods will mutually complement each other if their optimal actions in reducing host numbers are at different host densities. This is the situation for sterile releases, which performs the best at low host densities while parasitoid inundation performs better at higher host densities. Since that modelling was published, overseas studies have shown that SIT together with augmentative release of fruit fly parasitoids can suppress fruit fly populations to a greater extent than either technique alone (Wong *et al.,* 1992; Rendon *et al.,* 2006). The practical advantages of using parasitoids (and sterile insects) include the benefit of being self-dispersing so give wide coverage including areas where other techniques, such as spraying, cannot readily be applied. In Australia, a combination of both these techniques is likely to provide more economic and effective management of fruit fly outbreaks in the FFEZ and provide enhanced suppression of wild fly populations in the RRZ and endemic areas. In Australia, at least eight species of parasitoid are known to target Queensland fruit fly (Cochereau 1970; Quimio & Walter 2001; Snowball & Lukins, 1964), although their distribution through inland south-eastern Australia has not previously been established.

This project aimed to determine the feasibility of using native Australian parasitoids for augmentative release programs to control the Queensland fruit fly, *Bactrocera tryoni*. Specifically, this project aimed to i) determine through surveys in inland New South Wales and Victoria, in environments adjacent to some of our major horticultural production areas, the presence of fruit fly parasitoids, identifying prime candidates for augmentative release programs for the control of *B. tryoni* ii) establish and rear wild collected parasitoids in the laboratory, iii) determine the effect of mating status and size on potential fecundity of a candidate female parasitoid species identified in the surveys, iv) determine the effect of carbohydrate sources together with oviposition on lifespan and reproductive potential and v) identify through a literature review the potential for using augmentative biological control for the management of *B. tryoni*.

This augmentative parasitoid release technique is likely to provide more economic and effective management of fruit fly populations as part of an integrated pest management program in addition to providing a sound option for controlling exotic fruit fly incursions. This project has considerable practical relevance for management of a major Australian horticultural pest and offers a new fruit fly management tool for Australia, based on augmentative parasitoid release. This approach offers scope to markedly increase the efficacy of emergency plant pest incidents including the control of *B. tryoni* and incursion management of exotic fruit fly species that is target-specific and able to penetrate areas that chemical applications cannot penetrate.

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Introduction and Literature Review

Prospects for parasitoid-based biological control of Queensland fruit fly *Bactrocera tryoni* in Australia.

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Abstract
Augmentative release of the native and naturalised Australian parasitoids, especially the braconid *Diachasmimorpha tryoni*, may result in better management of the serious tephritid pest, Queensland fruit fly, *Bactrocera tryoni* in some parts of Australia. Mass releases are an especially attractive option for areas of inland eastern Australia around the Fruit Fly Exclusion Zone that produces *B. tryoni*-free fruits for export. *Diachasmimorpha tryoni* has been successful in other locations such as Hawaii for the biological control of other fruit fly species. Biological control could contribute to local eradication of isolated outbreaks and more general suppression of the *B. tryoni* population. Combining biological control with the use of sterile insect technique offers scope for synergy because the former is most effective at high pest densities and the latter most economical when the pest becomes scarce. Recommendations are made on methods for culturing and study of the four *B. tryoni* parasitoids present in Australia along with research priorities for optimising augmentative biological control of *B. tryoni*.

Keywords: Braconidae; Tephritidae; *Diachasmimorpha*; *Fopius arisanus*; sterile insect technique; integrated pest management; mass-rearing

Introduction

The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), is the major fruit fly pest for all of eastern Australia with literature on the species dating back more than 115 years (Clarke et al., 2011). It is a major economic pest as a consequence of its ability to survive in a wide range of climatic conditions, its polyphagous nature and its destructive damage to most cultivated fruits and fruiting vegetables. The original distribution of *B. tryoni* was considered to be tropical and subtropical coastal Queensland (QLD) and northern New South Wales (NSW), however, its distribution now extends along much of the eastern seaboard and areas of inland NSW and Victoria (Clarke et al., 2011). Within NSW, *B. tryoni* is best suited to the climate of the coastal and northern inland areas. It can, however, thrive in less suitable areas such as the south and south-west of the state during years of favourable rainfall, with distribution shrinking back to irrigated areas during dryer years (Dominiak, 2007). The majority of *B. tryoni* adults are believed to disperse up to 1 km, although larvae are readily transported in vehicles within infested fruit which poses a threat to many quarantined production areas within suitable climatic zones (Meats & Edgerton, 2008) such as those within NSW. *Bactrocera tryoni* also has the potential to spread internationally because of its tolerance of a wide range of climatic conditions and large host range, as well as its tendency to be dispersed by humans at the larval stage inside infested fruit (Meats & Edgerton, 2008).

The expansion of *B. tryoni* within Australia began as rainforests were cleared and large areas, including inland irrigation zones, were planted with susceptible imported fruit crops. The largely unrestricted interstate trade of fruits during the late 1890s also contributed to their spread. Infested fruits quickly become rotten and inedible causing considerable losses in production, often resulting in complete destruction of fruits rather than only cosmetic damage as is caused by many other insect pests (Plant Protection Service, 2001). Currently, *B. tryoni* is managed using mainly surveillance (trapping), public education, bait spraying, the sterile insect technique (SIT) and chemical control (Dominiak et al., 2003; Meats et al., 2003). However, with diminishing pesticide options for the control of *B. tryoni*, industries are increasingly looking at other alternatives. The aim of this review is to consider prospects for using augmentative biological control in the management of *B. tryoni*, an approach used for related pest species in other countries but not currently practiced in Australia.

Biological Control Strategies

Natural enemies when applied properly are promising, environmentally-friendly and effective tools for sustainable control of arthropod pests to the extent that biological control of insect pests is one of the most cost effective and environmentally sound methods of pest management (Wang et al., 2004). The basic principle in biological control is to use a natural enemy as the 'agent' to maintain the pest ('target') population below damage levels from year to year (Knipling, 1998a). In the past 120 years, more than 200 species of exotic arthropods have been introduced on more than 5000 occasions into 196 countries for the control of insect pests (Vreysen & Robinson, 2011). Such inoculative or classical biological control offers the advantage that well-chosen agents, compatible with the conditions into which they are released, have the ability to maintain self-perpetuating populations from generation to generation (Knipling, 1998a), providing good continuity of control. There is always a risk however, that the agent will attack non-target species. Parasitoids are, therefore, often considered a better option than predators, as the former rely on the host for development and are often more host specific (Cossentine, 2000) so reducing the risk of the agent attacking non-target species.

Parasitoids differ from parasites in that they are organisms that spend a significant portion of their life cycle in a parasitic relationship with a single host organism in which the host organism dies (Lazarovits et al., 2007). Parasitoids are categorised based on factors such as egg development pattern in the adult female (synovigenic vs pro-ovigenic), whether they develop within the host (endoparasitoid) or externally (ectoparasitoid), whether the host continues development (koinobiont) and feeding after parasitism or is arrested (idiobiont), and whether the adult parasitoid feeds upon the host (Lazarovits et al., 2007). Parasitoid wasps (Braconidae) have the advantage of being self-dispersing giving wide coverage in areas where other techniques such as spraying cannot be readily applied (Kitthawee & Dujardin, 2009). In addition, parasitoids are in no way dangerous to human health making them an attractive option for fruit fly control in urban areas such as those found in the Risk Reduction Zone (RRZ) (Wong et al., 1992).

Another response to the potential risks of exotic biological control agents is the use of conservation biological control. Conservation biological control aims to maximise the impact of existing natural enemies and has proven popular in many crop/pest systems (Gurr et al., 2012). There has been little research attention devoted to conservation biological control against fruit flies so the major focus of the present review is in other forms of biological control. A general limitation of all forms of biological control, however, is that the natural enemies alone will not typically provide adequate pest suppression alone, thus integration with other pest management tools such as the sterile insect technique (SIT) (Gurr & Kvedaras, 2010; Vreysen & Robinson, 2011) or bait sprays (Vargas et al., 2001) is required. The use of an integrated pest management (IPM) approach is especially important in eradicating local outbreaks of *B. tryoni* in Australia (Meats et al., 2003; Sutherst et al., 2000).

Historical Use of Biological Control for Fruit Flies in Australia

Of the eight opiine braconids that occur in Australia and are known to attack *B. tryoni* (Carmichael et al., 2005), there are four that are of particular interest for use in augmentative release, not just in Australia but worldwide due largely to their ease of rearing, range of target hosts, climatic/environmental tolerance and levels of parasitism achieved. These four opiine braconids attack a range of tephritid pest species in other locations (Table 1), including several species that are considered a biosecurity risk to Australia. *Fopius arisanus* (Sonan) is an egg-pupal parasitoid (Vargas et al., 2002); *Diachasmimorpha kraussii* (Fullaway) and *Diachasmimorpha tryoni* (Cameron) target late second to early third instar larvae, while *D. longicaudata* (Ashmead) target third instar larvae (Cancino & Montoya, 2004; Jessup & Walsh, 1997; Rungrojwanich & Walter, 2000) (Fig. 1). Of these, only *D. kraussii* and *D. tryoni* are native and have been detected from far north QLD (Carmichael et al., 2005) to southern inland NSW (Spinner et al., 2011). *Fopius arisanus* was introduced from Hawaii and ranges from far north QLD, as far south as Sydney (Carmichael et al., 2005); while *D. longicaudata* also introduced from Hawaii, has been recorded in far north QLD and Lord Howe island (Carmichael et al., 2005). The first effort to introduce braconids for pest control in Australia was in 1902 after unsuccessful searches for native natural enemies of another tephritid pest that occurs in Western Australia, the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) (Argov & Gazit, 2008). Between 1932 and 1938, the NSW Department of Primary Industries (DPI) attempted biological control of *B. tryoni* with introductions of several thousand *Tetrastichus giffardianus* Silv., and small numbers of *Opius humilis* Silv. and *O. fullawayi* Silv. (Noble, 1942). Later, large numbers (over 205,000 in NSW alone) of *Melitobia* (*Syntomosphyrum*) *indicum* Silv. (Noble, 1942) were released, however, all of these biological control attempts failed with none of the released species establishing (Noble,

1942). There is no published literature documenting augmentative release of mass-reared parasitoids, whether native or exotic, against *B. tryoni* in Australia.

Table 1. Host records of the opiine braconids, *Diachasmimorpha* spp. and *Fopius arisanus* parasitising major tephritid pests that occur worldwide. (- indicates no record).

Major Tephritid Pests	**Major Opiine Braconids**			
	D. longicaudata	*D. kraussii*	*D. tryoni*	*F. arisanus*
Bactrocera tryoni	(Carmichael et al., 2005)	(Carmichael et al., 2005)	(Carmichael et al., 2005)	(Carmichael et al., 2005; Rousse et al., 2005)
Ceratitis capitata	(Carmichael et al., 2005)	(Argov & Gazit, 2008; Messing & Ramadan, 1998)	(Carmichael et al., 2005; Vargas et al., 2002)	(Argov & Gazit, 2008; Carmichael et al., 2005)
Bactrocera latifrons	(Carmichael et al., 2005)	(Duan & Messing, 2000c; Messing & Ramadan, 1998)	-	(Carmichael et al., 2005)
Bactrocera cacuminata	-	(Carmichael et al., 2005; Rungrojwanich & Walter, 2000)	-	(Carmichael et al., 2005; Rousse et al., 2005)
Bactrocera dorsalis	(Carmichael et al., 2005; Snowball et al., 1962a)	(Rungrojwanich & Walter, 2000; Vargas et al., 2002)	(Carmichael et al., 2005)	(Carmichael et al., 2005; Snowball et al., 1962a; Vargas et al., 2002)
Bactrocera cucurbitae	(Carmichael et al., 2005)	(Carmichael et al., 2005)	-	(Quimio & Walter, 2001; Rousse et al., 2005)
Anastrepha suspensa	(Eitam et al., 2004)	-	-	(Rousse et al., 2005)
Bactrocera oleae	(Sime et al., 2006)	(Sime et al., 2006)	-	(Calvitti et al., 2002; Rousse et al., 2005)
Anastrepha ludens	(Cancino et al., 2009a)	-	(Cancino et al., 2009a)	(Cancino et al., 2009a; Rousse et al., 2005)
Bactrocera papaya	-	-	-	(Rousse et al., 2005)

Figure 1. A female *Diachasmimorpha* spp.

International Use of Biological Control for Fruit Flies

Utilising parasitic wasps for the control of fruit flies dates back to the early 1900s (Carmichael et al., 2005). Initial attempts were centred on classical biological control whereby parasitic species were inoculated into new geographical locations. For example, *D. longicaudata*, originally from south-east Asia (Wharton & Gilstrap, 1983), was successfully introduced into Hawaii (Wong et al., 1984) and subsequently into Australia from Hawaii in 1956-57 (Snowball et al., 1962b). This species is now widely established in the east of Australia, including Lord Howe Island (Carmichael et al., 2005), as well as in Florida and California (USA), the south of Central America and throughout South America (Aluja et al., 1990; Ovruski et al., 2000). *Fopius arisanus*, also native to south-east Asia (Wharton & Gilstrap, 1983), was established in Hawaii and later Australia (1956-1957) (Snowball et al., 1962a). Its introduction into other parts of the world including Israel, Mexico, and South America, however, was less successful, except in Costa Rica where it has achieved patchy distribution (Argov & Gazit, 2008; Ovruski et al., 2000; Rendon et al., 2006).

Other examples of parasitoid species used in classical biological control include the native Australian species *D. kraussii* and *D. tryoni* (Carmichael et al., 2005). *Diachasmimorpha kraussii* has been successfully introduced to Israel and Hawaii (Argov & Gazit, 2008; Bokonon-Ganta et al., 2007), while around 4.1 million *D. tryoni* were released in Maui, Hawaii (Vorsino et al., 2008) for control of *C. capitata* (Cancino et al., 2009a; Duan & Messing, 1999; Ramadan et al., 1994a). Shortly after its introduction, *D. tryoni* became the most abundant parasitoid in Hawaii (Duan et al., 1998), comprising up to 33% of total parasitoids in Kula, Maui (Ramadan et al., 1994a). Mass releases of *D. tryoni* in Mazapa de Madero Canyon in Mexico between 1987 and 1989 have also been successful, substantially reducing infestations in mangoes and oranges and greatly decreasing populations of *Anastrepha ludens* (Loew) and *Anastrepha oblique* (Macquart) (Ovruski et al., 2000).

In recent decades, increasing interest in the active integration of parasitoids into integrated pest management programs has replaced inoculative biological control. This is likely to reflect not only an increased awareness of the risk of non-target impacts but also significant advancements in rearing techniques and artificial diets for rearing hosts (Purcell, 1998; Purcell et al., 1994). These advances have allowed parasitoid wasps to be used as biological control agents against various fruit fly pests in augmentative release programs (Montoya et al., 2000; Sime et al., 2008; Wong et al., 1991). Augmentative biological control involves the supplemental release of parasitoids and relies on the mass-production of large numbers of parasitoids in a laboratory. Relatively few natural enemies may be released at a critical time of the season (inoculative release) sometimes with the expectation that reproductive populations will establish or literally millions may be released (inundative release). In addition, the crop/host plant system may be modified to support or augment the parasitoids, often known as habitat manipulation. Parasitoids have been used successfully in augmentative release programs in Hawaii, Mexico, Guatemala and Israel (Argov & Gazit, 2008; Messing et al., 1994; Ovruski et al., 2000). The major species utilised in augmentative research and control programs worldwide are *D. kraussii*, *D. longicaudata*, *D. tryoni* and *F. arisanus*. A particularly successful example of augmentative biological control of fruit flies occurred in Hawaii where parasitoids dramatically reduced fruit fly (mainly *B. dorsalis* (Hendel) and *C. capitata* densities within one year (Purcell et al., 1997). Trials of *C. capitata* control in Hawaii using the native Australian parasitoid, *D. tryoni* involved releasing 4.2million parasitoids, averaging 265,000 per week, resulting in significantly lower *C. capitata* per fruit compared to an untreated control area (Wong et al., 1991).

Introducing agent species to new habitats, especially in such large numbers, raises host specificity risks (Vreysen & Robinson, 2011). Imported parasitoids have been reported to form new host associations (Garcia-Medel et al., 2007), sometimes with other introduced species (Duan & Messing, 2000a). Since the introduction of *D. tryoni* to Hawaii for the control of *C. capitata*, this parasitoid has formed host associations with two gall forming tephritids, the lantana gall fly, *Eutreta xanthochaeta* Aldrich, and the Pamakani gall fly, *Procecidocharea utilis* Stone, introduced to Hawaii for the control of weeds (Duan & Messing, 2000a). *Diachasmimorpha kraussii* was also introduced to Hawaii for the control of *B. latifrons* (Hendel), the solanum fruit fly. Tests of possible host associations with native and introduced tephritid fruit flies in Hawaii found that gravid *D. kraussii* females would oviposit into all host larvae presented (Duan & Messing, 2000c). These studies were performed under confined laboratory conditions in choice and no-choice scenarios, and while it is not known whether such host associations occur in the field, the aforementioned findings illustrate the potential implications of introducing a species into a new habitat and the desirability of using native parasitoids over exotics.

Mass Production of Parasitoids

A limiting factor of mass-rearing most species of parasitoids (and all that attack fruit flies) is that it requires the use of live hosts, which in turn, increases production costs (Lopez et al., 1999). The quality (weight and size) of the host must be appropriately monitored as parasitoid cultures from low quality hosts tend to have a male biased sex ratio (Orozco et al., 2002). Hymenopteran parasitoids are haplodiploid with infertile eggs producing males and fertilised eggs producing females (Scarratt et al., 2008). Rearing facilities aim to optimise production of female parasitoids as females are the sex responsible for exerting biological control of the target species.

The size of host larvae is also a crucial factor for parasitoid emergence. High quality hosts that allow production of comparatively large fruit fly larvae increases parasitoid

emergence (Orozco et al., 2002). The age and condition of the larvae used during the rearing process is an important factor influencing both percentage emergence and sex ratio of mass reared parasitoids (Lopez et al., 2009; Messing et al., 1993). Measurements used to monitor the quality of mass reared parasitoids include: host weight, adult emergence, survival, fecundity, flight ability and searching behaviour (Cancino & Montoya, 2006). Such monitoring is important because the performance of mass reared insects produced for augmentative biological control can decrease over generations due to adaptation to laboratory conditions and behavioural changes such as host searching (Joyce et al., 2010). These changes may decrease their performance in the field and reduce the success of a parasitoid in a particular biological control program (Joyce et al., 2010). Therefore, various aspects of parasitoid biology need to be considered in order to optimise the rearing of parasitoids in large numbers.

Mating Behaviour

Like many insects, female parasitoid wasps respond to air- and substrate-borne male courtship signals (Duan & Messing, 2000b) and are pivotal to successful mating. Males usually emerge a few days before females so are ready to mate immediately when females emerge, although the optimum mating activity occurs when females are 3-7 days old (Purcell, 1998). Five species of male parasitoids including *D. tryoni* and *D. longicaudata* exhibit pulses produced by male wing fanning that are repeated numerous times during courtship (Joyce et al., 2010). A constraint in mass rearing is that the artificial rearing environment can affect the transmission and detection of these important courtship vibrations and thus impact rearing efficacy. Mass production can also lead to selection for mating traits that are adaptive in the rearing facility but that can adversely impact subsequent success of the female wasp when released in the field (Joyce et al., 2010).

Substrate Cues and Oviposition

Successful host location is a major problem for gravid parasitoids. Consequently, the exploitation of cues associated with the presence of hosts is very important in parasitoid foraging (Hoffmeister & Gienapp, 1999) and determines their success in both mass rearing and the wild. Both host larvae and their associated substrate (fruit), produce cues that trigger ovipositor-probing behaviour in gravid parasitoids (Duan & Messing, 2000b). The cues from host larvae are mainly vibrations and/or sound created by larvae feeding and crawling inside the fruit. The cues from the host substrate are thought to possibly be contact short-range volatile chemicals originating from the fermentation process caused by larval damage and/or the excrement of feeding larvae. Frugivorous tephritid larvae frequently cause bacteria-related decay of fruit, which subsequently emit chemical cues that may be used by parasitoids to search for hosts within the fruit. Chemical cues from the fermented host substrate are just as important as host vibration cues in the host searching process (Duan & Messing, 2000b). These chemical and/or physical cues associated with the infested fruit or produced by the feeding larvae are critical stimuli which allow female wasps to find and recognise infested fruit and available hosts (Duan & Messing, 1999). If these important host cues are absent, or not recognised as a result of the rearing environment, the parasitoids will not commence oviposition behaviour (Purcell, 1998).

Local arrestment of a foraging parasitoid occurs after a host-infested fruit has been located via chemical cues (Duan & Messing, 2000b). The parasitoid female then uses the antennae to locally perceive high concentrations of the chemical cues produced by the host infestation. A parasitoid may respond to the chemical cues by increasing their rate of turning or reducing their walking speed. Vibrotaxis involves the parasitoid standing stationary on the

fruit surface and using its legs to perceive vibrations caused by a feeding or moving host larva. In response to detecting vibrations, the parasitoid will probe that particular area with the ovipositor. Ovipositor probing is an essential element of host-searching behaviour (Ramadan et al., 1994a) and commences only after successful perception of other, preliminary cues. Accordingly, ovipositor probing is used as an indicator of the level of acceptance of a specific host-substrate (Duan & Messing, 1999).

Emergence and Transportation

Host pupal weight is positively correlated with emergence percentage (eg. *D. tryoni* (Purcell, 1998)), while high larval weights produce larger adult parasitoids (Ramadan et al., 1991). Larval weights greater than 4 mg are considered optimal in mass-rearing to ensure maximum emergence of fruit fly parasitoids (Purcell, 1998). A problem that can prevent parasitoid emergence is encapsulation. Encapsulation is the process whereby haemocytes form a multi-layered envelope around the invading organism (Ero et al., 2010). Egg encapsulation is a typical immune response by host insects in response to attack by parasitoids and has been recorded as occurring in larvae parasitised by a number of wasps (Ero et al., 2010). Parasitoid eggs laid in unnatural hosts are usually killed by encapsulation (Ramadan et al., 1994a). Advances in rearing techniques include the use of irradiated larvae which improves parasitism rates by compromising the hosts' immune system (Sivinski, 1996) reducing the likelihood that immature parasitoids will be killed. Irradiation of hosts also offers the advantage that only parasitised hosts will emerge and flies that are not parasitised fail to develop (Cancino et al., 2009b; Harris et al., 2010). The latter factor can be important because rates of parasitism are incomplete and the escape or release of adult hosts from a parasitoid rearing facility could contribute to pest impact. Purcell (Purcell, 1998) recommends a ratio of host larvae to female parasitoids of at least 15:1 in order to avoid hosts being attacked by multiple ovipositing wasps. Such super-parasitism can cause parasitoid mortality, low emergence rates and poor host quality in mass rearing (Wong et al., 1992), however, recent studies have demonstrated that moderate levels of superparasitism by *D. longicaudata* in the Mexican fruit fly, *A. ludens* (Loew) may result in a female-biased sex with few negative effects on the offspring (see Montoya et al. this special issue).

Mass-release of parasitoids involves transporting parasitoids to the intended area of release, a process that may take several days, during which parasitoids may suffer a decline in viability. In order to avoid this, parasitoids may be chilled (3.5 – 4.5°C). Larios et al. (Larios et al., 2002) reported that there was little or no adverse effect of chilling on parasitoid longevity, production of daughters or offspring sex ratio. Methods of release for *D. tryoni* now include chilling followed by aerial release, similar to the method used to dispense chilled sterile fruit flies for SIT (Sivinski et al., 2000). Chilling has no discernable effect on the short-term mortality of *D. tryoni* or on its ability to take flight immediately after aerial release (Larios et al., 2002).

The behaviour (eg. foraging) of a parasitoid is directly linked to their nutritional state (Sivinski et al., 2006; Wäckers, 1994). In addition, food is an essential component in maximising the reproductive success of adult female parasitoids (Faria et al., 2008). The pre-release environment provides opportunities for interventions, including pre-release feeding to maximise longevity and fecundity (Sivinski et al., 2006; Wäckers, 2001), and the exploitation of parasitoid learning ability to maximise the chance of locating and parasitising a host (Hoedjes et al., 2011; Meiners et al., 2003; van Baaren & Boivan, 1998). Pre-release feeding of parasitoids offers scope to maximise their performance following release, however, few studies have investigated the effect of different pre-release food types on parasitoid longevity in the laboratory or in the field. One such experiment used honey-water (50% solution by

volume) as the food source to test the mating status and oviposition of *D. kraussii* and *D. longicaudata* and determine the effect on parasitoid longevity (Sime et al., 2006). Zamek et al. (in press) provided 10% solutions of several diets to *D. tryoni*. Adult females fed white sugar had the highest lifespan whilst honey and golden syrup shared similar survivorship curves. This suggests that there is a need to compare white sugar at varying concentrations with the current common practice in many mass-rearing facilities worldwide of providing pure honey to fruit fly parasitoids. Learning in parasitoids is not a new area of study (for review, see (Hoedjes et al., 2011)), however, the exploitation of the learning abilities to enhance parasitoid searching success in a release program is a novel aspect requiring further investigation.

Release Rates and Monitoring

Release rate is also pivotal to successful augmentative release programs. Knipling (Knipling, 1998b) predicted a release ratio of parasitoid to pest at 3.3:1 would result in 96% parasitism, however, in practice, Sivinski (Sivinski, 1996) preferred a ratio of 10:1. Knipling (Knipling, 1998b) draws attention to the notion that different parasitoid/pest complexes will require different ratios. Control programs, therefore, need to be efficient and be partnered with adequate monitoring tools. Traditional monitoring methods for parasitoids rely on rearing and dissection of host material for identification and quantification of parasitism (Argov & Gazit, 2008; Greenstone, 2006; Wong et al., 1991). These tasks can be tedious, as *B. tryoni* does not emerge until fourteen days after pupation and parasitoids at least a further two days. Furthermore, related parasitoid species often have few or no morphologically distinguishable features in the immature stage (Greenstone, 2006), making it impossible for identification after dissection. In systems where quick identification and quantification of parasitism levels are required, such as in augmentative releases programs, molecular diagnostic tools would be of great use (Jenkins *et al*. this special issue). With rapid advancements of molecular genetics, a new wealth of techniques have been developed that are relevant (Mills & Kean, 2010) (Jenkins *et al*. this special issue) and applicable for augmentative release program monitoring. Data, such as current parasitism levels, could be quickly processed, and a decision made to release further parasitoids if required. Molecular techniques have been used to monitor the incidence of parasitism in other species (Mills & Kean, 2010), however, they have not been applied to the parasitoids of *B. tryoni* nor has molecular identification been implemented as a monitoring tool for *B. tryoni* parasitism in field collected samples (Jenkins *et al*. this special issue).

A final problem associated with mass-production and augmentative release of biological control agents is that the large numbers of released insects can modify the genetic structure of the wild population by mating with wild individuals. This “reverse bottleneck” is an instance in which the genetic structure of a population is diluted in the presence of a more numerous domesticated population of differing genetic structure (Vorsino et al., 2008). This effect can be overcome by regularly infusing laboratory cultures with fresh ‘wild’ specimens to ensure the genetic diversity of the original wild population is maintained.

Prospects for Parasitoid-based Biological Control of Fruit Flies in Australia

Despite the success of parasitoids as agents in international augmentative biological control programs, parasitoids have not yet been used for augmentative release in Australia against *B. tryoni* or *C. capitata*, the two key economic fruit fly pests in Australia. Mass-rearing facilities for *B. tryoni* and *C. capitata* are located in NSW and Western Australia, respectively. These facilities currently produce flies for the sterile insect technique (SIT), and could be a source or expand production to supply host material for parasitoid rearing. As 70% of the cost of

parasitoid rearing is associated with the production of host material (P. Montoya, pers. comm.) the existence of host rearing infrastructure and expertise in Australia is a benefit to the economics of parasitoid rearing.

Information on the biology of parasitoids is of great value in developing and improving mass-rearing techniques and augmentative release programs (Wang & Messing, 2003). Accordingly, to assess the potential utilisation of available parasitoid species for biological control of fruit flies in Australia, the following sections review key aspects of parasitoid biology, together with available information on the methods that are used for the study of fruit flies and their parasitoids, focussing particularly on the four major opiine braconids, *D. longicaudata*, *D. kraussi*, *D. tryoni*, and *F. arisanus* that are currently being considered for augmentative biological control.

Suitability of Parasitoid to Release into the Environment

Climate Matching

Information on the thermal biology of parasitoids is important in assessing their utility in augmentative biological control as this allows their effective range to be determined by using models such as CLIMEX. A complementary approach for assessing the zones in which a given parasitoid may have potential as an augmentative biological control agent is to study their spatial and seasonal abundance. This can indicate currently unoccupied areas into which the parasitoid could be released and is of particular relevance to Australia, where *B. tryoni* exists partly as a meta-population. This is a result of the large size of the country, highly variable climatic zones, presence of areas of irrigated horticulture and towns in otherwise uninhabitable arid vegetation, combined with high levels of human movement for commerce and leisure.

The distribution of *D. longicaudata* is temperature delimited to areas with mean temperatures above 10.5°C and below 30°C, despite the presence of its host in the region at these temperatures. The longevity of adult *D. longicaudata* is reduced to 3 days at constant temperatures above 30°C (Sime et al., 2006). At moderate temperatures (15°C to 25°C), longevity decreases with increasing temperature (from 19 days down to 7 days, respectively). Caution is, however, required in interpreting the results from such laboratory studies in which constant temperatures are used. Diurnal fluctuations in the field are likely to allow parasitoids respite and allow foraging for hosts (Sime et al., 2006). This is important because summer temperatures in some *B. tryoni*-infested zones of Australia can exceed 30°C. An additional point of caution regarding the longevities cited in the aforementioned study is that humidity was not regulated in the experiments. Future work should aim to match humidity levels to those likely to be encountered in the field as this can strongly affect longevity. For example, *D. kraussii* survived up to 30 days at 25°C and 60% relative humidity (Rungrojwanich & Walter, 2000), surviving significantly longer than when humidity was not controlled (Sime et al., 2006).

The longevity of *D. kraussii* also decreases with increasing temperatures over the range likely to be encountered in the field in Australia (eg. from 36 days to 11 days at 15°C to 25°C) (Sime et al., 2006). While both adult *D. longicaudata* and *D. kraussii* can survive for only 3 days at constant temperatures above 30°C, *D. kraussii* is more likely to be able to survive high temperatures than *D. longicaudata*, by resting in the heat of the day and foraging at cooler times (Sime et al., 2006). Information is less available for *D. tryoni* and *F. arisanus* but it has been reported for the latter that it does not survive below 15.5°C (Snowball & Lukins, 1964) although maximum temperatures have not been studied. The impact of high temperatures on all of these species could be ameliorated, however, by allowing adult parasitoids to feed and mate prior to releases being made in summer months. Potentially,

adults should also be held for several days under favourable storage conditions to allow egg maturation so that adult females are able to parasitise available hosts immediately upon release.

Field Sampling

The study of parasitoids and their potential as biological control agents is underpinned by field surveys in which the parasitoid fauna is determined. Fruit can be collected from research orchards (Jessup & Walsh, 1997), backyards (Aguiar-Menezes & Menezes, 1997; Aguiar-Menezes et al., 2001; Snowball et al., 1962a), or bushland (Aguiar-Menezes & Menezes, 1997; Aguiar-Menezes et al., 2001). Fruit has rarely been sampled from commercial orchards because pesticides used in these settings, along with the practice of collection and destruction of fallen fruit, are likely to lead to low parasitoid densities (Hernandez-Ortiz et al., 2006). Parasitoids can be collected by direct capture of adults in the field, utilising some form of aspirator (Jessup & Walsh, 1997) or rearing them from parasitised hosts within infested fruits. The latter collection technique is often preferred as this will also indicate the identity of hosts (Aguiar-Menezes & Menezes, 1997; Jessup & Walsh, 1997; Mkize et al., 2008; Wharton et al., 2000).

Due to the polyphagous nature of most tephritids, both native and exotic fruit trees need to be surveyed (Aguiar-Menezes & Menezes, 1997; Hernandez-Ortiz et al., 2006). As host plant species fruit at different times of the year and for different lengths of time, sampling must reflect such temporal trends. It is important, therefore, to build up a database of trees in the sampling area and revisit these throughout their fruiting season. Sampling needs to include fallen fruit as well as that on the trees as different host insects preferentially target these resources (Aguiar-Menezes & Menezes, 1997; Aguiar-Menezes et al., 2001; Sivinski et al., 2000). Lopez et al. (Lopez et al., 1999) advocate climbing trees or the use of a ladder, with fruit collected in a bucket attached to a pole. In a research orchard, this may be practical; however, it may be cumbersome when collecting in multiple locations throughout the day. Fruit ready to abscise will give the best indication of natural parasitism levels as the pest larvae in the fruit have been exposed to parasitoids for the maximum possible period of time (Lopez et al., 1999; Sivinski, 1996). In order to determine if fruit is ready to drop, tree branches can be gently shaken (Lopez et al., 1999; Sivinski, 1996). Picking fruit that is not completely ripe can lead to an underestimation of parasitism rates due to the decrease in the period where larvae are susceptible to attack, with some larvae having never been vulnerable to attack (dependent upon oviposition preferences of parasitoid species present) (Aguiar-Menezes & Menezes, 1997; Purcell et al., 1994). This also allows a shorter rearing time as the larvae are more likely to be near pupation (Lopez et al., 1999). In order to alleviate this issue, Wong *et al*. (Wong et al., 1991) dissected fruit at collection, removing mature larvae for rearing. In a different approach, Sivinski (Sivinski, 1996) considered only larvae which exited fruit within three days of sampling.

Collections have been made at weekly (Aguiar-Menezes & Menezes, 1997) to monthly intervals (Aluja et al., 2003) for periods of seven months (Spinner et al., 2011), twelve months (Hernandez-Ortiz et al., 2006) to two years (Aguiar-Menezes & Menezes, 1997). Sample size differed with quantity and fruit varieties available (Aguiar-Menezes et al., 2001; Garcia & Corseuil, 2004) or time spent sampling (Aluja et al., 2003). Sample size ranged from five per tree per week (Aguiar-Menezes & Menezes, 1997) to 2-100 pieces of fruit of each type of fruit available (Snowball et al., 1962a). The concurrent collection of site and management data is important for the full interpretation of trends. For example, any pesticide applications need to be noted (Lopez et al., 1999).

In tropical regions especially, native fruit trees may act as alternate hosts for pest fruit flies and therefore require sampling for parasitoids (Aguiar-Menezes & Menezes, 1997). Longer fruiting seasons in these regions are also likely to lead to higher numbers of parasitoids, as well as pests, and would be good locations for collections when the aim is to establish or supplement parasitoid colonies. In more temperate regions, such alternative host plants may be less readily available. There may however, be non-pest species of fruit flies that act as hosts for parasitoids when the major economic pest is not available.

Sentinel Fruits

The use of sentinel fruits, fruit items infested with host larvae and placed out in a sampling pattern and collected later for study, can be particularly useful for determining whether a parasitoid species prefers to forage within the canopy or on fallen fruit (Lopez et al., 1999). There is conflicting evidence in the literature as to whether or not sentinel fruits maximise the general efficiency of collection of parasitic wasps. It was suggested by Jessup and Walsh (Jessup & Walsh, 1997) that the use of sentinel fruits optimises the collection of wild parasitic wasps due to the extended amount of time the fruit remains in the field. However, Hernandez-Ortiz et al. (Hernandez-Ortiz et al., 2006) found that fruit picked directly at the site had parasitism rates more than twice as high as sentinel fruits (68.5% versus 31.5%).

Sentinel fruits should ideally be placed in the field within a layered container system, consisting of one vessel with holes above another containing a pupation medium. Protection from rain is provided by a final container or sheet of fiberglass or similar material. A gap of 50-100 cm will allow parasitoids to detect the fruit fly infested fruit and enter (Jessup & Walsh, 1997; Lopez et al., 1999). Adhesives such as Tanglefoot (Insect Tangletrap Coating, Tanglefoot; Tanglefoot Co., Grand Rapids, MI) will protect the sentinel fruits from ants (Lopez et al., 1999). The use of a cover is important to prevent sunburn of sentinel fruits (Jessup & Walsh, 1997). Generally, the use of sentinel fruits is best restricted to sites with controlled access, where human disturbance is unlikely.

Sample Processing

Samples can be returned to the laboratory in labelled paper bags (Aguiar-Menezes & Menezes, 1997), labelled plastic bags (P.S. Gillespie, pers. comm.) or directly into the vessels in which wasps will be reared-out (see below). Where possible, fruit should be transported to the laboratory on a daily basis (Lopez et al., 1999) in order to reduce the risk of spoilage. To determine the identity of host fruit flies, fruits should be placed in individual containers (Lopez et al., 1999) until the emergence of pests or parasitoids (Lopez et al., 1999; Sivinski et al., 2000). Moistened vermiculite (Aluja et al., 2003; Jessup & Walsh, 1997), sand (Mkize et al., 2008; Sivinski et al., 2000) or soil (Hernandez-Ortiz et al., 2006) can be used as a pupation medium, usually with a depth of 1cm (Aguiar-Menezes & Menezes, 1997; Lopez et al., 1999; Sivinski et al., 2000). Ventilation of containers can be achieved by cutting a hole in the container lid and covering with mesh (Jessup & Walsh, 1997; Lopez et al., 1999; Sivinski et al., 2000). This also prevents flies and wasps escaping and *Drosophila* spp. from entering and contaminating the culture (Sauers-Muller, 2005). Where only one species of fruit fly is expected or where host records are not of concern, fruit can be placed in bulk lots consisting of a single fruit type from each location (Aguiar-Menezes & Menezes, 1997; Mkize et al., 2008). Whether rearing out in individual or bulk containers, it is preferable to elevate fruit above the pupation medium (Jessup & Walsh, 1997; Mkize et al., 2008). This can be achieved by using sieves with holes large enough to allow larvae to pass through after exiting the fruit (Mkize et al., 2008), wire mesh (Jessup & Walsh, 1997), or suspending the fruit with netting (P.S. Gillespie, pers. comm.).

Sample Maintenance

Recommendations on sample maintenance vary from daily (Aluja et al., 2003), to once a week (Jessup & Walsh, 1997). Fruit should be checked for mould and moistness of media regularly, but may only need attention at longer intervals (Aguiar-Menezes & Menezes, 1997; Sivinski et al., 2000). Several researchers, including Sivinski et al. (Sivinski et al., 2000), Aguiar-Menezes et al. (Aguiar-Menezes et al., 2001) and Mkize et al. (Mkize et al., 2008), advocate sifting media every second day and counting or moving pupae to fresh vermiculite in a separate rearing container. This practice, however, is very time consuming for large scale survey work. At 26^{o}C, all host larvae will leave the fruit within two weeks (A. Jessup, pers. comm.), however, if the fruit is completely covered with mould it should be dissected earlier to determine if all larvae have emerged (Lopez et al., 1999; Sivinski et al., 2000). Earlier dissection will prevent fruit rotting fungi (e.g. *Penicilium* spp.) progressing to attack the pupae. If larvae are not found in the fruit, the fruit sample may be discarded. If immature larvae are found, the fruit, or the least non-rotten portion, should be returned to the container to allow the larvae to complete their lifecycle (Sauers-Muller, 2005). Artificial diets are useful for rearing larvae recovered from decomposing fruit, but if only a few fruits yield larvae, it may not be economical. Emergence of flies and wasps should be assessed every three days (Lopez et al., 1999) for approximately four weeks (Eitam et al., 2004; Jessup & Walsh, 1997). Many parasitoid species enter diapauses so uneclosed pupae should either be retained for up to twelve months (Sivinski et al., 2000) or dissected to identify the developing imago. Wing venation, however, is an important distinguishing feature between the braconid genera *Fopius* and *Diachasmimorpha* (Carmichael et al., 2005). Therefore, it is preferable to allow natural eclosion of adults so that this identification feature is well developed. Dead pupae can be reconstituted by soaking in water for 48-96 hours. Dissection of the re-hydrated pupae enables detection of at least basic features that will discriminate flies from wasps (Duan & Messing, 2000c). Alternatively, molecular analyses for identification can be used in this situation (Jenkins et al. this special issue).

Data Analysis

Four metrics for parasitism are used in the literature for field collections of parasitoids in infested fruits: parasitism rate, relative frequency, total index of parasitism and the infestation index. Of these, parasitism rate is the most widely used. Parasitism rates are calculated by assuming one parasitoid per fly and dividing the number of parasitoids by the number of parasitoids plus flies eclosed. Samples with no observed parasitism are not included (Aguiar-Menezes & Menezes, 1997; Hernandez-Ortiz et al., 2006; Mkize et al., 2008). Associations between parasitoids and fruit flies are calculated only where one species of fruit fly (Aguiar-Menezes et al., 2001) or parasitoid (Aluja et al., 2003) emerged per fruit sample. Further data analysis may comprise correlation and regression. Correlation analysis determines associations between fruit weight, the infestation index and parasitism rate (Hernandez-Ortiz et al., 2006). Regressions can be conducted using general linear models with parasitism rate (absolute abundance) and parasitism proportion (relative abundance) being the dependant variable. The latter enables determination of the best performing parasitoid species. Host plant is treated as an independent variable (Sivinski et al., 2000).

Host Range and the Risk of Non-target Impact

Fopius arisanus, D. kraussii, D. longicaudata and *D. tryoni* all attack a wide range of tephritid fruit flies, including species native to and exotic to Australia, not all of which are pests. Potentially a wide host range facilitates biological control by allowing a parasitoid to persist and reproduce in an area during times of local scarcity of the target (pest) by

exploiting other (non-pest) host species. Offsetting this, however, is the fact that risk to a non-target host can make regulatory authorities reluctant to allow active biological control. This is certainly the case when a classical biological control introduction is being considered but may also apply to augmentative biological control that will increase local parasitoid abundance and increase the magnitude of risk.

Non-target Impacts

Tephritidae are grouped into several sub-families and include fruit feeders (commonly referred to as fruit flies), gall-formers and flower-feeders (Duan & Messing, 2000a). Tephritid flies are parasitised by wasps in the subfamilies Opiinae (Fam. Braconidae), Dirhininae (Fam. Chalcidae), Euderinae, Tetrastichinae and Entedoninae (Fam. Eulophidae) (Commonwealth Scientific and Industrial Research Organisation, 1991). It is commonly assumed that the opine braconids coevolved with their frugivorous tephritid hosts (Wharton & Gilstrap, 1983), however, there are reports of opiine braconids also parasitizing gall formers (Table 2) and flower feeding tephritid flies (Table 3) .

Table 2. Non-target impacts of opiine braconids, *Diachasmimorpha*, *Fopius*, *Psyttalia*, and *Tetrastichus* spp., on gall-forming tephritids. (- indicates no record).

Host	Parasitoid	Oviposition recorded in gall	Oviposition into larvae in artificial diet	Parasitoid able to complete lifecycle	Threat	Reference
Phaeogramma lortnocoibon	*D. longicaudata*	×	×	-	Nil	(Duan et al., 1997b; Purcell et al., 1997)
Eutreta xanthochaeta	*D. longicaudata*	✓	✓	-	Very low	(Duan et al., 1997a)
Procecidochares alani	*D. longicaudata*	×	✓	×	Low	(Duan et al., 1997b; Purcell et al., 1997)
Procecidochares utilis	*D. kraussii*	✓	-	×	Low	(Duan & Messing, 2000c)
Eutreta xanthochaeta	*D. kraussii*	✓	✓	✓	Moderate	(Duan & Messing, 2000c)
Phaeogramma lortnocoibon	*D. tryoni*	×	×	-	Nil	(Duan et al., 1997b; Purcell et al., 1997)
Procecidochares alani	*D. tryoni*	×	✓	×	Low	(Duan et al., 1997b; Purcell et al., 1997)
Eutreta xanthochaeta	*D. tryoni*	✓	✓	-	Moderate	(Duan et al., 2000; Duan et al., 1997b)

Procecidochares alani	*F. ceratitivorus*	×	×	×	Nil	(Messing & Wright, 2006)
Phaeogramma lortnocoibon	*P. fletcheri*	×	×	-	Nil	(Duan et al., 1997b; Purcell et al., 1997)
Procecidochares alani	*P. fletcheri*	×	×	-	Nil	(Duan et al., 1997a; Purcell et al., 1997)
Eutreta xanthochaeta	*P. fletcheri*	✓	×	-	Very low	(Duan et al., 1997c)
Procecidochares alani	*T. giffardianus*	✓	✓	✓	Moderate	(Purcell et al., 1997)

Table 3. Non-target impacts of opiine braconids, *Diachasmimorpha*, *Fopius*, and *Psyttalia* spp., on flower-feeding tephritids (- indicates no record).

Host	**Parasitoid**	**Oviposition recorded in flowerhead**	**Oviposition into larvae in artificial diet**	**Parasitoid able to complete lifecycle**	**Threat**	**Reference**
Trupanea dubautiae	*D. longicaudata*	×	✓	×	Very low	(Duan & Messing, 1997)
Ensina sonchi	*D. kraussii*	✓	-	×	Very low	(Duan & Messing, 2000c)
Trupanea dubautiae	*D. kraussii*	✓	-	×	Low	(Duan & Messing, 2000c)
Trupanea dubautiae	*D. tryoni*	✓	-	×	Very low	(Duan & Messing, 2000a)
Ensina sonchi	*D. tryoni*	✓	-	✓	Low	(Duan & Messing, 2000a)
Trupanea dubautiae	*F. arisanus*	✓	-	×	Very low	(Wang et al., 2004)
Trupanea dubautiae	*F. ceratitivorus*	✓	-	×	Very low	(Messing & Wright, 2006; Wang et al., 2004)
Trupanea dubautiae	*P. fletcheri*	×	✓	×	Very low	(Duan & Messing, 1997)
Trupanea dubautiae	*F. caudatus*	✓	-	×	Very low	(Wang et al., 2004)

Most research on the non-target effects of opiine parasitoids has been conducted in Hawaii, where there are native and introduced gall-formers, the latter introduced for weed biocontrol (Duan & Messing, 2000a) and flower feeders which are important pollinators of rare and

endemic plants (Wang et al., 2004). Four gall-formers have been introduced into Hawaii from Mexico for weed biological control: *E. xanthochaeta* Aldrich, for the control of the woody weed lantana (*Lantana camara* L.), pamakani gall fly, *Procecidochares alani* Stekyskal, for the control of pamakani weeds, and *Ageratina riparia* (Regel) and *P. utilis* for the control of crofton weed, *Ageratina adenophorum* Spreng. The bidens gall fly, *Phaeogramma lortnocoibon* Asquith, is endemic to Kauai Island, Hawaii and is associated solely with the plant, *Bidens cosmoides* (A. Gray) Sherff (Duan et al., 1997a; Duan & Messing, 2000a; Duan et al., 1997c; Purcell et al., 1997). There have been a number of laboratory studies into non-target impacts of fruit fly parasitoids with such gall-formers (Table 2). It is suggested that some *Diachasmimorpha* spp. parasitise *E. xanthochaeta* galls due to the favourable ratio of ovipositor length to gall wall thickness (Duan et al., 1997b). However, in choice tests, *D. kraussii* and *D. tryoni* preferred frugivorous flies in fruit or artificial diet over gall flies (Duan & Messing, 2000c; Duan et al., 2000). When tested against their natal host, *D. tryoni* preferred to oviposit into coffee berries rather than lantana stem galls (Duan et al., 2000), although wasps emerging from *E. xanthochaeta* probed their natal host more frequently than those emerging from *C. capitata* (Jaenike & Papaj, 1992). Field studies have recovered *D. tryoni, Eurytoma tephritidis* Fullaway (Eurytomidae), and *Bracon terryi* (Bridwell) (Braconidae: Braconinae) from mature galls (Duan et al., 1998). Field releases of *D. longicaudata* in lantana patches resulted in 0.8% parasitism (Duan et al., 1997a). These results are supported by field surveys by Duan et al. (Duan et al., 1997c) on the parasitoid complex attacking *E. xanthochaeta*, showing *D. longicaudata* to be the least abundant parasitoid. These results and previous data confirm *D. tryoni* as the most abundant parasitoid of *E. xanthochaeta*, forming over 85% of the parasitoid complex (Duan & Messing, 1996; Duan et al., 1998; Wong et al., 1991). *E. tephritidis*, and *B. terryi* are also major parasitoids of *E. xanthochaeta* galls in Hawaii (Wong et al., 1991). *Psyttalia fletcheri*, introduced from India for the control of melon fly, *Bactrocera cucurbitae* Coquillett, has never been recorded parasitising gall-forming tephritids in Hawaii (Duan & Messing, 1996). *Eutreta xanthochaeta* galls harvested near *D. tryoni* releases and in non-release areas showed no significant increase in parasitism of galls when 100,000 *D. tryoni* adults were released per hectare per week (Wong et al., 1991). *D. tryoni* parasitised significantly more *E. xanthochaeta* as the elevation increased and land use moved from agriculture to native forest (Duan et al., 1998). Overall, *D. tryoni* was more abundant in the highland habitats areas (Wong et al., 1984; Wong & Ramadan, 1987). In this cooler, more humid region, lantana grew more vigorously, providing increased off-target opportunities, however, there was also an abundance of strawberry guava trees, *Psidium littorale* var. *cattleianum* (Sabine) which harboured *C. capitata* and oriental fruit fly, *Bactrocera dorsalis* (Hendel). Regardless of larval host, gravid females strongly favoured fruit hosts to lantana stem galls. The finding that *D. tryoni* was correlated with site but not with gall density (Duan et al., 1998), supports the theory of climate preference and presence of the usual host, rather than preference for gall flies. Duan et al. (Duan et al., 1998) suggest that the parasitoids are attracted to the habitats of the preferred host, where less preferred hosts may also be present.

Laboratory studies also show scope for non-target impacts of fruit fly parasitoids on flower-feeders (Table 3). Generally, flower-feeding tephritids are at less risk of attack by opine parasitoids than are gall-forming tephritids and it has been suggested that galls are more attractive to fruit fly parasitoids as they are more similar in shape and size to fruit than flowerheads (Duan & Messing, 1997). The number of visits to, and probing of flowerheads, was low and not significantly different in the presence or absence of normal fruit fly hosts (Duan & Messing, 2000a; Wang et al., 2004). The exception is *E. sonchi* and *D. tryoni*, with the presence of *C. capitata* significantly decreasing off-target interactions (Duan & Messing,

2000a). Whilst these braconids are not likely to affect *T. dubautiae* and *E. sonchi* in the field, the chalcids *Habrocytus elevatus* (Pteromalidae) and *Euderus metallicus* (Eulophidae) have been observed to attack *Trupanea* spp. in the wild (Duan & Messing, 1997).

Other than extrapolation from the international studies reviewed above, there is little information available on the risks of non-target effects in Australia. Flower-feeding tephritid genera in Australia comprise *Dioxyna*, *Tephritis* and *Trupanea* spp., which feed on Asteraceaous plants, and *Oedaspoides*, which feed on Goodeniaceae plants (Commonwealth Scientific and Industrial Research Organisation, 1991). There is no information, however, on attack, if any, by parasitoids. In the case of gall-forming tephritidae, there is anecdotal evidence of the parasitoids *D. tryoni* and *D. longicaudata* (both present in Australia) attacking *P. utilis* in Hawaii (Duan et al., 1997b). The latter tephritid was introduced to NSW and QLD for the biological control of crofton weed (Commonwealth Scientific and Industrial Research Organisation, 1991). Although there do not appear to be any records of attack in Australia, the risk of attack could be elevated if augmentative releases were made and is indicative of the more general need for caution in the use of these parasitoids in biological control. The vulnerability of a non-target host to a parasitoid depends upon the attractiveness of a host for oviposition and its physiological suitability for the completion of the parasitoids' lifecycle (Duan et al., 1997a). These biological factors, which also have a bearing on the more general suitability of the parasitoid for mass rearing and use in augmentative biological control, are examined for species of relevance to Australia in the following sections.

Parasitoid Biology

Fecundity

Diachasmimorpha longicaudata compares favourably with other parasitoids for reproductive output, regardless of host. When reared on *A. ludens*, *B. dorsalis* and *B. oleae*, *D. longicaudata* produced 187, 93 and 24 offspring, respectively. When reared on *A. ludens*, *F. arisanus* produced less than half of this number of offspring (71 offspring), whilst *D. tryoni* produced just under half as many offspring as *F. arisanus* (39 offspring). When reared on *B. dorsalis*, *F. arisanus* produced 199 eggs per female and *D. tryoni* produced 50 eggs per female on *C. capitata*. When reared on its ancestral host (*B. tryoni*) in an artificial diet, *D. kraussii* produced 112 offspring; on *B. oleae* in olives, the result was much lower, at 23 offspring. *Anastrepha ludens* trials were conducted in mango fruits, *B. oleae* trials in olives and *B. tryoni* on artificial diet (Cancino et al., 2009a; Rungrojwanich & Walter, 2000; Sime et al., 2006; Vargas et al., 2002).

Longevity

Adult longevity is an important attribute of parasitoid utility (Cancino & Montoya, 2004). At 25-26°C and 60% relative humidity, *F. arisanus* had the longest longevity (69 days) of the parasitoids potentially available for use in augmentative biological control in Australia. The next most long-lived species is *D. longicaudata* (51 days). The native species *D. kraussii* and *D. tryoni* are shorter lived at 30 and 26 days, respectively. Adult *F. arisanus* that emerged from *C. capitata* and *A. serpentina* had shorter life spans of 54 and 49 days, respectively, indicating host has an effect on longevity of the adult parasitoid (Cancino et al., 2009a; Rungrojwanich & Walter, 2000; Zenil et al., 2004). Pre-release feeding can increase adult longevity and subsequent parasitoid success in the field (Bautista et al., 2001). A related biological attribute to longevity is generation time. *D. longicaudata* and *D. tryoni* had similar generation times (23 and 22 days, respectively), whilst *F. arisanus* was almost double (42 days). The longer generation time of *F. arisanus* makes field establishment more difficult than for the other two species (Cancino et al., 2009a).

Ease of Rearing

Whilst the egg parasitoid *F. arisanus* may give better fruit fly control in the field due to attacking earlier in the lifecycle of the pest than do the other larval parasitoid species (Vargas et al., 2002), it is known to be difficult to culture (Ramadan et al., 1994b). *Diachasmimorpha tryoni* is known to be amenable to mass-rearing as large numbers (4.1 million/week) have been achieved in production facilities in Hawaii (Wong et al., 1991). Similarly, both *D. kraussii* (Rendon et al., 2006) and *D. longicaudata* (Ovruski et al., 2011) are suitable for mass-rearing. Furthermore, although mass releases of *D. tryoni* and *D. kraussii* have not been tested against *B. tryoni*, they have led to the successful control of other fruit flies including *C. capitata*.

Conclusion

Bactrocera tryoni poses an enormous threat to the sustainability of Australian horticulture. In particular, the Fruit Fly Exclusion Zone (FFEZ) which provides *B. tryoni*-free areas permitting Australian producers to export to areas that are climatically favourable to *B. tryoni* and therefore, are susceptible to outbreaks. If *B. tryoni* cannot be managed effectively in both the FFEZ and RRZ, Australia's economy may suffer from limited exports, as well as widespread crop damage. A solution to the over reliance on chemical insecticides is to develop a more integrated system to control populations in the RRZ and other endemic areas and stop their incursion into the FFEZ and other major endemic horticultural production areas. Parasitoids offer an attractive means of achieving this.

Of the four parasitoid species currently available in Australia for possible use in an augmentative biological control program against *B. tryoni*, *D. longicaudata* and *F. arisanus* have the longest adult longevity and highest fecundity. However, as they cannot survive below 15°C, they are probably unsuitable for Australia's major horticultural production regions within the FFEZ and surrounding RRZ in inland NSW, Australia. These species also parasitise a wide range of fruit fly species and are therefore more likely to constitute a risk to non-target tephritid species, including natives and any introduced for biological control of weeds. The native parasitoids *D. kraussii* and *D. tryoni* were the only *B. tryoni* parasitoids detected in a survey undertaken in inland NSW (Spinner et al., 2011) and are thus likely to be better suited climatically to this region. *Diachasmimorpha tryoni* appears to be better adapted to cooler climates than other parasitoids (Wong et al., 1991) (Table 4) and could therefore be released early in the season before large *B. tryoni* populations become established, or to deal with localised, early season outbreaks. Mature larvae of *D. tryoni* enter a winter diapause within host puparia (Ramadan et al., 1994a) and emerge at the beginning of the season at the same time that *B. tryoni* emerge. Surveys have shown that *D. tryoni* is more abundant at higher elevations (greater than 600 metres) where temperatures are generally lower than at sea level (Purcell, 1998). The distribution of *D. tryoni* is similar to that of *B. tryoni* and occurs in all commercial fruit crops (except pineapple and strawberries) and many vegetable crops (Drew & Lambert, 1986).

Table 4. Comparison of characteristics for the two Australian native parasitoids of *Bactrocera tryoni* (Adapted from (Carmichael et al., 2005; Duan & Messing, 2000c; Messing & Ramadan, 1998; Purcell, 1998; Rendon et al., 2006))

Characteristic	**Australian native parasitoids of *Bactrocera tryoni***	
	Diachasmimorpha tryoni	*Diachasmimorpha kraussii*
Life stage attacked	Larva	Larva
Temporal pattern	Detected early in the season (cold tolerance)	Detected late in the season (heat tolerance)
Geographical pattern	Found in areas of higher elevation.	Relatively large geographical range
Adult longevity	15-25 days	15-30 days
Previous use on augmentative release	Previous success in Hawaii and Mexico	Previous success in Hawaii.

Importantly for augmentative releases, *D. tryoni* is known to be amenable to mass-rearing (Wong et al., 1991). Additionally, although mass releases of *D. tryoni* have not been tested against *B. tryoni*, they have led to the successful control of other fruit flies such as *C. capitata*. Being suited to mass release in inland NSW is attractive because a widespread augmentative biological control program could decrease *B. tryoni* pest pressure on the FFEZ and also be used against isolated outbreaks within the FFEZ.

While the available information supports the viability of augmentative releases of *D. tryoni* against *B. tryoni* in inland NSW, there are large knowledge gaps concerning mass-rearing techniques, host specificity, and success rates. Although it is native to Australia, *D tryoni* has never been mass-reared for release in Australia or been field tested under Australian conditions. This information is critical to the success of biological control. Further, techniques that could optimise the mass-rearing process, such as food source for pre-release feeding and the influence of *B. tryoni* host size to maximise longevity and fecundity, have not been explored for *D. tryoni* although literature is available for closely related species that indicates appropriate methods to fill these knowledge gaps. There have been non-target effects from the release of *D. tryoni* on gall forming fruit flies introduced to Hawaii as biological control agents of the weed *L. camara* (Wang & Messing, 2004). Yet because *D. tryoni* is native to eastern Australia, where no gall forming insects have been recorded as hosts (Duan et al., 2000), they are unlikely to have equivalent non-target effects in Australia. *Lantana camara* is a pest weed in Australia, however, the biological control agents used to control it do not include gall-forming tephritids (Weed Management C.R.C. , 2003).

Like most parasitoids, *D. tryoni* is susceptible to insecticides even at exposure rates well under the recommended field application rates used for *B. tryoni* control (Purcell et al., 1994). Insecticides known to be harmful to *D. tryoni,* include carbaryl, permethrin and malathion (Purcell, 1998). The application of insecticides to control *B. tryoni* or other insect pests could significantly reduce the success of biological control against *B. tryoni* (Purcell et al., 1994) and this needs to be considered in integrated pest management strategies. Targeted bait spraying is potentially less harmful to natural enemies because widespread application of toxins is avoided, however, baits containing sugars are potentially attractive to adult parasitoids. The combination of GF-120 bait sprays and biological control using *D. tryoni* has been described as a compatible control option (Wang et al., 2011). The field effect of baits

such as GF-120 has never been tested with *D. tryoni*, although mortality is known from direct exposure in laboratory conditions (Wang et al., 2011). Thus, further research is required.

A more harmonious combination of control methods for *B. tryoni* is the release of parasitoids together with SIT (Barclay, 1987). These two techniques complement each other because they act on two different stages of *B. tryoni* (larvae and mating adult). A synergistic suppressive action can lead to local pest eradication (Knipling, 1998b). This occurs as parasitoids tend to have a greater impact on relatively dense host populations because hosts are easy to locate and the parasitoid is able to reproduce. In contrast, SIT is expensive to use against large, dense pest populations but becomes more cost effective at lower pest densities (Kaspi & Parella, 2008). Thus, parasitoids are generally released before sterile insects as parasitoids will suppress *B. tryoni* populations and reduce the number of sterile insects needed to achieve acceptable over-flooding ratios (Purcell, 1998). Success with SIT and biological control has been analysed in Mexico with *D. longicaudata* (Orozco et al., 2002). The use of both techniques helped to create fly free zones, which allowed access to new markets valued at US $15 million (Orozco et al., 2002). There is evidence of this synergistic relationship in studies with *F. arisanus* and *D. kraussii* (Rendon et al., 2006) and in the control of other insect pests such as codling moth (Cossentine, 2000). Research into IPM strategies involving SIT and *D. tryoni* (or indeed other parasitoid species), however, have not been conducted in Australia and should be further researched to establish the ability of combining SIT and other biological control methods to eradicate and/or suppress *B. tryoni* in the FFEZ and RRZ.

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Technical Report 1

Parasitoid fauna of Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera: Tephritidae) in inland New South Wales, Australia and their potential for use in augmentative biological control

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ABSTRACT
Augmentative releases of parasitic wasps may result in better management of the Queensland fruit fly, *Bactrocera tryoni* Froggatt, in inland NSW. To determine the fruit fly parasitoid fauna of inland NSW, a survey was conducted from October 2008 to April 2009. Fruit fly infested fruits were collected in Wagga Wagga, Cootamundra, Ganmain, Gundagai, Lockhart and Lake Cargelligo on the southwest slopes and plains of New South Wales and Albury-Wodonga on the NSW-Victorian border to detect the presence of parasitoids of fruit fly. Two species of opiine parasitoids were detected: *Diachasmimorpha kraussii* (Fullaway) and *D. tryoni* (Cameron); both species emerged from fruits that also yielded *B. tryoni* and island fly, *Dirioxa pornia* (Walker). Nine percent of fruit samples yielded parasitoids. We found statistically significant differences between fruit type, fruit species, sampling events and towns. Fruit fly parasitoids were most commonly detected in fig (27.2% of samples contained parasitoids), followed by stone fruit (11.5%), pome fruit (6.1%), loquat (4.3%) and citrus (2.1%). Parasitoid incidence varied throughout the fruit fly season, peaking in February-March 2009 (17.4%). Of the towns surveyed, Cootamundra had the highest incidence of parasitoids (28.8%), followed by Wagga Wagga (9.5%), Gundagai (10.2%) and Lockhart (1.2%) with no parasitoids detected in Albury-Wodonga, Ganmain or Lake Cargelligo. *Diachasmimorpha tryoni* was detected in all surveys excluding January-February 2009, during a heatwave. *Diachasmimorpha tryoni* was most prevalent in November-December 2008 (5.2%). *Diachasmimorpha kraussii* was most prevalent in February-March 2009 (14.5%), but was not detected in October 2008 or April 2009. *Diachasmimorpha tryoni* was detected in Wagga Wagga (6.1%) and Cootamundra (1.9%), with *D. kraussii* detected in Wagga Wagga (9.5%), Cootamundra (26.9%), Gundagai (10.2%) and Lockhart (1.2%) The presence of these parasitoid species in the region suggests they may be suitable for augmentative release in the control of *B. tryoni* in inland NSW.

Key words
Survey, fruit hosts, *Diachasmimorpha kraussii*, *Diachasmimorpha tryoni*, *Dirioxa pornia*.

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are the world's worst pests of edible fruit. Fruit fly surveillance, control, public awareness, research and market access issues cost Australian federal and state governments $129 million from 2003 to 2008. An additional $3.2 million was designated for research projects and action programmes which extended to June 2010. Included in the overall expenditure is the maintenance of the Fruit Fly Exclusion Zone (FFEZ) (Department of Agriculture Fisheries and Forestry, 2007). The FFEZ encompasses the major horticultural regions of Sunraysia, the mid Murray and the Goulburn Valley in Victoria, the Murrumbidgee Irrigation Area of New South Wales (NSW) and the Riverland of South Australia (Reynolds *et al.*, 2010). Surveillance in the FFEZ follows an internationally accepted Code of Practice (Standing Committee on Agriculture and Resource Management, 1996). This verifies area-wide freedom from the Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera: Tephritidae), allowing growers access to fruit fly sensitive domestic and international markets (Sutherst *et al.*, 2000). Bait sprays and the sterile insect technique (SIT) are the currently accepted practices for controlling outbreaks in the FFEZ (NFFS Implementation Committee, 2009) which occur during spring and summer, becoming less frequent in autumn (Yonow *et al.*, 2004). The region to the east of the NSW and Victorian sections of the FFEZ is known as the Risk Reduction Zone (RRZ). The RRZ does not extend into South Australia as this state is fruit fly free (Maelzer *et al.*, 2004). Several towns in the RRZ are under regular monitoring and chemical control programs in order to minimise the movement of *B. tryoni* from the RRZ into the FFEZ (Dominiak *et al.*, 2003a). The towns in inland NSW are ecological islands, separated from neighbouring towns by grazing or broadacre cropping, with few or no fruit fly host plants between towns. This makes it difficult and unattractive for the pest to move between the towns, unless aided by people carrying infested fruit from other areas (Fletcher, 1974; Dalby-Ball and Meats, 2000; Dominiak *et al*., 2003a, 2003b). Thus the major need for pest control is in urban and peri-urban settings (Dominiak *et al*. 2003a).

In several regions of the world, inundative releases of opiine wasps (Hymenoptera: Braconidae) as part of an integrated pest management (IPM) program have resulted in the effective management of fruit flies. For example, *Diachasmimorpha longicaudata* (Ashmead) has been successfully introduced to Mexico and Guatemala and *Fopius arisanus* (Sonan) and *D. kraussii* (Fullaway) have been introduced to Israel (Montoya *et al*., 2005; Argov and Gazit, 2008). Parasitoid wasps have the advantage of being self-dispersing, giving wide coverage in areas where other techniques, such as spraying, cannot be readily applied (Wong *et al*. 1992; Montoya and Cancino, 2004). In addition, parasitoids are innocuous to human health, making them an attractive option for fruit fly control in built- up areas (Cancino and Montoya, 2004). Despite this, and the importance of fruit flies as pests, parasitoids are not yet used in the management of fruit flies in NSW. The release of opiine wasps as part of an IPM program may provide more economic and effective management of fruit fly outbreaks in the FFEZ and provide enhanced suppression of wild fruit fly populations in the RRZ. They may also provide a sound option for controlling exotic fruit fly incursions. These possibilities, however, remain to be tested.

There are eight species of opiine braconids native to Australia and two that are introduced. These are predominantly tropical species, but three species have a known range which extends as far south as Sydney. These three are the endemic *D. kraussii* and *D. tryoni* and the introduced *F. arisanus* (Snowball *et al*., 1962; Carmichael *et al*., 2005). There are no published records of opiine braconids south of Sydney, although recently *D. kraussii* was recovered from table grapes collected in Wagga Wagga (O. Reynolds, unpublished data) and *D. tryoni* from peaches and plums in Albury (A. Jessup, unpublished data).

This study investigated which fruit fly parasitoids occur in the region to the east of the FFEZ. Towns in and near the RRZ were surveyed from October 2008 to April 2009 and the data were used to assess the species of parasitoid most likely to be successful in augmentative releases aimed at suppressing *B. tryoni* populations. To do this, we examined the differences in parasitoid prevalence between towns, across time and between fruit types. In addition, the stone fruit types were examined by species.

MATERIALS AND METHODS

Survey location

Seven towns in inland NSW, Australia were surveyed between October 2008 and April 2009: Wagga Wagga (35°07'S, 147°22'E), Cootamundra (34°39'S, 148°02'E), Ganmain (34°48'S, 147°02'E), Gundagai (35°05'S, 148°06'E), Lake Cargelligo (33°18'S, 146°23'E), Lockhart (35°14'S, 146°43'E) and Albury (36°06'S, 146°54'E). Wodonga (36°7'S, 146°53'E), the Victorian component of the Albury-Wodonga twin towns, was also included. There were six sampling events. Each event took place over a two week period (Oct 08), three week period (Nov-Dec 08, Jan 09, Jan-Feb 09, Feb-Mar 09) or a one week period (April 09) (Table 1). Lake Cargelligo was surveyed only once due to low numbers of *B. tryoni* and then replaced with Lockhart (Table 1). Ganmain and Lockhart were not surveyed after February as no and very few parasitoids were detected, respectively.

Table 1. New South Wales towns surveyed for fruit fly infested fruit on six sampling events in 2008 and 2009. (X indicates when sampling events were conducted for each town).

Sampling event	**1**	**2**	**3**	**4**	**5**	**6**
Date	**20-31 Oct 2008**	**20 Nov-19 Dec 2008**	**9-23 Jan 2009**	**27 Jan-6 Feb 2009**	**26 Feb-13 Mar 2009**	**28-30 Apr 2009**
Albury-Wodonga	X	X	X	X	X	X
Cootamundra	X	X	X	X	X	X
Ganmain	X	X	X	X		
Gundagai	X	X	X	X	X	X
Lake Cargelligo	X					
Lockhart		X	X	X		
Wagga Wagga	X	X	X	X	X	X

Survey method

Fruit with oviposition marks were removed from trees and vines and all were dissected to confirm the presence of fruit fly eggs and/or larvae. These infested fruit were collected from domestic and public gardens as commercial orchards are uncommon within the RRZ and those present used chemical pesticides. Mature fruit of various species (Table 2) were collected into plastic bags from on and below trees and tomato vines and kept in an insulated polystyrene box while in the field. Bags were left unsealed to prevent suffocation of larvae. All visibly fruit fly-infested fruit on a given tree or the ground beneath was collected. Where possible, fruit were processed in the field, otherwise, they were processed in the laboratory. Processing consisted of placing fruit into 285 – 850mL round plastic containers, covered with

velo voile (100% polyester) and secured with two rubber bands. A thin layer (10 mm) of vermiculite, moistened at a ratio of 4:1 vermiculite: water, in the bottom of the containers provided a medium for pupation, whilst absorbing fruit exudate. Wire stands 100cm in diameter and 1cm tall made from 0.56mm diameter galvanised wire, 13mm aperture mesh were used to suspend fruit above the vermiculite. This encouraged larvae to move freely into the vermiculite to pupate, minimising pupation on the underside of the fruit. Where possible, fruit were placed one per container to facilitate fruit fly host determination for each parasitoid species. Due to space constraints, figs were grouped four per container, while for both peaches and nectarines were grouped two to four per container. Within 36 h of collection, samples were returned to the laboratory and held in a controlled temperature (CT) room at 23±1ºC and 70±10% relative humidity for recovery of parasitised and unparasitised fruit fly puparia. As the egg and larval stage of *B. tryoni* takes ten days at 25^{o}C (Anderson, 1963), puparia were sifted from the vermiculite ten to twelve days after fruit collection. At this time, the fruit was dissected to check for any remaining larvae. If larvae were detected, the fruit was retained for a further week. Larval development took longer in apples (O'Loughlin *et al*., 1984) so sifting and dissecting of apples was conducted after four weeks. Emergence was thereafter checked daily. Because many parasitoid species undergo diapause of up to several months (Wong and Ramadan, 1992), remaining host pupae were dissected five weeks after the final sampling event to check for unemerged parasitoids. Adult parasitoids were identified using the key of Carmichael *et al*. (2005). Adult flies were identified using the key of Drew *et al*. (1982) by Rosy Kerslake and Michelle Rossetto of the Agricultural and Scientific Collections Unit of Industry and Investment NSW. Voucher specimens of fruit fly and parasitoid species reported from the present study were deposited in the Agricultural and Scientific Collections Unit of Industry and Investment NSW, Orange, NSW.

Table 2. The number of fruit samples in which the Queensland fruit fly, *Bactrocera tryoni* Froggatt were collected in inland NSW, Australia for each fruit type and species, and the number (and percentage) of those fruit samples that produced nil parasitoids or parasitoids of two *Diachasmimorpha* species.

Fruit type	Fruit species [Common name]	Number of fruit samples collected	Number of fruit without wasps	Number of fruit with *D. tryoni*	Number of fruit with *D. kraussii*
Fig	*Ficus* carica L. [1] [Fig]	44	32 (72.7)	2 (4.5)	10 (22.7)
Stone fruit	[all stone fruit types combined]	562	497 (88.4)	12 (2.1)	53 (9.4)
	Prunus persica L. [1] [Peach]	224	212 (94.6)	0 (0.0)	12 (5.4)
	Prunus armeniaca L. [1] [Apricot]	138	128 (92.8)	10 (7.2)	0 (0.0)
	Prunus persica var. nectarina (L.) [1] [Nectarine]	134	97 (72.4)	0 (0.0)	37 (27.6)
	Prunus cerasifera Ehrh. [Ornamental Plum]	42	39 (92.9)	2 (4.8)	1 (2.4)
	Prunus salicina 'Shiro' Lindl. [Yellow Plum]	11	11 (100.0)	0 (0.0)	0 (0.0)
	Prunus cerasifera Ehrh. X armeniaca L. [Plumcot]	9	9 (100.0)	0 (0.0)	0 (0.0)
	Prunus domestica L. [Plum]	4	1 (25.0)	0 (0.0)	3 (75.0)
Pome fruit	[all pome fruit types combined]	49	46 (93.9)	1 (2.0)	2 (4.1)
	Malus domestica Borkh. [1] [Apple]	30	27 (90.0)	1 (3.3)	2 (6.7)
	Pyrus pyrifolia Burm. [Nashi Pear]	11	11 (100.0)	0 (0.0)	0 (0.0)

	Cydonia oblonga Mill. [Quince]	5	5 (100.0)	0 (0.0)	0 (0.0)
	Pyrus communis L. [Pear]	3	3 (100.0)	0 (0.0)	0 (0.0)
Loquat	*Eriobotrya japonica* Thunb. [Loquat]	115	110 (95.7)	5 (4.3)	0 (0.0)
Citrus	[all citrus types combined]	146	143 (97.9)	1 (0.7)	2 (1.4)
	Citrus sinensis (L.) [1] [Orange]	75	72 (96.0)	1 (1.3)	2 (2.7)
	Fortunella japonica (Thunb.) [Cumquat]	34	34 (100.0)	0 (0.0)	0 (0.0)
	Citrus x paradisi Macfad.[1] [Grapefruit]	19	19 (100.0)	0 (0.0)	0 (0.0)
	Citrus limon (L.) [Lemon]	18	18 (100.0)	0 (0.0)	0 (0.0)
Berry	[all berry types combined]	9	9 (100.0)	0 (0.0)	0 (0.0)
	Lycopersicum esculentum L. [Tomato]	8	8 (100.0)	0 (0.0)	0 (0.0)
	Feijoa sellowiana Berg [Feijoa]	1	1 (100.0)	0 (0.0)	0 (0.0)

[1] denotes fruit varieties which were also hosts to the island fly, *Dirioxa pornia*, in this study.

Data analysis

Data were categorised into the number of fruit fly infested pieces of fruit which contained no wasps, or contained wasps of a given species. Seasonal fruit phenology meant oranges were the only fruit to be found in all six sampling periods. Other fruits were available for one to four sampling events. In order to reduce this imbalance, fruit species were grouped for analyses into stone fruit, pome fruit, citrus and berry (tomatoes and feijoas) with loquat and fig remaining single species groups. Parasitism rates (percent) for each fruit species were calculated by dividing the total number of adult parasitoids emerged by the total number of adult parasitoids emerged plus the total number of adult flies emerged.

Wasp data were analysed in GenStat 12.1 (Payne *et al*., 2009) using separate binomial generalised linear models of presence/absence of the wasp on fruit type, time (sampling event) and location (town). The data were then restricted to remove those data where wasps were absent and the species composition was analysed again using a binomial generalised linear model. Separate models were fitted due to the non-availability of certain fruits in certain towns and the seasonal nature of fruits across time. For example, fruit fly infested apricots were detected only in Cootamundra, Ganmain, Lockhart and Wagga Wagga in sampling event 2 and in Gundagai in sampling event 3. Fruit fly infested apricots were not found in Albury-Wodonga. The tests for a difference within a factor (i.e. fruit type, sampling event or town) were based on the change in deviance (Δ dev) and Fisher's exact test was used when comparing two groups within a factor, with Bonferroni correction for multiple paired comparisons. Parasitism rate was calculated as the number of wasps emerged divided by the number of wasps plus the number of flies emerged. Parasitism rates were analysed using a binomial generalised linear model, with data restricted to those fruit for which there were 10 or more samples. Pairwise differences were calculated using two-sample binomial tests with Bonferroni correction for multiple paired comparisons.

RESULTS

Altogether, 1349 fruit fly infested fruit were collected from nineteen different fruit species. *Bactrocera tryoni* was detected in nineteen fruit species and the non-economic fruit fly *Dirioxa pornia* was detected in seven of these (Table 2). As figs, peaches and nectarines numbered 2-4 per sample (other fruits were held individually), this equated to 925 samples. *Bactrocera tryoni* was detected in 913 of these, with *D. pornia* infesting 124 samples. Both species were found together in 115 samples. No other fruit fly species were detected.

The parasitoids *Diachasmimorpha kraussii* and *D. tryoni* were detected in nine of the nineteen fruit species. *Diachasmimorpha kraussii* was found in 67 samples and *D. tryoni* in 21 (Table 2). Parasitoid counts per sample were always low (maximum for *D. kraussii* = 11; *D. tryoni* = 2).

Parasitoids were found in the presence of *B. tryoni* alone and when *B. tryoni* and *D. pornia* co-infested the same fruit sample but were not found in samples infested with *D. pornia* alone. No other parasitoids were detected. Vinegar flies (Diptera: Drosophilidae) and their parasitoid *Leptopilina* sp. (Hymenoptera: Cynipoidea), the metallic-green tomato fly, *Lamprolonchaea brouniana* (Bezzi) (Diptera: Lonchaeidae) and dried fruit beetles, *Carpophilus* sp. (Coleoptera: Nitidulidae) were also present (results not presented).

Fruit type and fruit species effects

Diachasmimorpha kraussii and *D. tryoni* were detected in all fruit types with the exception of berry. There were differences between fruit types in the probability of presence of parasitoids (Δ dev = 34.24, df = 5, $p<0.001$). Parasitoids were most frequently found in figs, followed by stone, pome, loquat, citrus and berry. When parasitoids were recovered from fruit,

significantly higher numbers were recovered from citrus compared with stone fruit ($p<0.001$), citrus compared with fig ($p<0.001$), and fig compared with loquat ($p<0.001$) (Table 2).

Where parasitoids were present, the prevalence of the two species was significantly different across fruit types (Δ dev = 16.08, df = 4, p=0.003). *Diachasmimorpha kraussii* was detected in orange, peach, plum, ornamental plum, apple and fig. *Diachasmimorpha tryoni* was reared from orange, apricot, ornamental plum, apple, loquat and fig. The two wasp species were never found together in the same piece of fruit. The prevalence of *D. kraussii* in loquat was significantly lower than in stone fruit ($p < 0.001$) and fig ($p = 0.003$).

Within the most commonly parasitized fruit type 'stone fruit' there were significant differences between fruit varieties in the probability of detecting parasitoids (Δ dev = 53.22, df = 6, $p<0.001$). Parasitoids were more commonly found in peach and apricot than nectarine or plum. No parasitoids were detected in yellow plum or plumcot. Of the fruit with greater than or equal to 10 samples, the parasitism rate was highest in peach, followed by apricot, nectarine and fig (Table 3). Strongly significant differences in parasitism rates were detected between the four fruit species with greater than or equal to ten fruits (Δ dev = 61.32, df = 3, $p<0.001$). There was no difference in parasitism rates between nectarine, apricot and peach but all three fruits were highly significantly different from figs ($p<0.001$), which had the lowest parasitism rate.

When wasps were present, the prevalence of the two species was significantly different between fruit species in the fruit type 'stone fruit' (Δ dev = 58.36, df = 4, p=0.003). *Diachasmimorpha kraussii* was more commonly found in apricot than nectarine, peach and plum. The prevalence of *D. kraussii* was significantly higher in nectarine than ornamental plum.

Table 3. Rates of parasitism of Queensland fruit fly, *Bactrocera tryoni* Froggatt and Island fly, *Dirioxa pornia* by two *Diachasmimorpha* species across fruit type.

Fruit	**Number of fruit samples containing parasitoids**	**Number of *D. tryoni***	**Number of *D. kraussii***	**Total number of fruit flies**	**Parasitism rate (%)**
Nectarine	37	0	107	811	11.66
Fig	12	2	12	563	2.43
Peach	12	0	23	100	18.70
Apricot	10	13	0	95	12.04
Loquat	5	5	0	40	11.11
Orange	3	2	2	85	4.49
Apple	3	2	3	42	10.64
Ornamental Plum	3	3	1	26	13.33
Plum	3	0	8	5	61.54

Sampling event effects

The probability of the presence of wasps differed throughout the survey period (Δ dev = 19.59, df = 5, p = 0.001). Wasps were most frequent in sampling event 5 (Feb-Mar 09), followed by sampling event 3 (Jan-09), sampling event 4 (Jan-Feb 09), sampling event 2 (Nov-Dec 08), sampling event 6 (April-09), then sampling event 1 (Oct-08) (Table 4). The only significant pair-wise difference in the probability of presence of wasps was between Collection 1 (Oct-08) and Collection 5 (Feb-Mar 09). When parasitoids were present, the prevalence of the two species differed between sampling events (Δ dev = 57.24, df = 5, p < 0.001) *Diachasmimorpha tryoni* was the only species found in sampling event 1 (Oct-08) and sampling event 6 (April-09), and was the most common species in sampling event 2 (Nov-Dec 08). *Diachasmimorpha kraussii* was more prevalent from sampling event 3 (Jan-09) to sampling event 5 (Feb-Mar 09).

Table 4. The number of fruit samples in which the Queensland fruit fly, *Bactrocera tryoni* Froggatt was collected in inland NSW, Australia for each sampling event, and the number (and percentage) of those fruit samples that produced nil parasitoids or parasitoids of two *Diachasmimorpha* species.

Sampling event	**Date**	**Number of fruit samples collected**	**Number of fruit without wasps**	**Number of fruit with *D. tryoni***	**Number of fruit with *D. kraussii***
1	Oct-08	98	95 (96.9)	3 (3.1)	0 (0.0)
2	Nov-Dec 08	271	253 (93.4)	14 (5.2)	4 (1.5)
3	Jan-09	348	303 (87.1)	1 (0.3)	44 (12.6)
4	Jan-Feb 09	108	99 (91.7)	0 (0.0)	9 (8.3)
5	Feb-Mar 09	69	57 (82.6)	2 (2.9)	10 (14.5)
6	Apr-09	31	30 (96.8)	1 (3.2)	0 (0.0)

Town effects

Opiine wasps were detected in Cootamundra, Gundagai, Wagga Wagga and Lockhart but were undetected in Albury-Wodonga, Ganmain and Lake Cargelligo. The probability of presence of wasps differed significantly between towns (Δ dev = 107.57, df = 6, p<0.001) with wasps most frequent in Cootamundra, followed by Gundagai, Wagga Wagga and Lockhart (Table 5). Where parasitoids were present, the prevalence of the two species was significantly different between towns (Δ dev = 38.17, df = 3, p<0.001) (Table 5). Both species of wasps were found together at one location on only one sampling event, during Feb-Mar 09 (sampling event 5); here *D. tryoni* was found in two figs, and *D. kraussii* was found in ten figs. In all other cases, only one species was found at any one residence or public garden.

Table 5. The number of fruit samples in which the Queensland fruit fly, *Bactrocera tryoni* were collected in inland NSW, Australia for each town, and the number (and percentage) of those fruit samples that produced nil parasitoids or parasitoids of two *Diachasmimorpha* species.

Town	Number of fruit samples collected	Number of fruit without wasps	Number of fruit with *D. tryoni*	Number of fruit with *D. kraussii*
Wagga Wagga	295	267 (90.5)	18 (6.1)	10 (3.4)
Albury-Wodonga	218	218 (100.0)	0 (0.0)	0 (0.0)
Gundagai	167	123 (89.8)	0 (0.0)	14 (10.2)
Cootamundra	156	111 (71.2)	3 (1.9)	42 (26.9)
Lockhart	84	83 (98.8)	0 (0.0)	1 (1.2)
Ganmain	32	32 (100.0)	0 (0.0)	0 (0.0)
Lake Cargelligo	3	3 (100.0)	0 (0.0)	0 (0.0)

DISCUSSION

This survey presents data for the first records of *D. kraussii* in Cootamundra, Gundagai and Lockhart and of *D. tryoni* in Cootamundra and Wagga Wagga. *Diachasmimorpha kraussii* was previously detected parasitising *B. tryoni* in table grapes from Wagga Wagga in 2007 (O. Reynolds, unpublished data) and were also detected in Wagga Wagga in this survey. Although *D. tryoni* were previously detected in peaches and plums from Albury-Wodonga in 2005 (A. Jessup, unpublished data), they were not detected in the current survey, despite the detection of *B. tryoni* in these fruits. Nectarine, plum, apricot and ornamental plum are recorded here for the first time as fruit hosts of *D. kraussii* parasitising *B. tryoni*. Apple, apricot, ornamental plum and fig are recorded here for the first time as fruit hosts of *D. tryoni* parasitising *B. tryoni*. The identification of *D. kraussii* and *D. tryoni* reared from fruit collected on the south-west slopes and plains of NSW suggests that these two species are potential candidates for successful augmentative releases in the region.

Fruit fly parasitoids were found in association with *B. tryoni* alone and together with *Dirioxa pornia* (syn. *D. confusa*), a non-economic fruit fly known only to attack overripe and damaged fruit on the ground (Lloyd *et al*., 2010). However, parasitoids were not found with *D. pornia* alone, suggesting that *Diachasmimorpha kraussii* and *D. tryoni* are unable to parasitise *D. pornia*. *Dirioxa pornia* is parasitised by *F. arisanus* but not known to be parasitised by any other opiine species (Clausen, 1956), however experimentation is needed to confirm this. *Bactrocera cacuminata* (Hering) and *B. newmanii* (Perkins) are the other potential alternate fruit fly hosts in the region, both of which are native, non-economic species (Drew, 1989) but neither were detected in this study. Laboratory studies by Ero (2009) showed that *D. kraussii* was unable to complete development in *B. cacuminata*, however the capability of *D. tryoni* as a host of this species has not been reported. Further, the ability of *D. kraussii* and *D. tryoni* to parasitise *B. newmanii* has not been studied. Alternative host insect species may be important reservoir hosts between outbreaks of *B. tryoni* (Ero, 2009), thus these fruit fly species deserve further research as potential hosts.

Differences in total parasitism across fruit types could be due to a number of factors including fruit size, fruit phenology or both. It has been suggested that smaller fruits are more

favourable fruit hosts for larval parasitoids than larger fruits as the fruit fly larvae are more accessible to the parasitoid in a smaller fruit (Sivinski, 1991 ; Lopez *et al*. 1999; Aluja *et al*., 2003). This may account for the higher proportion of parasitoids in stone fruit compared with citrus in our survey, as stone fruit were observed to be commonly smaller than the citrus species collected. However it does not explain the differences between parasitism in figs and loquats. Figs had the highest wasp incidence with loquats lowest, despite both fruit being small thin-skinned fruit of similar size. Loquats fruited during sampling events, Oct-08 and Nov-Dec 08, when parasitoids, particularly *D. kraussii* were rare, whilst figs fruited in Feb-Mar 09, when both parasitoid species were detected. Hence, there may be confounding effects of fruit phenology which may explain the difference in wasp incidence between loquats and figs. Parasitoids were found more often in figs than in any other fruit type (Table 2). However, the rate of parasitism was lowest in figs (Table 3). This is because the proportion of unparasitised fruit flies was much higher in figs. We were unable to compare figs and loquats on a percentage parasitism basis as the number of loquat samples containing parasitoids was too low (less than ten). Laboratory studies of fruits grown in this region would complement those conducted on tropical and subtropical fruits (Sivinski, 1991; Lopez *et al*., 1999; Aluja *et al*., 2003) and assist in determining the usefulness of parasitoids of fruit fly for biological control in this region.

Since the ovipositors of *D. kraussii* and *D. tryoni* are of similar length (pers. obs., 2008) there is no oviposition advantage to either species in competing for fruit fly larval hosts in fruits of any skin thickness. Despite previous records of *D. kraussii* from loquats and lemons (Rungrojwanich and Walter, 2000), it is likely that *D. kraussii* was not found in these fruits because when they were collected there was also low fruit fly activity (from October to December). Apricots also fruited mainly in Nov-Dec 08 (data not shown), when D. *kraussii* were found in very low numbers, whereas all other stone fruit predominated in Jan-08 (data not shown), when the maximum *D. kraussii* prevalence occurred (Table 4). Our results showed that stone fruit were more likely to be infested with parasitised *B. tryoni* than either pome or citrus, and that when present, the parasitoid species was more likely to be *D. kraussii* (Table 2). Similarly, in a laboratory choice study, Ero (2009) demonstrated that *D. kraussii* had the greatest preference for peaches (stone fruit), followed by (in order) pears (pome), apple (pome) and lastly orange (citrus). In a no choice situation, *D. kraussii* parasitise equally across peach, pear, apple and orange (Ero, 2009). This suggests that in a domestic garden, or urban town where multiple fruit types are present, *D. kraussii* may demonstrate a preference for stone fruit and thus may not provide high levels of control in less preferred fruit types. Fruit preference is therefore likely to be affected by a complex of factors.

It is possible that differing thermal requirements affected the prevalence of the two *Diachasmimorpha* species. The long term average annual temperature range in the region is approximately 15 to 33^{o}C (Bureau of Meteorology 2009a). However, daily maximum temperatures in Jan-Feb 09 were extreme with a record heatwave of thirteen consecutive days over 36^{o}C (Bureau of Meteorology, 2009b, 2011). *Diachasmimorpha kraussii* was most prevalent in the warmer months (Jan-09 to Feb-Mar 09), including during the heatwave, but was not detected in the cooler months (Oct-08 and Apr-09) (Table 4). Conversely, *D. tryoni* was rarely detected in the warmest months and not at all during the heatwave, but was the only species detected early and late in the season (Oct-08 and Apr-09). The prevalence of *D. kraussii* in the warmer months including during the heatwave suggests that *D. kraussii* may be more heat tolerant than *D. tryoni*. Laboratory studies confirm that *D. kraussii* is tolerant of temperatures up to 32^{o}C (survival 3 days), although longevity is greatest at 15^{o}C (36 days) (Sime *et al*., 2006). Such studies have not been conducted for *D. tryoni* and would be useful before determining the more appropriate species for the control of *B. tryoni* in this region at

different times of the year. In addition, studies to determine lower and upper lethal and flight thresholds of both species to verify the seasonal extent of their usability would be valuable. The current survey results suggest that *D. tryoni* may complement current practices for gaining early season control of *B. tryoni*, whilst *D. kraussii* may be required to maintain this control through the warmer months until *D. tryoni* is active again in the autumn. Further seasonal sampling needs to be conducted to determine if the trends observed in this study are consistent.

Parasitoid prevalence across the region is likely to differ due to the means of parasitoid dispersal, which has possibly occurred by humans transporting infested fruit which also contain parasitised fruit fly larvae. This is feasible, as many of the incursions of *B. tryoni* into the FFEZ are thought to be from humans transporting infested fruit and is why the transport of fruit into the region is prohibited without a permit (Yonow and Sutherst, 1998; Dominiak *et al*,. 2000). The parasitoids may also have been distributed through the region via wind dispersal (Greany *et al*., 1977; Messing *et al*., 1997).

Parasitoids complement current SIT practices. Indeed, parasitoids may have a synergistic effect when used in conjunction with SIT (Barclay, 1987). This synergistic effect is realised by sterile fruit flies first reducing the number of viable eggs produced, followed by augmentative release of parasitoids to suppress adults emerging from any remaining viable eggs (Wong *et al*., 1992; Knipling, 1998). Therefore, augmentative biological control may be useful as part of an integrated pest management program or systems approach against *B. tryoni*. As *D. tryoni* and *D. kraussii* were found in the RRZ, they can presumably persist there, and as such might be considered further for research on augmentative release.

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Technical Report 2

Carbohydrate diet and reproductive performance of the fruit fly parasitoid *Diachasmimorpha tryoni* (Cameron)

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ABSTRACT
Augmentative releases of parasitoid wasps are often used successfully for biological control of fruit flies (Tephritidae) in programs worldwide. The development of cheaper and more effective augmentative releases of the parasitoid wasp *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae) may allow its use to be expanded to cover Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), a serious pest of many vegetables and most fruit production in Australia. This demands a fuller understanding of the parasitoid's reproductive biology. Mating status, fecundity and size of female *D. tryoni* were determined under laboratory conditions. A range of pre-release diets, 10% concentrations of honey, white sugar and golden syrup, were also assessed in the laboratory. Mature egg loads and progeny yields of mated and unmated parasitoid females were statistically similar, demonstrating that mating status is not a determinant of parasitoid performance. Female lifespan was not negatively impacted by the act of oviposition though larger females carried more eggs than smaller individuals, indicating a need to produce large females in mass-rearing facilities to maintain this trait. White sugar gave the highest adult female lifespan whilst honey and golden syrup shared similar survivorship curves; all were significantly greater than control females provided with water only. Pre-release feeding of *D. tryoni*, particularly with white sugar, may enhance the impact of released parasitoids on Queensland fruit fly. Findings are important because honey is currently the standard diet for mass-reared braconids, yet white sugar is less than one third the cost of other foods. Further work is required to assess post-release performance of the parasitoid.

Key words: fruit fly, biological control, sugar, honey, diet, ovigeny

INTRODUCTION

The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), is the major fruit fly pest of eastern Australia, with literature on its impact and control dating back more than 115 years (Clarke *et al.*, 2011). Decreasing availability of allowable insecticides has led to the need to explore additional control tactics, including incorporating biological control in an integrated pest management system for Queensland fruit fly. The parasitoid *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae) is endemic to Australia and was successfully introduced into Hawaii in 1913 (Duan and Messing, 1999). *Diachasmimorpha tryoni* was later used successfully on Maui, Hawaii, in an augmentative release (Wong *et al*., 1991) and subsequently in a concurrent parasitoid and sterile fly release program (Wong *et al*., 1992) to suppress a wild population of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). It is also used effectively along the Mexican/Guatemalan border (Sivinski *et al*., 2000) for the control of *C. capitata*.

Lack of suitable sugar sources for adult parasitoid wasps is recognised as an important cause of failure in biological control programs (McDougall and Mills, 1997; Bautista *et al*., 2001). Sugar (carbohydrate) consumption is known to increase the lifespan and fecundity of many parasitoid species (Siekmann *et al*., 2001) and is why many mass-rearing facilities rear adults on honey or honey solutions (Cancino and Montoya, 2006). Many parasitoids including *D. tryoni* require carbohydrates as a source of energy (Jervis *et al*., 1993; Wäckers, 2001), which are provided by the consumption of sugar-rich foods (Wyckhuys *et al*., 2008). The carbohydrate food sources provided to parasitoids are important for increasing adult lifespan, which directly influence the effectiveness of these parasitoids in biological control programs (Jacob and Evans, 2000).

The effect of different food sources on lifespan and fecundity have not been explored for *D. tryoni*, however honey is known to increase lifespan in other braconids including *Fopius arisanus* (Sonan) (Wu *et al.*, 2008), *D. longicaudata* (Ashmead) (Sivinski *et al.*, 2006) and *D. kraussii* (Fullaway) (Duan, 2000). However, it is important to gain species-specific information especially in terms of food source, in order to support mass-rearing systems and optimise biological control outcomes. Another potentially important factor that has not been investigated for *D. tryoni* is the effect of oviposition on female lifespan. Research indicates that there is an energetic cost to the act of reproduction (Wu *et al*., 2008) which may shorten the lifespan of female *D. tryoni*.

Similarly, knowledge of the reproductive biology and ecology of parasitoids is crucial when developing biological control programs based on augmentative releases (Eliopoulos *et al.*, 2003). *Diachasmimorpha tryoni*, like all wasps from the subfamily Opiinae has a haploid-diploid sex setermination mechanism in which unfertilised eggs are haploid (and males) and fertilised eggs are diploid (and female) (Wharton 1997), so it is highly preferable if the wasps are mated. *Diachasmimorpha tryoni* is a synovigenic species (Ramadan *et al.*, 2002), so it requires nutrients for gamete production potential to be reached (Cicero *et al*., 2011). Specific requirements of food, mates and suitable hosts may be needed in order for females to mature additional eggs (Wang and Messing, 2003). Subsequent egg maturation is not instantaneous in synovigenic species and eggs cannot be produced and laid immediately after finding a host (Ellers *et al*., 2000). Generally among parasitoid wasps there is little difference in the number of offspring produced between mated and virgin females (King, 2002; Riddick, 2005) but braconid parasitoids, including *Diachasmimorpha* spp., engage in complex courtship behaviour that may be disrupted by mass-rearing conditions (Joyce *et al*., 2010). Knowledge of the effect of mating status on egg load might be useful for estimating production potential of *D. tryoni* in mass-rearing systems.

Although not documented for *D. tryoni*, it is a common finding in parasitoids that large females live longer, have more eggs immediately available for laying, and produce more progeny than their smaller counterparts (Cloutier *et al*., 2000; Doyon & Boivin, 2005). Research provides strong support that fitness of females increases with size for parasitoid wasps in general (Lauziere *et al*., 2000). A larger size provides several physiological and behavioural advantages such as increased lifespan, fecundity and progeny production (Sagarra *et al*., 2001).

Studies were conducted to understand the effect of mating status and size on potential fecundity of female *D. tryoni* under laboratory conditions and the effect of three carbohydrate sources (honey, white sugar and golden syrup (56% invert syrup (glucose and fructose), 44% sucrose) at 100mL/L water concentrations) together with oviposition on lifespan and reproductive potential. The experiments addressed (1) whether virgin or mated females produce more offspring and/or matured more eggs, (2) the effect of oviposition on the lifespan of females, (3) the carbohydrate source that best promotes female lifespan and fecundity, and (4) whether female size positively affects egg load and lifespan. Information gained from these experiments will assist in developing mass-rearing protocols for *D. tryoni* in Australia and will have direct relevance to its use in overseas programs against other targets, in addition to having significance for the rearing other hymenopteran biological control agents.

METHODS

Insect cultures

Cultures of *D. tryoni* and *B. tryoni* were established from infested peaches collected at Gosford Horticulture Institute, Gosford, New South Wales (NSW), Australia in January 2011. The parasitoid and fruit fly cultures were kept in a growth room (22 ± 2°C, 65 ± 15% RH, L: D 16:8) at the Elizabeth Macarthur Agricultural Institute (EMAI), Menangle, NSW. Parasitoids were reared on the offspring of field collected *B. tryoni* larvae and supplemented when necessary with *B. tryoni* larvae from the Fruit Fly Production Facility at EMAI. Adult parasitoids were fed a standard diet of pure honey (streaked on a 50 mL cup using a paintbrush) and water (provided from a dental wick soaked in water) unless used in experiments that specified other treatments. All *B. tryoni* larvae were reared on a standard rehydrated carrot medium diet (Christenson *et al.,* 1956; Snowball *et al*., 1961) made on site. Adult flies were fed white sugar cubes, yeast hydrolysate (MP Biomedicals Australasia Pty Ltd, PO Box 187, Seven Hills, NSW, Australia) and water.

Mating status, fecundity and size

Two treatments were evaluated under laboratory culture conditions: 1) cages with five *D. tryoni* females and five *D. tryoni* males (mated) and 2) cages with five *D. tryoni* females only (virgin). Females were assigned to plastic 175x120x60 mm cages (model C500, WF Plastic, Sydney, NSW, Australia) upon eclosion and paired with males aged one to 10 days. All cages were provided with the standard diet of honey and water and were covered with synthetic gauze mesh secured with two rubber bands. Groups of parasitoids (rather than individuals) were kept in the cages so that conditions for mating and oviposition closely resembled conditions experienced during mass rearing. There were ten cages for the mated treatment (n = 50 females in total) and seven cages for the virgin treatment (n = 35 females in total); this experiment used parasitoids from three generations (F5-7) of *D. tryoni*.

After ten days in which males and females were allowed to mate and feed, males were removed from the mated cages. Each cage, including the unmated female cages, was then presented with an ovipositional unit on one occasion, which comprised a petri dish containing

28g of carrot media and approximately 100 third instar *B. tryoni* larvae as hosts (as well as honey and water) for 24 hours. After this time, each petri dish was removed and placed on a bed of moistened vermiculite (4:1 vermiculite to water) for ten days, which allowed enough time for all the host larvae present to burrow into the vermiculite and pupate, and then sieved. The number of eclosed parasitoids per treatment was recorded.

To determine the number of progeny per female (progeny yield), the total number of progeny recovered from each ovipositional unit was divided by the number of females that were still alive on the exposure date. After the parent females from both treatments had been exposed to the host larvae, they were killed (frozen) and stored in a -4 °C freezer to allow later hind tibia measurements and mature egg load counts (described below).

Carbohydrate sources, oviposition and female lifespan

Upon eclosion of F7 parasitoids, *D. tryoni* females were separated and placed individually into plastic cages (as described above). Each female was paired with one *D. tryoni* male aged one to 10 days old (i.e. male: female ratio of 1:1) and provided with one of four treatments: honey (100mL/L water), golden syrup (56% invert syrup (glucose and fructose), 44% sucrose) (100mL/L water), white sugar (cane sugar; 100% sucrose) (100mL/L water) or water only (control). These carbohydrate sources were selected because they are either typically provided in mass-rearing programs (honey, e.g. Wong and Ramadan, 1993; Sivinski *et al*., 1996), are a potentially cheaper form of carbohydrate (sugar) source, or they have been reported in the literature as prolonging the survival and enhancing the reproduction of female parasitoids (maple syrup; (primarily sucrose with small amounts of other sugars including fructose and glucose) and molasses (sucrose, glucose and fructose in a ratio of approximately 2:1:1) e.g. Bautista *et al*., 2001; in this study golden syrup was used as a readily available alternative in Australia). There were 10 females for each treatment. When the females were six days old, half of the females (five from each carbohydrate source) were presented with an oviposition unit containing a petri dish with 28g of carrot media and approximately 20 third instar *B. tryoni* larvae as hosts. Host material was exposed to the parasitoids for 24h commencing at 10:00 am AEST every three days until the females were 12 days old (i.e. days 6, 9 and 12; three occasions). The remainder of the cages (five from each carbohydrate source) were not exposed to an ovipositional unit.

Survival of females was recorded daily until death. Females were stored in a -4°C freezer until hind tibia measurements were made.

Hind tibia and egg load measurements

To determine *D. tryoni* female parasitoid size, hind legs were extended to expose the tibia for measurement with a microscope eyepiece graticule. Females were further analysed for their egg loads (under the same microscope settings) by removing the abdomen from the rest of the body with tweezers. Small holes were prodded into the abdomen using an entomological pin (0.53 mm diameter). One drop of water was placed over the abdomen to allow for suspension, and then a cover slip was used to squash the abdomen to release the eggs. Eggs were counted as above with a microscope and numbers recorded.

Statistical analyses

Separate one-way ANOVAs were used to analyse the effect of mating status on progeny yield and egg load, and the effect of oviposition on lifespan. Linear regression was used to examine the relationship between egg load and female size (hind tibia length). Survival analysis (non-linear regression, Weibull fit) was used to describe the shape of the mortality-time relationship in females from the different carbohydrate treatments. All analyses were

conducted using GenStat version 13 (Payne *et al*., 2010) except the survival analysis, which used JMP (SAS, 1995).

RESULTS

Mating status, fecundity and size

Mating status of female *D. tryoni* (10 days old) did not have a significant effect on the mature egg load available for oviposition ($F_{1, 83}$ = 0.06, P = 0.801) with mated females holding an average (±SE) of 28.3 ± 4.0 mature eggs and virgin females holding an average of 27.8 ± 4.7 mature eggs. The mature egg load of female *D. tryoni* (both mated and virgin) increased with increasing female body size (10.6 eggs for every 0.1 mm increase in hind tibia length, $F_{1, 83}$ = 26.64, P < 0.001, Fig. 1).

The mating status of females did not have a significant effect on progeny yield ($F_{1, 83}$ = 0.58, P = 0.448), with mated females producing per female an average of 0.39 ± 0.05 progeny with a 1.2:1 male:female ratio and virgin females producing per female an average of 0.29 ± 0.05 male progeny.

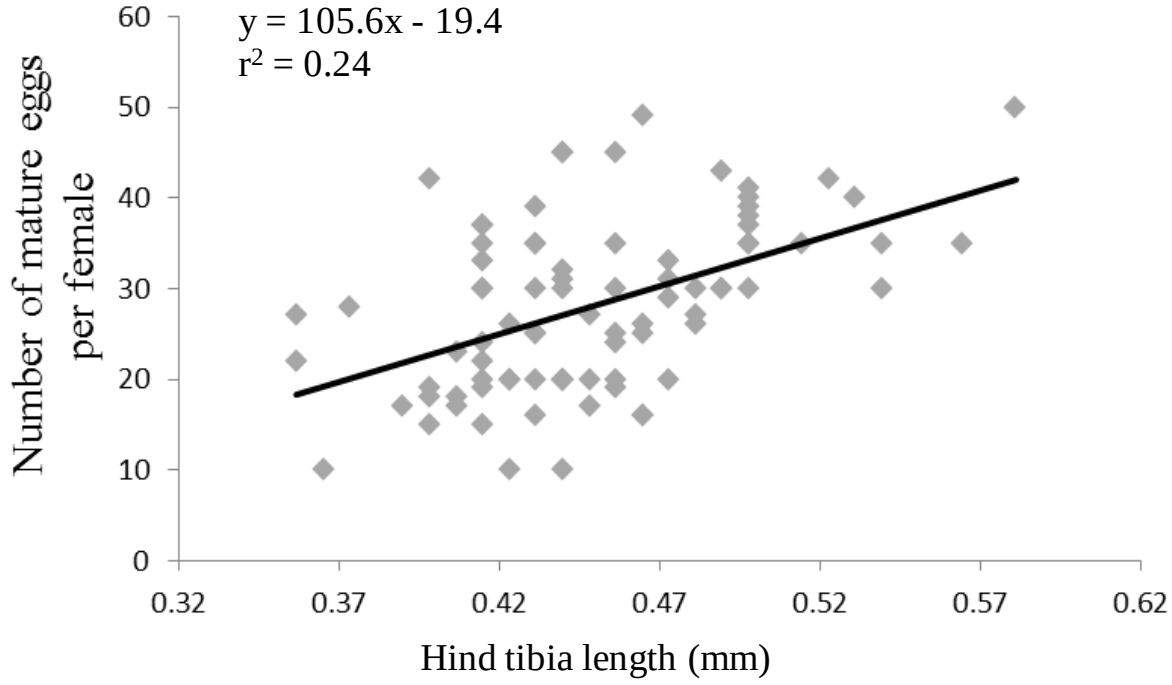

Fig. 1. Relationship between adult female *Diachasmimorpha tryoni* size (hind tibia length) and egg load.

Carbohydrate sources, oviposition and female lifespan

The act of oviposition did not have an effect on female lifespan ($F_{1, 38}$ = 0.02, P = 0.89); the average (±SE) lifespan of egg laying females was 11.3 ± 2.53 and non-egg-laying females was 10.8 ± 2.41 days. Data across the oviposition treatments was then pooled to analyse the effect of the carbohydrate treatments on lifespan. Females provided water only (the control) lived for 2.0 ± 0.6 days compared to honey: 12.0 ± 3.8 d, golden syrup: 11.8 ± 3.7 d and sugar: 17.9 ± 5.7 d (mean ± SE). Survival analysis across all four treatments proved impossible because of the very short lifespan of the parasitoids from the control (water) treatment (Fig. 2). Therefore, only the three carbohydrate treatments were included in the

survival analysis. The shape of the survival curves differed between the three carbohydrate sources ($\chi^2 = 7.90$, d.f. = 2, $P = 0.02$, Fig. 2) and showed that white sugar gave maximum survival. There was no significant relationship between female size (pooled across the three carbohydrate treatments) and lifespan ($F_{1, 28} = 0.03$, $P = 0.87$).

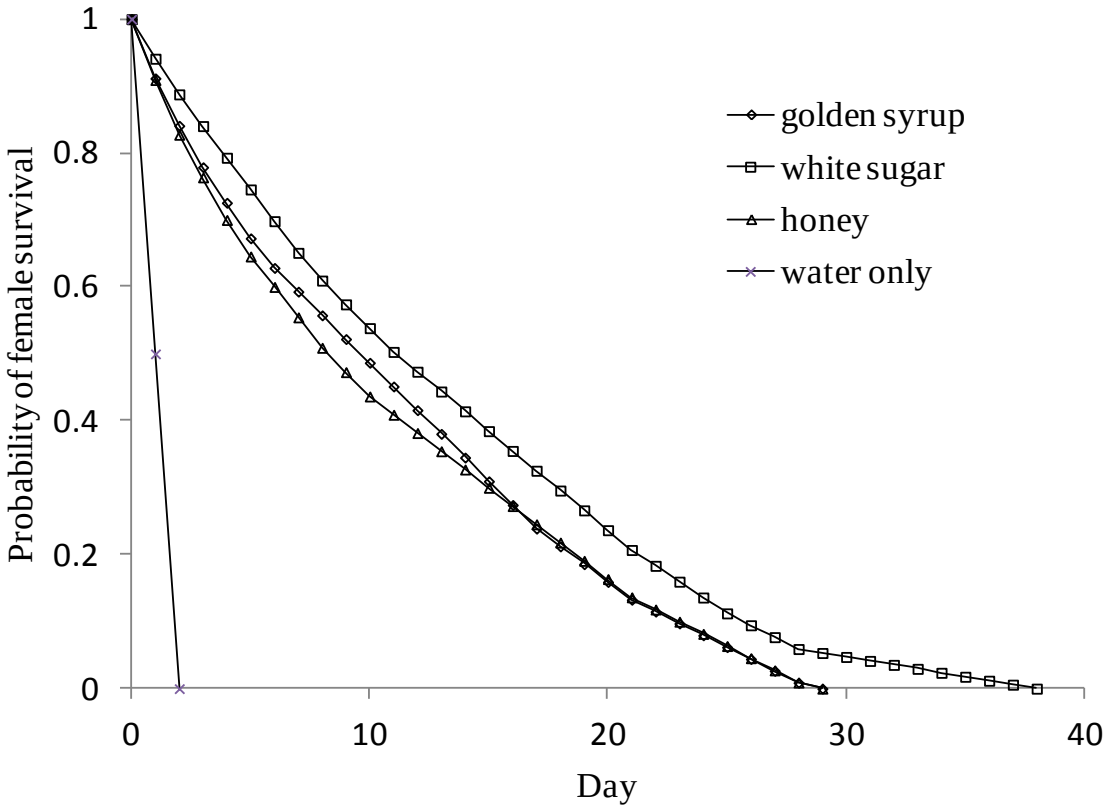

Fig. 2. Survivorship curves of female *Diachasmimorpha tryoni* fed solutions 100mL/L water) of golden syrup, white sugar, honey and water only.

DISCUSSION

White sugar maximised the survival of female *D. tryoni* under laboratory conditions. Carbohydrates are critical for survival and fecundity of synovigenic parasitoids (Stuhl *et al*., 2011) such as *D. tryoni.* Another braconid, *Cotesia glomerata* (L.), as well as the ichneumonid *Bathyplectes curculionis* (Thomson), lived longer when fed glucose and other simple monosaccharides compared with more complex carbohydrate solutions (Wäckers, 2001; Jacob and Evans, 2004). The carbohydrate concentration that maximised the lifespan of four braconid species varied from 25-75% (Azzouz *et al*., 2004; Wu *et al.,* 2008; Tompkins *et al*., 2010; Lightle *et al*., 2010) and the relationship between carbohydrate concentration and lifespan was not always linear (Wu *et al.,* 2008). Only a comparatively low concentration (100mL/L) was used in this study; higher sugar concentrations are expected to increase lifespan further in *D. tryoni* but the optimal concentration has not yet been determined. Indeed, if sugar benefits overall performance of the parasitoid (including age-specific

fecundity) as effectively as either honey or golden syrup, savings could be made in the cost of materials used in rearing. At US$0.21/100g, sugar is a far cheaper substrate (c.f. honey: US$1.16/100g and golden syrup: US$0.72/100g).

The act of oviposition did not shorten the lifespan of *D. tryoni* but the females in this study were only exposed to hosts three times during their lifetime and few progeny emerged. For *Meteorus pulchricornis* (Wesmael) the sugar concentration that maximised lifespan also maximised lifetime progeny production (Wu *et al.*, 2008). Further investigation of the interaction between diet, oviposition and lifespan is warranted for *D. tryoni* to determine the carbohydrate source that optimises both oviposition and life span, as adult insect feeding allows the utilisation of carbohydrates that may be required for the development of the reproductive system (Jordao *et al*., 2010). An optimal carbohydrate source such as white sugar will be important to the success of augmentative biological control in conjunction with high quality (large) females (Jacob and Evans. 2000; Wäckers. 2001).

Female size under laboratory conditions did not have a positive correlation with female lifespan but was positively correlated with egg load for *D. tryoni*. Similarly, the bethylid parasitoid, *Cephalonomia stephanoderis* (Betrem) showed a positive correlation between size and egg load but not lifespan (Lauziere *et al*., 2000). It is common for larger female parasitoids to live longer (e.g. Sagarra *et al*., 2001; Doyon and Boivin, 2005). The relationships between female size, lifespan and egg load are strongly influenced by the amount and type of food available and by access to hosts (Godfray, 1994) but the nature of these relationships (positive or negative) varies from species to species (Bautista *et al*., 2001; Eliopoulos *et al.*, 2003; Wang and Messing, 2003). Female size accounted for 24% of the variation in mature egg load for *D. tryoni*, indicating that size is only one of the factors to determine egg load in this synovigenic species.

The mating status of *D. tryoni* influenced neither egg maturation nor progeny yield (a proxy measure for attack/parasitism rate). This implies that mating status will not affect the initial efficacy of augmentative biological control as both virgin and mated females have the capacity to produce similar numbers of mature eggs and therefore progeny. For ongoing biological control it is important that females mate successfully and are able to produce female offspring but from this study there did not appear to be any advantage of parasitoids mating prior to release. Again, the relationships between mating status, egg maturation and progeny yield are species-specific and the effects can be quite subtle (Wang and Messing, 2003).

Experiments into improving mass-rearing techniques are vitally important to the success of the augmentative release of biological control agents. The use of white sugar increased female lifespan in the laboratory, which may indicate a need to compare white sugar at varying concentrations with the current practice of providing pure honey. These carbohydrate sources also need to be tested on parasitoids from a wider sampling range and for their effect on other aspects of *D. tryoni* behaviour such as flight (which is known to be energetically expensive for parasitoids (Hausmann *et al.*, 2005)) and foraging in order to gain a better understanding of the carbohydrate source that will optimise the performance of this biological control agent in the field.

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Technical Report 3

Longevity of *Diachasmimorpha tryoni* (Cameron) fed different fruit-based diets

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INTRODUCTION

Bactrocera tryoni Froggatt (Diptera: Tephritidae) is the most significant pest of the fresh fruit and fruiting vegetable industries. Key pesticides used to control *B. tryoni*, have either recently been withdrawn from most uses (dimethoate), or are under review (fenthion) by the Australian Pesticides and Veterinary Medicines Authority (APVMA) and soon likely to have the same fate. It is therefore very timely that alternative control methods are explored, and this area of research is gaining momentum for *B. tryoni* control. One alternative method is the augmentative release of parasitoids. Augmentative release involves the mass-rearing and release of additional numbers of a natural enemy, when too few are present to control a pest effectively. *Diachasmimorpha tryoni* (Cameron) is a larval parasitoid of *B. tryoni* and is native to Australia. Recent surveys have demonstrated its presence in inland New South Wales (NSW) near some of our major horticultural production areas, although only in low numbers (Spinner et al., 2011). This suggests this species is able to persist in this area and might therefore be suitable for augmentative release. In addition, this parasitoid has been released overseas for the control of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Wong et al. 1991; Wong et al. 1992; Sivinski et al. 2000). Longevity is pivotal in augmentative release programs, as the released parasitoid benefits from a longer life period in order to locate its host and reproduce. Interventions pre-release can provide a parasitoid with the best chance of survival once released. The provision of food is one such intervention. Studies have shown that feeding parasitoids pre-release with some form of sugar source can significantly increase longevity (Siekmann et al. 2001; Cancino and Montoya 2006). However, this has not been studied for *D. tryoni*. Here we aim to compare the use of honey, which is the most common sugar source used in mass-rearing (Cancino and Montoya 2006) with different fruit types commonly encountered by the parasitoid throughout much of its range in inland NSW. A related parasitoid, *Diachasmimorpha longicaudata* (Ashmead) reportedly consumes juices oozing from wounded citrus and other fruits (Sivinski et al. 2006).

MATERIALS AND METHODS

Insect cultures

Cultures of *D. tryoni* and *B. tryoni* were established from infested peaches collected at Gosford Horticulture Institute, Gosford, New South Wales (NSW), Australia in January 2011. The parasitoid and fruit fly cultures were kept in a growth room (22 ± 2°C, 65 ± 15% RH, L: D 16:8) at the Elizabeth Macarthur Agricultural Institute (EMAI), Menangle, NSW. Parasitoids were reared on the offspring of field collected *B. tryoni* larvae and supplemented when necessary with *B. tryoni* larvae from the Fruit Fly Production Facility at the Elizabeth Macarthur Agricultural Institute. Adult parasitoids were fed a standard diet of pure honey (streaked on a 35 mL plastic cup using a paintbrush) and water (provided from a dental wick soaked in water) unless used in experiments that specified other treatments. All *B. tryoni* larvae were reared on a standard rehydrated carrot medium diet (Christenson et al. 1956; Snowball et al. 1961) made on site. Adult flies were fed white sugar cubes, yeast hydrolysate (MP Biomedicals Australasia Pty Ltd, PO Box 187, Seven Hills, NSW, Australia) and water.

Longevity of D. tryoni

Upon eclosion of F11 parasitoids, 15 *D. tryoni* adults (10 females and 5 males) were placed into each of 30 mesh cages (30 x 30 x 30 cm, Bugdorm, Taiwan). Each cage was provided with one of six treatments: honey (streaked on three inverted plastic cups), quartered orange (two quarters/cage), orange juice (from half an orange), whole apricot (halved, seed removed), whole macerated apricot (halved, seed removed and the flesh macerated), or water

only (control). Whole, halved or quartered fruit were placed on a 100mm diameter plastic petri dish within each cage. Orange juice (navel oranges, halved and juiced) was poured into 35-mL clear plastic containers (Solo, P101M, Urbana, Illinois, USA) accessed via a cotton wick, with one provided per treatment cage. All cages were provided with water soaked cotton wicks. Honey was selected as it is commonly provided in mass-rearing programs (e.g. Wong & Ramadan 1993; Sivinski et al. 1996), while fruit juice and pulp were chosen as several parasitoid species have demonstrated survival on orange pulp and juice that often equalled that obtained on a honey and water diet (Stuhl et al. 2011). There were five cages for each treatment. Survival of males and females was recorded daily until death.

Statistical analysis

Data was analysed using a parametric survival regression analysis (Kalbfleisch and Prentice, 1980) with the hazard function fitted with Weibull distribution. The survivor function of parasitoids for each treatment group (combination of diet and gender) was considered as follows:

$$S(t) = \exp(-\lambda t^{\alpha})$$

With link function being

$$\log_e(\lambda) = \text{Treatment effect}$$

Where λ is a treatment constant (representing the daily mortality rate) used in the Weibull hazard function and α is Weibull distribution shape parameter. The number of days when a proportion ($\boldsymbol{p}$) of the parasitoids subjected to the various diets would survive was calculated using the following inverse survival function:

$$Z(\boldsymbol{p}) = \{-(1/\lambda)\log_e(\boldsymbol{p})\}^{1/\alpha}$$

All analyses were performed on GenStat version 14 (Payne et al., 2011). Least significant difference (LSD) limits at 5% level were calculated for each group to compare the effects of diet by gender on the daily mortality rates.

RESULTS AND DISCUSSION

Carbohydrates are critical for survival and fecundity of synovigenic parasitoids (Stuhl et al., 2011) such as *D. tryoni*. The carbohydrate honey (H) maximised the survival of female *D. tryoni* under laboratory conditions (Table 1). Parasitoids fed honey had at least 10% survival beyond 24 days, compared to the other diet treatments with 90% of parasitoids surviving a maximum of 11 days. The coefficient of the survivor regression was highest for parasitoids (female and male) supplied water only, which corresponds to the highest mortality rate per day (Table 1). Daily mortality rates of the parasitoids fed with either cut apricot (HA) or Orange juice (OJ) did not differ significantly from those fed with water only. The predicted survivorship curves are shown in Figure 1. Stuhl et al., (2011) compared the mean longevities of three species of parasitoids which attack *Anastrepha* spp., *Diachasmimorpha longicaudata* (Ashmead), *Doryctobracon areolatus* (Szepligeti) and *Utetes anastrephae* (Viereck) fed either honey, guava juice, guava pulp, orange juice, orange pulp or water only. The authors showed that for all three species, when provided with the pulp and juice of guava, these did not differ from those provided water alone. The lifespan of male *D. areolatus* and *U. anastrephae* fed orange juice were not significantly different to those given guava juice. In

male and female *D. areolatus* and *U. anastrephae*, survival on orange pulp was greater than on juice, however there was no difference between pulp and juice for male and female *D. longicaudata*. In general, survival of all three species fed orange diets were better than either water alone or guava diets, with longevity often similar to those fed a honey diet.

Given so little is known about the dietary requirements or indeed potential food sources in the field of braconids parasitoids, an assessment of their natural foods could lead to diet improvements. Although the fruits tested in this study did not lead to increased longevity compared with honey, further testing is required on a range of fruit/plant sources and on other life history parameters. Such dietary enhancements could produce a healthier mass-reared parasitoid with greater fecundity and survival and ultimately a more effective biological control agent (Stuhl et al. 2011).

Table 1. Survival regression coefficients, standard error and scale parameter (λ) for treatment groups; Weibull shape α = 1.71; λ = exp (Coefficient); OJ=Orange juice, HO= Cut orange, W=Water, HA=Cut apricot, MA=Macerated whole apricot, H=Honey

Group	Parameter	SE	Student t value	Coefficients	λ †	Days at least 10% survived
OJ male	-2.315	0.200	*	-2.315	0.0988 abc	6.3
OJ female	-0.201	0.245	-0.82	-2.516	0.0808 bcd	7.1
HO male	-0.493	0.283	-1.74	-2.808	0.0603 cde	8.4
HO female	-0.702	0.245	-2.87	-3.017	0.0489 de	9.5
W male	0.343	0.283	1.21	-1.972	0.1392 ab	5.2
W female	0.522	0.246	2.12	-1.793	0.1665 a	4.6
HA male	0.093	0.280	0.33	-2.222	0.1084 abc	6.0
HA female	-0.152	0.247	-0.62	-2.467	0.0848 abcd	6.9
MA male	-1.008	0.275	-3.66	-3.323	0.0360 e	11.4
MA female	-0.564	0.248	-2.28	-2.879	0.0562 cde	8.8
H male	-1.813	0.285	-6.36	-4.128	0.0161 f	18.2
H female	-2.292	0.244	-9.39	-4.607	0.0100 f	24.1

† values with the same letter are not significantly different at 5% level

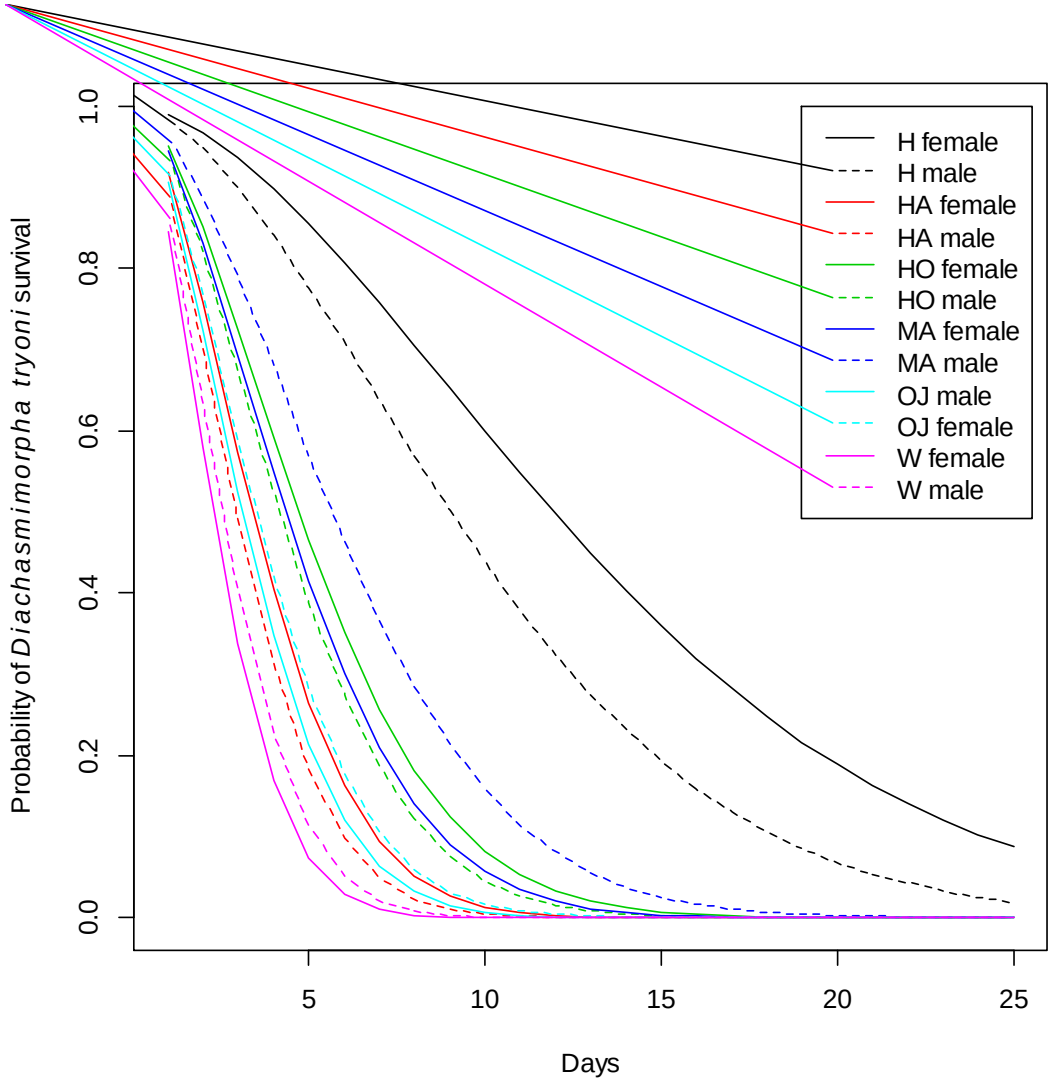

Figure 1. Survivorship curves of male and female *Diachasmimorpha tryoni* parasitoids fed various diets. OJ=Orange juice, HO= Cut orange, W=Water, HA=Cut apricot, MA=Macerated whole apricot, H=Honey

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Review 1

Synergizing biological control: Scope for sterile insect technique, induced plant defences and cultural techniques to enhance natural enemy impact

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ABSTRACT
When used alone, only a minority of biological control programs succeed in bringing the target pest population under sufficient control. Biological control is, therefore, usually employed with chemical, cultural, genetic or other methods in an integrated pest management (IPM) strategy. The interactions between different pest management methods, especially conventional pesticides and host plant resistance, is an area of growing research interest but relatively little consideration is given to novel combinations. This paper reviews the interactions between biological control and other forms of pest management, especially induced plant defences and the novel, non-toxic plant protection compounds that may boost these defences; and sterile insect technique. We also cover the cultural methods that offer scope to support synergies between the aforementioned methodological combinations. We conclude that despite the sometimes negative consequences of other pest management techniques for biological control efficacy, there is great scope for new strategies to be developed that exploit synergies between biological control and various other techniques. Ultimately, however, we propose that future use of biological control will involve integration at a greater conceptual scale such that this important form of pest management is promoted as one of a suite of ecosystem services that can be engineered into farming systems and wider landscapes.

Key words: integrated pest management, induced plant defences, herbivore induced plant volatiles, silicon, induced defenses, landscapes, ecosystem services, ecological engineering.

INTRODUCTION

It is human nature to reduce complexity to simplicity, seek to 'pigeon hole' and categorize; indeed the very process of science is reductionist. Ultimately, however, the real world is complex and attempts to manage a system rarely succeed when an overly simple approach is taken. Biological control has developed into a large and diverse field but remains just one of several pest management methods. According to Way and van Emden (2000), "the IPM toolbox has never been fuller". The global increase in genetically modified crop varieties with insect resistance is one new "tool" and its interactions with biological control – both negative and positive – have been reviewed by Altieri (2004) and continue to be the subject of empirical research (eg Chen *et al*., 2007). But the last decade has also witnessed a high level of interest in conservation biological control, sterile insect technique and induced plant defenses. It is timely, then, to review the nature of interactions between varying pest management approaches and consider their compatibility for IPM. It is appropriate that the compatibility of differing pest management approaches be considered from a biological control perspective because biological control is often considered to be the foundation for pest management systems (Van Driesche and Bellows, 1996) (Figure 1).

Interactions between biological control, host plant resistance and pesticides.

A great deal of research attention has been devoted to the impact of conventional pesticides on natural enemies and this is the topic of Gentz *et al*. (this volume) so reviewed only briefly here in relation to the interactions of pesticides with host plant resistance and biological control. Interactions involving novel plant protection compounds are covered more fully. Host plant resistance may make pests more susceptible to insecticides by slowing their growth such that they are smaller and less well developed or less well nourished at any given time. Such an effect may be especially powerful when penetration of plant tissue by boring or mining pests is delayed (e.g. Kvedaras and Keeping, 2007). An increase in pest susceptibility may also allow the use of an insecticide concentration that is low enough to allow many natural enemies to survive whilst still conferring a high level of mortality of the pest (van Emden, 1990). Such a relationship between host plant resistance, biological control and dose- adjusted pesticide use would allow the application of a product to bring a pest outbreak under control whilst maintaining the within-crop community of natural enemies to persist and provide ongoing protection from future pest establishment. Despite the attraction of this system we are unaware of it being actively practiced in any agricultural system to the extent that pesticide doses are actually reduced. A likely explanation for this is the challenge of reliable data capture from the field with rapid feedback to the farmer of robust management recommendations. If the conceptually ideal pest management system requires complex sampling or lengthy laboratory analyses, the time delays and costs may render the most elegant of theoretical systems impracticable. An illustration of this is provided by recent attempts to rationalize pesticide use for cotton aphid control. Steinkraus (Steinkraus, 2007) report a system whereby crop monitoring involved returning aphids to the laboratory for light microscopy to check for presence of capilliconidia (the infective stage) of the entomopathogenic fungus *Neozygites fresenii* (Nowak). If the proportion of individuals with these minute, lemon-shaped spores on their cuticle was more than 15% a recommendation not to spray was communicated to the farmer. Though the system was technically sound and resulted in effective pest management with lower intensity of insecticide applications and reduced costs were employed, a constraint to its wider adoption was human in nature. Processing of samples required a moderate level of technical skill and laboratory processing, and the workload was highly concentrated into only a few weeks of each year. Thus human and physical infrastructure were not used for most of the year and

struggled to meet demand for the critical time period. Future solutions to such impediments to better use of biological control may come from technological or human system developments. In the short term, the processing of aphid samples could be carried out by large public organizations such as State departments of agriculture that could use the human and physical capacity for other tasks for the remainder of the year. In the longer term, a bioassay that could provide immediate results in the field such as with the enzyme-linked immunosorbent assay (ELISA) method or with more advanced forms of DNA barcoding that lead to field test kits would put analysis of samples and decision making in the hands of (quite literally) farmers or crop scouts.

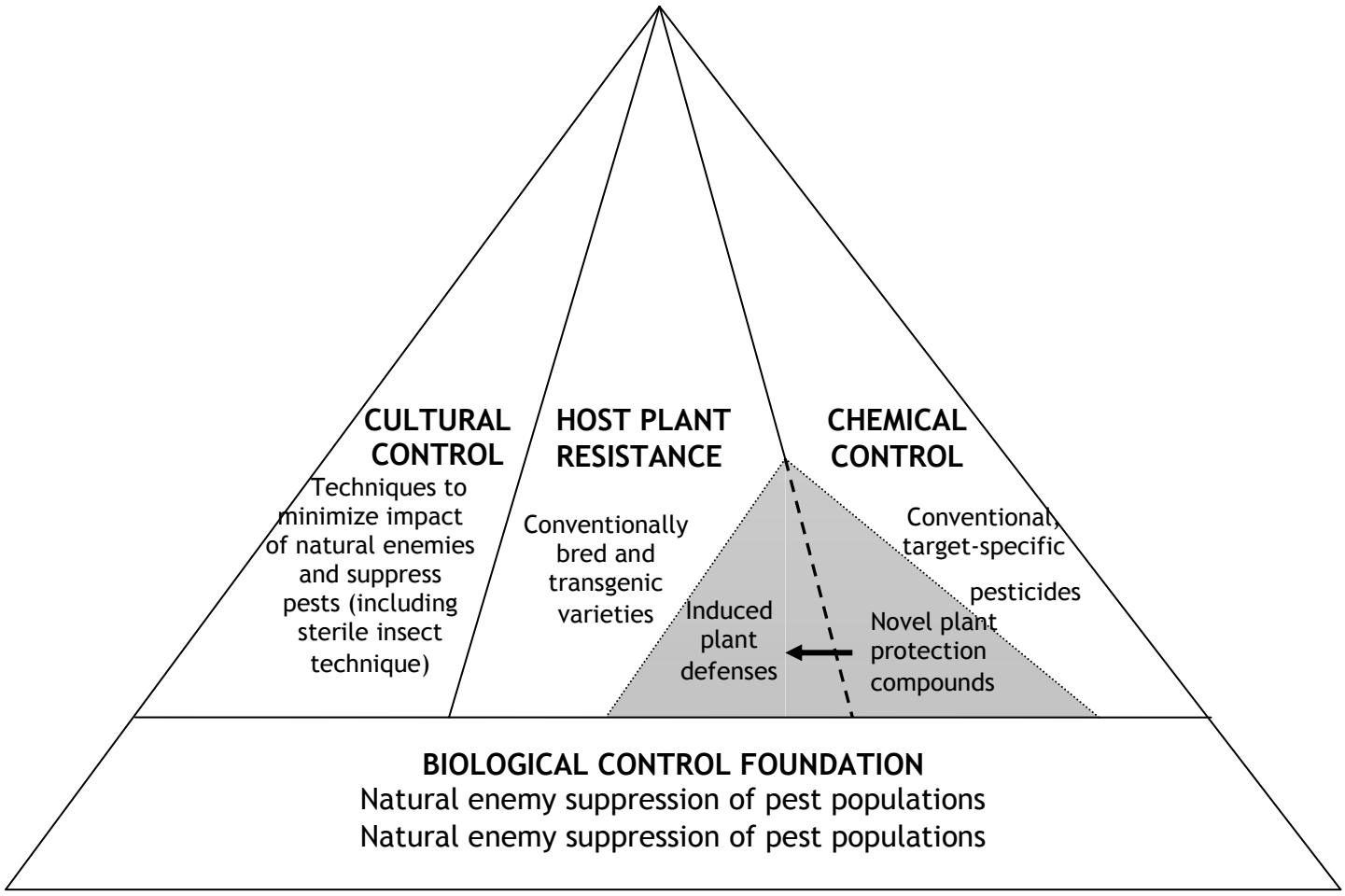

Figure 1. Biological control conceptualized as the foundation of integrated pest management and illustrating some of the positive interactions with other pest management approaches. Adapted from van Driesche & Bellows (1996) *Biological Control*, Chapman and Hall, New York, p. 297.

Biological control and host plant resistance

The direct interactions of biological control with host plant resistance have received much research attention and, notwithstanding the many cases of negative interactions (Simmons and Gurr, 2005), synergies are often evident whereby the combination of both methods results in superior pest suppression than when either biological control or host plant resistance are used alone. For example, population growth of the aphid *Schizaphis graminum* (Rondani) has been investigated on susceptible and (conventionally bred) partially resistant varieties of barley with and without the parasitoid *Lysiphlebus testaceipes* (Cresson) (Waterhouse, 1999). In that system, biological control alone resulted in slower population growth of the aphid than occurred on either variety of barley, but the combination of biological control with the partially resistant variety resulted in much lower population growth. Even in cases where detrimental effects of host plant resistance are recorded the combined effect of biological control and resistant crop variety results in fewer pests than does either method alone. For example, in work with *Aphis fabae* (Scopoli), aphids reared on a partially resistant faba bean variety adversely affected the embryonic larval development, pre-oviposition period, fertility and fecundity of the predator *Coccinella septempunctata* (Linnaeus) (Shannag and Obeidat, 2008). Importantly, however, a significant decrease in the number of aphids was achieved compared with any other combination (Figure 2).

Novel plant protection compounds and induced plant defences

The phenomenon of host plant resistance 'breakdown' (actually a change in the pest population rather than any change in the plant) has threatened many traits that originally gave highly effective pest control (e.g. Shen *et al*., 2003). One response to this is exploration of completely novel host plant resistance traits. Amongst the most exciting of these are the induced defense mechanisms of plants. Far from being 'sit there and take it' victims of herbivory, plants have evolved a range of defenses that can be 'switched-on' by herbivore feeding or even oviposition (Khan *et al*., 2008). Induced defenses are widely recognized as an important type of plant defense strategy, particularly in cases where defenses are costly or the threat of herbivore attack is intermittent and predictable from prior exposure (Arimura *et al*., 2005). Amongst these defense mechanisms is the ability to release volatile compounds that recruit predators and parasitoids of pests. These herbivore-induced plant volatiles (HIPVs) are released in response to herbivore damage to aid location by predators and parasitoids of plants where their prey or hosts, respectively, are present. HIPVs and their potential use in IPM have recently been reviewed by Khan *et al*. (2008) so this section focuses chiefly on novel aspects of their use.

Known HIPVs include methyl salicylate, methyl anthranilate, methyl jasmonate, benzaldehyde, cis-3-hexenyl acetate and cis-hexen-1-ol. The qualitative and quantitative characteristics of HIPV blends vary according to the herbivore involved and the plant species (Turlings & Tumlinson, 1993; Takabayashi *et al*., 1994). Work in the USA (Khan *et al*., 2008) has shown that plant-derived or synthetic versions of these chemical cues will attract beneficial insects into treated crops. Current field studies in Australia have shown attraction of parasitoids such as *Trichogramma* spp. to grapevines and brassicas treated with methyl anthranilate and benzaldehyde (Simpson, M. R., personal communication, 15 November 2008). Thus, HIPVs offer potential for manipulating natural enemy populations in a manner that is far more precise than is the norm in biological control.

Much, however, remains to be resolved before HIPVs can be used commercially to enhance biological control. A key issue that needs to be resolved is the relative importance of direct and indirect effects. Exogenous HIPVs may function as direct attractants, that is, by

constituting a signal recognized by natural enemies immediately after application to a plant. HIPVs may also act indirectly on natural enemies by causing plants to emit endogenous volatiles which are then detected by natural enemies. The latter, as well as being a potentially more effective signal, would also be longer lasting than would the influence of artificially applied HIPVs alone, making novel plant protection products based on HIPVs more viable. The likelihood of exogenous compounds triggering production of endogenous compounds is supported by a significant body of recent results. Airborne or topically applied

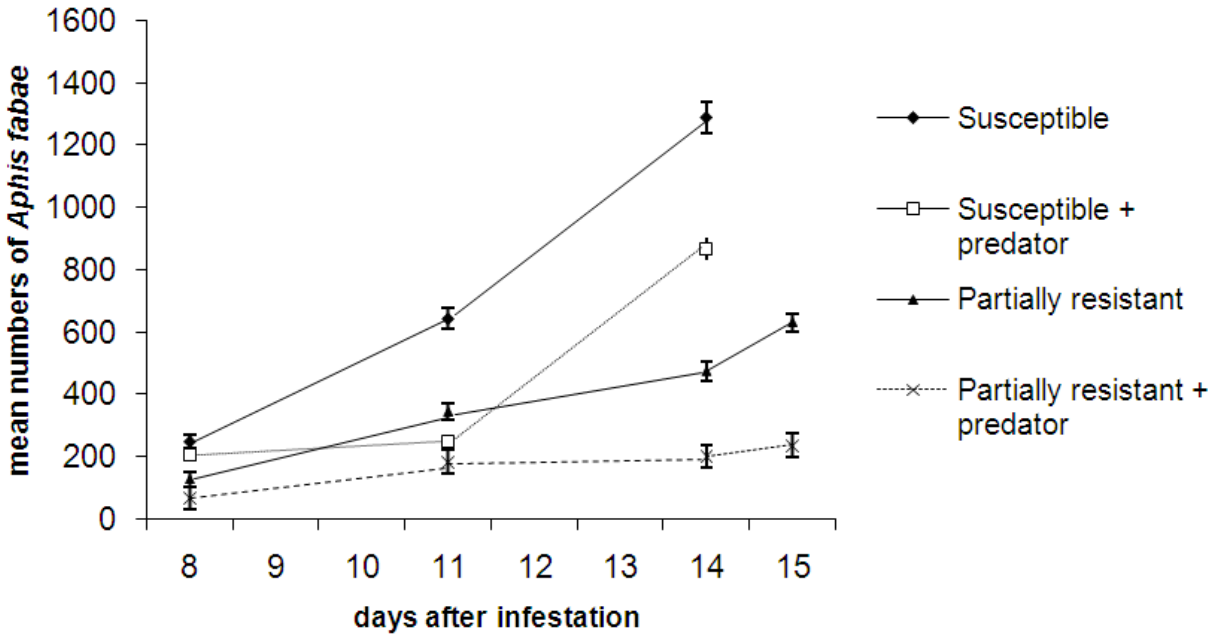

Figure 2. Example of a positive interaction between a host plant resistance and biological control: effect of partial plant resistance faba bean (variety 7954) and a predator (*Coccinella septempunctata*) on numbers of *Aphis fabae*. Drawn using data from Shannag and Oneidat (2008). *Annals of Applied Biology* **152**, p. 334.

methyl jasmonate (MeJA), for example, can cause the emission of volatiles in some plants similar to those produced in response to herbivore damage (Hunter, 2002). There is evidence that methyl salicylate and hexenyl acetate also function as elicitors of plant signaling (Shulaev *et al*., 1997; Ozawa *et al*., 2000; Engelberth *et al*., 2004). Work on rice demonstrated a role of ethylene signaling in induced defenses against arthropod herbivores (Lu *et al*., 2006). Plants attacked by *N. lugens* produced ethylene 2-24hr after infestation along with HIPVs and *Anagrus nilaparvatae* (Pang & Wang), a parasitoid of *N. lugens*, was attracted to emitting plants. Further, exogenous application of ethephon (a compound that breaks down within the plant to produce ethylene) resulted in a similar HIPV profile to that produced by rice brown planthopper-infested plants as well as attraction of its parasitoid. The same authors also considered it likely that *N. lugens* activates other - most notably the salicylate - signaling pathways. In other work, exogenous applications of jasmonic acid to rice plants have led to dramatically elevated levels of several volatiles including aliphatic aldehydes, alcohols, monoterpenes, sesquiterpenes, methyl salicylate and *n*-heptadecane (Lou *et al*., 2005). The potential for such chemical ecology to be developed into a practical pest management strategy is evident from a doubling of parasitism of *N. lugens* eggs by *A. nilaparvatae* on control rice plants that were surrounded by rice plants to which jasmonic

acid had been applied. It is likely that other parasitoids, as well as rice pest predators, make use of such plant-provided chemical cues. The same cues may also affect pest behavior, making treated plants less attractive to planthoppers (Karban and Chen, 2007).

A further complication in the use of HIPVs in practical pest management is the interaction between plant defenses against differing taxa of pests. Crops are often attacked simultaneously by pests as differing as arthropods and fungi. Recent work illustrates synergies between the metabolic pathways controlling systemic acquired resistance (SAR) and natural enemies that may result. In a study of pathogens of maize plants and their attraction of parasitoids, application of a salicylic acid mimic led to SAR against the pathogen *Setosphaeria turcica* (Luttr.). Moreover, when benzo-(1,2,3)-thiadiazole-7-carbothionic acid *S*-methyl ester (BTH) was applied to maize seedlings prior to damage by *Spodoptera littoralis* Boisduval caterpillars, treated plants that were under attack from caterpillars were far more attractive to the parasitoid *Microplitis rufiventris* (Kok) than were caterpillar-damaged but untreated plants (Rostás and Turlings, 2008). Thus SAR, whether natural or artificially-induced with compounds such as BTH, may not only be compatible with indirect defenses based on natural enemy attraction but actually enhance biological control.

Other issues that require research before commercial use of exogenously-applied compounds that trigger plants' induced defenses include the possibility that these defenses may be so metabolically costly that yield reductions occur. Such fears are not supported by recent authors (Aharoni *et al*., 2005; Turlings and Ton, 2006). Work on maize by Engelberth *et al*. (2004) suggested that HIPVs at low concentrations have no effect until a treated plant is subsequently attacked by pests (see also Turlings and Ton, 2006). The prior application of an HIPV then gives an augmented level of natural HIPV production.

An additional concern is that artificially-induced production could interfere with the short-range detection of pests by natural enemies and the longevity of their response. That is, the ubiquity of the chemical signal may erode a predator or parasitoid's response to specific prey or host presence either spatially or temporally. Though there is field evidence against such negative consequences from maize intercropping systems (Khan *et al*., 1997), future work will need to use methods such as sentinel baits to determine whether such an effect operates to any significant degree.

A final potential problem with the use of HIPVs to attract natural enemies into a specific crop is that they could starve or leave unless suitable prey or hosts are available. Over time, the response of natural enemies to the chemical cues could diminish unless HIPV applications were well timed. One strategy that would reduce the risk of this potential effect is to employ effective monitoring. Since this is a foundation of IPM and monitoring protocols exist for most major pest species, all that would be required is to develop appropriate thresholds to guide the timing of HIPV application. A further, perhaps complementary, 'attract and reward' strategy has been postulated (Khan *et al*., 2008). The 'reward' component of this approach aims to maximize the fitness and performance of attracted natural enemies by providing appropriate sources of nectar, pollen and shelter. A still wider scale ecological engineering approach could also be used whereby the use of HIPVs and reward treatments to make crops powerful sinks for natural enemy populations is coupled with manipulation of the nearby non-crop habitat. This should aim to provide refuge areas and either 'corridor' or 'stepping stone' vegetation to facilitate movement of biological control agents into crop sinks.

Of course, HIPVs may not be the only type of compound with scope to enhance biological control. A growth in the level of research interest in the effects of silicon on plants illustrates one research avenue that has barely been explored by biological control

researchers. Whilst silicon is the second most abundant element in soil (Ma and Yamaji, 2006), plant available forms of silicon are often deficient, especially in old, leached soils or areas with a long history of cropping. Application of silicon fertilizer may have several agronomic benefits including improved plant growth and increased yield (Epstein, 1994; Ma, 2004). Silicon is taken up by plants as soluble silicic acid ($Si(OH)_4$) and deposited in various parts of the plant as solid amorphous silica ($SiO_2 \cdot nH2O$) (Raven, 1983). McNaughton *et al.* (1985) suggested that silicon could be an important anti-herbivore agent in agricultural systems and its accumulation in plant leaves could increase leaf tissue toughness and thus potentially reduce herbivore damage. Recent work (Kvedaras and Keeping, 2007; Kvedaras *et al*., 2007) showed the application of calcium silicate significantly reduced the growth rate, survival and penetration of the borer, *Eldana saccharina* (Walker) in sugarcane. It is long recognized that silicon can enhance the constitutive plant defenses (i.e. those that are expressed continually even in the absence of biotic or abiotic stressors), but there is now evidence for enhancement of induced chemical defenses (Ma, 2004; Hammerschmidt, 2005). Available studies of the role of silicon in induced resistance are largely confined to plant pathogens, a fact that is surprising given the wealth of evidence from non-silicon-related studies for induced resistance being important in plant defense against arthropod pests (Gatehouse, 2002). Silicon-accumulating plants supplemented with silicon, translocate silicic acid throughout their tissues and, when attacked, produce systemic stress signals such as salicylic acid and jasmonic acid (Fauteux *et al*., 2005) that are key to plant induced defenses (Gatehouse, 2002). Silicon has been postulated to play two important roles in plant chemical defense: (i) enhanced signal transduction at the cellular level leading to an increase in induced systemic resistance and (ii) modulation of the generation of systemic signals (Fauteux *et al*., 2005). Work by Gomes *et al*. (2005) demonstrated the significance of silicon for induced plant defences. Application of calcium silicate to wheat plants that were exposed to the aphid, *S. graminum*, elevated the activity levels for three plant enzymes involved in plant defense and suppressed aphid reproduction. Only one study, however, has tested for the effect of silicon on pests via the activity of predators and parasitoids (Moraes *et al*., 2004). That work showed no effect of silicon on natural enemies, but it employed non-choice conditions in which parasitoid wasps were confined at a small scale on individual plants that were not widely spaced. The experiments that Moraes *et al*. (2004) conducted with predators were still less conducive to detection of induced plant defences involving HIPVs; aphids were removed from the test plants and fed to predators that were not exposed to plants at all. Work recently commenced in Australia by the authors is methodologically and conceptually more advanced in the use of choice tests in which parasitoids and predators range over widely spaced plants (so that effects of HIPVs are evident). Under these more natural conditions, any effect of silicon on the plants' ability to mount an induced response by attracting natural enemies will be apparent.

The potential for exogenously-applied compounds such as silicon amendments and HIPVs acting as elicitors of induced plant defenses is an exciting possibility for manipulating biological control agents in pest management. Further, if the promising results to date translate to the availability of novel plant protection compounds that promote host plant resistance traits operating via the third trophic level it may prove to be a durable strategy. Conventional pesticides and currently used host plant resistance traits operate directly on pests by mechanisms such as direct toxicity, antifeedant or antixenotic mechanisms. In contrast, HIPV- or silicon-based plant protection compounds that operate via an enhancement of natural enemy activity are likely to be less prone to a diminution of efficacy as a result of genetic adaptation of the target pest population. This is so because

pest suppression is likely to be via more than one biological control agent, possibly multiple guilds of agents that would be able to adapt in response to any shift in pest phenotype.

The foregoing sections illustrated ways in which host plant resistance traits may operate in synergistic ways with biological control, especially if integrated with novel plant protection compounds. It is recognized, however, that plant defenses can exert lethal and sub-lethal effects on natural enemies (Simmons *et al*., 2006). One reason for this is because plant breeding to date has strongly favored host plant resistance mechanisms that act directly upon pests with a corresponding neglect of the mechanisms that operate via natural enemy activity. By development of strategies that exploit more fully the subtleties produced from the millions of years of co-evolution by angiosperms and arthropods, both herbivorous and entomophagous, new and more sustainable synergies could be achieved in IPM. In the short term, however, biological control will be best served by adopting approaches that reduce the effects of conventional pesticides. Mitigating the adverse indirect effects of pesticides on natural enemies could employ some of the cultural techniques detailed later in this paper. For example, the preservation of non-crop vegetation near (or connected by suitable corridors) to crops could be important sources of biological control agents (Schellhorn *et al*., 2000; Tscharntke *et al*., 2008) Further, nectar or pollen sources within crops could support natural enemies during periods of prey scarcity following the use of a narrow-spectrum insecticide.

Interactions between biological control and the sterile insect technique (SIT)

Sterile insect technique is an environmentally-friendly option to suppress insect pest populations and even eradicate geographically isolated outbreaks. It uses mass-reared insects that are irradiated before release to render them infertile. The success of SIT relies on sterile releases 'overflooding' the wild population, minimizing the possibility of wild males and wild females mating to produce viable eggs. This biologically-based approach has enjoyed significant success around the world (e.g. Alphey, 2007). A drawback, however, is that SIT can be expensive, especially when used against dense or widely dispersed pest populations (Parker and Mehta, 2007). Biological control using inundative or augmentative release of parasitoids is an alternative or extension to SIT that has resulted in effective suppression of target species in several regions of the world, for example, opiine braconid wasps to control members of the family Tephritidae in Latin America and parts of the United States (Ovruski *et al*., 2000), several biological control agents for the management of various pest insects in diverse crops in Latin America (see review by Van Lenteren and Bueno (2003) and the native pupal endoparasitoid, *Chouioia cunea* (Yang) for the control of the fall webworm, *Hyphantria cunea* (Drury), in areas of China (Yang *et al*., 2006). Of greater relevance to this review, however, is that empirical studies and population modeling strongly suggest that a synergistic interaction between released parasitoids and SIT may give more rapid and cheaper pest suppression or eradication (Sivinski, 1996). Here we deal with only those studies that have combined the two methods of control and only those insects that are plant pests.

An augmentative release program is an attractive extension of SIT as the mass-reared sterile pests contribute the supply of hosts for mass-rearing the natural enemies (Thomas, 2007). Unlike SIT which is most economical for small pest populations (that are easily 'overflooded' by released 'steriles'), parasitic wasp releases work best against a high pest population where wasps can readily locate pests. Put simply, combining these two methods together could avoid the limitations of each individual method: parasitoids used to bring a high pest population down to a level where SIT becomes effective. Parasitoids and sterile

insects share the advantage of being self-dispersing so give wide coverage including areas where the other techniques, such as chemical spraying, cannot readily be applied.

This logic is supported by theory. Population modeling has demonstrated that the combined use of SIT and parasitoids would be much more efficient than either method alone for suppressing or eradicating a host species (Barclay, 1987). That paper proposed that the greater combined efficiency of SIT and parasitoid release, as opposed to use of either singularly, was an example of a broader principle: that two pest control methods will mutually complement each other if their optimal actions in reducing host numbers are at different host densities. This is the situation for SIT which performs best at low host densities while parasitoid inundation performs better at higher host densities.

Figure 3. Factory scale rearing of the fruit fly parasitoid *Diachasmimorpha longicaudatus* in conjunction with production of sterile fruit fly in Mexico. (Photographs by kind permission of Andrew Jessup, International Atomic Energy Agency, Vienna).

Since the modeling of Barclay (1987), several studies have shown that SIT together with augmentative or inundative release of biological control agents can be effective. An example is the successful eradication from New Zealand of the Australian painted apple moth, *Teia anartoides* Walker. That program employed widespread spraying of the entomopathogenic bacterium *Bacillus thuringiensis* (Berliner) subsp *kurstaki* to reduce the pest density. In this instance, widespread spraying of the agent compensated for its poor inherent dispersal capacity. Sterile insect technique was implemented in 2003 when trap catches of moths were only 1% of their 2001-2 levels (Suckling *et al*., 2007). Most cases of combined use of SIT and biological control, however, involve dipteran targets and hymenopteran agents.

In Hawaii, augmentative releases of *Diachasmimorpha tryoni* (Cameron) for the control of the Mediterranean fruit fly, *Ceratitis capitata* (Cameron) raised rates of parasitism to 47% compared with 14.2% in the control area, with a significant reduction in both the adult and larval population of *C. capitata* achieved (Wong *et al*., 1991). However, when combined with sterile adult *C. capitata* a significant decrease in the number of male *C. capitata* trapped per day and the mean percentage egg hatch was recorded (Wong *et al*., 1992). Overall, this led to a nine-fold decrease in the number of *C. capitata* recovered from fruit in the region compared with the control area indicating that the two control techniques, when used together, were more effective at reducing fruit fly populations than either alone. The same effect was evident in a study of the leaf miner, *Liriomyza trifoli* Burgess, a pest of ornamental and vegetable crops (Kaspi and Parrella, 2006). In a greenhouse containing

chrysanthemums the combined release of sterile adult leafminers and its parasitoid *Diglyphus isaea* (Walker) gave a significant reduction in mine production and the adult leafminer population. Furthermore, a synergistic interaction between these methods was demonstrated such that SIT used with biological control gave better pest control than did either technique alone. A model based on observed data indicated that only the combined use of these methods would effectively eradicate the pest population.

In the early 1990's, as part of an area-wide campaign against *Anastrepha* spp. from Mexico, SIT and augmentative biological control were selected as the main methods of control as they are economically feasible and have minimal or no effect on non-target organisms (Orozco, 2004). Subsequently, a mass-rearing facility to produce 50 million *Anastrepha* spp. and 30 million parasitoids (*Diachasmimorpha longicaudata* (Ashmead)) per week (presently 50 million parasitoids per week; A. Jessup pers. comm. 2007) was built (Figure 3). As part of this integrated management campaign, the states of Senora, Chihuahua and Coahuila are now recognized as fruit fly-free areas. In Costa Rica, combined SIT and parasitoid release is successfully funded by grower groups (Messing, 1996), who clearly see the benefit of a combined release. In a coffee plantation in Guatemala, effective suppression of *C. capitata* was attained using combined releases of the parasitoid *D. tryoni* and sterile adult *C. capitata* (Cancino *et al*., 1996). In another study which involved the aerial release of these same species along the border between Guatamala and Mexico, the combination of tactics was reportedly synergistic in effect (Sivinski *et al*., 1996). However, in neither of these studies, was the degree of control indicated. Another study showed that at various sites in Guatamala, caged F1 *C. capitata* populations were suppressed using a combination of two parasitoids, *Fopius arisanus* (Sonan) and *Diachasmimorpha kraussi* (Fullaway) and sterile male *C. capitata* than the latter technique alone (Rendon *et al*., 2006). The authors suggested that the release of multiple species of parasitoid may be advantageous as they each have clear habitat preferences and therefore differ in their ability to exploit environments within and surrounding agroecosystems.

A recent evaluation in Hawaii showed that SIT and biological control as part of an IPM program over recent years has proven very effective at reducing fruit fly populations (Kaplan, 2008). In fact, various fruit fly parasitoids are reared for mass release into the field around the world, often in conjunction with SIT. For example, in addition to Mexico and Hawaii, there are mass production facilities for fruit fly parasitoids in Brazil, Peru, and Guatemala (A. Jessup pers. comm. 2007).

There have also been a number of successful studies combining SIT and biological control for lepidopteran targets. The combined use of inheritedly sterile (sterile F_1 adults) potato tuber moth, *Phthorimaea operculella* (Zeller) and *Trichogramma* spp. (oophagous parasitoids) in a laboratory trial was more effective in reducing fertile F_1 *P. operculella* progeny than either technique used alone. Furthermore, the level of suppression attained by the combined releases was thought to be additive in effect (Saour, 2004). The authors predicted that because this reflected a single release, when multiple releases of sterile insects and *Trichogramma* occur, that synergism of treatment effects may be obtained and concluded that further work on the integration of these two control strategies was warranted. Field cage studies of sterile adult codling moth, *Cydia pomonella* (L.) along with the parasitoid *Trichogramma platneri* led to less apple damage than when either tactic was used alone (Bloem *et al*., 1998). In an earlier study, *T. platneri* were released in apple orchards using SIT against codling mothin British Columbia, Canada (Cossentine and Jensen, 2000). Combined use of parasitoids and SIT led to significantly lowercodling moth damage compared with plots where *T. platneri* was not released. A further benefit of this integrated

strategy was that the non-viable codling moth eggs produced by released steriles were suitable hosts for *T. platneri* so contributed to persistence of the parasitoid population.

Despite such encouraging findings, indeed factory scale commercialization in several countries, there is still great scope to realize the full utility of the synergies between SIT and biological control. A major, industry-funded project exploring SIT and braconid releases against fruit flies recently commenced in Australia, where a combination of these techniques is likely to provide more economic and effective management of tephritid outbreaks. In surrounding zones, which are usually managed to minimize the pressure that pest populations exert on pest free areas, a combination of both these techniques could provide enhanced suppression of pest populations. It is thought by some (Sivinski, 1996; Wharton, 1989) that natural enemies may be most compatible with SIT in suburban and native settings rather than in the monocultures typical of commercial orchards because the former provide better availability of nectar and other alternative food sources, lower pesticide application intensity and moderated microclimatic extremes. The habitat manipulation approaches described under the following cultural techniques section offer scope to make intensive monocultures more conducive to natural enemies released to complement SIT. Further, switching from disruptive, broad spectrum pesticides to novel compounds such as those in the preceding section could help alleviate mortality of mass released parasitoids and sterile insects.

However, what becomes apparent in a number of these situations is the lack of documentation of the degree of control exerted and the economic benefits achieved from the combined release of sterile insect pests and their parasitoids. Despite studies in this aspect of the integrated use of biological control, spanning more than two decades, relatively little is currently available in the peer-reviewed literature. The majority of studies have focused on Tephritidae, reflecting the economic importance of this taxon. Against these pests especially, there is good scope to make wider use of SIT/biological control synergies. A constraint to expand use to other pest taxa is the generic, biological requirements of SIT; that females can mate only once and that irradiation can produce sterility without adversely affecting mating success with 'wild' pests. In practical terms, a facility for producing large numbers of pests is also required both to produce sterile pests and hosts for parasitoid rearing. Despite these constraints there is great potential for future applied ecological research in this area.

Interactions between biological control and cultural practices

It has been accepted for many years that cultural methods such as tillage and fire can have negative effects on biological control agents as well as upon pests. Sometimes these can be idiosyncratic and difficult to predict in advance. For example, mass trapping of olive fruit fly led to capture of large numbers of parasitoids of scale insects (Neuenschwander, 1982). But a dominant phenomenon within biological control over the last decade has been a growth in the level of research interest in conservation techniques whereby the release of exotic or mass reared agents is replaced by practices that conserve and make more effective the existing natural enemy fauna of a region. Cover crops of various types have been employed in conservation biological control to provide nectar and pollen (forms of tropic supplementation, *sensu* Daugherty *et al*., 2007), moderate the microclimate and support non-pest herbivores that serve as alternative host/prey (Jonsson *et al*., 2008). If not managed carefully, however, cover crops can also behave as weeds by competing with the crop for water and nutrients (Bugg and Waddington, 1994; Meyer *et al*., 1992; Nyczepir *et al*., 1998). Cover crops can also increase the cost of production, or decrease yields (Brown and Glenn, 1999), as they require extra maintenance, water and/or fertilizer beyond that required by the

crop (Horn, 2000). Non-crop plants can also favor at least some pest species, a risk that was identified in very early work on the potential for habitat manipulation in rice (Lim and Hong, 1977). In order to minimize the potential negative consequences of increasing plant diversity in a hit-and-miss manner, "ecological engineering" has been proposed as a framework for use of biodiversity and habitat structure that is characterized by a series of methodical steps aimed at identifying the "right kind of diversity" (Gurr *et al*., 2004).

Cultural practices can favor biological control; for example the use of strip harvesting of alfalfa (*Medicago sativa*) advocated at the dawn of IPM (Stern *et al*., 1964). In more recent years the utility of this method has been assessed in Australian alfalfa hay production (Hossain *et al*., 2002) Releasing paint spot or fluorescent dye marked predators into alfalfa plants immediately before passage of a tractor-mounted mower showed that the majority of predator individuals survived cutting and relocated only a short distance to uncut strips. Subsequently, predators would move from these refuges to re-growing plants in adjacent strips and exert more effective control of *Helicoverpa* spp. pest larvae than in areas where strip harvesting was not used. Still more recently Pearce and Zalucki (2005) have explored the potential for using lucerne to promote natural enemy activity in field crops. Generally, however, the availability of studies demonstrating the importance of shelter to arthropod natural enemies has not resulted in many rigorous studies showing benefits in terms of reduced pest densities and increased crop yield (Griffiths *et al*., 2008).

Crop residue retention too can dramatically influence the performance of natural enemies and shelter is likely to be one mechanism by which this operates. The classic example of this is the sugar cane pest *Pyrilla perpusilla* (Walker) for which markedly improved control was achieved in unburned crop residues compared to those burned (Mohyuddin, 1991). This was attributable to the egg parasitoid, *Parachrysocharis javensis* (Girault), the activity of which was enhanced by the moderated microclimate provided by the crop residue. It is surprising that crop residues have not been more thoroughly investigated in more recent years as a means of enhancing biological control of arthropod pests. This is highlighted by the recent work on the benefits of trash retention for biological control of other taxa including weeds, plant parasitic nematodes and plant pathogenic fungi. Field experiments on weed seed predation by arthropods suggested good scope for use of crop residues (Cromar *et al*., 1999). Epigeal invertebrates were found to be the dominant predators of the weeds, common lambsquarters and barnyard grass, responsible for 80-90% of all seeds consumed. Predation was favored by avoidance of tillage and a groundcover of corn residue. The benefits of crop residues in plant disease suppression is also well recognized (Whipps and Davies, 2000). A recent study illustrated potential for this work on the important wheat disease, head blight, caused by *Fusarium graminearum* Schwabe. A study by Perez *et al*. (2008) indicated that green manures (i.e. sorghum–sudan grass hybrid or buckwheat plants tilled into the soil along with wheat residue) promoted the development of indigenous soil microorganisms that were antagonistic to the survival of the fungal pathogen. Other work, on plant parasitic nematodes (Stirling *et al*., 2005), showed that incorporation of sugar cane trash in a field experiment subsequently resulted in a reduction in pest nematode densities of between 71% and 95%, depending on species, compared with an unamended control treatment. Amendment increased readily oxidizable carbon, microbial biomass, microbial activity and numbers of free-living nematodes. Though none of the known predators of nematodes were enhanced, an unidentified predatory fungus was found only in amended soil.

The preceding examples illustrate that the mechanisms by which crop residues may favor predation, parasitism and suppression of pest taxa are likely to me manifold. One that appears a priority for future research is enhancing levels of organic matter, a phenomenon

that has been best studied in tropical rice. Since the original work by Settle *et al*. (1996), in which composted cow manure was added to plots of rice and natural enemy densities were increased by the availability of detritivore prey, other workers have sought to measure the benefits of organic matter supplementation. Jiang and Cheng (2004) investigated this approach for enhancing biological control of whitebacked planthopper (*Sogatella furcifera* (Horváth)) in China. Composted barnyard manure was added to plots of rice and synthetic fertilizer added to the control plots at rates equivalent to the nutrients present in the manure. Abundance of collembola was enhanced by the manure treatment and, though no benefits to rates of predation or parasitism were evident for the first 40 days after rice was established, after this time activity of predators especially was enhanced (Figure 4). Ecologically, this strategy infuses the "detrital shunt" of the food webs (Polis and Strong, 1994) (Figure 5) with the allochthonous organic matter constituting a resource subsidy that enhances numbers of detritivores. These additional prey species decouple populations of natural enemies from reliance on pest herbivores, so allowing generalist predators in particular to establish and remain in crop, ready to provide immediate control of immigrating pests. In at least some

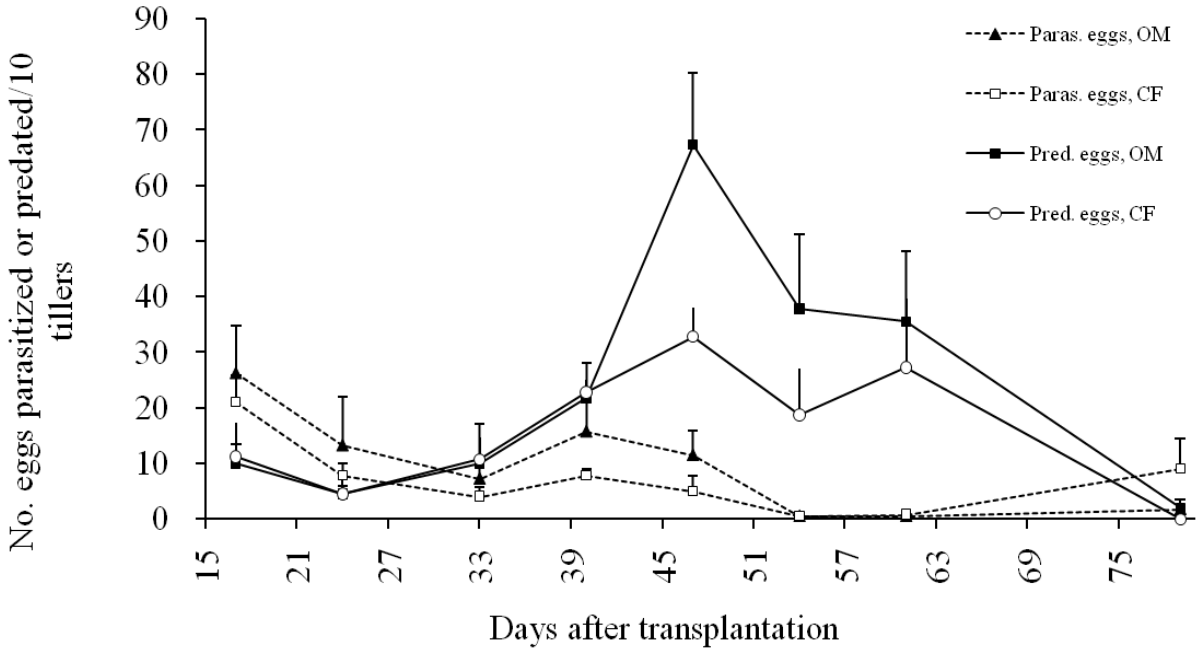

Figure 4. Example of a positive interaction between a cultural treatment and biological control: effect of organic matter (OM) versus chemical fertilizer (CF) on numbers of parasitized or predated whitebacked plant hopper (*Sogatella furcifera*). Redrawn using original data from authors: Jiang and Chen (2004) *Journal of Pest Science* **77**, pp. 185–189.

cases, however, detritivores may play a still more critical function. Feeding studies on *Atypena formosana* (Oi) by Sigsgaard *et al*. (2001) demonstrated that alternative prey is 'an absolute necessity' for the linyphiid spider (*A. formosana*). Spider survival on diets consisting solely of rice brown planthopper *Nilaparvata lugens* (Stål) or the green leafhopper

(*Nephotettix virescens* (Distant) was poor. In contrast, a mixed diet of the hemipteran plus collembola and drosophila improved development time and survival of spiders. This illustrates that availability of prey such as collembola is essential for the performance of this linyphiid, rather than being only an early season, alternative food resource. This phenomenon may apply more widely to the use of generalist predators in agriculture whereby the key pest species (when the only prey species present) is not a suitable diet for the development of the potentially efficacious predator.

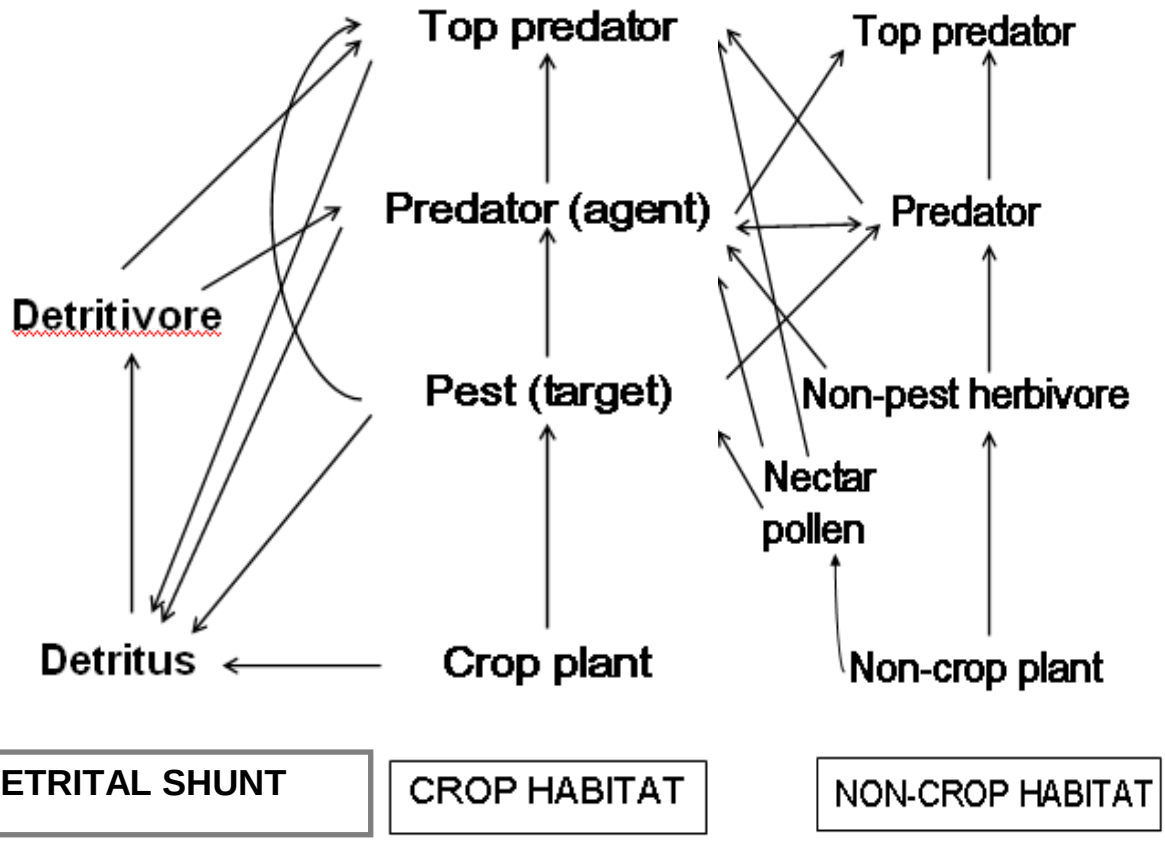

Figure 5. Schematic representation of an agricultural system food web showing the potential importance of non-crop habitat and detritus. The latter can be augmented by application of organic matter to support predators, allowing populations to develop early in the season before pests arrive.

Other than the food-web related effects of organic matter on biological control dealt with above, it has recently been suggested that soil organic matter content may make soils a more favorable structure for burrowing arthropods such as Coleoptera (Pywell *et al*., 2005). Of course not all such burrowing species will be beneficial and this is a further illustration of the need to carefully assess the benefits of any form of habitat manipulation on pests as well as on natural enemies. Ultimately, the use of organic matter enrichment in biological control is likely to be an important future direction. Cultural practices such as crop residue retention, green manures or other amendments will be promoted by those concerned with sequestering

atmospheric carbon dioxide. Farmers and land managers are likely to receive 'carbon credits' for adoption of these methods. How best to simultaneously support biological control is an exciting avenue for research.

Conclusion

This review had deliberately emphasized the potential positive interactions between biological control and other forms of pest management. Notwithstanding the potential negative interactions, and the obvious need to avoid them, it is research into the additive and especially synergistic interactions that will yield the pest management strategies required if humanity is to meet the challenges of the future. Our population is expected to expand from the current level of 6.5 billion to 9 billion by 2050 and agriculture will have to meet the resulting increased demand for food and fiber. This challenge is compounded by loss of agricultural land to urbanization and land degradation (soil acidification, erosion, desertification and salinization), by water scarcity and by increasing use of croplands to produce bio-fuels. Essentially, agriculture is about the management of ecosystem services 'benefits that people obtain from ecosystems' (Millenium Ecosystem Assessment, 2005), to produce food, fuel and fiber. These services include pollination, nutrient cycling, carbon storage, land stabilization, nitrogen fixation and conservation of threatened wildlife as well as biological control. In total, the global value of the ecosystem services has been estimated at US$2.6x10^9 (Costanza *et al*., 1997) . But the sustainability of 'industrial agriculture' (characterized by high inputs of synthetic fertilizers and pesticides, mechanical tillage and other technologies) is increasingly questioned. The Millennium Ecosystem Assessment (2005) highlighted the state of global ecosystems and their role for human well-being. That study examined 24 ecosystem services and found that only four (global climate regulation and production of aquaculture, crops and livestock) had been enhanced over the last 50 years. Fifteen, including biological control of pests had been degraded.

Accordingly, threats to agricultural sustainability such as environmental pollution, pest resistance to pesticides, dependence on fossil fuels and other non-renewable resources have led to research into alternative approaches that aim to promote ecosystem services. The value of biological control of crop pests alone has been estimated at $100 billion worldwide per annum (Costanza *et al*., 1997). Despite the action of biological control, insect pests still destroy an estimated 15% of world food production and lead to annual applications of approximately 3 million tonnes of pesticides. Maybe some of the ideas sketched out in this review will enhance biological control, mitigate these unacceptable levels of pest damage and simultaneously support still broader ecosystem services. An example is provided by the Wetland Integration and Sustainable Expansion into Rice approach. This 'WISER Approach' being developed in Laos offers scope to enhance biological control of rice pests by increasing the numbers of and (especially) early season density of native aquatic and amphibious predators (Jahn, G. pers com., 27 June 2008). In the Mekong basin, rice fields close to the river system flood early in the wet season leading to rapid colonization and breeding in rice paddies of fish and other predators (such as copepods) that can help suppress rice pests and mosquitoes (Fernando, 1993; Vromant *et al*., 1998). In contrast, rice paddies remote from the river system, though filled with water from local rains, are colonized by larger species, such as fish, one or two months later. This, in turn, results in a long delay in predatory species contributing to pest suppression. At the same time this phenomenon also reduces the extent to which rice paddies contribute to the conservation of aquatic biodiversity on farmland. Scope for rural communities to harvest "wild" foods is also reduced. A solution to these interrelated problems is the construction of deep pits on rice farms that serve as refuges, particularly for fish, during the dry season. By mutual

agreement within the human community, harvesting of fish from these is strictly regulated (e.g. on one day of the year only). As soon as the rainy season commences, aquatic predators are able to colonize rice paddies from the pits and rapidly breed to exploit the expanded aquatic habitat. In tropical rice production, the paddies may be connected to, or in very close proximity to, a network of human-made, natural and semi-natural aquatic habitats through which not only vertebrates but many invertebrates and prey of invertebrates (e.g. plankton) may readily move with beneficial consequences for biological control.

More generally, there is significant research interest in the relevance to biological control of connectivity and permeability of terrestrial vegetation features in farmlands (Tscharntke *et al*., 2008). Many agricultural systems other than rice are irrigated or exist in proximity to natural and semi-natural aquatic habitats and the influence of these on natural enemy activity has been little studied. Extending the spatial analysis and metapopulation approaches from terrestrial work to understand and manipulate the aquatic component of agricultural landscapes is an exciting prospect. In the case of the WISER approach, agronomic practices are being explored through collaboration between the International Rice Research Institute and the World Wildlife Fund as a means of reducing harvesting pressure on wild populations and using farms themselves as habitat to complement biodiversity conservation in formal refuges. In the future, measures could be introduced to improve spatial and temporal connectivity of aquatic habitats in agricultural landscapes; a fashion analogous to the use of shelterbelts, hedgerows and 'beetle banks' in temperate farmland. Such features may be as useful to biodiversity conservation as they are to biological control enhancement.

Ultimately, the contribution of biological control to meeting the pest management challenges to sustainable agricultural production will depend not only on its strategic use with other forms of pest management, but promotion of biological control to farmers and policy makers as one of a suite of ecosystem services. These can be enhanced by ecological engineering at scales that transcend individual fields and farms and encompass catchments and provide benefits, including carbon cycling at scales as large as the entire biosphere.

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Technology Transfer

Throughout the life of the project, several activities were undertaken to disseminate information and highlight the potential for augmentative biological control using fruit fly parasitoid wasps. A list of these is provided below.

Publications

Journals

Zamek, AL, Spinner, JE, Micallef, JL, Gurr, GM & Reynolds, OL (in press). Parasitoid fauna of Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera: Tephritidae) in inland New South Wales, Australia and their potential for use in augmentative biological control. *Insects.*

Zamek, AL, Reynolds, OL, Mansfield, S, Micallef, JL and Gurr, GM. (in press) Carbohydrate diet and reproductive performance of the fruit fly parasitoid *Diachasmimorpha tryoni* (Cameron). *Journal of Insect Science.*

Spinner, JE, Cowling, AM, Gurr, GM, Jessup, AJ and Reynolds, OL (2011). Parasitoid fauna of Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera: Tephritidae) in inland New South Wales, Australia and its potential for use in augmentative biological control. *Australian Journal of Entomology* 50, 445-452.

Gurr GM and Kvedaras OL. (2009). Synergizing biological control: Scope for sterile insect technique, induced plant defences and cultural techniques to enhance natural enemy impact? *Biological Control* 52 (3): 198-207.

Newspaper, magazine, farmer/grower articles

Olivia Reynolds, Geoff Gurr, Andrew Jessup (2010) Biological control enters the fight against 'Qfly' threat. *Australian Citrus News*, 86: 20-21.

Olivia Reynolds, Geoff Gurr, Andrew Jessup and Jennifer Spinner (2009) Combating the fruit fly threat for Australian fruit and vegetable growers using biological control. *Vegetables Australia and Australian Fruitgrowers.*

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Field Days

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Research Study Tours

Florida, Mexico, Guatemala and Hawaii, Central and North America. June 2009. Collaborators and colleagues visited included: Dr's Tim Holler, John Sivinski, Pedro Rendon, Jorge Cancino, Don Mcinnis and Eric Jang. Observed and studied sterile insect and parasitoid rearing facilities and participated in sterile male fly release.

Recommendations

1. This project has advanced our knowledge of the biology and pre-release feeding requirements of *D. tryoni* and should be considered when further developing augmentative release programs; research findings have been validated by the publication of completed research.
2. Ashley Zamek graduated with second class Honours in 2011 and produced a peer-reviewed journal publication from her thesis which should be commended.
3. Studies to understand what food sources braconid parasitoids feed on in nature and how their natural foods could be utilised in mass-rearing to make diet improvements; dietary enhancements could produce a healthier mass-reared parasitoid with greater fecundity and survival and ultimately a more effective biological control agent
4. Detailed pre-release feeding studies should be completed to confirm the food source that will maximise a parasitoids performance while minimising rearing costs.
5. Continued development of mass rearing techniques of selected parasitoids to minimise costs, while maximising efficiencies and parasitoid performance is warranted.
6. Parasitoids can associatively learn host- and food-finding cues, suggesting that post-release orientation towards microhabitats and host choice specificity could be manipulated by pre-release experiences, and this deserves further investigation.
7. Identification of ways in which we can manipulate landscapes (i.e. composition and connectivity of landscapes) to ensure that parasitoids can readily find and exploit 'islands' of fruit fly habitat.
8. Determine the effectiveness of the combined use of parasitoids and the sterile insect technique in controlling *B. tryoni* populations, compared with either technique alone.

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Appendix 1

Biological Control 52 (2010) 198–207

Review

Synergizing biological control: Scope for sterile insect technique, induced plant defences and cultural techniques to enhance natural enemy impact

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A b s t r a c t

When used alone, only a minority of biological control programs succeed in bringing the target pest population under sufficient control. Biological control is, therefore, usually employed with chemical, cultural, genetic or other methods in an integrated pest management (IPM) strategy. The interactions between different pest management methods, especially conventional pesticides and host plant resistance, is an area of growing research interest but relatively little consideration is given to novel combinations. This paper reviews the interactions between biological control and other forms of pest management, especially induced plant defences and the novel, non-toxic plant protection compounds that may boost these defences; and sterile insect technique. We also cover the cultural methods that offer scope to support synergies between the aforementioned methodological combinations. We conclude that despite the sometimes negative consequences of other pest management techniques for biological control efficacy, there is great scope for new strategies to be developed that exploit synergies between biological control and various other techniques. Ultimately, however, we propose that future use of biological control will involve integration at a greater conceptual scale such that this important form of pest management is promoted as one of a suite of ecosystem services that can be engineered into farming systems and wider landscapes.

Appendix 2

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Parasitoid fauna of Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera:Tephritidae) in inland New South Wales, Australia and their potential for use in augmentative biological control

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Abstract

Augmentative releases of parasitic wasps may improve management of the Queensland fruit fly, *Bactrocera tryoni* Froggatt, in inland New South Wales (NSW). A survey was conducted from October 2008 to April 2009 to detect the presence of parasitoids of fruit fly. Fruit fly-infested fruits were collected in Wagga Wagga, Cootamundra, Ganmain, Gundagai, Lockhart and Lake Cargelligo on the south-west slopes and plains of NSW and Albury-Wodonga on the NSW-Victorian border. Two species of opiine parasitoids were detected: *Diachasmimorpha kraussii* (Fullaway) and *D. tryoni* (Cameron); both species from fruits that also yielded *B. tryoni* and island fruit fly, *Dirioxa pornia* (Walker). Nine per cent of fruit samples yielded parasitoids. There were statistically significant differences between fruit type, fruit species, sampling events and towns. Fruit fly parasitoids were most commonly detected in fig (27.2% of samples), followed by stone fruit (11.5%), pome fruit (6.1%), loquat (4.3%) and citrus (2.1%). Parasitoid incidence varied throughout the fruit fly season, peaking in February–March 2009 (17.4%). Of the towns surveyed, Cootamundra had the highest incidence of parasitoids (28.8%), followed by Wagga Wagga (9.5%), Gundagai (10.2%) and Lockhart (1.2%), with no parasitoids detected in Albury-Wodonga, Ganmain or Lake Cargelligo. *Diachasmimorpha tryoni* was detected in all surveys except January–February 2009, during a heatwave. *Diachasmimorpha tryoni* was most prevalent in November–December 2008 (5.2%). *Diachasmimorpha kraussii* was most prevalent in February–March 2009 (14.5%), but was not detected in October 2008 or April 2009. *Diachasmimorpha tryoni* was detected in Wagga Wagga (6.1%) and Cootamundra (1.9%), with *D. kraussii* detected in Wagga Wagga (9.5%), Cootamundra (26.9%), Gundagai (10.2%) and Lockhart (1.2%). The presence of these parasitoid species in the region suggests they may be suitable for augmentative release in the control of *B. tryoni* in inland NSW.