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Integrating spatially explicit molecular and ecological methods to explore the significance of non-crop vegetation to predators of brassica pests

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ABSTRACT

There is mounting evidence that non-crop vegetation can promote natural enemies of crop pests but most studies use only one or a few approaches to explore key processes. Here we integrate field sampling, insect marking, insecticide disruption, and molecular gut content analysis to explore the potential value of non-crop habitats to predators of brassica pests in temperate Australia. Twelve monthly surveys of 13 farms established that an exotic ladybird (*Hippodamia variegata*) and a native lacewing (*Micromus tasmaniae*) were numerically dominant predatory arthropod species in brassica crops and present in adjacent perennial pasture, bushland and riparian vegetation. We applied dye to non-crop vegetation on three sites and subsequently sampled predators from adjacent brassica crops. Relatively large proportions of *H. variegata* and *M. tasmaniae* were marked, especially close to the non-crop vegetation though also extending 100 m into the crop, indicating predator immigration into the crop. In a third study, predators were monitored in three brassica crops after the host farmers sprayed insecticide to control what they considered to be excessive pest densities. Within two days, *H. variegata* and *M. tasmaniae* adults were present in the crops and numbers increased significantly over 12 days showing rapid crop recolonisation. Finally, molecular gut analysis indicated large proportions of both predator species sampled from non-crop (non-brassica) vegetation contained DNA of brassica-specialist herbivores suggesting predator movement from crop to non-crop vegetation, possibly to access nectar. Findings demonstrate *H. variegata* and *M. tasmaniae* are likely to be important predators of brassica pests in the region and expand our understanding of the significance of non-crop vegetation for coccinellids and lacewings.

1. Introduction

The very recent Report by the International Panel of Experts on Sustainable Food Systems (IPES-Food, 2016) acknowledged the success of current farming systems in supplying large volumes of agricultural products but bemoaned the multiple negative outcomes including loss of biodiversity and reliance on inputs including pesticides. Agricultural productivity needs to be increased to meet the burgeoning needs of the human population, but seeking to achieve this by still greater reliance on non-renewable, synthetic inputs and eroding the natural resource base is unsustainable (Godfray, 2011). Accordingly, there have been calls for ecological intensification in which ecosystem services (ES) play a stronger role in suppressing pests, maintaining soil fertility, protecting water cycles and so on (Bommarco et al., 2013). Biological control is one such ES that has been valued at US$4.5 billion per annum in the USA (Losey and Vaughan, 2006).

Brassica crops such as cabbage and broccoli are attacked by various herbivorous arthropods, particularly aphids and

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caterpillars (Cole and Horne, 2006; Zalucki et al., 2009), and their control relies heavily on the use of broad spectrum insecticides (Devine and Furlong, 2007). In the case of diamondback moth, *Plutella xylostella* L., the combined cost of its impact and control is estimated at $4-5 billion per annum globally (Zalucki et al., 2012). Such costs, associated with ever increasing levels of insecticides resistance, are driving interest in integrated pest management (IPM) and especially biological control for this and other pests (Zalucki et al., 2009).

The potential of natural enemies to exert effective biological pest control is strongly affected by farming practices that determine the availability of key resources such as alternative prey, shelter, and plant foods such as nectar and pollen (Schellhorn and Silberbauer, 2003; Burgio et al., 2006; Lu et al., 2014; Gurr et al., 2017). Many experiments have shown that manipulating the crop habitat, for example by strips of flowering plants to the crop margin, as intercrop rows, or as a groundcover, can increase the abundance of predators and other natural enemies and lead to reduced pest densities and damage (Matteson, 2000; Lu et al., 2014). However, a key challenge with the use of this approach is that it can interfere with normal crop management and even reduce the yield (Letourneau et al., 2011). Accordingly, much research attention has been given to what can be considered a broad alternative: the use on non-crop habitats that are already present on or near croplands and that might be managed, or at least preserved, to serve as source habitat for natural enemies and to provide key ecological resources that are absent from the crop (Schellhorn and Sork, 1997; Matteson, 2000; Liu et al., 2005, 2014; Gentz et al., 2010). Understanding the characteristics of non-crop habitats is important in the manipulation of vegetation that provides shelter and food for natural enemies (Macfadyen et al., 2015b; Parry et al., 2015; Gurr et al., 2017). For example, McEwen et al. (2001) noted that hedgerows, wind breaks, weedy strips and riparian areas could serve as a reservoir or ecological corridors for brown lacewings and other natural enemies. Specific plants can provide key resources for natural enemies (Lu et al., 2014) which may increase their activity at field edges (Bowie et al., 1999; Pywell et al., 2015). Natural enemies can move from non-crop areas into crops (Matteson, 2000) and therefore provide potential ES to growers. Particular attention has been given to the significance of non-crop vegetation because this can provide the aforementioned resources and compensate for temporal or complete absences in crops themselves, serving a source habitat from which natural enemies spill over into crops and strengthen biological control (Morandin et al., 2014; Inclán et al., 2015; Ramsden et al., 2015; Wilson et al., 2015).

The ability of a predator to suppress a focal pest also depends on temporal dynamics. The presence of sufficiently high numbers of predators in a given crop is key to checking pest population build-up, especially for herbivores such as aphids with the capacity to reproduce rapidly (Schellhorn et al., 2014). Many predators do not reproduce as rapidly as their prey (Dixon, 2000). Accordingly, effective control of pests by predators relies on them being able to persist locally year-round so sufficient numbers are present when pests first arrive; thereby preventing pest increase. This, in turn, reinforces the need of a farm landscape to provide continuity in the availability of essential resources including suitable foods and habitats, noting that crops themselves may be scarce or even absent for periods (Landis et al., 2000).

Generalist predators can be especially useful in pest management (Symondson et al., 2002). Compared with specialist species whose local persistence is dependent on the availability of pest prey, generalists can persist by using prey species other than the focal pest and may also be able to exploit plant-derived foods such as pollen (Symondson et al., 2002), though they are still vulnerable to disturbance by crop management practices (Thorbek and Bilde, 2004). Establishing an understanding of the spatio-temporal dynamics of key predator species – especially their association with various habitats on farms – is essential for understanding their biology and how their impact as ecosystem service providers might be enhanced (Macfadyen et al., 2015a; Schellhorn et al., 2015; Zalucki et al., 2015).

We surveyed brassica farms in temperate Australia to identify key predator species that might be promoted to enhance control of pests. A numerically dominant ladybird beetle and lacewing were selected and then monitored in multiple vegetation types on 13 farms and over 12 months to provide a tempo-spatial understanding of habitat use. A second study applied a fluorescent pigment dye to dominant non-crop vegetation types and subsequently sampled predators from adjacent crops. A third study monitored the recolonisation pattern of crops by predators in the days after host farmers used a disruptive insecticide. A final study used molecular gut content analysis and spatially explicit field sampling, including non-crop vegetation, to explore patterns in consumption of brassica pests.

2. Material and methods

2.1. Study one: spatio-temporal distribution of predators

Monthly surveys were conducted of 119 sites representing crop and non-crop habitats on 13 brassica producing farms in the Central West region of New South Wales, Australia close to the city of Bathurst (33°25′12″S 149°34′40″E). Crops sampled were broccoli, cauliflower and cabbage (*Brassica oleracea*) whilst non-crop habitats were perennial pasture, riparian vegetation and bushland (native, perennial woody vegetation). On each farm a vacuum sample (see below) was taken on each sample date from a previously unsampled area of each type of non-crop vegetation (except for one farm that lacked riparian habitat) as well as from a previously unsampled area of each crop type present at the time. Crops were defined by type (broccoli, cauliflower or cabbage) and by date: early-planted (Sept-Oct), mid-season (Nov-Dec) and late-planted (Jan-Feb). Farming practice in this region involves sequential plantings such that brassica crops are present virtually year-round. Each sample consisted of the catch from a position within the vegetation type consisting of four 20×1 m strips of vegetation that were sampled using a motorised vacuum device (STIHL® BG 85, Andreas Stihl Ag & Co. Waiblingen, Germany). The inlet was fitted with a removable net bag to intercept arthropods as described in Schellhorn and Silberbauer (2003); Hamilton et al. (2004). Arthropods within each sample were immediately killed with chloroform to prevent predation, labelled and held in a 12 v refrigerator. In the laboratory, arthropods were separated from debris by sieving and brushing and transferred to Petri dishes and stored in a freezer. A stereo-binocular dissecting microscope (10×) (Leica, SE305-A, Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) was used for sorting and identification.

2.1.1. Data analysis

Insect count data for each of the two predator species were analysed by regression analysis using a Poisson distribution and log link function with date, vegetation type and interaction as fitted terms using Genstat Release 18.1. The non-significant interaction term was dropped for the final analysis and predictions.

2.2. Study two: predator movement from non-crop to crop vegetation

Three brassica fields were selected on each of three of the farms from study one to give on each site, one field bordered by pasture, one by bushland, and one by riparian vegetation. A 10 m-wide strip of the non-crop vegetation running the full length of the border