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Measuring natural enemy dispersal from cover crops in a California vineyard

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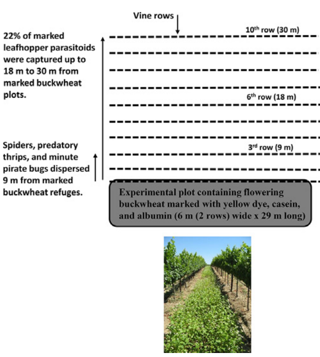
GRAPHICAL ABSTRACT



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ABSTRACT

Dispersal of natural enemies from buckwheat cover crop plots embedded within a southern California vineyard during spring and summer was investigated by using an arthropod mark-capture technique. Specifically, arthropods were marked in flowering buckwheat plots by spraying plants with a "triple mark" solution containing yellow dye, casein protein, and albumin protein. In turn, we recorded the abundance of marked and unmarked natural enemies at a gradient of distances from the treated buckwheat plots into the vineyard. Natural enemies marked with yellow dye were identified visually, while the presence of casein and albumin protein marks were detected using anti-casein and anti-albumin enzyme-linked immunosorbent assays (ELISA). The percentage of natural enemies marked with yellow dye indicated that spiders, predatory thrips (Aeolothripidae), and minute pirate bugs (Anthocoridae) dispersed 9 m (i.e., 3 rows) from marked buckwheat refuges over a six day period. The percentage of leafhopper parasitoids (*Anagrus erythroneurae* S. Trjapitzin and Chiappini) marked with yellow dye indicated that 22% of marked parasitoids were captured up to 18 m (i.e., six rows) to 30 m (i.e., 10 rows) from buckwheat plots up to six days after marks were applied to cover crops. Up to 17% of natural enemies marked with yellow dye, albumin, or casein were captured in non-treated control plots, suggesting that parasitoids, spiders, minute pirate bugs and predatory thrips were able to cross the 36 m buffer zones used to separate marked buckwheat plots and unmarked control plots. Results comparing the percentage of parasitoids and 'other beneficials' marked with a double mark (where any two of the three marks were detected) between distances in buckwheat plots indicated that double marked parasitoids were found up to 30 m (i.e., 10 rows) from buckwheat refuges, while no double marked parasitoids were captured in control plots. No triple marked arthropods were captured. To exploit the dispersal capabilities of natural enemies, these results suggest that buckwheat refuges planted in California vineyards could be planted every 6th (i.e., 18 m) or 10th (30 m) row to gain potential benefits from providing natural enemies with flowering buckwheat refuges.

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1. Introduction

Floral and extrafloral nectar can maximize the longevity, fecundity, searching activity, and attack rates of natural enemies, and an increase in female-biased sex ratios of progeny of parasitoids and predators may result from access to these resources (Berndt and Wratten, 2005, Kost and Heil, 2005, Irvin et al., 2006, Hogg et al., 2011). Carbohydrate sources are important as adult parasitoids need to locate food at least once a day to avoid starvation (Azzouz et al., 2004, Idris and Grafius, 1995, Siekmann et al., 2001). Searching for food resources and hosts/prey involves metabolic costs and natural enemies need to minimize foraging time for food if reproductive success is to be maximized (Lewis et al., 1998). The time that natural enemies spend looking for carbohydrate resources in crops can be reduced by deliberately providing floral subsidies in the form of nectar and pollen (Wilkinson and Landis, 2005). Nectar can be provided to natural enemies in vineyards by sowing flowering plants as a cover crop or by tolerating flowering weed species (Barbosa, 1998). Cover crops also help maintain soil quality and contribute to erosion prevention, and their use is encouraged by the Californian wine industry which promotes sustainable practices through the Code of Sustainable Winegrowing Workbook (CSWW) (Dlott et al., 2002). The purported benefits that arise from the provisionment of cover crops that act as food sources for natural enemies in agroecosystems is a key component of conservation biological control (Gurr et al., 2004).

The use of buckwheat, *Fagopyrum esculentum* Moench, as a cover crop has been evaluated in vineyards in New Zealand (Berndt et al., 2002), Australia (Simpson et al., 2011) and California (Irvin et al., 2016), and is recommended as a cover crop plant for enhancing natural enemies in crops grown in arid soils in the southwestern USA (Grasswitz, 2013). Buckwheat can enhance natural enemy reproduction which may concomitantly reduce pest densities (Nicholls et al., 2000; Berndt et al., 2002, English-Loeb et al., 2003, Irvin et al., 2014). Other attributes favoring the selection of buckwheat as a cover crop are inexpensive seed that is readily available and germinates easily, short sowing to flowering times, and tolerance of poor growing conditions (Angus et al., 1982, Bowie et al., 1995, Grasswitz, 2013).

Natural enemies that utilize cover crops can disperse into adjacent crops and provide varying levels of pest control (Powell, 1986, Lővei et al., 1993, Freeman-Long et al., 1998). Despite potential benefits, habitat diversification through cover crop plantings in some instances may impede natural enemy movement and host/prey location efficiency (Sheehan, 1986, Frampton et al., 1995, Mauremootoo et al., 1995). Further, cover crops may act as 'sinks' for some species of natural enemies, which negatively affects pest suppression (MacLeod, 1999). To determine whether natural enemies will disperse from a cover crop into a high value crop it is important to determine the distances over which natural enemies will move (Gurr et al., 2005, Wratten et al., 2007). Consequently, understanding natural enemy dispersal dynamics from cover crops helps determine the size and spacing of cover crop patches in cropping systems (Landis et al., 2000).

Effective arthropod marking and tracking techniques are essential for evaluating the movement of natural enemies in an agroecosystem (Lavandero et al., 2004). Hagler et al. (1992, 2002) first described and applied mark-release-recapture methods to mark arthropods with foreign proteins (e.g., vertebrate IgGs). In turn, the protein marks were detected on field-collected specimens using anti-protein specific enzyme-linked immunosorbent assays (ELISA). Over a decade ago, an effective mark-capture method was described for marking arthropods directly in the field using inexpensive food proteins (e.g., chicken egg albumin, bovine milk, soy milk) with standard spray equipment (Jones et al., 2006). The ELISAs used to detect these food products are simple, inexpensive, sensitive, and have been standardized for large-scale processing (Hagler & Jones, 2010, Hagler et al., 2014). Irvin et al. (2012) demonstrated the potential of using albumin and casein proteins in combination with a fluorescent dye (a triple mark) to mark *Cosmocomoidea* (formerly *Gonatocerus*) *ashmeadi* (Girault) (Hymenoptera: Mymaridae [Huber, 2015]), an egg parasitoid of the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae). A double- or triple-marking system has the potential to reduce the rate of false positives that occurs using a single mark and this occurs when some insects are incorrectly identified as being marked when they are not (Irvin et al., 2012).

Here, we investigated the dispersal of natural enemies from buckwheat cover crop plots into surrounding grape vines by spraying flowering buckwheat plants with a triple mark containing yellow dye, casein, and albumin. The goal was to determine what types of natural enemies disperse from cover crops, and the distances over which they move. Beneficial insects that may be present in vineyards and enhanced through nectar cover cropping include parasitoids (e.g., *Gonatocerus* spp., parasitoids of sharpshooter eggs, and *Anagrus erythroneurae* Triapitzyn and Chiappini, a parasitoid of leafhopper eggs; both are mymarids) and generalist predators (e.g., anthocorids, coccinellids, chrysopoids and arachnids) (Van Driesche et al., 2008, Irvin et al., 2014). Minute pirate bugs (Anthocoridae) are generalist predators of thrips, spider mites, psyllids, mealybugs, aphids, white flies, insect eggs, and small caterpillars (Daane et al., 2008, Patterson and Ramirez, 2017). Predatory mites and thrips are the most significant predators of spider mites on grapevines (Hanna et al., 1997). Key pests of grapes in California include leafhoppers (Hemiptera: Cicadellidae), mites (Acari: Tetranychidae) and thrips (Thysanoptera: Thripidae) (CSWA, Wine Institute, and CAWG, 2012). Sharpshooters (Hemiptera: Cicadellidae) are significant pests of grape in California due to their ability to vector *Xylella fastidiosa* Wells et al., a xylem-dwelling plant pathogenic bacterium that causes Pierce's disease, a lethal malady of grapes (Blua et al., 1999). Other herbivore pests such as honeydew producing hemipterans like mealybugs (Pseudococcidae), psyllids (Psyllidae) and aphids (Aphididae) can be pestiferous in vineyards (Bettiga, 2013), especially if they develop mutualisms with ants which disrupt biological control (Navarrete et al., 2013, Schall and Hoddle, 2017). Information on natural enemy dispersal would enable optimization of cover crop plantings for conservation biological control of key grape pests in commercial vineyards in southern California.

2. Methods

2.1. Experimental design

In 2008, thirteen plots (28.7 m × 4.8 m [2 rows] separated by at least 36 m) were selected in four vineyard blocks of Cabernet Sauvignon grapes in a commercial organic vineyard in Temecula, CA, USA (GPS coordinates: 33° 3′26.18″N x 117° 00′52.12″W; elevation: 1637 feet). One or two buckwheat plots and control plots (vineyard plots that did not contain buckwheat) were randomly allocated per block, for a total of seven buckwheat and six control plots. The control plots were maintained according to vineyard management practices, which comprised of machine and hand cultivation between rows to remove unwanted weed vegetation. On May 1, 2008, buckwheat seed (Outsidepride, Salem, OR) was sown at recommended agricultural sowing rates, which translated to 336 g of buckwheat seed per 28.7 m plot, on a randomly allocated side of the row of each buckwheat plot. The other side of the row in the buckwheat plots was cultivated and sown with buckwheat on June 11, 2008. Buckwheat sowing was staggered to increase the length of time flowers were available for natural enemies. Buckwheat seed was re-sown in buckwheat plots 2–3 times between late May and mid-July 2008 at approximately 4 w intervals.

Sprinkler irrigation was installed on existing irrigation lines (drip irrigation for the vines which is common in southern California vineyards) to provide water to the buckwheat plots. Irrigation consisted of 5 sprinklers (blue Micro Bird Spinner sprinkler heads per plot, 45 L/h, 360° × 3.66 m diameter coverage; Temecula Valley Piping and Supply, Temecula, CA) each attached to 7 mm tubing which was supported by