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Reduced efficiency of tropical flies (Diptera) in the decomposition of snail cadavers following molluscicide poisoning

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ABSTRACT

Decomposition is a key ecosystem service that reduces non-living organic matter, returning nutrients to the soil. In this study, we examine the responses by fly (Diptera) communities to molluscicide-poisoned apple snail (*Pomacea canaliculata*) cadavers in a tropical rice production landscape. Fewer molluscicide-poisoned cadavers were colonised by decomposer flies (niclosamide = 61% of cadavers with fly larvae; methaldehyde = 53%; *Camellia* sp. seed extract [saponin] = 50%) compared to control (freezer-killed) cadavers (81%). Metaldehyde, niclosamide and saponin reduced the abundance (average 51% reduction), biomass-density (average 63% reduction) and species richness (average 38% reduction) of flies emerging from the snail cadavers. The decay of control cadavers was generally faster (57% more tissue removed over 3 days) than molluscicide-treated cadavers. We suggest that poisoned carcasses potentially affect ecological communities across a range of trophic levels.

1. Introduction

Trade in pesticides has dramatically increased since the early 2000s, particularly in Asia (Horgan, 2017). Current trends in pesticide use are worrisome given their often lethal and sublethal effects on non-target organisms (Langan and Shaw, 2006; O'Hea et al., 2010; Goulson et al., 2015; Verdú et al., 2015; Horgan et al., 2017). Pesticides can also have detrimental effects on human and animal health (Pingali and Roger, 1995; Qiao et al., 2012) and have a negative impact on ecosystem services, particularly on pollination (Goulson et al., 2015) and herbivore regulation (Hardin et al., 1995; Horgan et al., 2017). It is tempting to project from observations of pesticide-related changes in the abundances, diversities or behaviours of key biocontrol or pollination agents to declines in ecosystem services. However, observed pesticide effects on non-target organisms are not always correlated with their effects on associated ecosystem services, particularly in agricultural systems (Wilby and Thomas, 2002; Horgan et al., 2017; but see Rosenfeld, 2002). Further, direct evidence of pesticide impacts on ecosystem services will help determine the environmental consequences of recent increases in pesticide use.

Regional and global campaigns aimed at managing or eradicating invasive molluscs or the mollusc hosts of zoonotic diseases have been associated with increasing molluscicide use in several countries (Waller et al., 1993; Takougang et al., 2006; Coelho and Caldeira, 2016; Newton et al., 2017; Horgan, 2018). Among the most widely used molluscicides are metaldehyde and niclosamide (Takougang et al., 2006; Coelho and Caldeira, 2016; Horgan, 2018). The latter is also a potent lampricide (killing lamprey larvae before they develop into parasitic adults) and is widely used as a defouling agent as well as a World Health Organization (WHO) recommended treatment against *Schistosoma mansoni* Sambon, 1907 (the causative agent of schistosomiasis) and its intermediate snail hosts (Waller et al., 1993; Coelho and Caldeira, 2016; Newton et al., 2017). Both products are toxic to a range of non-target organisms in terrestrial (Sparks et al., 1996; Langan and Shaw, 2006) and aquatic habitats (Calumpang et al., 1995; Zidan et al., 2000; Cruz et al., 2000; Attademo et al., 2016). In response to these non-target effects, researchers have investigated a wide range of plant-extracts as organic alternatives to synthetic molluscicides (Monkiedje et al., 1991; Hammond et al., 1994; San Martin et al., 2008, 2009). A number of plant-based products, including saponins from endod berry (*Phytolacca dodecandra* L.), quinoa (*Chenopodium quinoa*, Willd.) and tea seeds (*Camellia* spp.) are commonly recommended as self-delivery or

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commercially available molluscicides (e.g., Abebe et al., 2005; San Martin et al., 2008; Kijprayoon et al., 2014). The potential environmental consequences of such organic molluscicides have received relatively little research attention; however, some molluscicidal plant extracts cause mortality of non-target organisms including native molluscs, fish, and frogs at the concentrations recommended for managing snail pests (Monkiedje et al., 1991; Oliveira-Filho and Paumgartten, 2000; Mosta-Fa et al., 2005).

Research into the relative toxicities of molluscicides to pest (target) molluscs and associated non-target organisms (Waller et al., 1993; Francis-Floyd et al., 1997; Mosta-Fa et al., 2005) has been mainly conducted in laboratories. Relatively few published field studies have examined the potential non-target impacts of molluscicides in natural or agricultural systems. We are aware of only eight such studies (i.e., Johnson et al., 1991; Purvis and Bannon, 1992; Calumpang et al., 1995; Shore et al., 1997; Mutze and Hubbard, 2000; Iglesias et al., 2003; Ferguson, 2004; Salvio et al., 2011) compared to > 150 publications based on laboratory studies (from Web of Science and Google Scholar using molluscicide types and a range of non-target organisms as search criteria). Furthermore, we are aware of only four studies that directly assess the potential effects of molluscicides on ecosystem services; however, these were also based on laboratory bioassays. Langan and Shaw (2006) reported that iron phosphate reduced the burial of leaf litter by earthworms (*Lumbricus terrestris* L.) in laboratory arenas. Khalil et al. (1991) reported a delay in methanogenesis by cultures of *Methanosarcina barkeri* Balch et al., 1979 (bacteria) treated with niclosamide. Oliveira-Filho and Paumgartten (2000) reported declines in oxygen metabolism among bacterial colonies treated with niclosamide and with *Euphorbia milli* Des Moul. latex. In a study by Monkiedje et al. (1991), endod berry extract actually stimulated heterotrophic bacterial activity, but niclosamide caused a decline in activity.

Flies (Diptera) form a functionally significant component of soil macrofauna. Whereas flies often represent only a small percentage of the major animal groups in soils, particularly in tropical regions (e.g., 0.5% in tropical grasslands: Giller, 1996) they often occur in high numbers (e.g., thousands of individuals m^{-2}) and are among the most diverse of soil arthropod groups (Frouz, 1999). Dipteran species make up a large proportion of all saprophytic, necrophagous, and coprophagous soil invertebrates. Frouz (1999) indicated three major categories of Dipterans association with soils; these are (a) species that are soil dwelling during their entire life-cycles; (b) species that are soil dwelling only during immature stages; and (c) species that pupate in the soil. Many necrophagous and coprophagous species will only pupate in soils, with the eggs and larval stages occurring predominantly within decaying substrates above the soil. Nevertheless, the migrating larvae and buried pupae of necrophagous flies can represent a valuable food source for ground dwelling arthropod predators such as carabid and staphylinid beetles (Giller, 1996; Gardiner et al., 2014). Furthermore, a wide range of soil arthropods, including ants, predatory beetles, and mites (Putman, 1978; Giller, 1996), visits carcases to consume dipteran eggs and larvae. Necrophagous flies (both adults and larvae) also contribute to the fine structure of topsoils (directly) and mineral soils (indirectly) through faecal deposition, thereby facilitating the release of carrion materials during decomposition (Putman, 1978; Stork and Eggleton, 1992).

In the present study, we examine the effects of molluscicides on the utilization of snail cadavers by decomposer flies. Invasive molluscs often occur at high densities (Hall et al., 2006; Sousa et al., 2008) and can represent an appreciable biomass in affected ecosystems (e.g., > 40 d g m^{-2} in stepped rice; 2–8 d g m^{-2} in irrigated rice: Stuart et al., 2014; Horgan, 2018). Poisoning of invasive snails can therefore result in large pulses of dead and decaying matter. Efficient decomposition will reduce unpleasant odours and return nutrients to the soil (Stork and Eggleton, 1992; Carter et al., 2007). However, given the noted effects of molluscicides on bacteria (Khalil et al., 1991; Monkiedje et al., 1991; Oliveira-Filho and Paumgartten, 2000) and reports of the insecticidal properties of some molluscicides (Pery et al., 2004; Büyükgüzel and Kayaoğlu, 2014), molluscicide-poisoned cadavers could have potentially lethal or sublethal effects on decomposer organisms. Such effects could ultimately reduce rates of nutrient cycling in the soil. We therefore examined the effects of the molluscicides niclosamide, metaldehyde and *Camellia* tea seed extract (saponin) on decomposition of apple snail (*Pomacea canaliculata* Lamarck) cadavers. We hypothesised that the utilization of molluscicide-treated snails by decomposer flies will be lower than for snails killed by other means (i.e., freezing). Because apple snails are a considerable pest of rice and attain high numbers in tropical rice fields (Horgan, 2018), we also examined the effects of habitat as influenced by different stages in rice production (land preparation, early tillering, mid/late tillering and fallow) on fly communities. Finally, we included cadaver size as a factor in two of our experiments to examine potential dose-related effects (assuming greater quantities of poison associated with larger snails) on the fly communities. To our knowledge, this is the first study to examine the indirect effects of molluscicides on decomposer fauna.

2. Materials and methods

2.1. Study organisms

We used carcases of the apple snail, *P. canaliculata* in our experiments. Snails were collected from lowland irrigated rice fields at the International Rice Research Institute (IRRI) Experimental Station at Los Baños in the Philippines (14°11′N, 121°15′E). Although several species of apple snail have been introduced to Asia, only *P. canaliculata* occurs at our study site (Stuart et al., 2014). We focused on flies as an indicator of decomposer communities. Because of difficulties in accurately identifying many of the flies in our study, we separated adult files that emerged from the cadavers into morphospecies. In some cases, it was possible to associate larvae and pupae with their adult morphospecies before emergence of the adults. We also noted any parasitoid wasps (Chalcididae and Diapriidae) emerging from the cadavers.

2.2. Molluscicides and treatments

We used snails that were killed with niclosamide, metaldehyde, or a tea seed extract in our experiments. Snails were collected by hand picking from paddy fields at IRRI. The snails, of undetermined sex, ranged in shell height from 15 to 35 mm and included juvenile and reproductive life stages (see Table S1 for further details). The snails were returned to a laboratory (26 °C) and placed in plastic basins with c. 15 cm of water treated with molluscicides on the day of collection. Niclosamide (Bayluscide 250 EC® [25%], Bayer, Philippines) was applied to the basins at the rate of 15 ml/L. Metaldehyde (Snailkil 6% P®, Agchem Manufacturing Corp., Philippines) was applied at a rate of 0.5 g/L. We used *Camellia* tea seed extract (min. 15% saponin) as a natural molluscicide; the powdered extract was applied at a rate of 2 g/L. The former two molluscicides were applied within recommended rates, albeit at the high end of recommendations. The tea seed powder was applied at 1.5× the maximum recommended rate. Dead snails, verified by prodding with a forceps, were collected 24 h after treatment and frozen at −4 °C until required. A group of snails, without molluscicide treatments, were directly frozen on the day of collection as experimental controls.

2.3. Study site and crop production stages

Field experiments were conducted at the Experimental Station of IRRI. The site, located in southern Luzon Island has a tropical climate: temperatures ranged between 22 and 36 °C during the years of this study, and annual rainfall was 5493 mm in 2009 and 5981 mm in 2010 (Fig. S1). Double cropping of rice is practiced in the region with the first, dry season crop usually between December and June and the