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Experimental approaches to test pesticide-treated seed avoidance by birds under a simulated diversification of food sources

Ana Lopez-Antia, Manuel E. Ortiz-Santaliestra, Rafael Mateo *

Instituto de Investigación en Recursos Cinegéticos (IREC) CSIC-UCLM-JCCM, Ronda de Toledo s/n, 13071, Ciudad Real, Spain

HIGHLIGHTS

- Partridges avoid pesticide treated seed when alternative food is available.
- Avoidance of imidacloprid treated seeds is due to post-ingestion distress.
- Refusal to thiram treated seeds decreases over time without alternative food.
- Diversity of food sources increases the risk of pesticide treated seed ingestion.
- Imidacloprid poisoning still occurs when alternative food is present.

GRAPHICAL ABSTRACT

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ABSTRACT

Pesticide coated seeds are known to be potentially toxic for birds, but the risk of poisoning will depend on how likely the individuals are to consume them. To refine the risk assessment of coated seed consumption by birds we studied the consumption and avoidance of seeds treated with imidacloprid, thiram, maneb or rhodamine B under different scenarios of food unpredictability (diversity or changes in food sources). In a first set of experiments, we examined during four days the amount of ingested food by red-legged partridges (*Alectoris rufa*) when offered untreated seeds, treated seeds or both. In the latter case, we also assessed the effect of a daily interchange in the position of feeders containing treated and untreated food. A second experiment, conducted with imidacloprid only, consisted of offering, during 27 h, fixed overall amounts of treated and untreated food, equally distributed in a different number of feeders per pen (1, 2, 4 or 8 feeders of each type of food) in order to diversify food sources. All the tested pesticide-treated seeds were avoided in two-choice experiments, and imidacloprid and thiram were also avoided in one-choice experiments. We found that imidacloprid treated seeds were avoided, probably as a consequence of a conditioned aversion effect due to the post-ingestion distress. However, under a diversification of two-choice food sources with multiple feeders, imidacloprid-treated seeds were ingested by partridges at increasing amounts that can produce sublethal effects or even death. Thiram treated seeds were also initially avoided in one-choice experiment, but probably mediated by a sensory repellence that progressively decreased with time. Our results reveal that the risk of pesticide exposure in birds may increase by unpredictability of food resources or prolonged availability of coated seeds, so pesticide registration for seed coating should consider worst-case scenarios to avoid negative impacts on farmland birds.

* Corresponding author at: IREC, CSIC-UCLM-JCCM, Ronda de Toledo s/n, 13071, Ciudad Real, Spain. Tel.: +34 926295450.
E-mail address: Rafael.Mateo@uclm.es (R. Mateo).

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1. Introduction

Pesticide coated seeds can be used as food resource by farmland birds during sowing periods, when alternative food sources are usually scarce. Depending on the pesticide, this route of exposure can be a risk of direct and severe intoxication for birds. The risk assessment related to treated seed consumption by birds under the first tier approach considers a worst case scenario in which all the food consumed by birds is treated, but higher tier approaches taking the possible avoidance of the treated food into account are also needed to determine the risk (European Food Safety Authority, 2009). The avoidance of a toxic food item could happen in two ways: (1) a rejection due to the physical characteristics of the food such as taste, odour and colour (Avery, 1984; Luttik, 1998; Tucker, 1965) or (2) a rejection due to sickness produced by a sublethal intoxication through a mechanism of conditioned aversion (Avery et al., 1997; Blackwell et al., 2001; European Food Safety Authority, 2009). In the former scenario, avoidance will occur before the bird ingests a significant amount of toxic, whereas in the latter one it may occur after the ingestion of a given amount of toxic which is generally expected to be non-lethal, although sometimes the bird can die if aversion takes too long to occur (Bennett, 1989a) or the bird eats too fast due to, for example, starvation, competition or predation risk (M'Kay et al., 1999; Pascual et al., 1999). Furthermore, although avoidance of pesticide-treated seeds could prevent the death of birds, important sublethal effects could still occur.

Eighteen pesticides or pesticide mixtures are currently approved in Spain for cereal seed treatment (MAGRAMA, 2013), fourteen of which are fungicides (e.g. thiram and maneb) and the remaining four are insecticides. Starting in December 2013, the European Union has banned the use of the three neonicotinoid insecticides (i.e. imidacloprid, thiamethoxam and clothianidin) for seed coating, soil treatment and foliar treatment due to its toxicity on pollinators, but its use for winter cereals sown during the autumn will continue to be authorized (Regulation 485/2013). Previously, we have found that feeding on wheat seeds treated with imidacloprid or thiram can produce adverse effects on the immune function and ultimately cause the death of red-legged partridges (*Alectoris rufa*), as well as reduce chick survival as a consequence of parental exposure (Lopez-Antia et al., 2013). Therefore, the risk of poisoning associated with seeds treated with these pesticides can be diminished only if coated seeds have a repellent effect on birds. Avoidance by birds of toxic compounds present in their food has been observed with several pesticides (e.g. Bennett, 1989a,b; Kononen et al., 1987). Furthermore, the colour coating that must be applied to pesticide treated seeds may also have the potential to repel birds (Hartley et al., 2000). However, avoidance is not equally effective for all pesticides and can be reduced under conditions of food shortage (Pascual et al., 1999). Although some studies have proven that results of experimental avoidance tests cannot be extrapolated to field conditions (Mineau and Palmer, 2013; M'Kay et al., 1999; Pascual et al., 1999), these experiments may contribute to elucidate whether birds reject or not each given pesticide, and to understand the avoidance behaviour under unpredictable scenarios and the importance of the availability of alternative food resources.

Here we have experimentally tested the avoidance by red-legged partridge of one insecticide used for seed treatment with known effects on birds' health (imidacloprid); and two fungicides (thiram and maneb) of lower acute toxicity. Moreover, we also tested the potential effect on visual avoidance produced by the most used seed dye, rhodamine B. We wanted to determine if partridges selected the untreated seeds against the treated ones and how this selection/avoidance varied depending on the unpredictability of the environment (diversity or changes in food sources) or the habituation during prolonged exposures. We hypothesize that birds are less prone to avoid treated seeds when their presence is subjected to higher variations and that any avoidance of treated seeds, especially when there is no alternative food, might decrease over time if primary repellence is not followed by a conditioned aversion mechanism mediated by toxic effects.

2. Material and methods

2.1. Chemical selection

For this experiment we selected three products commonly used as seed treatment: one insecticide (imidacloprid) and two fungicides (thiram and maneb). Imidacloprid and thiram produce proven harmful effects on birds' health (Lopez-Antia et al., 2013), whereas maneb is supposed to be less toxic for birds. We also tested the most used seed dye, rhodamine B, also present in the pesticide-coated seeds.

Imidacloprid is one of the most commonly used insecticides worldwide, belonging to the family of neonicotinoids that have dominated the seed protection market with a share of the 77% (Elbert et al., 2008). Despite the recent ban for some uses in the European Union (Regulation 485/2013), its use for seed coating of winter cereal sown during the autumn is still authorised. It acts binding to specific nicotinic acetylcholine receptors, thus interfering with the transmission of nerve impulses. Oral median lethal doses (LD_{50}) for birds available in the literature vary from 31 mg/kg in the Japanese quail (*Coturnix japonica*) to 152 mg/kg in the bobwhite quail (*Colinus virginianus*) (Tomlin, 2006). In the field there are some documented cases of wild bird mortalities due to the ingestion of seeds treated with imidacloprid (Berny et al., 1999; Bro et al., 2010; De Snoo et al., 1999) and some cage studies have proven that exposure to this insecticide has direct effects on biochemical and oxidative stress parameters, immune system or reproduction in birds (Balani et al., 2011; Lopez-Antia et al., 2013; Siddiqui et al., 2007). Some studies suggest the possible repellent effect of imidacloprid for birds (Avery et al., 1993, 1994, 1997).

Thiram is a dithiocarbamate fungicide known to negatively affect avian growth, physiology and reproduction (Guitart et al., 1996; Lopez-Antia et al., 2013; Subapriya et al., 2007a,b). Oral LD_{50} for birds has been found to vary from 673 mg/kg in the ring-necked pheasant (*Phasianus colchicus*) to more than 2800 mg/kg in the mallard duck (Hudson et al., 1979). Thiram has been widely studied and used as a bird and mammal repellent (Kennedy and Connery, 2008; Nolte and Barnett, 2000; Werner et al., 2010).

Maneb is a dithiocarbamate widely used as fungicide in agriculture and suspected to be an endocrine disruptor (Vandenberg et al., 2012) with effects such as the inhibition of the thyroid function (Cocco, 2002; McKinlay et al., 2008) or the decrease in plasma testosterone levels (Manfo et al., 2011) occurring at very low doses. Oral LD_{50} for birds is less than 1500 mg/kg during 14 days for common starling (*Sturnus vulgaris*) or pheasant, and more than 5000 mg/kg during 8 days for mallard duck (USEPA, 2000). To date, nothing has been published about the repellent effect of maneb for birds.

Rhodamine B is a red dye widely used in treated seeds as legislation requires these kinds of seeds to be coloured to prevent human consumption. In a field experiment performed by de Almeida et al. (2010), seeds treated with rhodamine B were consumed by wild birds in equal amounts as untreated seeds. However, other studies have described a neophobic response to dyed food in birds used to eat the same non-coloured food (Hartley et al., 2000).

2.2. Seed treatment

For the experiments, we purchased the following pesticides: 1) Escocet® (imidacloprid 35% w/v, Bayer CropScience, Alcácer, Spain) 2) Pormasol® Forte (thiram 80% w/w, Bayer CropScience, Alcácer, Spain) and 3) Sembral maneb col® (maneb 40% w/v, Cequisa SA, Barcelona, Spain). The second product is not specifically for seed treatment because thiram-based products for this purpose were not available at the time we purchased the pesticides. Seeds were sprayed with the recommended doses for cereal seed coating