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MORTALITY OF THE YELLOW MEALWORM *TENEBRIO MOLITOR* EXPOSED TO FERTILIZERS AND HERBICIDES COMMONLY USED IN AGRICULTURE

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YELLOW MEALWORM
GLYPHOSATE
2,4-D
NITRATES
FARMING MANAGEMENT
INSECT CONSERVATION
FOOD AVAILABILITY
WILDLIFE CONSERVATION

ABSTRACT. – A preliminary screening test was performed to examine whether fertilizers or herbicides commonly used by farmers affect the development and mortality of the grain beetle *Tenebrio molitor* (mealworms). Mealworms (n = 300) were exposed for four weeks to four different treatments: organic liquid fertilizer (pig manure), organic solid fertilizer (turkey litter), mineral fertilizer (nitrates), and herbicides (a mixture of glyphosate and 2,4-D). After four weeks in direct contact with all treatments, mealworm mortality ranged from 74 % to 88 %. Surprisingly, control mealworms placed in the same room with the other treatments also experienced high mortality (72 %) while mortality of control-isolated mealworms was low (8 %), suggesting that volatile compounds from tested products can be noxious to larval insects. Because *Tenebrio* larvae and other insects are the main food source for many birds, lizards and other wildlife, organic fertilizers from farms should be adequately treated before being dispersed in the field. Also, mineral fertilizers and herbicides should be used with moderation and in the prescribed proportions.

INTRODUCTION

Over the past 40 years many countries have experienced an agricultural revolution. This revolution has occurred at two scales. First, fields have been amalgamated to allow efficient and large scale farming. Secondly, changes in agricultural practice have allowed an increased production through the use of fertilizers, herbicides and other pesticides. In the second half of the 20th century many zones throughout the world have doubled the use of inorganic nitrogen, causing important negative changes for the wild flora and fauna (Vickery *et al*. 2001). Poultry (e.g., broiler, turkey) litter is extensively applied to agricultural lands as organic fertilizer because it contains high levels of elements that favour plant growth (Ponder *et al*. 2005). However, trace elements (*e.g*. copper and zinc) are often added to poultry diets to increase resistance to diseases, and therefore the litter also contains a large variety of trace elements which are potentially toxic to living systems when present in sufficient concentrations (Kpomblekou *et al*. 2002). Also other organic fertilizers such as pig manure can be toxic due to high concentrations of arsenic (Li & Chen 2005). Organic fertilizers also have a large variety of microbial communities, including the bacteria *Esherichia coli* and *Salmonella* sp.

In many regions of Spain where farming activity is high and constitutes an important economical source for the population, the use of organic fertilizers such as manure from animal farms is intense. They are used to increase cereal growth, but occasionally manure from animal farms is also discarded in fields. In many cases farmers eliminate much larger quantities of organic fertilizers than are allowed by law. This poses a considerable threat to many species. As has been described in other areas in Europe, intensive farming practices in Catalonia, NE Spain are associated with decreasing populations and biodiversity.

The present study was designed as a preliminary screening to assess the impact that different agrochemicals have on terrestrial insects. We examined the effects of commonly used mixtures of organic and mineral fertilizers, and herbicides on the mortality of the yellow mealworm (*Tenebrio molitor*). The yellow mealworm was chosen as an assay animal because it is commercially available and very easy to reproduce in captivity. Moreover, *T. molitor* is a frequently used model species in scientific studies (Vijver *et al*. 2003, Armitage & Siva-Jothy 2005).

MATERIAL AND METHODS

Treatments. - Two types of organic fertilizers (turkey and pig manure), one mineral fertilizer, and one herbicide mixture, commonly used in farming practices in Catalonia, were tested. The turkey manure came from 2-month old birds that were not medicated at that time and was obtained from a local farm. The pig manure was obtained from young and adult animals that were medicated against the itch infection. At the time of the study, we used the available products that were being applied in the field, despite the differences in medication. Unfortunately, a detailed analysis of both organic fertilizers was not available.

We used a mixture of two types of synthetic herbicides (1 liter of the isopropylamine salt of Glyphosate (Logrado, Masso Divission Agro), 36 % p/v (360g/l), and 100 cm^3 of 2,4-D (Agrodan), 80 %, in four liters of water), both herbicides commonly used in the zone, were also examined. Using a binary mixture makes difficult to deduce the individual effect of each herbicide to the insect. However, we wanted to assess the effect of the herbicides actually used in the field in our study area on *T. molitor* mortality. The farmers there mix two types of herbicides and apply such mixture in the field.

The mineral fertilizer consisted of dry nitrate pellets dissolved in water (27 %). The analysis of the turkey excrements used in this study showed very high levels of pathogenic microorganisms, and comprises *Escherichia coli* = 3,300,000 UFC/g, aerobic bacteria = 480,000,000 UFC/g, anaerobic bacteria (*Clostridium perfringens)* = 13,000 UFC/g, *Salmonella* = positive, mucor fungi with filaments = 700,000 colonies/g, and yeast = 15,000,000 colonies/g.

Animals and experimental design. - We used 300 healthy mealworms of similar size. They were placed in manufactured soft aluminium open boxes (16 x 11 x 3 cm). A set of 5 boxes with mealworms were employed for each treatment (10 individuals in each; total = 50 larvae). We also used 2 control treatments. One control was placed in the same room as the other treatments (control non-isolated), and the other was isolated in a different room (control–isolated).

All mealworms were kept under similar artificial environmental conditions (dark room with a mean temperature of 25 ºC, range: 24-26 ºC), at the Agriculture School of Solsona (Catalonia, Spain). All were fed on the first and 21^{th} day of the experiment, with similar type and quantity of food: 12 g of white wheat, 3 g of dry powdered cat food, 10 g of green lettuce and ca.10 g slice of white bread. Each box was covered with a piece of cellulose paper (10 x 8 cm). Water (10 ml) was added once a week to each slice of bread.

The different treatments were applied only once at the beginning of the experiment, following the procedure used by the farmers in the zone. For each treatment we added in each box 20 g of turkey excrements or 12 g of pulverized nitrate pellets dissolved in water.

The response of mealworms to different treatments was measured in each box once a week during 4 weeks. Number of live and dead mealworms, number of moults, and number of pupae were recorded. The presence of morphological anomalies was also noted.

Statistical analysis. - We employed a survival analyses to measure the probability of death after a period of 4 weeks (e.g., Charalambidou *et al*. 2003). Differences in death time between treatments were tested by fitting a Cox proportional hazards regression model. Treatments were considered as a fixed effect, and to account for the putative differences between boxes, a replicate effect was added as a random effect (as referred to in literature on survival analysis). Survival analyses were completed using the survival module of the R package (http://www.r-project.org/).

RESULTS

Mealworm mortality

Mealworm mortality was high and significantly different between treatments after 4 weeks (Table I). The probabilities of death over time were found to be significantly higher for the control non-isolated mealworms compared to the control-isolated mealworms ($P < 0.001$). Also, mealworms in contact with herbicides and nitrates showed shorter lifespan than that of control non-isolated ones ($P = 0.02$ and 0.03 respectively). In contrast, survival probabilities of mealworms in contact with pig or turkey excrements were not significantly different from control non-isolated ones.

Moults and pupae

The number of moults produced by the mealworms after one week was different among treatments (control isolated = 13, control not isolated = 23, nitrates = 7, herbicides = 6, turkey excrements = 12, pig excrements = 15) and decreased due to the high mortality of mealworms after the first week. In all treatments the development of pupae during the first week was very low (n = 3) and only started in nitrates (n = 2) and herbicides (n = 1). After 4 weeks the percentage of pupal development was still low in all treatments (Table II). Pupal mortality after 4 weeks was high in nitrates and herbicides (50 % respectively) and in pig excrements (40 %), but no dead pupae were found in the other treatments.

We did not find any abnormal appearance of the mealworms or pupae during the study, in contrast to the findings reported by other authors (García *et al*. 2003).

DISCUSSION

Direct effect of the products

Our study demonstrates that all the products tested,

Table I. – Number (n) and percentages (%) of mealworm mortality after 1 and 4 weeks under different treatments. The number of boxes for each treatment was 5 with 10 mealworms in each. Total mealworm sample size for each treatment was similar (n = 50). The results of Cox proportional hazards regression model for mealworm mortality, are also indicated. Chi-squared values (χ^2), degrees of freedom (df) and probability values (P) correspond to the comparisons between the control-non isolated treatment and the other five treatments employed after a period of 4 weeks. Mealworm mortality was significantly lower in control isolated in relation to the other treatments.

Treatments	Mealworm mortality				Survival analysis			
	1 week		4 weeks		4 weeks			
	%	n	%	n	χ^2	df	P	
Control-isolated	2	1	8	4	27.19	1	< 0.001	***
Control-non isolated	18	9	72	36				
Nitrates	36	18	86	43	5.06	1	0.024	*
Herbicides	34	17	88	44	4.35	1	0.037	*
Turkey excrements	18	9	74	37	2.04	1	0.150	n.s.
Pig excrements	20	10	84	42	0.1	1	0.750	n.s.
Box (random) (boxes*treatment, 5*6)					0.02	0.01	0.660	n.s.

Table II. – Cumulative number of pupae developed after 1 to 4 weeks (bold) for different treatments. The number of boxes for each treatment was 5 with 10 mealworms in each.

Treatments	**1**	**2**	**3**	**4**
Control-isolated	0	0	6	16
Control-non isolated	0	1	2	8
Nitrates	2	5	6	6
Herbicides	1	3	4	6
Turkey excrements	0	2	6	10
Pig excrements	0	0	2	5
Total	3	11	26	51

which are commonly used in agricultural practices, are noxious for the larvae and pupae of *T. molitor*. The high mortality of control non-isolated mealworms has been quite surprising. Contamination during manipulation seems rather unlikely because in that treatment mortality was high in all independent boxes of this group (from 50 % to 90 %) after 4 weeks.

After 4 weeks, larvae mortality was very high in all treatments. Herbicides and nitrates appear to be the most toxic, as can be seen after one week exposure. The strong negative effects that herbicides and other pollutants have on wildlife have been already demonstrated (Mañosa *et al*. 2000). In our study, we tried to match as much as possible the natural conditions and we used a binary mixture of herbicides. However, it is still necessary to conduct further studies examining the effect of each herbicide separately on mortality.

Bird excrements from aviaries can be dangerous when spread outdoors, as they may contain fungi, bacteria and small insects. In fact, it has been shown that *T. molitor* can be parasitised by many species of invertebrates (Oliveira *et al*. 2004, Warr *et al*. 2004) including those found in poultry manure. In many barns with infected ruminants, *Mycobacterium avium* has been isolated, and *T. molitor* larvae can be infected with these bacteria in naturally contaminated bran and peat (Fischer *et al*. 2004). Consequently, infected beetles could mechanically transmit mycobacteria to their predators, that could also be infected, and increase the chain of diseases.

Volatile effects

The present study showed that larvae might die solely from fertilizer or herbicide exposure from air. This suggests that the products we used are very potent toxicants for the larvae. This might imply that *Tenebrio* larvae (and potentially also other insect larvae) could be affected in larger areas, other than the fields treated. However, we do have to point out that the experiment was conducted under laboratory conditions in the absence of ventilation or rainfall, which might have increased the effects of aerial exposure.

Conservation aspects

The effect of farming on biodiversity is a considerable threat to many organisms, including plants, insects and birds. The decrease of many farmland birds, such as the partridge (*Alectoris rufa*) and their food sources, is a problem in many developed countries and is usually attributed to the use of high levels of fertilizers and pesticides (Potts 1980, 1986, Rands 1986, Moreby & Southway 1999). In one area of NE Spain (Sanaüja, Lleida, Catalonia), the population of the red-legged partridge (*Alectoris rufa*) has dramatically declined in the last 20 years (Castilla & Martínez in press), and there is a great controversy amoung managers, hunters and farmers about the causes that have contributed to their decline. However, partridges appear to be able to find food rich in calcium in the same study area (Castilla *et al*. in press).

The present study shows that herbicides and nitrates are especially toxic to the *Tenebrio molitor* larvae, and tenebrionids are the most preferred food items for many farmland bird species. The decrease in invertebrates associated with farming practices might therefore affect the survival and successful reproduction of birds (Savory 1989, Hart *et al*. 2006). Because agrochemical products negatively affect biodiversity, they should be spread with caution and adequately treated before being dispersed in the field. It has been proved that low additions of organic fertilizers benefit some invertebrate prey species (Vickery *et al*. 2001). Several authors have suggested that management to conserve and increase wild bird numbers should concentrate on improving foraging habitat quality, i.e. increasing the abundance of nutritious invertebrate chick-food (Browne *et al*. 2006). Thus, a higher control of agricultural practices should be conducted by the local administrations, and more founding of projects focused on the relationships between farming and biodiversity should be encouraged (see, for similar claims: Berry *et al*. 2005, Chhatre & Saberwal 2005).

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