

Mid-term effects of exposure to fungicide-treated seeds in feral rock pigeons (*Columba livia*)

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Abstract

The feeding response of feral rock pigeons (*Columba livia*) to wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) seed treated with the fungicide «Maneb» (manganese ethylene-bisdithiocarbamate) was assessed under aviary conditions. Bird preferences for dry or moist seed were also recorded. In all cases, non-treated (49.14 g bird⁻¹ day⁻¹) rather than treated seed (32.97 g bird⁻¹ day⁻¹) was selected, and wheat rather than barley (48.16 compared to 33.95 g bird⁻¹ day⁻¹ respectively). Dry seed was consumed in preference to moist seed (47.53 g bird⁻¹ day⁻¹ compared to 15.08 g bird⁻¹ day⁻¹) — a preference noted both when treated and non-treated seed was consumed. The birds fed exclusively on treated seed showed a reduction in body weight; no significant differences were recorded in those fed either untreated or mixed seed. Water consumption was three times greater in pigeons fed exclusively on treated seed.

Additional key words: diet, fungicide, Maneb, seed treatment.

Resumen

Efectos a largo plazo de la exposición de semillas tratadas en aves: un estudio experimental con paloma bravía salvaje (*Columba livia*)

Este trabajo evalúa la respuesta alimenticia que presenta la paloma bravía (*Columba livia*) a una dieta de semillas de trigo (*Triticum aestivum*) y cebada (*Hordeum vulgare*) tratadas con el fungicida Maneb (etilen-bisditiocarbamato de manganeso). La exposición al alimento se efectuó en períodos prolongados de tiempo y bajo las condiciones de un aviario. Aparte del efecto del tratamiento también se consideró la condición (seca o húmeda) de la semilla consumida. En todos los casos, las aves escogieron semillas no tratadas (49,14 g por ave y día) antes que las tratadas (32,97 g). El consumo de trigo fue más alto que el de cebada (48,16 g y 33,95 g, respectivamente). Las semillas secas (47,53 g) de ambas especies de cereal fueron consumidas más que las semillas húmedas (15,08 g), y esta preferencia persistió con el tratamiento o no de las mismas. Las palomas que se alimentaron exclusivamente de semillas tratadas mostraron una reducción en el peso corporal, pero no se encontraron diferencias estadísticamente significativas del peso entre aves que se alimentaron de semillas sin tratamiento y las que consumieron una mezcla de ambas clases. El consumo del agua fue tres veces más alto en palomas alimentadas exclusivamente con semillas tratadas.

Palabras clave adicionales: dieta, fungicidas, Maneb, tratamiento de semillas.

Introduction

Fungicides are widely used for protecting crops. These can be applied to plants in the form of solutions of wettable powder, sprays or dusts. Seeds are often treated to provide pre-emergence protection.

Maneb, Propineb, Ziram and Mancozeb are commercial carbamate fungicides used worldwide (Mineau *et al.*, 1999). Maneb is of moderately low toxicity to birds (Gosselin *et al.*, 1984) although acute doses can induce severe symptoms such as hypotonia, slow breathing and bradycardia, functional abnormalities of the thyroid and liver, hyperactivity with lack of coordination, loss of muscular tone, nausea, vomiting, loss of appetite, weight loss, delayed reflexes, respiratory paralysis and death (Smith, 1992; Fryday *et al.*, 1996).

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Birds exposed to chronic doses of Maneb show weight loss, weakness of the hind legs and increased mortality (Hill and Camardese, 1986), thyroid abnormalities, delayed reflexes, paralysis, impaired kidney function and benign lung tumours (Smith, 1992). Adverse effects on fertility and foetal development have also been recorded, such as the suppression of egg formation, eggshell thinning, impaired incubation behaviour (Maci and Arias, 1987; Parker and Goldstein, 2000), increased embryo mortality and early foetal death, stillborn offspring, incapable newborns, and abnormalities of the eye, ear, body wall, central nervous system and skeletomuscular system (Hudson *et al.*, 1984). However, all this information has come from laboratory research using lethal or sublethal concentrations of the fungicide. No information is available on its effects at environmental concentrations or over time.

The Iberian Peninsula is the winter home of a large number of migratory birds from northern and central Europe (Tellería *et al.*, 1988). The main food source of these visitors is the cereal seed sown between October and February. Most of this seed will have been treated with pre-emergence fungicides, usually Maneb. The aim of the present study was to investigate the selection preferences of the rock pigeon (*Columba livia*) for untreated or treated wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) seed. Preferences for dry or moist seed (simulating recently sown seed and seed that has been in the soil for several days) were also recorded. Finally, attempts were made to determine whether the consumption of treated seed causes mid-term behavioural problems in these birds.

Material and Methods

Twenty four feral rock pigeons (*Columba livia* L.) were captured in the north-eastern corner of the province of Salamanca (central-western Spain), an area of extensive cereal croplands. These birds were captured in early August using a mist nest; all were weighed at the time of capture. No discrimination was made among colour varieties. After a short trip (5-15 min), the birds were housed in an outdoor aviary (2.75 x 1 x 2 m) with cages for individual birds. The backs and sides of these cages were lined with thick paper to visually isolate the birds from one another. All birds were individually colour-ringed and fed a mixture of poultry feed, corn, beans, bread and cooked rice. An

adjustment period of three weeks was allowed before any experiments were undertaken. All experiments were performed between September and February.

Three groups of eight pigeons were established: group 1 - birds fed on untreated seed only; group 2 - birds fed on treated seed only; group 3 - birds fed on treated and non-treated seed.

In treated-seed experiments, all seeds (both of wheat and barley) were treated with 40% w v⁻¹ Maneb (manganese ethylene-bisdithiocarbamate) at a concentration of 3 ml per kg. This concentration is recommended by the manufacturer and is commonly used in Spanish croplands.

The three groups were simultaneously offered their corresponding seed types in separate feeders (dimensions 10 x 15 x 8 cm) made of transparent plastic with a 4 cm circular opening at the top to prevent seed being thrown out during consumption. The number of feeders in the cages varied from 4-8, with sufficient space between them to allow easy access. All feeders were placed on wooden platforms (0.92 x 0.4 m) at a height of 30 cm in the cage so that no choices would be made on the grounds of space constraints. These feeders maintain the relative humidity of the feed provided, thus preventing seed moisture changes from influencing selection (Fryday *et al.*, 1998). Maneb evaporation has been described as negligible at 20°C (Hornsby *et al.*, 1996); since the experimental temperature range was from 4 to 16°C none should have evaporated from the treated seed.

Consumption was calculated by weighing the seed offered to the pigeons and by weighing what was left 7 days later. The experiments were then repeated every week during the experimental period, changing the locations of the feeders each time to ensure that true seed selection was made.

Experiments on preferences for dry and moist seed were performed only in December. The four combinations of seed (treated or non-treated, wheat or barley) were weighed and then placed in water for two days to moisten them, thus imitating the ground moisture conditions of the sowing season. All pigeon groups received moist and dry seed *ad libitum* for two days. After this time, all seed was removed and replaced by fresh seed. The moist seed not eaten was dried in an oven at 90°C to compare the total weights consumed.

The weight of the pigeons was checked individually every 15 days in the early morning. Water consumption

was measured using a calibrated drinking trough; no other water sources were available. The mobility of each group of birds was recorded daily over 30 min periods. The sequence, intensity and duration of different behaviours (resting, feeding, drinking, nesting, flight, settling, flapping, grooming and preening) were recorded.

The average food consumption of groups 1 and 2 were compared by one-way ANOVA. In group 3, consumption was analysed (in terms of seed species and treatment) by two-way ANOVA. Differences in the behavioural variables between groups and months were compared by one-way ANOVA. Inter-group differences were examined by post-hoc analyses using the Scheffé test (Sokal and Rohlf, 1994).

Results

Consumption of treated and non-treated seeds

Significantly more untreated than treated seed was consumed ($49.14 \text{ g bird}^{-1} \text{ day}^{-1}$ compared to $32.97 \text{ g bird}^{-1} \text{ day}^{-1}$; $F=14.337$, $P<0.001$ for groups 1-2 and $F=29.248$, $P<0.001$ within group 3). Dietary selection between seed species was also statistically significant ($F=21.565$, $P<0.001$ and $F=5.019$, $P<0.05$ between groups 1-2 and within group 3 respectively): wheat was preferred over barley, with average consumptions of $48.16 \text{ g bird}^{-1} \text{ day}^{-1}$ compared to $33.95 \text{ g bird}^{-1} \text{ day}^{-1}$. No significant interaction was found between treatment and seed type ($F=0.674$ for group 3).

Selections made between treated/non-treated seeds and barley/wheat showed the birds preferred non-treated seeds, regardless of the cereal consumed. However, wheat was preferred over barley (Table 1).

Table 1. Quantities ($\text{g bird}^{-1} \text{ day}^{-1}$) of the different types of seed consumed (species, treatment).

Group	Species	Seed	Mean	SE
1	Barley	Untreated	49.87	3.11
1	Wheat	Untreated	64.35	3.65
2	Barley	Treated	32.69	2.89
2	Wheat	Treated	52.90	4.11
3	Barley	Untreated	32.68	3.32
3	Wheat	Untreated	49.65	2.97
3	Barley	Treated	20.55	2.97
3	Wheat	Treated	25.73	2.99

Consumption of dry/moist, treated/non-treated seed

Significant differences were seen in seed consumption depending on its moisture condition ($F=64.666$, $P<0.001$ between groups 1-2 and $F=66.845$, $P<0.001$ within group 3), with the birds selecting dry seed ($47.53 \text{ g bird}^{-1} \text{ day}^{-1}$) in preference to moist seed ($15.08 \text{ g bird}^{-1} \text{ day}^{-1}$). When treated/non-treated seed species were compared, dry seed was always consumed in preference to moist seed (regardless of treatment) ($F=6.049$, $P<0.05$ for dry seed species, $F=3.912$, $P<0.05$ for dry treated seed between groups 1-2, and $F=4.696$, $P<0.05$ for dry seed species and $F=4.330$, $P<0.05$ for dry treated seed within group 3). No significant interaction was observed between treatment and seed type or moisture condition ($F=2.609$ for *moisture condition x seed type* within group 3 and $F=0.107$ for *treatment x moisture condition* within group 3). Moist, treated barley seeds were the least consumed (Table 2).

Behavioural changes and water consumption

Significant differences were seen in body weight variation between the three groups ($F=15.197$, $P<0.01$). Post-hoc ANOVA analyses showed significant differences between groups 2 and 3, and between groups 1 and 2, but no differences were seen between groups 1 and 3 (Fig. 1).

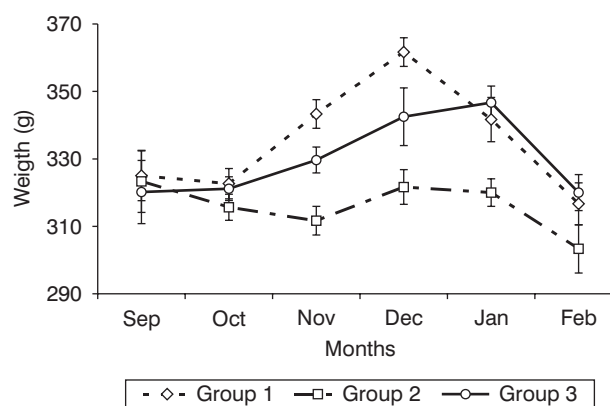


Figure 1. Average weight variation in the different groups. Vertical bars represent standard errors

Table 2. Quantities (g bird⁻¹ day⁻¹) of the different types of seed consumed (species, treatment, condition)

Group	Species	Seed	Condition	Mean	SE
1	Barley	Untreated	Dry	54.10	5.70
1	Barley	Untreated	Moist	19.08	6.86
1	Wheat	Untreated	Dry	63.60	8.17
1	Wheat	Untreated	Moist	35.51	6.07
2	Barley	Treated	Dry	47.10	4.32
2	Barley	Treated	Moist	9.25	3.13
2	Wheat	Treated	Dry	67.15	6.12
2	Wheat	Treated	Moist	20.12	3.68
3	Barley	Untreated	Dry	34.50	3.28
3	Barley	Untreated	Moist	11.49	3.18
3	Wheat	Untreated	Dry	54.33	4.23
3	Wheat	Untreated	Moist	16.33	3.33
3	Barley	Treated	Dry	26.41	3.31
3	Barley	Treated	Moist	6.70	2.38
3	Wheat	Treated	Dry	33.08	3.97
3	Wheat	Treated	Moist	2.19	1.67

Mean water consumption was 87.91 ml bird⁻¹ day⁻¹, and was greatest in the group fed on treated seed (25.43 and 33.60 ml bird⁻¹ day⁻¹ for groups 1 and 3 respectively; $F=268.452$, $P<0.001$). No significant differences were observed in behavioural patterns (resting $F=1.743$, feeding $F=0.972$, nesting $F=0.899$, flight $F=1.773$, settling $F=0.486$, flapping $F=1.955$, grooming $F=0.973$ and preening $F=2.285$).

Discussion

Toxin-related bird deaths are commonly caused by a combination of insecticides, fungicides and pesticides, and are more common where large hectares are treated (Odderskaer and Sell, 1993; Hunt *et al.*, 1995; Mineau *et al.*, 1999), where acute dermal exposure is high, and where heavy arthropod mortality requires that a change to a more substantial seed diet be made (Moreby and Southway, 1999).

In recent decades, pre-emergence fungicides have been used prophylactically against crop diseases and as a pest repellent (i.e., to stop animals from foraging on sown seeds) (Avery *et al.*, 1993; Clark, 1997). The risk of acute poisoning associated with this practice has been widely discussed (De Snoo *et al.*, 1999; Pascual *et al.*, 1999b). The present results show that the consumption of a mixture of treated and untreated seed leads to no serious mid-term behavioural problems in

rock pigeons. The feed mixture of treated and non-treated seed reflects the situation birds face in the field, with treated seed available in croplands and non-treated seed supplied by natural vegetation. The groups fed with either the non-treated seed or the mixture of treated and non-treated seed showed similar behaviour. This supports the claim that birds with a varied diet in the wild may tolerate the relatively high toxin concentrations taken in when feeding on agricultural seed (McKay *et al.*, 1999). In fact, the results show that these birds preferentially select non-treated seed, and it has been reported that the toxicity associated with treated seed can be reduced through regurgitation (Pascual *et al.*, 1999a). In the present work, however, this kind of behaviour was not recorded.

The birds fed exclusively on treated seed showed a reduction in body weight. Given the increase in croplands sown with treated seed (>80% of the total agricultural area of Spain is now sown with treated seed), and the likely future increase in this practice, it is possible that pigeons in the wild will show similar weight losses.

Ethylene-thiourea is an important degradation product of ethylene-bisdithiocarbamate fungicides. At suitable temperatures this product degrades quickly in the presence of oxygen (Jacobsen and Bossi, 1997), which might explain the high water consumption recorded in the birds fed only on treated seeds. It may be

that the birds need this water to degrade the fungicide more easily. Also, since ethylene-thiourea is polar, it can be dissolved and eliminated via the excretory system (Karasov, 1990); water would be essential in this.

Wheat was preferred under all experimental conditions, probably because of its greater energy value (Wiens and Dyer, 1975). Although moist seeds are more digestible than dry seeds (Karasov, 1990), wild birds may also prefer the latter since moist, germinating seeds probably still contain fungicide. In the experimental situation, the greatest differences in weight among the different groups were recorded when the moist seeds had begun to germinate.

The present results differ from those of other studies which report increased bird locomotor activity after fungicide ingestion (Fryday *et al.*, 1996; Parker and Goldstein, 2000), probably because the doses involved were very different. The latter authors used near-lethal doses of a wettable powder solution (Parker and Goldstein, 2000) or a liquid form diluted with corn oil (Fryday *et al.*, 1996). In addition, the birds were poisoned through both dermal contact and dietary exposure. The highest degree of exposure to the fungicide was recorded just after its application to their feed.

The present results suggest that wild birds probably prefer untreated seeds. This should be taken into account when evaluating the potential risks to bird species in croplands sown with treated seeds. The results also establish that environmental concentrations of Maneb do not result in serious behavioural alterations to medium or large granivorous birds in the mid-term, especially if other food resources such as grain from natural flora or non-treated agricultural seed is available. However, the decrease in body weight detected in the birds that consumed the treated seed may be mortal to smaller bird species. In conjunction with other ecological factors (cold winter temperatures, etc.), such a weight loss might increase the risk of death by hypothermia. The possible risks to wintering avifauna should be examined.

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