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Short Communication

Herbicides and pinching off in birds of prey – pilot study

Peter C Leendertse[1]*, Bas Tinhout[1] and Arnold van den Burg[2]

[1]CLM Research and Advice, Gutenbergweg 1, P.O Box 62, Culemborg, 4104 BA, 4100 AB, Netherlands

[2]Zoological Museum Netherlands, Netherlands

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***Corresponding author:** Peter C Leendertse, Senior Adviser, CLM Research and Advice, Gutenbergweg 1, P.O Box 62, Culemborg, 4104 BA, 4100 AB, Netherlands, Email: pele@clm.nl

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Abstract

In this pilot study the herbicide tembotrione was detected in three dead nestling goshawks that suffered from excessive feather loss (also known as "pinching-off") and other internal anomalies. Tembotrione is used in maize cultivation and it blocks an essential enzyme in most plants, thereby preventing photosynthesis. It also blocks this enzyme in birds, which may lead to aberrant chick development. The three goshawk nests were located near farms with maize cultivation and it is likely that the nestlings consumed tembotrione-contaminated prey. Further study is needed to clarify any causal relationship.

Introduction

Since the 1990s, the phenomenon of "pinching-off" has been observed in birds of prey. Between 2010 and 2020 the number of cases reported by volunteers has sharply increased (unpublished data Dutch Raptor Working Group). In such cases, nestlings lose their feathers, especially the primary and secondary flight feathers and tail feathers, but more recently also incidences have been reported in which all feathers are lost (Figure 1). The cause of pinching off is not known [1,2], although a connection to a non-specific symptom of infectious diseases such as West Nile virus is sometimes suggested [3,4]. Recently, nestlings have also been found that have lost all their feathers [5]. The chicks cannot fledge and typically die from starvation, and sometimes as a result of hypothermia. In the affected nestlings, the skin is yellowed and the autopsy image shows blackening of the tendons and connective tissues, a twisted position, and pale colouration of the heart with darker spots on the ventricles.

This syndrome resembles ochronosis (or alkaptonuria), which can also occur in mammals and humans, and often results from genetic malfunctioning of the enzyme homogentisate 1,2-dioxygenase (or homogentistic acid dehydrogenase). This

Figure 1: A goshawk *Accipiter gentilis* nestling without flight feathers and down (photo Arnold van den Burg).

enzyme is important in one of the steps in the breakdown of the amino acid Tyrosine. One step upstream of this enzyme there is the enzyme 4-hydroxyphenylpyruvate dioxygenase (4-HPPD), which is the target of 4-HPPD herbicides. In animals, blocking 4-HPPD results in disruption of the breakdown of tyrosine, similar to malfunctioning of the enzyme homogentisate

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1,2-dioxygenase, but not necessarily with the same secondary effects, because of accumulation of other intermediates.

4-HPPD is also found in plants, where it plays an important role in providing carotenoids needed for photosynthesis [6]. 4-HPPD herbicides are used in agriculture to control weeds. They block this enzyme and thereby disrupt photosynthesis, which kills plants that are unable to detoxify the herbicide. Some crop plants like maize contain cytochromes that can render tembotrione harmless. It is possible that these herbicides also cause 4-HPPD disruption in birds of prey. Pesticides are often found in birds as is also shown by earlier studies in the Netherlands. Guldemond, et al. 2021 [7] reported 14 different pesticides in eggs, young and adults of barn swallows *Hirundo rustica*. Tembotrione was found in one of the adults.

As a first step to investigate a possible relationship, we conducted a pilot study to measure pesticide residues in five dead birds with poor feather development (including pinching off) and blackening of connective tissue.

Methods

In the period 2021-2023, seven dead birds were collected: three goshawk *Accipiter gentilis* nestlings from the Netherlands, one white stork *Ciconia ciconia*, and three house sparrows *Passer domesticus* from Portugal. The birds were visually selected based on characteristics of pinching off and ochronosis, like poor feather development and at least some blackening of tendon or connective tissue. In general, the internal symptoms were more severe in the Goshawks than in both other species. After storage in the freezer, muscle, kidney, liver, and blackened connective tissue were collected and commercially analysed on the presence of pesticides by Eurofins The Netherlands, using gas (GC-MSMS) and liquid LC-MSM chromatography combined with improved mass spectrometry. A total of 764 pesticides are analysed using this technique, including tembotrione and four other 4-HPPD herbicides. Samples were homogenized and extracted to prepare the samples. The standard addition method was used to identify specific pesticides. The detection limit (LOQ) of the analysis was 0,01 mg/kg.

Results and discussion

No pesticides were found in the Portuguese white stork and house sparrows and therefore the symptoms are likely to be explained by other factors, possibly heavy metal exposure or nutritional inadequacies. Some persistent and long-banned pesticides such as DDT and dieldrin were detected in the goshawks (Table 1). Furthermore, the herbicide tembotrione was found in all three goshawks. The concentration varied between 0,064 to 0,57 mg/kg.

The herbicide tembotrione has been used in the Netherlands since 2010, mainly for weed control in maize cultivation. During the past 10 years, sales and use of tembotrione have increased to between 7,000 and 9,000 kg per year (equalling an estimated 30% - 50% surface area of maize-cultivated fields). The pesticide is sprayed upon the land when weed seedlings start to develop and can be used until the maize closes the canopy or gets too high for agricultural machines. The nests of the three goshawks were located near farms with maize cultivation and it is likely that the nestlings consumed prey that were contaminated with tembotrione. Seed and seedling-eating birds like pigeons are often the staple food of goshawks during the nesting season and are likely to get exposed to this herbicide.

Table 1: Results of multi-pesticide analysis in the birds. Shown are the pesticides detected and their concentrations (mg/kg). Certain pesticides were detected qualitatively but the concentration was so low that it could not be determined with certainty.

Code	Bird species	Tissue type	Pesticide	Concentration (mg/kg)
1,1	Goshawk	liver/kidney	Tembotrione	0,064
1,2	Goshawk	muscle	P,p'-DDE Dieldrin	0,15 0,013
2,1	Goshawk	liver	Tembotrione	0,57
2,2	Goshawk	Connective tissue	P,p'-DDE	0,042
3,1	Goshawk	liver/kidney	Tembotrione	0,07
3,2	Goshawk	connective tissue	Geen	0,0
3,3	Goshawk	abdominal fat	P,p'-DDD/o,p'-DDT Antrachinon P,p'-DDT Dieldrin Cis-heptachloor-exo-epoxide (isomeer B) Pentachlooraniline	Qualitatively Qualitatively Qualitatively 0,014 Qualitatively Qualitatively
4,1	House sparrow	liver/kidney	none	< LOQ
5,1	White stork	Liver/kidney	none	< LOQ
5,2	White stork	connective tissue	none	< LOQ

Besides tembotrione, four other herbicides with active ingredients that block 4-HPPD have been placed on the market in the Netherlands since the late 1990s. Residues of these herbicides were not found in the birds.

Based on this pilot study, it cannot be determined whether tembotrione causes pinching off and ochronosis in goshawks. The mechanism in the birds seems to deviate from the exact mechanism in humans, in which tembotrione can be administered to patients with genetic defects of homogentisate 1,2-dioxygenase to prevent some of the ochronosis symptoms (pers. comm. A. Thijs, physician of Amsterdam UMC), that does prevail in the birds. To further investigate a possible relationship between 4-HPPD herbicides, ochronosis, and pinching-off, we recommend that measurements be made in a larger sample of nestlings of raptors with and without symptoms. To further clarify any causal relationship, the possible route of contamination of nestlings of birds of prey due to agricultural use of the active substance should also be investigated. Such a study should focus on the use of tembotrione (and possibly other 4-HPPD-inhibiting herbicides) in the vicinity of the nests and contamination in the food chain (prey animals and their food). Furthermore, the upstream and downstream physiological consequences of the blocked 4-HPPD enzyme should be clarified in birds.

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Conclusion

The herbicide tembotrione may play a role in the phenomenon of ochronosis-related pinching-off in nestling goshawks. Further study is needed to clarify any causal relationship.

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