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IMPACT OF NATURALLY CONTAMINATED SUBSTRATES ON INSECTS: BIOACCUMULATION AND EXCRETION

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Bio-based Industries Consortium

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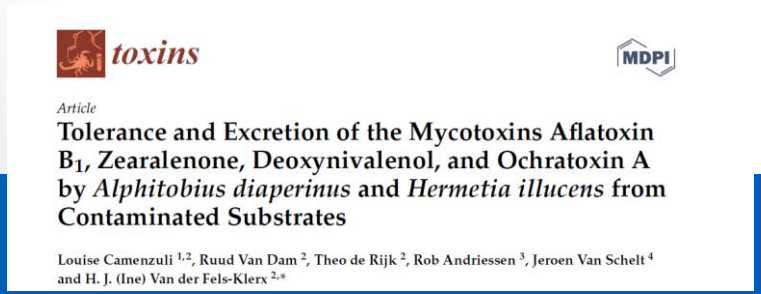
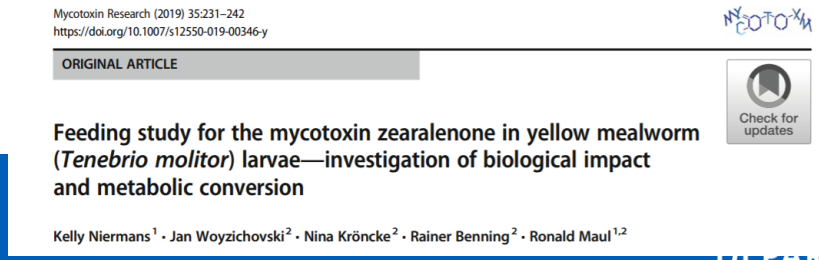
Background

Insects more and more used for feed applications, also following the recent legislative openings

Impact of feeding with contaminated (agrochemicals and mycotoxins) substrates being investigated by several groups, still much is not known

Few papers already indicated a potential ability of insects to degrade and excrete mycotoxins, and low to none level of mycotoxins overall are usually found in insects
Thus, insects seems to be particularly tolerant to mycotoxin contamination





The present study was performed on Black Soldier Fly (BSF, *Hermetia illucens*) and Lesser Mealworm (LM, *Alphitobius diaperinus*) larvae grown on naturally contaminated substrates (agrifood waste)

Substrates:

- Wheat middlings
- Corn distillation residues
- Corn gluten feed
- Rice Bran
- Rapeseed wastes
- Olive pomace
- Apple pomace
- Chopped carrots

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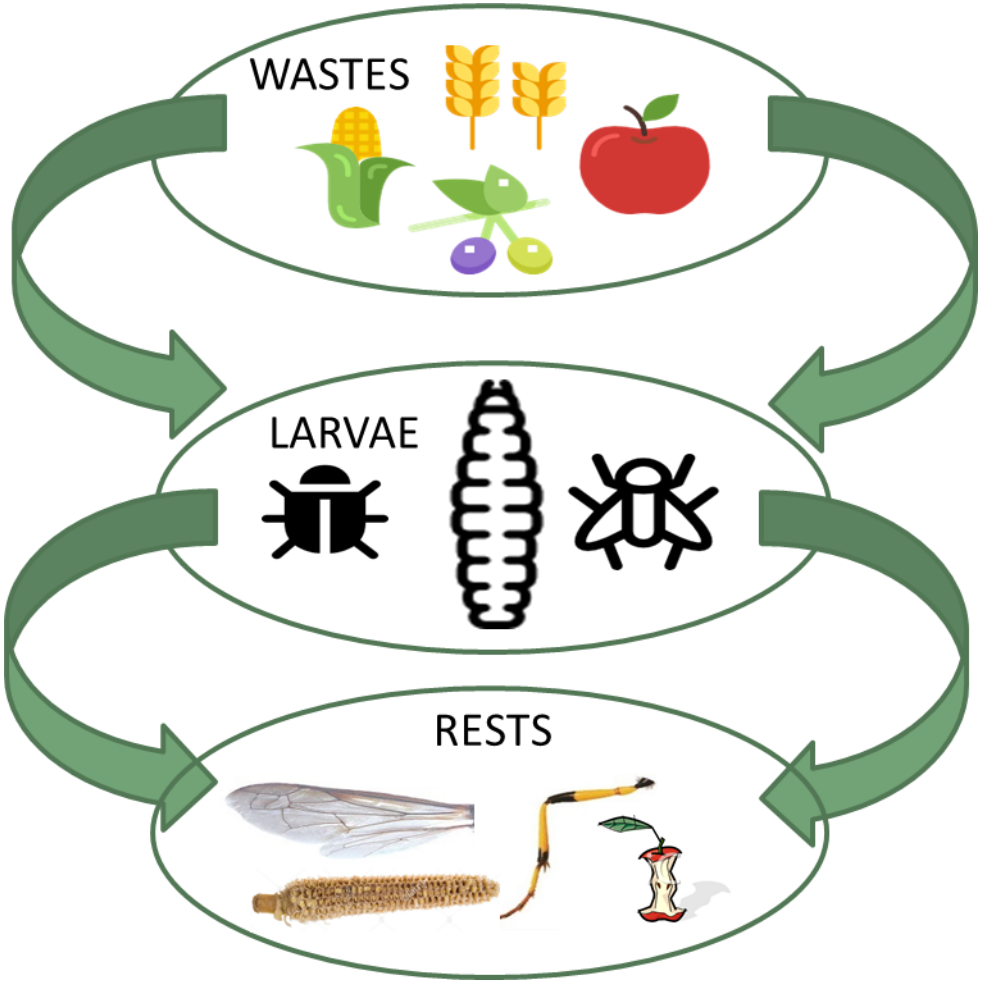
Mycotoxins and agrochemicals screened in the agrifood waste

Among mycotoxins:

	Method	LOD (µg/kg)
Aflatoxin B1 (AFB1)	LC/Fluo	0.5
Aflatoxin B2 (AFB2)	LC/Fluo	0.125
Aflatoxin G1 (AFG1)	LC/Fluo	0.5
Aflatoxin G2 (AFG2)	LC/Fluo	0.125
Ochratoxin A (OTA)	LC/MS	20
Patuline (PAT)	LC/MS	100
Deoxynivalenol (DON)	LC/MS	10
3-Acetyldeoxynivalenol (3ADON)	LC/MS	20
Nivalenol (NIV)	LC/MS	10
Fusarenon X (FUSX)	LC/MS	20
Diacetoxyscirpenol (DAS)	LC/MS	10
T2	LC/MS	10
HT2	LC/MS	10
Fumonisin B1 (FB1)	LC/MS	25
Fumonisin B2 (FB2)	LC/MS	25
Zearalenone (ZEN)	LC/MS	10

Mycotoxins in green never found in any sample

Micotoxins determined in:



Agrifood waste substrates

	Mycotoxin amount (µg/Kg)			
Description	DON	FB1	FB2	ZEN
Wheat middlings	938 ± 100	< LOD	< LOD	< LOD
Corn distillation residues	779 ± 5	573 ± 3	441 ± 3	< LOD
Corn gluten feed	1207 ± 43	727 ± 6	294 ± 5	173 ± 4
Rice Bran	< LOD	< LOD	< LOD	< LOD
Rapeseed wastes	< LOD	< LOD	< LOD	< LOD
Olive pomace	< LOD	< LOD	< LOD	< LOD
Apple pomace	< LOD	< LOD	< LOD	< LOD
Chopped carrots	< LOD	< LOD	< LOD	< LOD

DON, FB1, FB2 and ZEN detected in corn and wheat samples, still below the legal limit for feed

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Larvae

Different formulations of the agrifood waste were prepared in order to optimize larvae growth, using the SAME batches which have been analyzed in the previous tests.

For BSF larvae grown on:

- 100% Corn distillation residues
- 79% Corn distillation residues, 10.5% olive pomace, 10.5% Apple pomace

DON, FB1, FB2, ZEN always <LOD

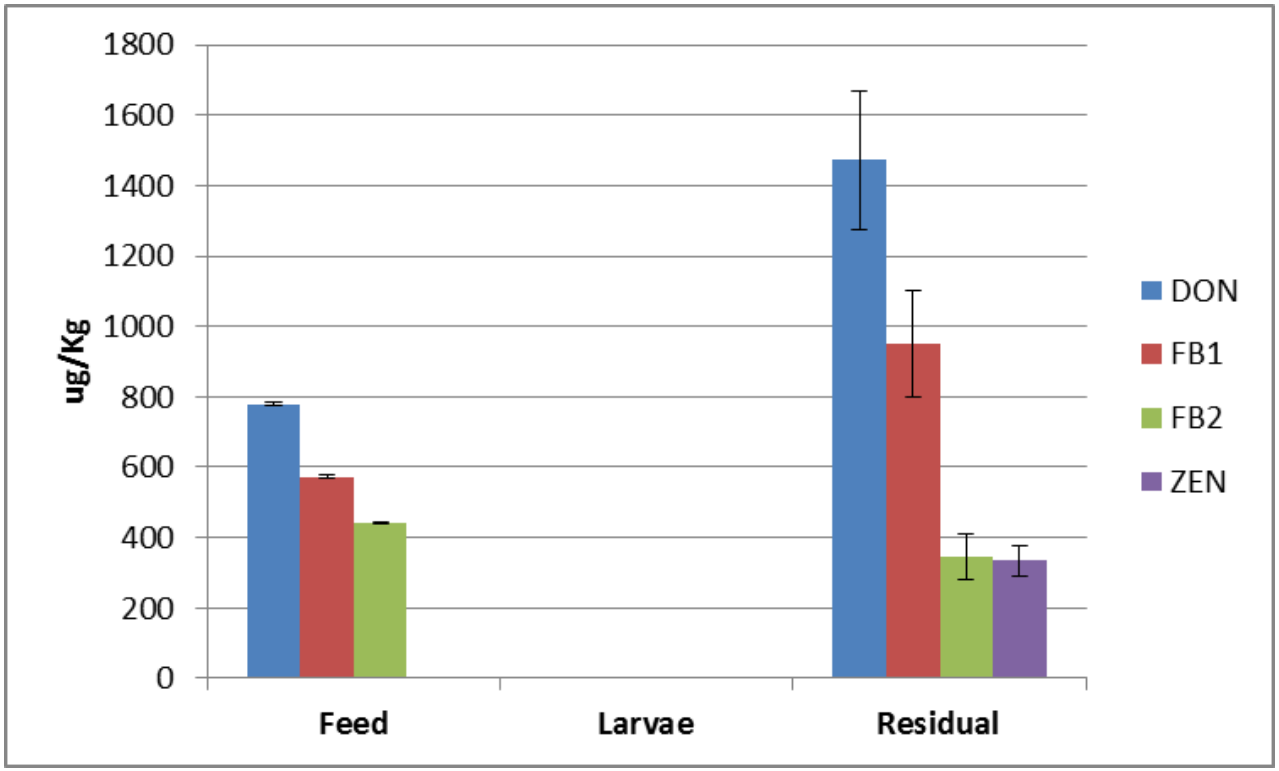
For LM larvae grown on:

	Mycotoxin amount (µg/Kg)			
Description	DON	FB1	FB2	ZEN
100% Wheat Middlings	416±28	<LOD	<LOD	< LOD
75% Wheat Middlings, 25% Corn Gluten Feed	608±59	<LOD	<LOD	< LOD
50% Wheat Middlings, 50% Corn Gluten Feed	<LOD	<LOD	<LOD	< LOD
100% Corn Gluten Feed	726±164	127±6	<LOD	< LOD
100% Corn Distillation Residues (+ chopped carrots)	468±181	<LOD	<LOD	< LOD
95% Wheat Middlings, 5% Rice Bran	<LOD	<LOD	<LOD	< LOD
90% Wheat Middlings, 10% Rice Bran	755±134	<LOD	<LOD	< LOD
85% Wheat Middlings, 15% Rice Bran	<LOD	<LOD	<LOD	< LOD
80% Wheat Middlings, 20% Rice Bran	<LOD	<LOD	<LOD	< LOD
95% Wheat Middlings, 5% Rapeseed Wastes	<LOD	<LOD	<LOD	< LOD
90% Wheat Middlings, 10% Rapeseed Wastes	<LOD	<LOD	<LOD	< LOD
85% Wheat Middlings, 15% Rapeseed Wastes	557±237	<LOD	<LOD	< LOD
80% Wheat Middlings, 20% Rapeseed Wastes	<LOD	<LOD	<LOD	< LOD

DON, FB1, FB2 and ZEN detected in larvae grown on contaminated substrates, still below the legal limit for feed

Feed/Larvae/Residual fraction

BSF grown on:
100% Corn Distillation Residues



Total Mass Balance (mycotoxin in larvae+residual/mycotoxin in feed):

DON: 81%

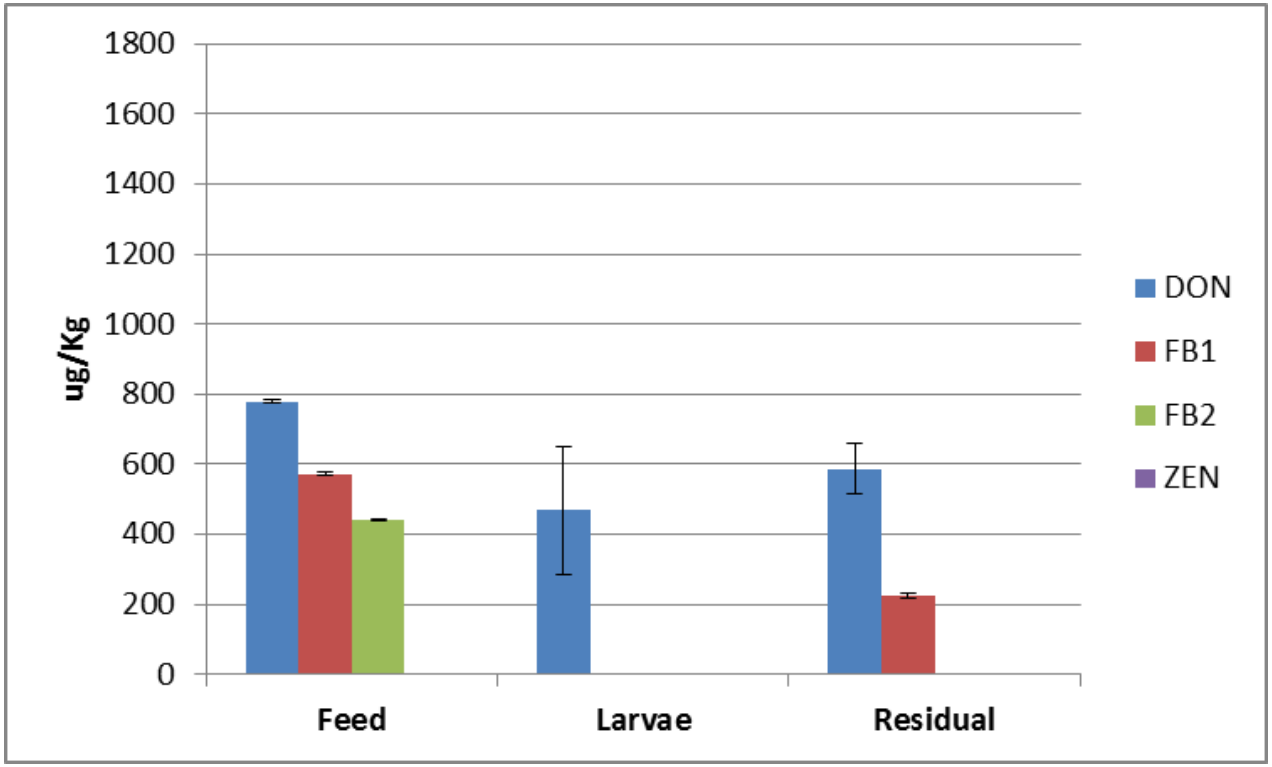
FB1: 72%

FB2: 4%

ZEN: ?

Feed/Larvae/Residual fraction

LM grown on:
100% Corn Distillation Residues



Total Mass Balance (mycotoxin in larvae+residual/mycotoxin in feed):

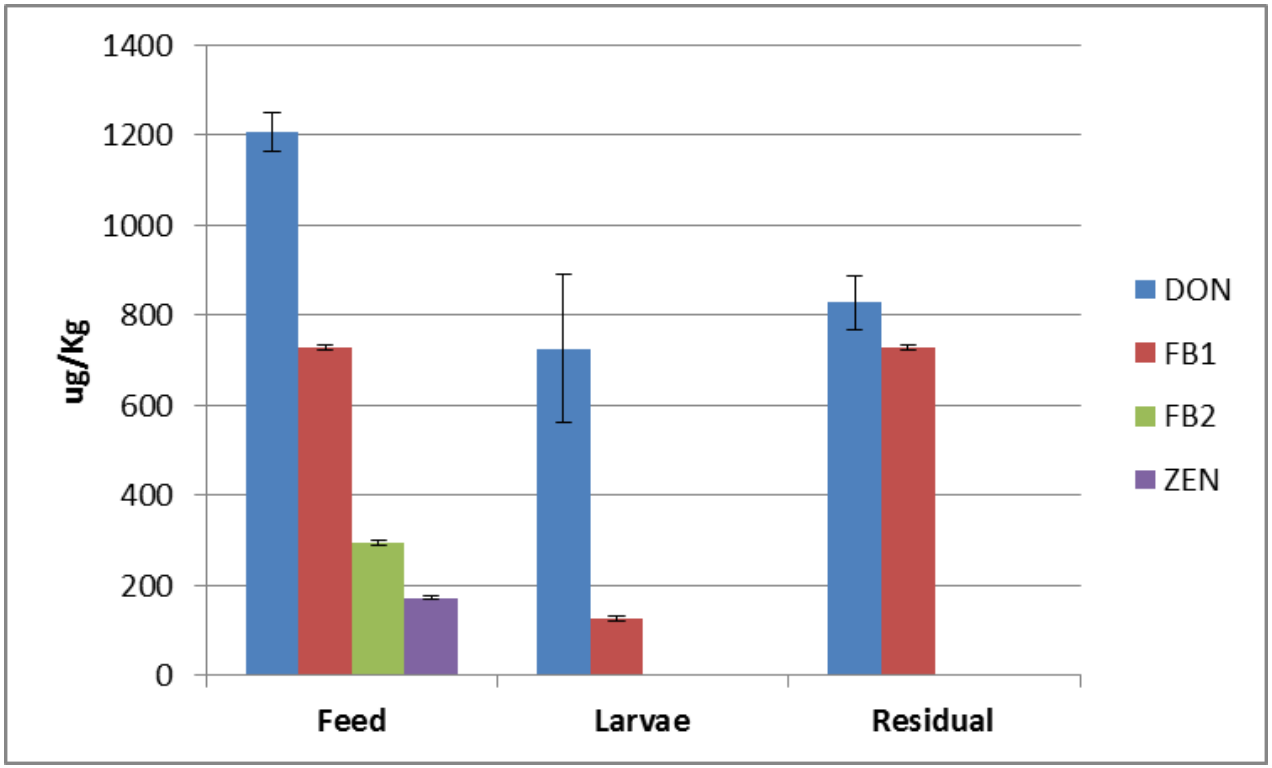
DON: 23%

FB1: 12%

FB2: 2%

Feed/Larvae/Residual fraction

LM grown on:
100% Corn Gluten Feed



Total Mass Balance (mycotoxin in larvae+residual/mycotoxin in feed):

DON: 43%

FB1: 57%

FB2: 5%

Conclusions (and open issues)

Insects do not accumulate mycotoxins when growing in mycotoxin-contaminated substrates

BSF is able to completely get rid of mycotoxins, mostly relying on excretion, even if the uncomplete mass balance, and also the appearance of previously undetectable ZEN, implies the presence of some biotransformation

LM is less efficient, and has some mycotoxin accumulation inside the body. The largely incomplete mass balance seems to point to biotransformation of mycotoxins in metabolized undetectable forms

If the feeding substrate for insects is into legal limits for mycotoxins, very likely insects will be as well

More investigation on mycotoxin metabolites (and eventual release of mycotoxins after ingestion when insects are used as feed) and on the ability of insects to «extract» mycotoxins from the matrix certainly needed

Insects as a potential source of enzymes able to degrade mycotoxins?

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All the results shown in this presentation are included in the manuscript:

«Impact of naturally Contaminated Substrates on *Alphitobius diaperinus* and *Hermetia illucens*: Uptake and Excretion of Mycotoxins» accepted for publication on ***Toxins***

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