Dear Feed Microscopy specialists,

Another very active year is behind us. The main activities were the organization of the annual meeting hosted by Landesbetrieb Hessisches Landeslabor in Bad Hersfeld and participating at the Feed Conference in Milano with a poster.

It is a pleasure to present to you our annual Newsletter.

The IAG-Board

Genevieve Frick, Roland Weiss, Jeroen Vancutsem, Julia Dietz, Manuel Zadravec

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President's address

As the president of the IAG feed Microscopy Association, I am pleased to address this greeting to all our members.

Last year can be presented as a quite successful and interesting time which gives us a glimpse at more for 2024!

Effectively, the board and all active members managed to keep up the association with such essential performances as organizing and highlighting two Proficiency Tests (on undesirable seeds and on animal proteins), hosting a rewarding Annual Conference (in Bad Hersfeld) and gathering data for a poster and a publication on packaging material residues. Also, the workshop "Implementing Regulation 2022/893: methods of analysis for the detection of constituents of terrestrial invertebrates for the official control of feed" was offered to all IAG members by Jeroen Vancutsem, which was much appreciated.

Reviewing the past year brings me to remember the presentations that some of us could follow at the EURL-AP Workshop 2023 (online on 23 and 24 May 2023) where the results of the "Combined microscopy-PCR EURL-AP Proficiency Test 2022" were presented. This PT showed, among other information, a poor sensitivity score for the disclosure of milk powder. This is an authorized ingredient with little characteristic features, it must be detected and reported, which is a difficult task for microscopists. Other interesting subjects were the presentation of the developments of the detection methods for peptides of animal origin by Mass Spectrometry methods, after the success of established PCR methods in the last decades.
Invertebrates (of terrestrial origins such as insects and marine origins such as crustaceans, molluscs, or cephalopods) also acquire increasing importance and attention, and their features must be studied by feed specialists.

The IAG Annual Meeting and workshop, from 13 to 15 June, was hosted in Landesbetrieb Hessisches Landeslabor (LHL) - Standort Bad Hersfeld, Schloss Eichhof. It allowed almost 40 of us to exchange opinions and experiences face to face and enjoy a very enriching social program. Thank you to the organizers!!

Among others, interesting parts of the meeting were:

- Visualising a video on insect meal production and discussing the correlation with animal feed
- Reporting on the 2023 IAG Animal Protein PT and discussion. For the first time, insect meal and the method recommended by the EURL-AP (double sedimentation) were part of the proficiency testing. Also, an aquafeed with processed terrestrial vertebrates' meal was part of the sample set. The results were globally satisfying, and the expertise of the group is increasing.
- The Ring Test on undesirable substances (weed seeds and ergot sclerotia) in a seed mix was reported and discussed with a lot of gained knowledge.
- Several cases of impurities detailed in the past year were also reported, and attention has been drawn to special cases and how to detect them by microscopy.
- Further work of the association on method writing, workshop, and proficiency testing was discussed, as usual.

You will find a more detailed summary of the IAG Annual meeting on the next pages of the newsletter!

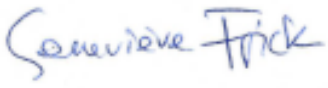
Exclusively for IAG-Members a full report of the IAG meeting is available on the IAG homepage. There you can also find the presentations of the meeting as also from the workshop.

Thanks to my colleagues of the board and other active members, we are an attractive and useful association, we present a nice and up-to-date homepage, we follow the new regulations, and anticipate arising challenges.

Please enjoy reading of this Newsletter and be sure that we will be in contact in the coming year!

See you in Namur in June 2024

Yours sincerely



Geneviève Frick, IAG president

IAG proficiency test on animal protein detection 2023

For decades the International Association for Feedingstuff Analysis (IAG) section Feed Microscopy has been organising collaborative studies for the evaluation of composition and the detection of animal constituents in feed.
Over the years, the organization of these studies has successively been delegated to the Danish Plant Directorate and Wageningen Food Safety Research. In line with these successful past collaborations, in 2023, the IAG board decided to subcontract this organisation to the Walloon Agricultural Research Centre CRA-W (Gembloux, Belgium).
The CRA-W is notably hosting the European Union Laboratory for Animal Proteins Detection in feedingstuff (EURL-AP), is ISO/CEN 17025 accredited for light microscopy and PCR detection of animal constituents in feed and ISO/CEN 17043* accredited for the organisation of proficiency tests for the detection of animal constituents.

The present proficiency test was organised in the next framework. The use of processed animal by-products as ingredients for animal feedingstuffs within the European Union is regulated by the TSE Regulation EC N°999/2001 (European Commission, 2001), as amended. In particular, Article 7 prohibits to use of processed animal proteins (PAPs) in the feeding of farmed animals (extended feed ban).
The objective of the present study was to assess the performance of the participants to detect the presence of PAPs in feed by the reference method using light microscopy as stated in Annex VI of Regulation EC 152/2009 (European Commission, 2009) imposing the methods of analysis for the determination of constituents of animal origin for the official control of feed as recently amended by Commission implementing Regulation (EU) No 2022/893 (European Commission, 2022) and related SOPs.

The listing of laboratories was established by the organiser and combined with the existing IAG one received from the board of the organisation.
The invitation included the next schedule:

- Announcement: 6 January 2023
- Sending of sample boxes and communication to the instructions: 24 February 2023
- Deadline for results delivery: 24 March 2023
- Publication of report: end May 2023

In agreement with the IAG board members, the organisers foresaw a maximum of 60 participants. This maximum number was reached. From the 60 registered participants (Annex 1), all participants except one delivered their results.

Four different sample materials were prepared for the study. Each participant received one entity of 40g of each material. The sample set consisted of:

- 1 = Ruminant feed I
- 2 = Ruminant feed II + 2 % of insect PAP from *Hermetia illucens*
- 3 = Aquafeed
- 4 = Aquafeed + 2 % of porcine PAP

Following the current legal European requirements for the light microscopic method, three parameters were assessed, based on identification markers:

- Terrestrial vertebrates
- Terrestrial invertebrates
- Fish

It is the first time that a proficiency assessment has been simultaneously conducted for the three parameters since the modification of Annex VI of regulation EC 152/2009 as regards the use of light microscopy for the detection of terrestrial invertebrates' constituents (European Commission, 2022).

Assessment of the ability to identify particles of each parameter (terrestrial vertebrates, terrestrial invertebrates and fish) is a binary data treatment (presence or absence) which was expressed in terms of sensitivity (*SE*), the probability of positive test given the real presence of a parameter into a matrix, specificity (*SP*), the probability of negative test given the real absence of a parameter into a matrix, and accuracy (*AC*), the fraction of correct positive and negative tests.

The global accuracies range from 0.929 to 0.970 which are more than optimal levels of performance and demonstrate the ability of a large majority of participants to implement the light microscopic method for the detection of animal remains in feed. Considering the accuracies per parameter, for the present study the disclosure of fish material obtained the best score (*AC* of 0.991 with only 2 errors), followed by the disclosure of terrestrial vertebrates' material (*AC* of 0.941 with 14 errors) and finally the detection of terrestrial invertebrates' material (*AC* of 0.896 with 22 errors).

The total number of errors was 38 which represents a total rate of 5.6 % over all delivered results. Among this total number of errors, specificity issues were predominant: 12 *PD* for terrestrial vertebrates and 13 *PD* for terrestrial invertebrates, while only 1 *PD* for fish.
Sensitivity issues were mainly related to terrestrial invertebrates, with 9 *ND*. For the two other parameters, failure to disclose the presence of terrestrial vertebrates and fish remains was limited (2 *ND* and 1 *ND* respectively).

It could be concluded that the first IAG PT in the organisation of CRA-W was very successful, novel, and with the most participants. We are looking forward to another PT in their organization.

IAG Ring Test 2022 for Ambrosia seeds, Datura seeds and Ergot sclerotia in bird feed

Summary

The ring test on undesirable substances in bird feed was aimed at exercising the detection of ***Ambrosia*** spp. seeds, ***Datura*** sp. seeds and **Rye Ergot** sclerotia (*Clalviceps purpurea*) in a kernel-mix matrix resembling a bird feed (Directive 2002/32/EC). 24 participants took part in the Ring Test.

Sample set:

3 portions of 250 g of seed mix were prepared. Materials 1 and 3 contained 1.5 kg of each of Dari, Millet, Sorghum, Buckwheat, Sunflower and Hemp and 0.450 kg of Spinach (*Spinacia oleracea*) seeds ("mimicking" *Ambrosia*). Material 2 had the same basic composition except for the addition of 0.450 kg of cereals (wheat and barley) instead of Spinach seeds.

Ambrosia seeds were added into samples of Material 1 (20 units in each sample) and samples of Material 2 (5 units in each sample). ***Datura*** seeds (14 units) were added in samples of Material 2. **Ergot sclerotia** was added only in samples of Material 3 (mean of spiking = 175 mg / sample).

Mimicking contaminants were also added into the samples. ***Aeschynomene indica*** seeds (20 units) were added into each sample of Material 2. These weed seeds are often found in rice and show similar size, shape and colour than *Datura* seeds, but have a smooth and

shiny surface. They may also be confused with *Crotalaria* spp seeds which are in the list of harmful botanical impurities. **Sclerotia** from fungi (not *Claviceps purpurea*, but possibly ***Sclerotinia sclerotiorum***) were added into Material 1 and Material 3 (appr.100 mg/sample) to mimic ergot.

Recovery performance:

	Material 1			Material 2				Material 3		
	Ambrosia	Datura	Ergot	Ambrosia	Datura	Ergot	Crotalaria	Ambrosia	Datura	Ergot
	N° Seeds	(not	(not	N° Seeds	N° Seeds	(not	(not	(not	(not	Weight (g)
No	Recovery (%)	added)	added)	Recovery (%)	Recovery (%)	added)	aded)	added)	added)	Recovery (%)
1	90			100	86			PD		88
2	100			100	93		PD			97
4	80			80	86					77
5	85		PD	100	100		PD			476
6	90			80	100					101
7	85			100	100					94
8	95			100	100					96
9	70		PD	20	86		PD			128
11	100			100	100					98
12	35		PD	60	93		PD			131
13	35			60	57					75
14	100			100	93		PD			100
15	75			80	86		PD			101
16	90		PD	100	93					84
17	90			80	71					85
18	90			80	100		PD			87
19	100		PD	80	100	PD	PD			143
20	35		PD	60	64					77
21	85			100	93					88
22	90			40	93					44
23	90			120	86					106
24	90		PD	120	107					140
25	90			100	93					80
26	55			60	100					90

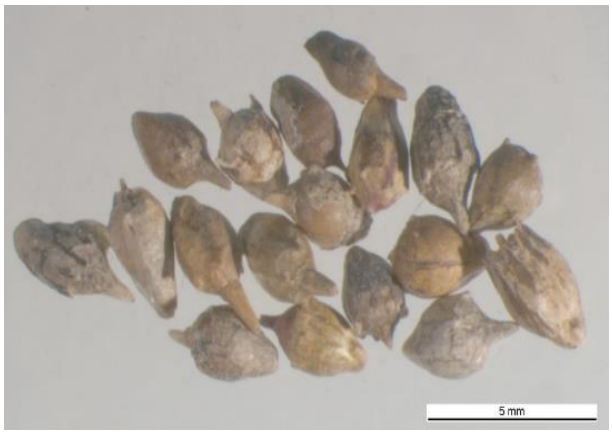
Dark green cells = excellent results
Light green cells = satisfying results
White cells = unsatisfying results
PD = Positive Deviation (satisfying)

Ambrosia fruits were recovered by a large majority of the participants. The presence of Spinach seeds (*Spinacia oleracea*) did not induce specificity problems but may have an influence on the sensitivity ("masking effect"). The legal limit for *Ambrosia* is 50 ppm. With the levels of spiking chosen (between 280 ppm and 400 pm in Material 1 and between 80 and 120 ppm in Material 2) all excellent or satisfying results (21 from 24) were found above the legal limit and would have been declared as non-complying. From the unsatisfying results, only one in Material 2 (the lowest spiking level) would have led to an error in the compliance of the sample.

Datura detection was excellent or satisfying for all participants and the level of spiking (around 350 ppm) was far below the legal limit (1000 ppm). 20 seeds of *Aeschynomene indica* were added in Material 2 (approx. 180 mg; mean of spiking = 730 ppm). This species is not listed as an undesirable substance but was added as a mimicking contaminant. It was mentioned (recognized) by 2 participants but was misidentified as *Colutea* sp. once and *Crotalaria* spp. 8 times (each time over the legal limit of 100 ppm). The presence of *A. indica* seems not to have disturbed the detection of *Datura* seeds by the performers.

Target contaminants

Ambrosia fruits (or seeds)

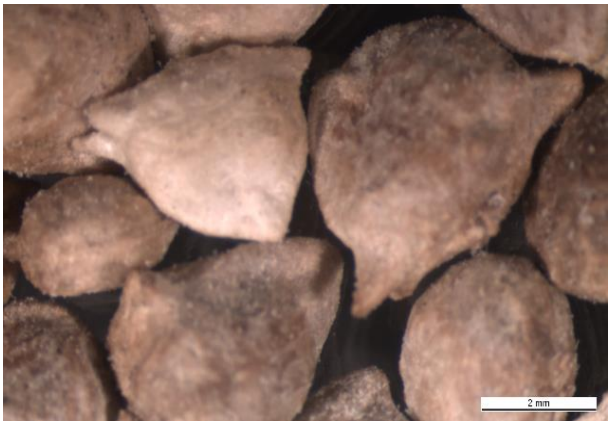


Datura seeds

Ergot sclerotia (*Calviceps purpurea*)

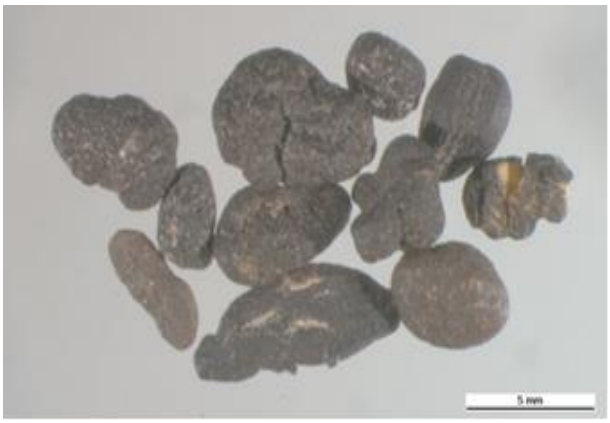
Mimicking ingredients or contaminants

Spinach (*Spinacia oleracea)*



Indian jointvetch (*Aeschynomene indica*)

Sclerotia *(Sclerotinia spp.)*



Other: Devil-bean (*Crotalaria retusa)*

Conclusions:

The performed ring test allowed the evaluation of proficiency of the participants in the frame of detection of undesirable entities such as toxic or undesirable bodies in a kernel mix. Especially, the ability to specifically detect and select *Ambrosia* seeds, *Datura* seeds and Ergot sclerotia was challenged, even in matrices containing mimicking or masking material.

The performance of the network of participants was excellent or satisfying in most cases. The presence of a masking ingredient such as Spinach seeds had an effect on the performance for *Ambrosia* detection and thus should be assimilated.

Some improvement of the capacities can easily be reached through training, in particular for the specificity problems: "Positive Deviations" for:

a) *Claviceps purpurea* sclerotia in presence of *Sclerotinia sclerotiorum* particles and

b) *Crotalaria spp*. seeds in presence of *Aeschynomene indica* seeds.

The few unsatisfying results questioning individual proficiency is not alarming, but for participants showing low recovery in general, we recommend a more careful examination of the samples. While handling cases from their monitoring and depending on how close the findings are towards the legal limit, the analysts may have to take into account the above discussion and invest more attention in the observations and selection of material, in order to evaluate the compliance correctly.

The Annual Meeting 2023

In 2023 annual IAG meeting was held in Bad Hersfeld in the organisation of Landesbetrieb Hessisches Landeslabor (LHL). A few brief meeting notes are included below.

13/06/2023

Presentation LHL

An introduction on food and feed control in Hessen was presented. The agency has 5 departments (central service, veterinary medicine, food, agriculture and import (airport Frankfurt).

Round Table, Feed Survey (G. Frick)

A round table was organised with cases and experiences of the participants.

Video on Insect meal production (J. Vancutsem)

Some fragments from the film 'bugs' were presented, e.g. on the rearing of insects.

Technical discussion about liquid products for packaging materials (T. Stojkovski)

A protocol for the analysis of packaging materials in wet bread was presented (drying). Also a protocol for candy syrup was presented. In an experiment 100 ml syrup was wet sieved at different sieve diameters (1 mm; 0,3 mm and 0,05 mm).

14/06/2023

IAG- AP PT results (P. Veys)

A PT with 4 samples was organised (Sample 1: Ruminant Feed I, Sample 2: Ruminant Feed II + 2% *H. illucens*, Sample 3: aquafeed, Sample 4: aquafeed + 2% PAP).

Following results were obtained:

Sample	**Vert.**		**Invert.**		**Fish**		**Global**
	SE	SP	SE	SP	SE	SP	
1		0.98		0.87		0.98	0.95
2		0.95	0.84			1	0.93
3		0.88		0.92	1		0.93
4	0.97			0.96	0.98		0.97
Total	0.94		0.90		0.99		

This is the first PT containing insects. Also some pictures of participants were evaluated.

Practical implementation of the EURL-AP SOP Operational protocol for the combination of light microscopy and PCR (J. Vancutsem)

A proposal of the flow of different matrices for the analysis of constituents of animal origin was presented.

Invertebrates from marine origin in TCE sediments (P. Veys)

According to EU-regulation 1069/2009 aquatic invertebrates and mussel meal and starfish meal are animal by-products. Shells from crustaceans and molluscs are considered as minerals.

Photographs of krill meal, oyster shells and starfish meal were presented.

15 years EURL-AP Light Microscopy trainings: Feedback and Perspectives (A. Anselmo)

The feedback of the EURL-AP microscopy training was presented. The onsite trainings are evaluated as excellent. For online trainings the conclusion is mixed as the interactivity is more limited. Currently the participation of OCLs is limited.

Transfer of microplastics from packaging materials to insect larvae for food and feed (G. van der Borg)

An experiment was set up in which larvae of black soldier fly were fed with microplastics. Samples were analysed by polarisation. For macroplastics, PE 300 and PE 125 there was no accumulation. For PET there was a high accumulation. Currently other plastics are added.

PLASBo - Harmonized Method for Plastics and Microplastics in Soil (R. Weiss)

Over 100 locations in Austria were sampled for the monitoring of microplastics. Samples were wet sieved at 1, 2, 5 mm. The fractions > 1 mm were visually analysed by 3 laboratories. The fraction 0,05-1 mm was analysed by FT-IR. As a conclusion it was stated that there was no correlation between the microscopic and FT-ITR result, but in general there is an increasing contamination of microplastics with the intensity of use of the soil.

Ring Test Undesirable substances in Seed Mix, presentation of the results (G. Frick)

A PT was organised for the analysis of undesirable substances (*Ambrosia*/*Datura*/ergot). 3 samples of 250g were prepared.

The following results were obtained:

Sample N°	Undesirable substances	Other added	Excellent/satisfying results
1	*Ambrosia*	spinach seeds + sclerotia of *Sclerotinia sclerotiorum*	21/24
2	*Ambrosia*	*Aeschynomene indica*	22/24
3	*Datura*	spinach seeds + sclerotia of *Sclerotinia sclerotiorum*	21/24

The presence of spinach seed had a masking effect on the detection of *Ambrosia* seeds. The presence of *Sclerotinia* sclerotia led to a lower specificity in the absence of ergot sclerotia. *Aeschynomene indica* was misidentified by 8/24 laboratories as *Crotalaria* seeds.

Challenges in Barcode sequencing of botanical content (L. Hougs)

In barcording there are error sources at the level of DNA-purification, PCR-amplification and sequencing. 10 feed mixes were prepared with classical ingredients. The microscopic analysis results were fine, but the barcoding results were not good at this moment.

Experiences with the Leica DM 2000 LED microscope (J. Vancutsem)

The experiences with the Leica DM 2000 LED microscope were presented as well as an introduction to polarisation microscopy.

Impurities, Several case studies (P. Czajkowski)

Some examples of impurities in raw materials and mixed feed were shown.

15/06/2023

IAG-A10 Method for the detection and determination of macroscopically/microscopically detectable foreign substances in feedingstuff (R. Weiss)

A first reading of the method IAG-A10: Method for the Detection and Determination of macroscopically/microscopically detectable foreign Substances in Feedingstuff, based on the VDLUFA method was presented.

Determinator (G. van der Borg)

The advantages of the use of Determinator were presented. There exist several Determinator subjects such as: Senecio, pollen, starch and animal particles determination. Identification of constituents can be done following a series of questions based on photographs and description of features.The results are produced by an overlap/redundancy calculator that calculates the overlapping characteristics. In the future other models will be developed.

Method-cascade (R. Weiss)

According to EU-Regulation 625/2017 a cascade system for the choice of analysis methods is implemented where the method of preference is: EU-method > ISO/EURL-method > NRL-method

Following methods were presented:

Animal proteins: EU-Regulation 152/2009, composition: IAG-method A2 / VDLUFA 30.7, *Datura*: IAG A3 / VDLUFA 30.3, Ergot: EURL-method, *Ambrosia*: IAG-A5 or VDLUFA 30.8, packaging materials: VDLUFA 30.9 or (IAG-A10)

Social life during IAG Meeting

We have entertaining social programs in addition to highly engaging scientific and professional ones. We received a guided tour of Bad Hersfeld on our first day there to familiarize ourselves with the host town.

Second day we visited Point Alpha Memorial. The memorial is an authentic scene of the Cold War era. This is where the inner-German border ran. We toured the complex of the memorial, consisting of the U.S. Camp, House on the Border, border reconstructions, and Road of Hope. Following the excursion, we enjoyed an excellent dinner.

Only for members: all the lectures in detail can be found on the IAG-Homepage: www.iag-micro.org

What's new in legislation in food/feed microscopy?

Commission Implementing Regulation (EU) 2024/771 – Feed control

29/02/2024 Commission Implementing Regulation (EU) 2024/771 amending Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for the official **control of feed** has approved and enters into force from 04/04/2024.

In this implementing regulation the sampling methods and preparation of feed samples for the examination by visual inspection or by microscopy are written down.

Details concerning the sampling are found in ANNEX I with a specific reference to the final sample in point 7: In case of examination by visual inspection or by microscopy, the amount of the final sample for examination shall be 1 kg.

Details concerning the preparation of samples are written down in ANNEX II, A. with the description of a specific procedure in case of examination by visual inspection or by microscopy:

- In case of an examination by visual inspection (without making use of microscope), the whole aggregate or final sample is used for examination.
- In case of a microscopic examination, the laboratory may reduce the aggregate sample, or further reduce the reduced sample. The final samples for defence and possibly reference purposes are taken following a procedure equivalent to the procedure followed for the final sample for enforcement.
- For the determination of rye ergot and harmful botanical impurities, the final sample has to be divided into 2 subsamples of equal weight of approximately 500 grams. One subsample is examined. In case the result of the subsamples is equal or below 50 % (analytical threshold) of the maximum level, the sample is compliant with the maximum level. If the result is above 50 % of the maximum level, another subsample needs to be examined and the average of the result of the 2 subsamples is used for checking compliance with the maximum level.

Details concerning the reporting of samples are written down in ANNEX II, C. 5. This section contains the details concerning the correction for moisture content: "In case of the determination of rye ergot or harmful botanical impurities by visual/microscopic examination correction to the moisture content is not necessary."

Details concerning the measurement uncertainty are written down in ANNEX II, C. 6. These existed already but are now clarified: "This procedure is only applicable in cases where the method of analysis enables the estimation of the expanded analytical measurement

uncertainty and correction for recovery (e.g. not required in case of visual/microscopic examination).

→ Implementing regulation - EU - 2024/771 - EN - EUR-Lex (europa.eu)

Commission Implementing Regulation (EU) 2023/2782 – Food control

On 14/12/2023 Commission Implementing Regulation (EU) 2023/2782 laying down the methods of sampling and analysis for **the control of** the levels of mycotoxins in **food** has been approved and enters into force from 01/04/2024.

In this implementing regulation the sampling methods of food samples for the analysis of ergot sclerotia are written down.

Details concerning the sampling are found in ANNEX I, PART I, A.1 to A.5 with a specific reference to the sampling of very small lots (≤ 0,5 tonnes) for the analysis of ergot sclerotia: a lower number of incremental samples may be taken, but the aggregate sample combining all incremental samples shall also be in that case at least 1 kg (or 0,25 kg in the case of small particle cereals and oilseeds) and for the determination of ergot sclerotia, at least 1 kg. For bigger lots, the general descriptions can be followed.

Details concerning the protocol for the analysis of ergot sclerotia are found in ANNEX I, PART I, A.6: "Acceptance of a lot or sublot: Control of ergot sclerotia:

From the aggregate sample, 2 subsamples of at least 0,5 kg shall be taken for examination. One subsample shall be examined. In case the result of the subsamples is equal or below 50 % (analytical threshold) of the maximum level, the sample is compliant with the maximum level. If the result is above 50 % of the maximum level, another subsample needs to be examined and the average of the result of the 2 subsamples is used for checking compliance with the maximum level. The following outcomes shall be derived:

- acceptance if the first subsample contains less than 50 % of the maximum level of ergot sclerotia or if the average of two subsamples conforms to the maximum level.
- rejection if the average of two subsamples exceeds the maximum level."

→ Implementing regulation - EU - 2023/2782 - EN - EUR-Lex (europa.eu)

Case report

The presence of Ambrosia trifida in soybean samples

J. Vancutsem (FAVV-FLVVT) & R. Weiss (AGES)

FLVVT received three soybean samples for the official control of botanical impurities in the months September/October 2023. In the samples the presence of *Ambrosia trifida* was confirmed, together with *A. artemisiifolia*.

The whole analysis sample was analysed according to IAG method IAG-A5 "Method for the Determination of Fruits and Seeds of *Ambrosia spp.* in Animal Feedingstuff". After a first analysis, the samples were sent to AGES for confirmation.

The following results were obtained:

Sample N°	Weight (g)	*A. trifida*		*A. artemisiifolia*	
		# seeds	Conc. mg/kg	# seeds	Conc. mg/kg
1	951,21	8	342	1	5
2	1721,01	7	241	3	8
3	2072,25	8	118	3	6

The mean weight of *A. trifida fruit* is around 40 mg, much more than the mean weight of *A. artemisiifolia* fruit that has a weight of around 5 mg (Frick *et al.*, 2011).

Pic © J.Vancutsem: *A.trifida* vs *A.artemisiifolia*

This case should give you a short impression how good and effective the IAG-network is working and should animate you – if you have any problems, questions or simply samples to check for their results confirmation - please get into contact with the IAG-association.

→ Contact: www.iag-micr.org

General Information about *Ambrosia. trifida*:

A. trifida is an annual weed. It reproduces by seeds. Its biology is similar to that of *A. artemisiifolia*, but it is more frost-resistant, develops faster, and its mature seeds appear earlier.

A. trifida has an upright stem reaching 100 cm and more (exceptionally 300 cm). The leaves are opposite, 3-palmately lobed (lower leaves may be 5-palmately lobed, higher leaves may be non-lobed), petiolate. The inflorescences are similar to those of *A. artemisiifolia*, but the male inflorescence is longer (up to 20 cm) and the female heads are larger (2-4 mm in diameter).

The fruits of *A. trifida* are 7-8 mm long, 3-4 mm wide and like those of *A. artemisiifolia*, with 4 to 8 small lateral thorns.

Pic 2 © R. Weiss (AGES): *A.trifida*

Reference:

Frick G., H. Boschung, G. Schulz-Schroeder, G. Russ, I. Ujčič-Vrhovnik, B. Jakovac-Strajn, D. Angetter, I. John, J.-S. Jørgensen (2011). Biotechnol. Agron. Soc. Environ. 2011 15(S1), 39-44

IAG-A5 "Method for the Determination of Fruits and Seeds of Ambrosia spp. in Animal Feedingstuff" in its actual version

→ IAG-Homepage (www.iag-micro.com)

StopDatura: Strategies for the prevention of Datura stramonium and its alkaloids in soybean

E. Reiter [1], S. Follak [2], I. Strnad [1], P. Riegler-Nurscher [3], M. Treiblmeier [4], R. Weiss [1]

[1] AGES - Austrian Agency for Health and Food Safety, Institute for Animal Nutrition and Feed, [2] AGES -Austrian Agency for Health and Food Safety, Institute for Sustainable Plant Production, [3] Josephinum Research, [4] BLICKWINKEL-digital service

Introduction

The common datura (*Datura stramonium*) is an important agricultural weed and contains toxic secondary plant constituents, namely the two tropane alkaloids scopolamine and atropine. It is commonly found in field crops harvested late in the season like maize sunflower, millet, or soybean. In addition to the poisonous seeds, the plant itself contains toxic plant juice.

At present, in animal feed, maximum levels are set in directive 2002/32/EG for 1000 mg/kg for harmful botanical impurities, while for food maximum permissible levels for the alkaloids are in force since September 2022 as laid down in Regulation EC 2023/915.

Tropane alkaloids	**max. level µg/kg**	
	Atropine	**Scopolamine**
Baby food and processed cereal-based food for infants and young children containing millet, sorghum, buckwheat, maize or their derived products	1	1

max. level since 1.9.2022	**Sum of Atropine and Scopolamine**
Unprocessed millet grains and sorghum grains	5
Unprocessed maize grains - except unprocessed maize grains for which it is evident e.g. through labelling, destination, that it is intended for use in a wet milling process only (starch production) - except unprocessed maize grains for popping	15
Unprocessed buckwheat grains	10
Maize for popping millet, sorghum and maize placed on the market for the final consumer milling products of millet, sorghum and maize	5
Buckwheat placed on the market for the final consumer milling products of buckwheat	10

Tab: max. levels for tropane alkaloids according to Regulation EC 2023/915 Annex 1

Due to the increased appearance of datura in field crops, it is necessary to keep the risk of contamination with datura seeds and tropane alkaloids as low as possible.

This includes more than just sorting out the seeds, as the poisonous plant juice also contaminates the harvest. Prevention strategies in the field as well as after harvest have to be established.

Pic. 1 Datura plant on field

Pic. 2 Datura plants

Following strategies for datura control are being developed as part of the national Austrian research project **StopDatura** → https://dafne.at/projekte/stopdatura.

- First a drone-based datura detection tool with high-resolution UAV images was developed to support the control of datura in the field before harvest.
- Secondly, screening tests for tropane alkaloids in soybean by microscopy and LC.MS/MS are being investigated to improve product safety.
- The third and most important part of the project is raising awareness of datura and the risk of tropane alkaloid contamination along the entire production chain.

In a first step, drone flights were used to detect datura infestation in soybean fields. In the next step, the harvested material from these fields was sampled and subsequently analyzed both microscopically and by LC-MS/MS (after removing datura seeds).

Pic 3 cracked capsule with black seeds

Pic 4 seeds of Datura stramonium (sample 6)

Results

The results so far show that the use of drones enables the accurate detection of datura in soybean fields. However, the results also show that soybean fields contaminated with numerous datura plants have high concentrations of tropane alkaloids.

The table below shows the analytical results of a highly datura contaminated field in detail. The results show that highly contaminated batches may contain levels of datura seeds significantly above the maximum level for datura seeds in feed and that even after removal of the toxic seeds, alkaloid levels far above 100 µg/kg could be measured.

Sample ID	Datura-seeds found in sample [mg/kg]	No. of seeds	Sum atropine & scopolamine after cleaning [µg/kg]
Sample 1	1945	354	143
Sample 2	2078	389	146
Sample 3	2597	526	165
Sample 4	2727	510	238
Sample 5	2439	495	259
Sample 6	2092	395	141
Sample 7	2224	440	97
Sample 8	1871	378	152
Mean	**2247**	**436**	**168**

Tab 2: Results of a highly datura contaminated field

Conclusions

The project offers the possibilities to raise awareness of the contamination with datura and implement actions to reduce it. In brief:

- High-resolution UAV images enable successful on-field-detection.
- Farmers know about the datura infestation and can systematically remove it.
- Contamination with toxic plant juices can be reduced.

References:

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- Begemann J, Ostovar S, Schwake-Anduschus C (2021): Facing Tropane Alkaloid Contamination in Millet – Analytical and Processing Aspects. Quality Assurance and Safety of Crops and Foods 13, 79–86.

- Follak S, Schleicher C, Schwarz M, Essl, F (2017): Major emerging alien plants in Austrian crop fields. Weed Research 57, 406–416.

IAG member's article at the Feed conference 2023

During 2023 our members were very active. At the Feed conference 2023, a group of authors presented the poster titled:

Monitoring on packaging material residues in feed – survey among IAG feed microscopy members and other European official control units - impact on the safety of feeds using former food products.

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Abstract:

Macro- and microscopic evaluation of feed includes detection of animal proteins, botanic impurities, label control, customs and of prohibited ingredients such as packaging materials (PM; Regulation (EC) 767/2009). In addition, detection of micro-plastics (possible degradation products of some of the PM) is getting attention. PM can harm animals or disturb their feed intake, pollute the environment and can be considered as undesired impurities in feeds. These materials do not consist of a definite molecule, group of molecule, living species or definite bodies. They can be plastic foil, hard plastic, metal pieces, paper, wood, or some combination of materials. Their features (sharp, pointed) can be as important as the material itself. This is a typical topic for microscopy detection and evaluation. In this poster presentation, a review of the work done on detection of PM in 14 monitoring entities (institute, laboratories) is presented:

- since 2012 some institute analyze more than 20 samples each year, the incidence of non-compliant samples will be presented.
- a majority of the entities have an active monitoring, whereas others have passive surveillance (done while performing other microscopy analyses), the results are compared.
- protocols used depend on sample types and analysts, harmonization is sought.

With these results, an overview of the situation and the opportunities for a safe utilization of former food products in Europe will be discussed.

The poster is also published on the IAG -Homepage: → www.iag-micro.org

SAVE THE DATE - Next IAG Meeting

Annual **IAG meeting and practical workshop "Botanical impurities"** will take place **on June 5-7, 2024 in Namur, Belgium,** hosting by Walloon Agricultural Research Centre. All useful information can be found in the official invitation letter on IAG website. Registrations will be done online only (by clicking on the "registration form" hyperlink into the invitation letter) and **until May 3**.

→ www.iag-micro.org

Take the opportunity to establish connections with colleagues and take part in discussions and information sharing about important subjects related to a crucial area of monitoring.

Closing remark

Dear reader,

We hope you found the items in this newsletter to be interesting to read. Furthermore, we hope that the knowledge provided will be helpful for all of your endeavours. Your inquiries will be assisted by the board members of IAG section Feed Microscopy as well as, of course, by all other members. Thus, feel free to get in touch with us.

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