Beauveria bassiana – how does the understanding of its endophytic activity and other non-lethal effects on pest species affect its use as a biocontrol agent?

Edith Ladurner – CBC (Europe) S.r.l., BIOGARD Division

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The strain ATCC 74040 of *Beauveria bassiana*

The strain ATCC 74040

- **Obtained from cotton boll weevil,** *Anthonomus grandis*, at USDA-ARS Crop Insect Research Center, Texas, USA (not genetically modified)
- Owner of Intellectual property rights: **CBC (Europe) S.r.l.**
- **Annex I inclusion (Reg. EU 540/2011): 2009**
- Formulated product: **Naturalis**
- Formulation: **OD (oil dispersion)**
- Concentration: **0,0185% w/w (2.3 x 10^7 CFU/mL)**
- Hazard symbol: **not classified (NC)**
- **Pre-harvest interval (PHI): 0 days** and **MRL not requested**
- Currently registered in 16 EU countries and 4 non-EU countries

Primary mode of action

Adherence of conidia to host's cuticle

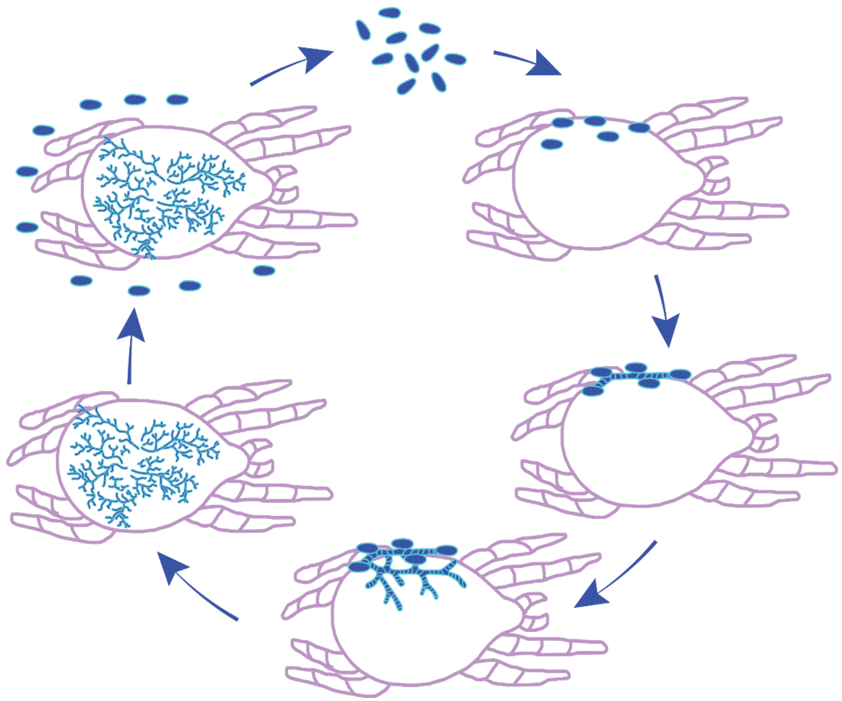
Germination of conidia

Production of germ tube and appressorium

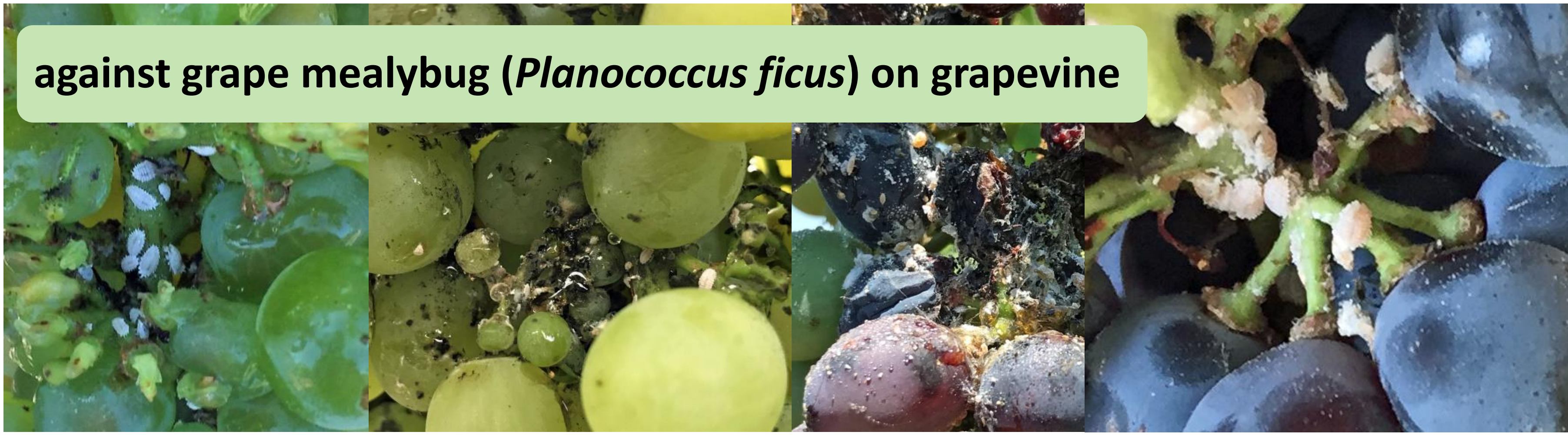
Penetration of host's cuticle by mechanical pressure and action of cuticle-degrading enzymes.

Vegetative growth in host's haemolymph

Production of external conidia upon death of host



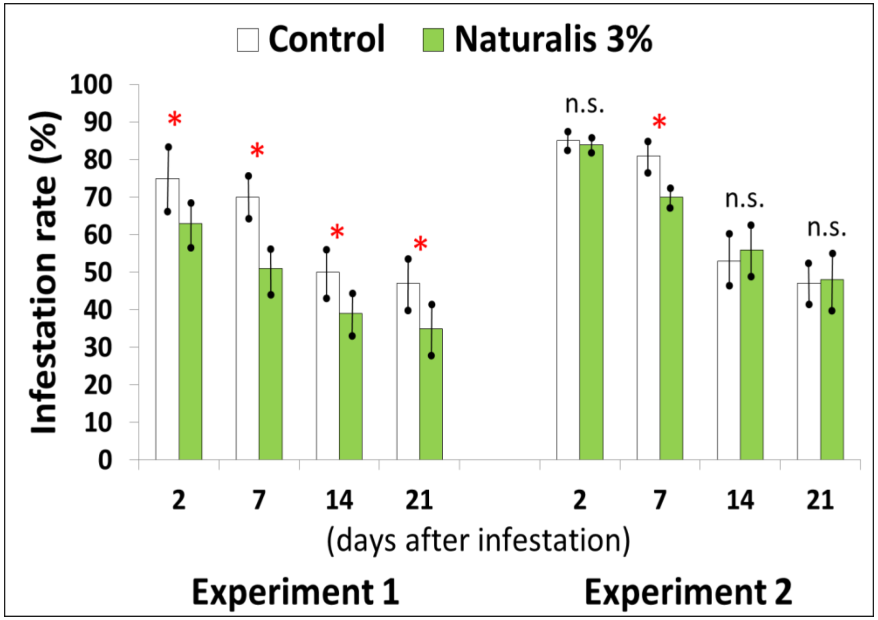
Putative endophytic establishment & endophytic activity:

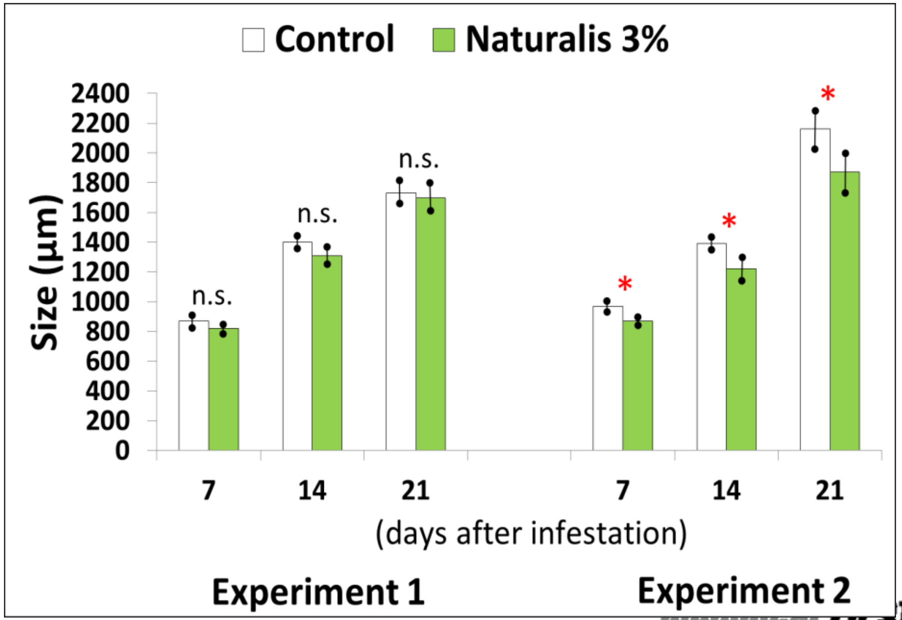


Grape mealybug on grapevine - observed effects:

- **Reduced infestation rate**
- **Reduced size increase**

of grape mealybug larvae in laboratory bioassays on first *B. bassiana*-treated, and then surface-sterilized leaves *versus* control leaves.

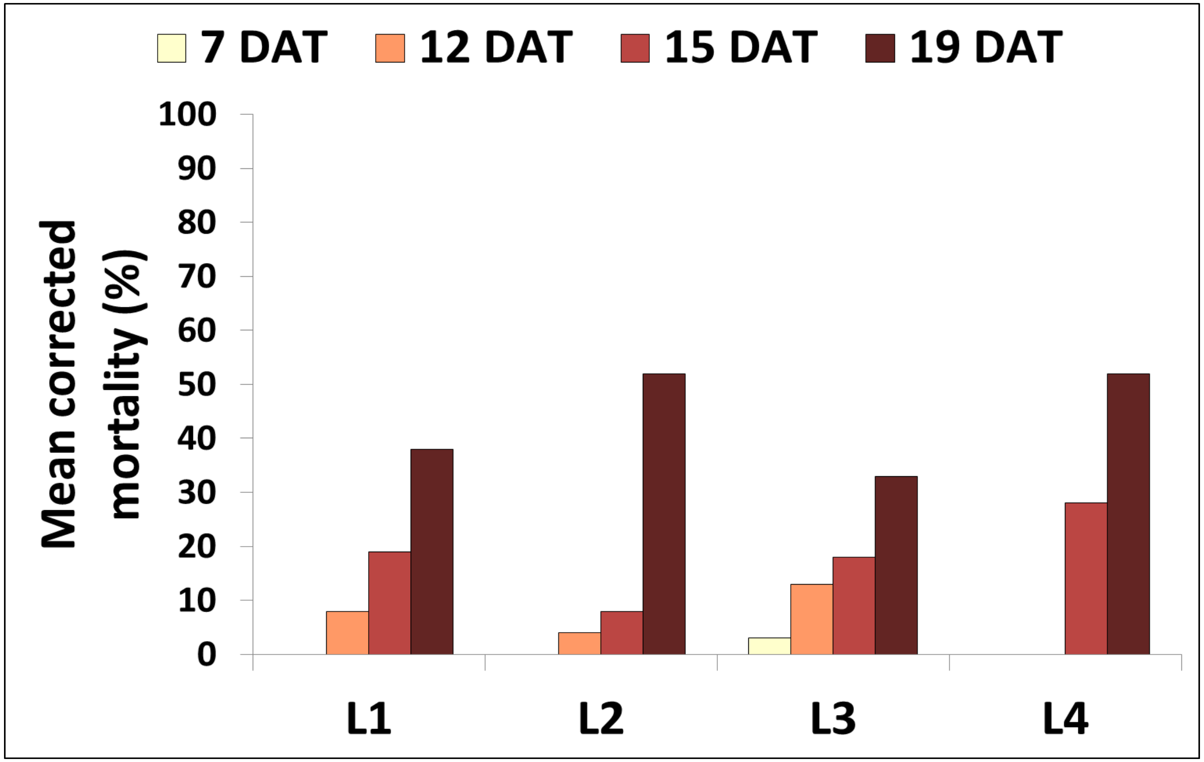




Rondot & Reineke (2016)

Tomato leaf miner on tomato - observed effects:

- **33–52% corrected mortality for all larval stages after 19 days of feeding on first *B. bassiana*-treated, and then surface-sterilized leaves.**



Considerations concerning endophytic activity

Concerns & doubts related to:

- **Tested rates. 3–244 times higher than max. authorized field rates. Economically feasible?**
- **Test conditions in bioassays: 21-25°C and 50-70% RH. Do these conditions resemble field conditions?**
- **Efficacy. Overall low efficacy** (0-27% against Grape mealybug; 33–52% against *T. absoluta*: after 19 days of feeding, but only 0-13% after 12 days of feeding). NB: total duration of larval development of *T. absoluta:* approx. 10–12 days at 25°C.
- **Endophytic establishment on leaves. Notwithstanding the high application rates, low on grapevine** (0–30% in field)**, better on tomato** (>60% in lab)**.**
- **What about other plant parts?**
- **Other varieties?**

… last, but not least: can we be sure that *B. bassiana* actually endophytically colonizes leaves?

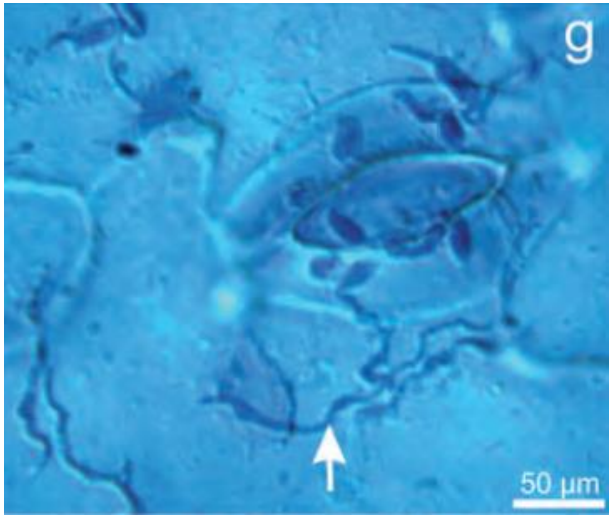
Endophytic establishment of strain ATCC 74040

Endophytic establishment in leaves

- **Light microscopic studies failed to show systemic endophytic growth of inoculated entomopathogenic fungi, including strain ATTC 74040,** in leaves of different host plants (Ullrich et al., 2017; Koch et al., 2018).

The Authors conclude that:

- reason for inability to grow endophytically is not known;
- specific combinations of fungal strains and host cultivars may have given other results;
- **the results indicate a saprotrophic rather than an endophytic life style of the fungi studied, strain ATCC 74040 included.**



Source: Ullrich et al., 2017

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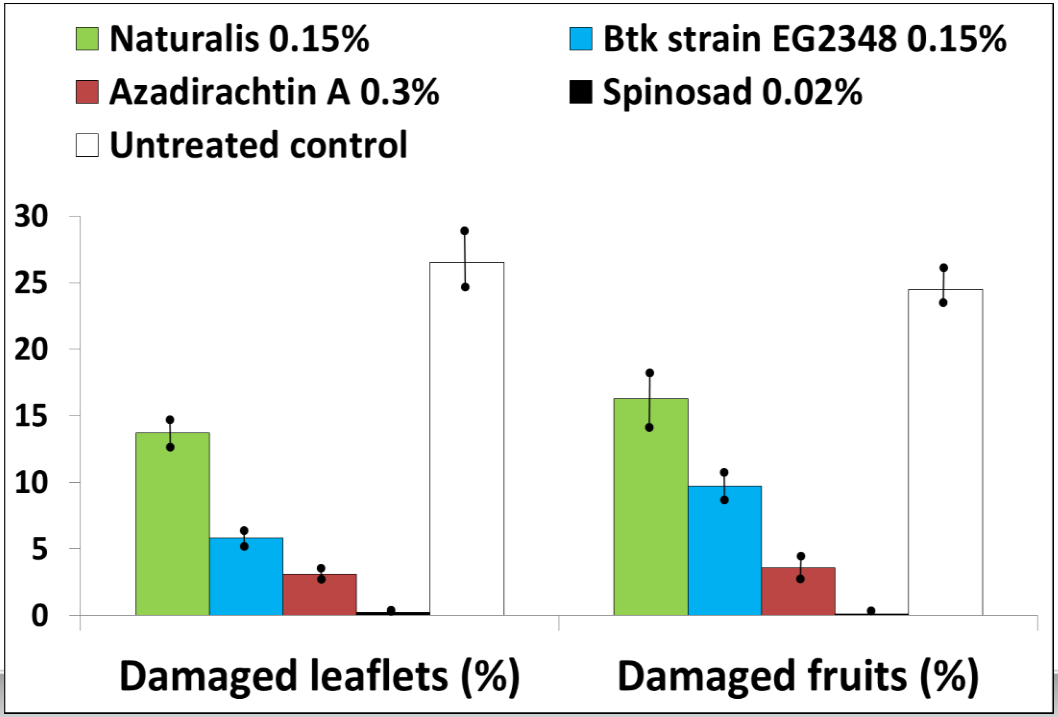
Efficacy of epiphytic *B. bassiana against T. absoluta*

Efficacy of epiphytic *B. bassiana* in bioassays

- **92-100% after 15 days of feeding, 3-66% after 7 days of feeding (Klieber & Reineke, 2015)**

Efficacy of epiphytic *B. bassiana* in field trials



Mean efficacy of Naturalis®

approx. 50% in leaf damage reduction

approx. 35% in fruit damage reduction

Mean efficacy of reference products:

78-99% in leaf damage reduction

60-99% in fruit damage reduction

GEP trial Eboli, Italy, 2010
Tested rate: Naturalis at 0.15% (1.5 L/Ha);
4 appl.s at weekly intervals/treatment
Final assessment: 7 DAT4

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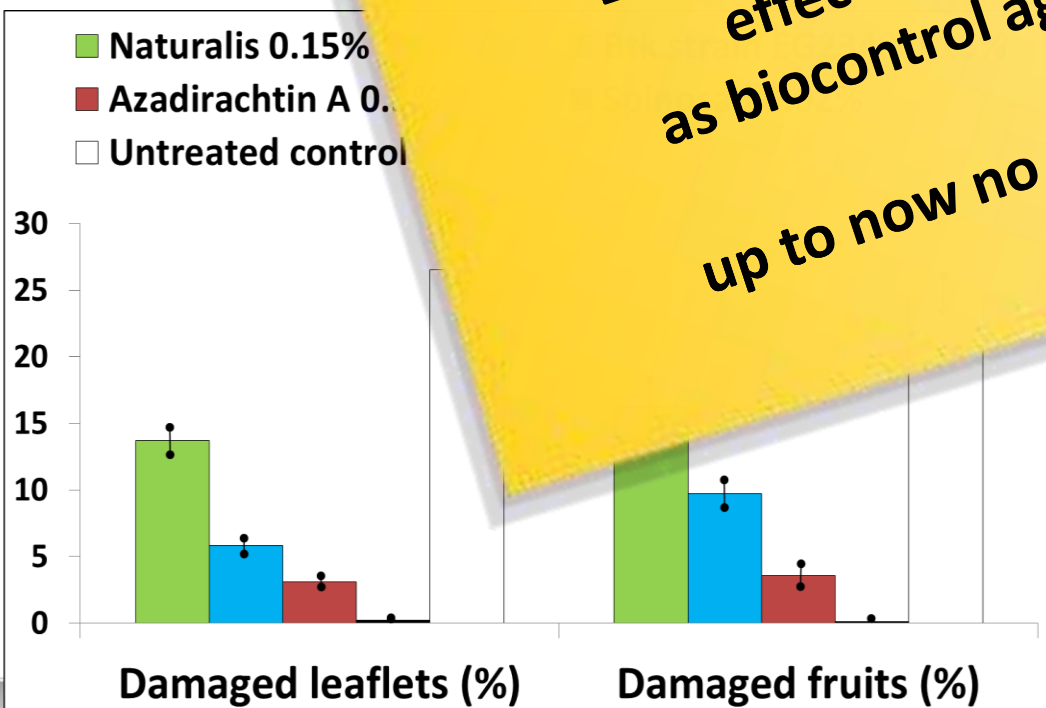

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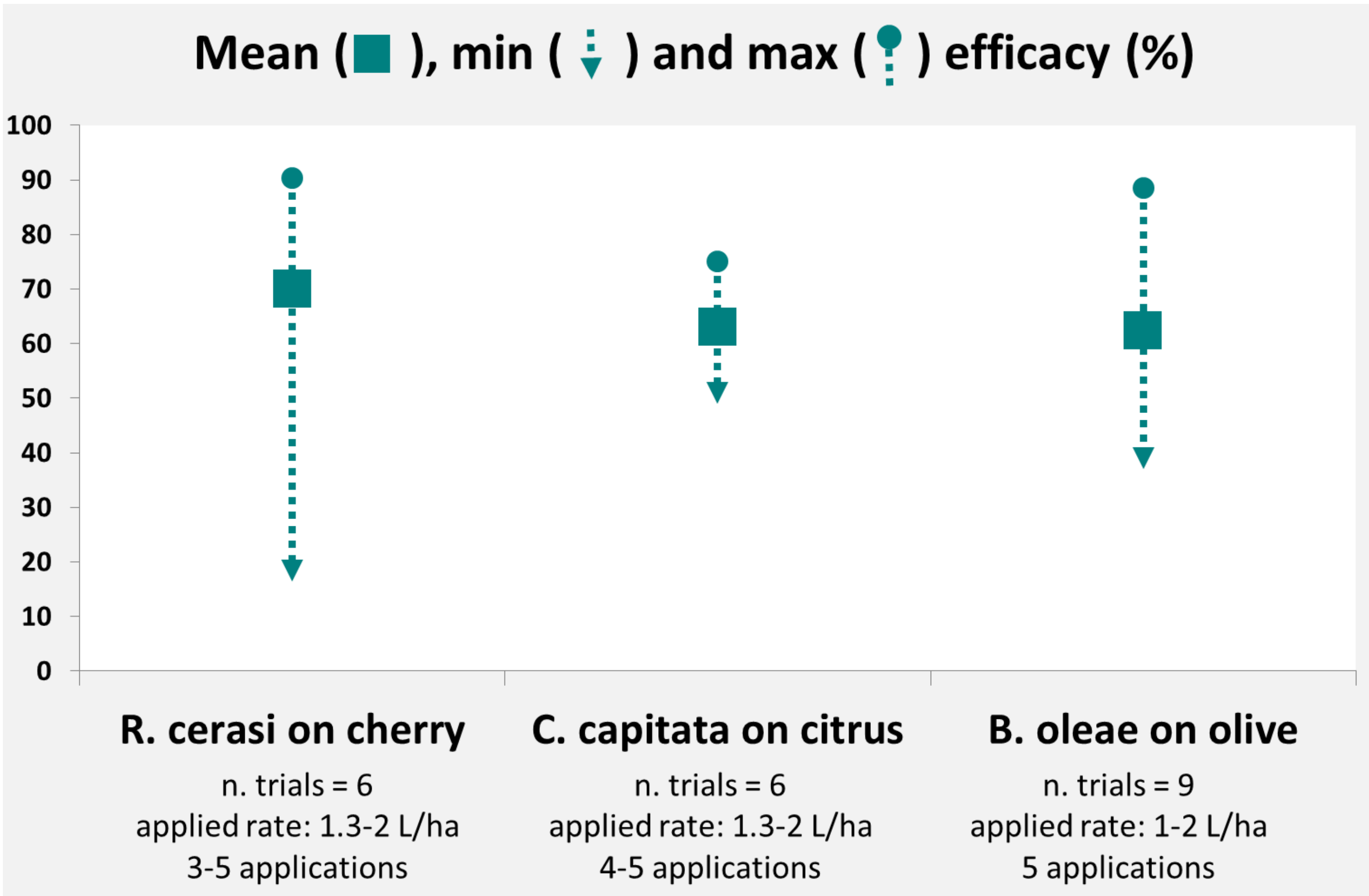
Endophytic activity & effect on use as biocontrol agent:

up to now no effect!

Oviposition-deterrent activity on fruit flies

Efficacy of *B. bassiana* strain ATCC 74040 against fruit flies



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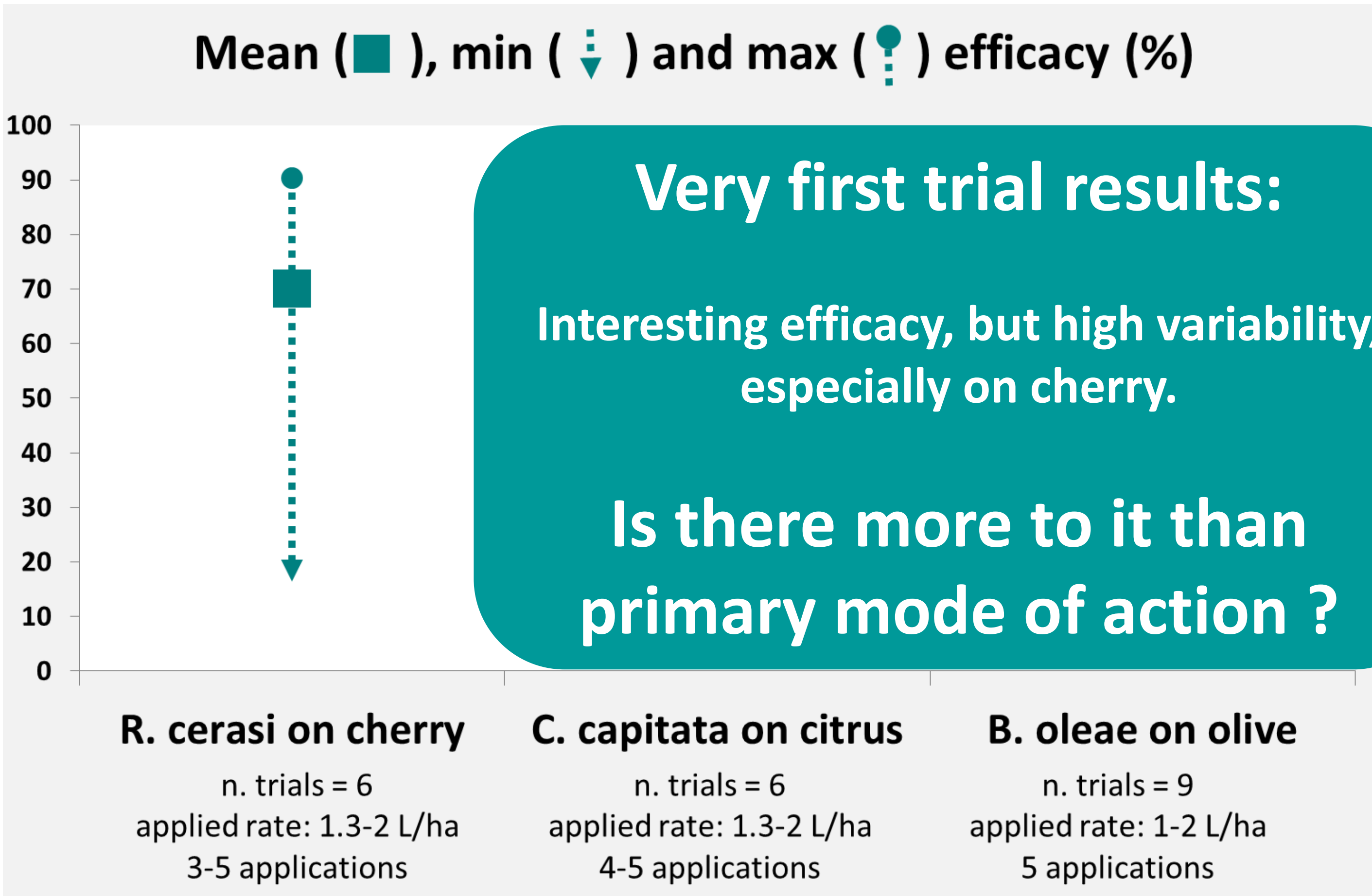


Very first trial results:

Interesting efficacy, but high variability, especially on cherry.

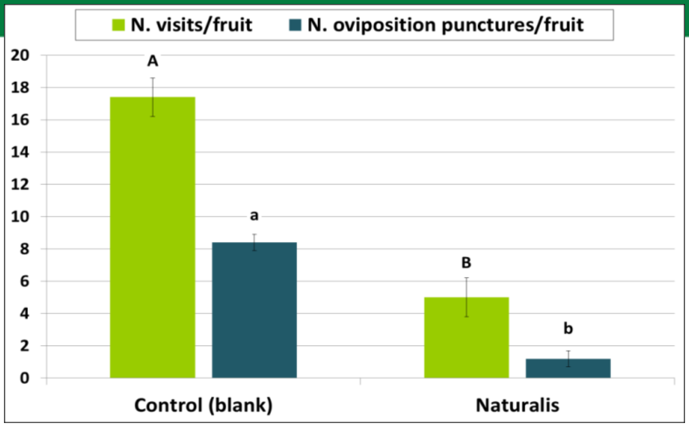
Is there more to it than primary mode of action ?

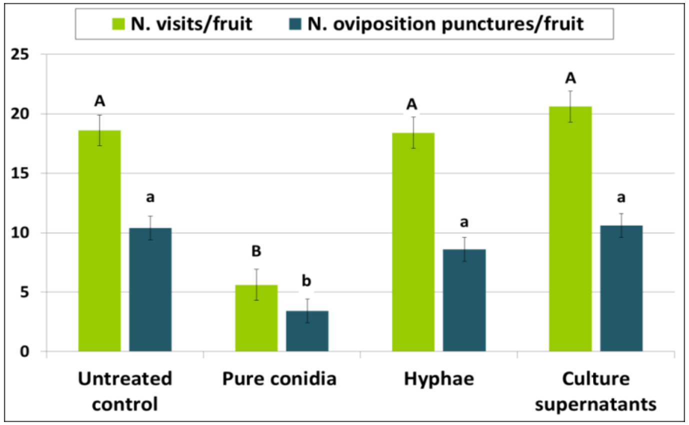
Oviposition-deterrent activity on fruit flies

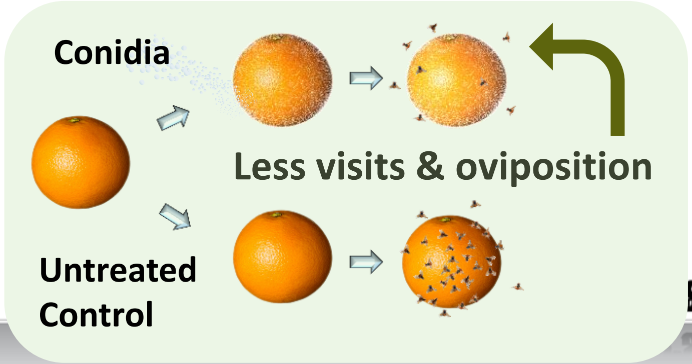
Oviposition-deterrent activity

- **Oviposition-deterrent effect of formulated product and pure conidia suspension**.
- **Intact conidia, and not other fungal fractions, seem to be responsible for observed effect.**
- **Identification of two hydrophobins**, small proteins known to form a hydrophobic coating (rodlet layer) on conidia of strain ATCC 74040.
- **Hydrophobic layer of conidia on fruit surface seems to impair ability of fruit flies to detect fruit-derived stimuli.**

NB: the lower the pest pressure, the higher the efficacy. Under conditions of too high a pest pressure, females will eventually lay eggs also on treated fruits.







Ortu et al. (2011), Ruiu et al. (2013)

Oviposition-deterrent activity on fruit flies

- **Past recommendations on cherry** :

1st application at **beginning of fruit colouring** (BBCH 81). Under Southern EU zone climatic conditions fruit colouring usually starts 10-14 days after beginning of flight of *R. cerasi,* **but may also start later, when flight has already started**.

R. cerasi adults go through a maturation period of gonads of 6-13 days during which they need to feed on carbohydrates, proteins and water. **Once gonads are mature, females will start laying eggs, even if fruit colouring has not yet started**.

- **Updated recommendations on cherry** :

1st application approximately 1 week after beginning of flight, irrespective of BBCH.

Oviposition-deterrent activity on fruit flies

- **Past recommendations on cherry** :

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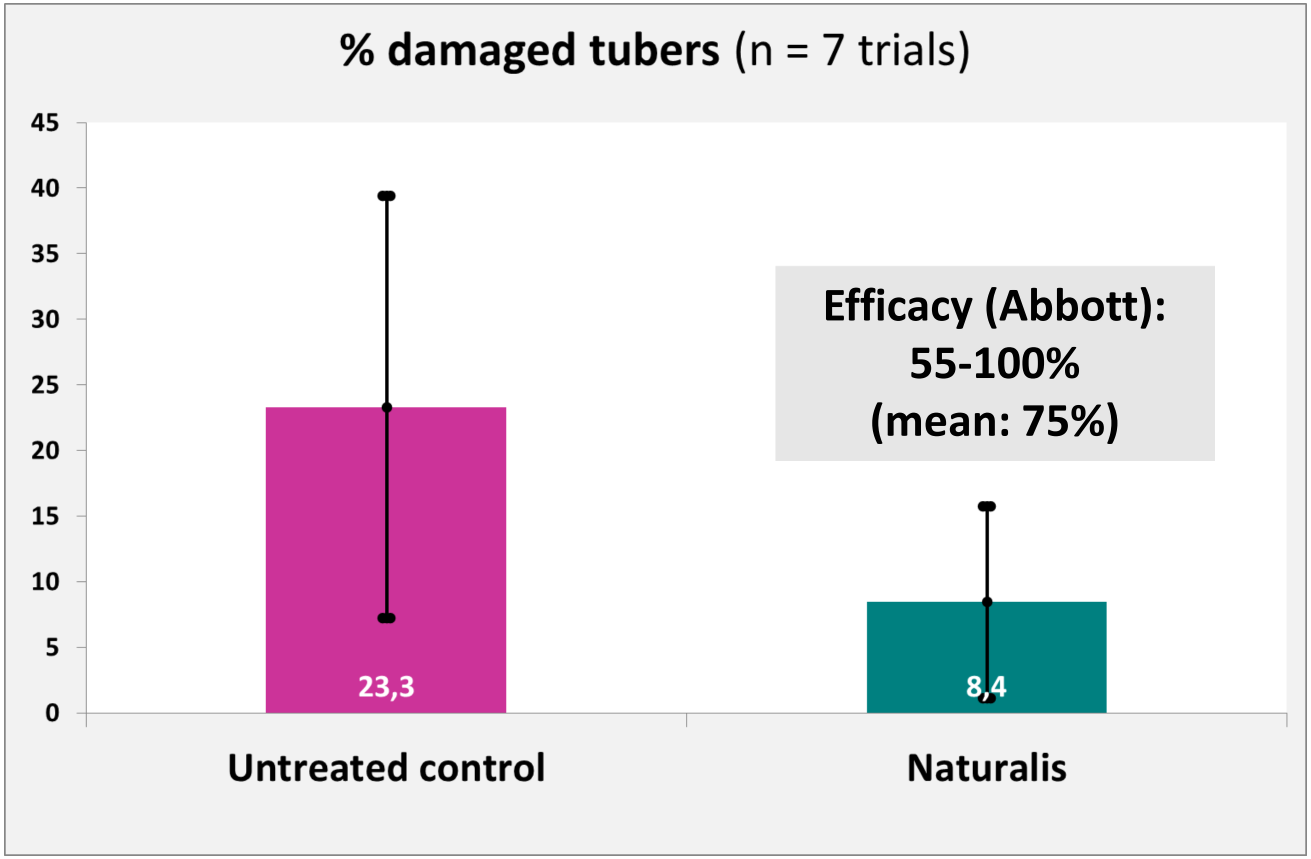
Oviposition-deterrent activity & effect on use as biocontrol agent:

improved and more consistent efficacy thanks to appriopriate instructions for use!

- **Updated recomme**... **...ns on cherry** :

1st application approximately 1 week after beginning of flight, irrespective of BBCH.

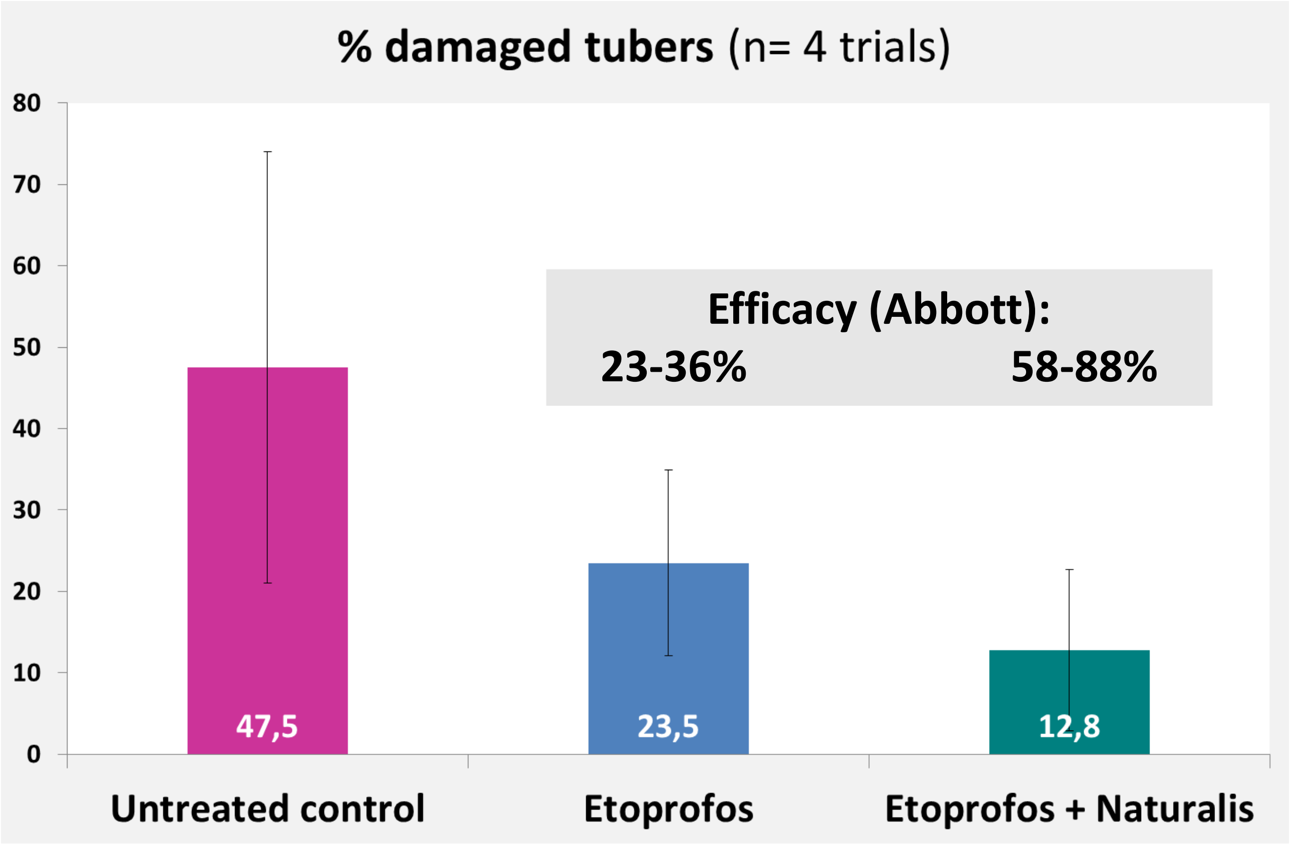
Activity of strain ATCC 74040 against wireworms on potato



Naturalis (3 L/ha) applied at sowing into the furrow and around the tubers.

Ladurner et al. (2009)

Activity of strain ATCC 74040 against wireworms on potato



Etoprofos (30 kg/ha) applied at sowing

+

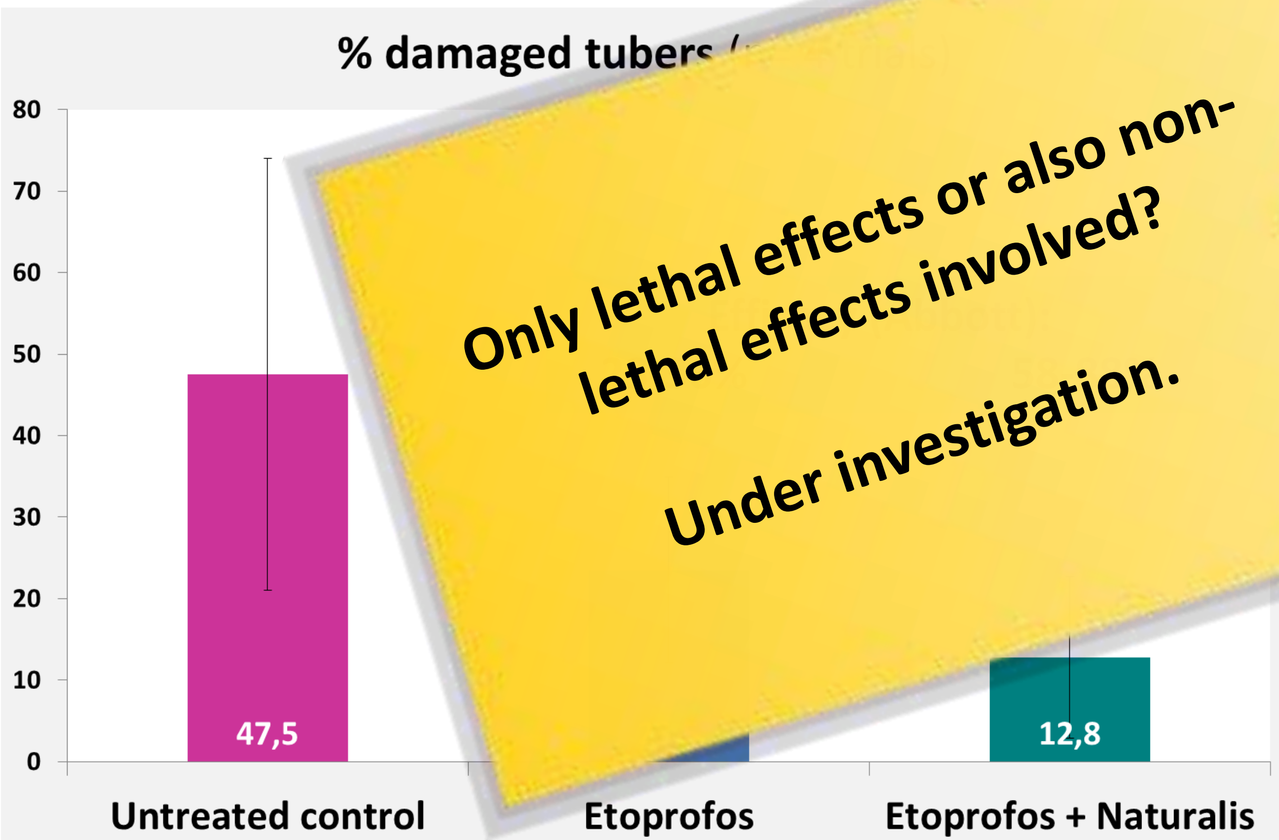
Naturalis (0.5 L/ha) applied at weekly intervals via irrigation system during last part of crop cycle (total n. appl.s = 6)

Naturalis applied via irrigation system during last part of crop cycle.

Bariselli et al. (2018)

Is there more to it than this?

Activity of strain ATCC 74040 against wir… on potato



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Naturalis applied via irrigation system during last part of crop cycle.

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Bariselli et al. (2018)

Conclusions

- **Question:** **does the understanding of its non-lethal effects affect the use of *Beauveria bassiana* as a biocontrol agent?**
- **Answer:** **it definitely does (see oviposition-deterrent activity), but only if application conditions are feasible under practical cultivation conditions!**
- **To keep in mind:** **in order to gain insight into potential non-lethal effects of microbial control agents, basic scientific research is of sound importance, but …**

any published study on findings concerning the mode of action of a microbial control agent automatically translates to additional data and/or information requirements by competent regulatory authorities (EFSA, etc.).

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