Development of a plant protection agent based *on Lysobacter enzymogenes* – progress and challenges (*mikroPraep + OptiLyso*)

Ada Linkies, Yvonne Rondot

Way Forward in Organic Plant Health Care Strategies
Online Conference
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Project *mikroPraep* (03/2019-06/2022) and follow-up project *OptiLyso* (04/2023-03/2026)

- goal: development of a marketable sustainable biopesticide for organic and integrated crop protection
- bacterial genus *Lysobacter* sp. (Xanthomonadaceae) with described antagonistic activity
- application range: oomycetes and fungi (i.e. downy mildew, *Phytophthora infestans,* apple scab) on different crops (cucumber, grape, tomato, potato, apple)
- integration in a copper reduction strategy

Project consortium *mikroPraep* and *OptiLyso*

Julius Kühn Institute,
Institute for Biological Control;
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Hochschule Geisenheim (HGU),
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Technical University of Berlin,
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(*mikroPraep* only)

Bio-Protect GmbH;
Konstanz, Germany

E-nema GmbH,
Schwentinental, Germany
(*OptiLyso* only)

Efficacy and host spectrum

Production and fermentation

Application strategies

Resilience against abiotic stress

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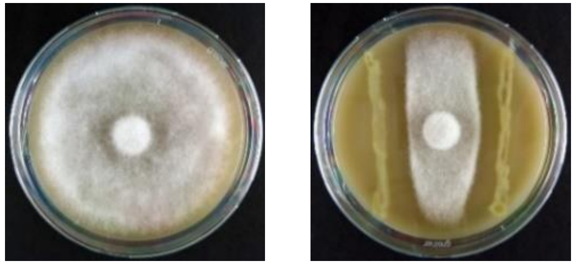
Identification of potent isolate of *Lysobacter* sp.

Lysobacter Species	Isolate	*Ascochyta fabae*	*Alternaria radicina*	*Alternaria solani*	*Botrytis cinerea*	*Bipolaris sorokiniana*	*Fusarium culmorum*	*Phoma lingam*	*Rhizoctonia solani*	*Phytophthora infestans*	*Pythium ultimum*
		Fungal/Oomycetal Pathogen									
		Diameter of Inhibition Zone (mm)									
Lysobacter sp.	BI-6067	28 ± 3	0 ± 0	21 ± 1	17 ± 2	16 ± 2	24 ± 0	27 ± 1	20 ± 0	31 ± 5	10 ± 0
L. enzymogenes	BI-6432/1 Kg	11 ± 0	0 ± 0	13 ± 2	17 ± 5	11 ± 1	10 ± 0	20 ± 4	15 ± 0	36 ± 3	10 ± 1
L. enzymogenes (LEC)	BI-6432/2 Kc	43 ± 1	0 ± 0	31 ± 7	42 ± 1	16 ± 1	32 ± 2	40 ± 3	37 ± 2	47 ± 5	12 ± 1
L. enzymogenes	BI-6434	39 ± 5	0 ± 0	30 ± 10	45 ± 2	32 ± 2	30 ± 7	31 ± 1	22 ± 3	37 ± 1	10 ± 2
L. enzymogenes	BI-6445	29 ± 9	0 ± 0	0 ± 0	28 ± 2	0 ± 0	6 ± 8	0 ± 0	11 ± 15	29 ± 2	0 ± 0
L. enzymogenes	BI-6447	35 ± 6	9 ± 13	40 ± 7	37 ± 6	14 ± 0	47 ± 7	43 ± 2	20 ± 4	34 ± 2	10 ± 0
L. enzymogenes	BI-6457	42 ± 4	14 ± 2	35 ± 6	37 ± 8	22 ± 6	34 ± 1	47 ± 2	21 ± 1	46 ± 4	12 ± 0
Lysobacter sp. [1]	Nr. 31, Wolf	32 ± 0	0 ± 0	0 ± 0	36 ± 3	20 ± 4	24 ± 2	30 ± 7	0 ± 0	30 ± 1	0 ± 0

[1]: similar probability for L. gummosus, L. ginsengisoli, L. antibioticus and L. capsici.

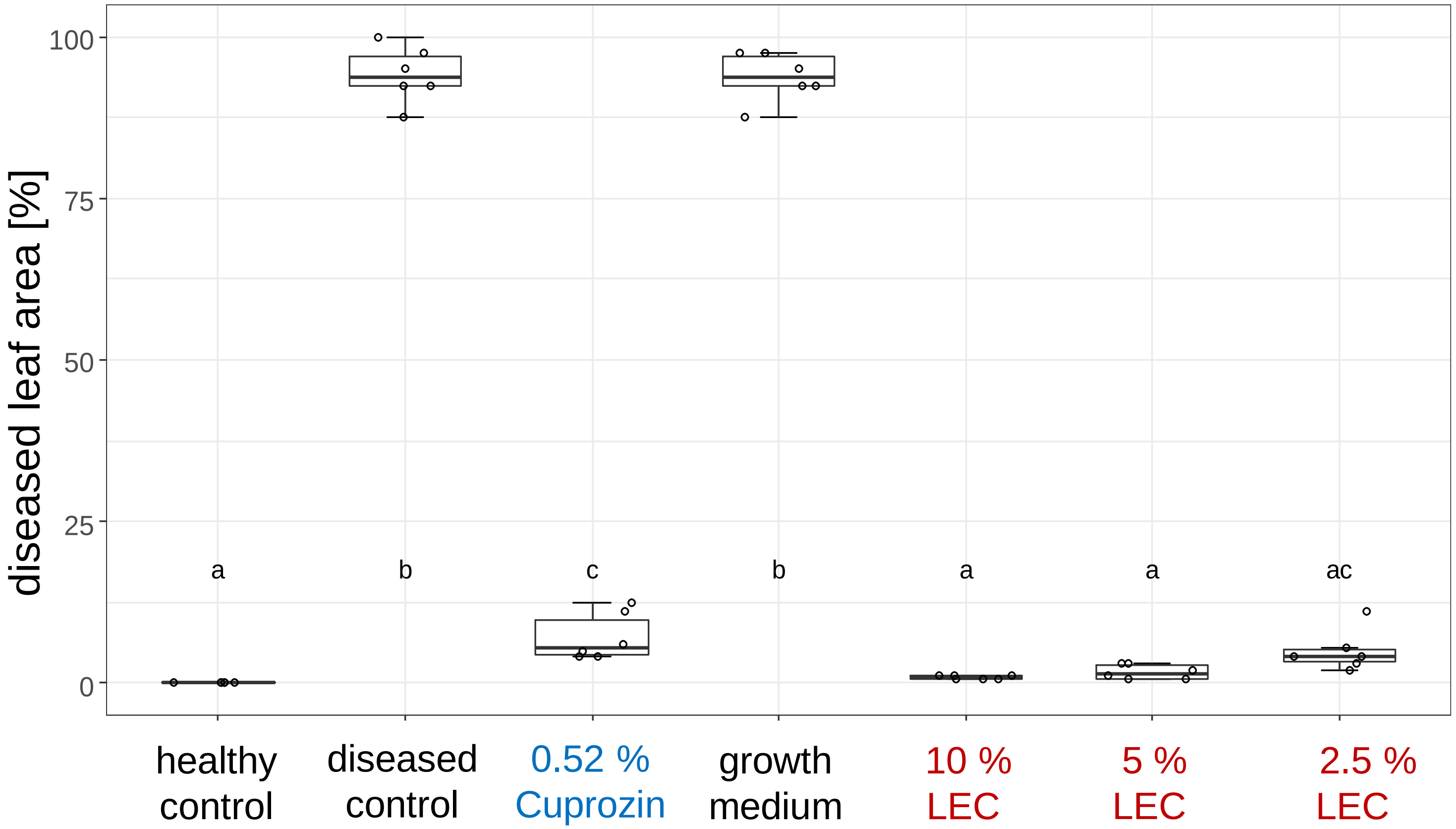


P. infestans in dual culture with *L. enzymogenes*

- diverse *in vitro* activity against several fungal and bacterial pathogens
- broad enzymatic activity
- *L. enzymogenes* isolate LEC used for further investigations

Drenker, C.; El Mazouar, D.; Bücker, G.; Weißhaupt, S.; Wienke, E.; Koch, E.; Kunz, S.; Reineke, A.; Rondot, Y.; Linkies, A. (2023) Characterization of a Disease-Suppressive Isolate of *Lysobacter enzymogenes* with Broad Antagonistic Activity against Bacterial, Oomycetal and Fungal Pathogens in Different Crops. *Plants 12*, 682. https://doi.org/10.3390/plants12030682

Effectiveness against downy mildew on cucumber (liquid culture, protective application, climate chamber)

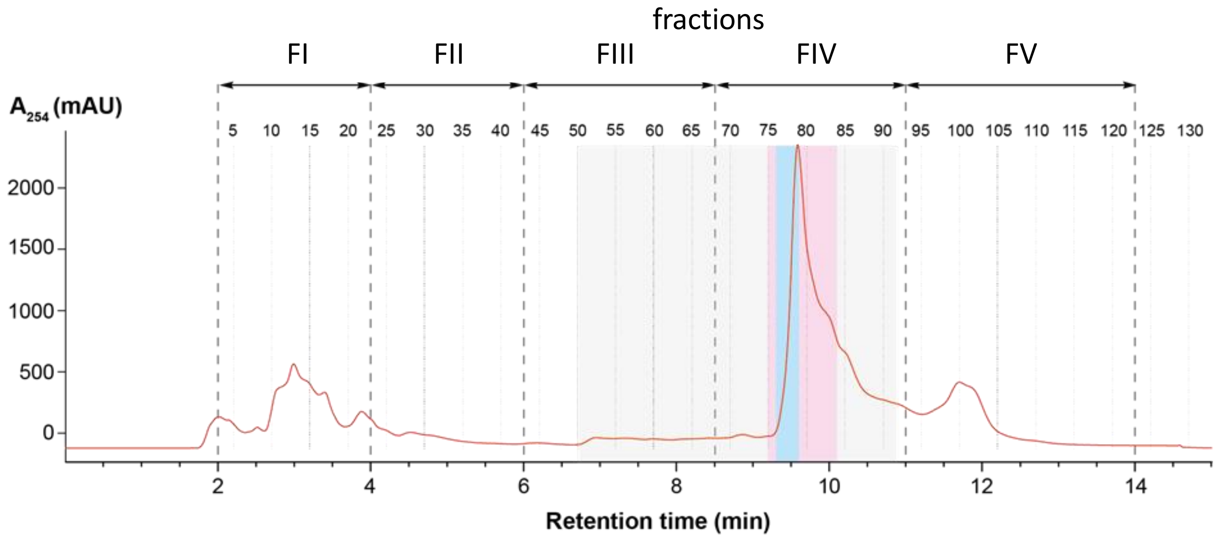


n=9; Tukey-Test (p<0.05)

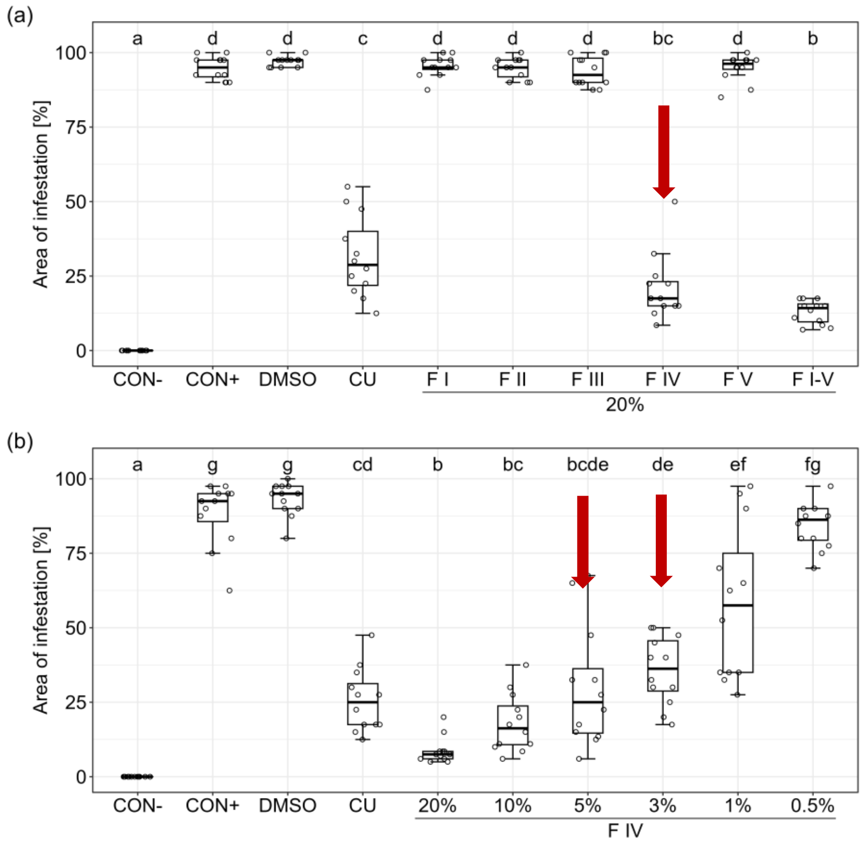
LEC = *L. enzymogenes* U407

- dose dependent effect observable
- 2.5% treatment comparable to chemical control (Cuprozin)
- *ad planta* activity confirmed (e.g. downy mildew on grapevine; apple scab and *P. infestans* on potato and tomato)

Determination of active compounds (HPLC; 130 fractions)



- *in vitro* activity: *Bacillus* sp. grey; *F. culmorum* pink; *P. ultimum* blue
- pooled fraction IV: Heat Stable Antifungal Factor (HSAF) and related compounds
- *in vivo* activity of fraction IV in dose-dependent manner
- less activity than intact broth (comp. to 3-5% LEC liquid culture)



Efficacy and host spectrum

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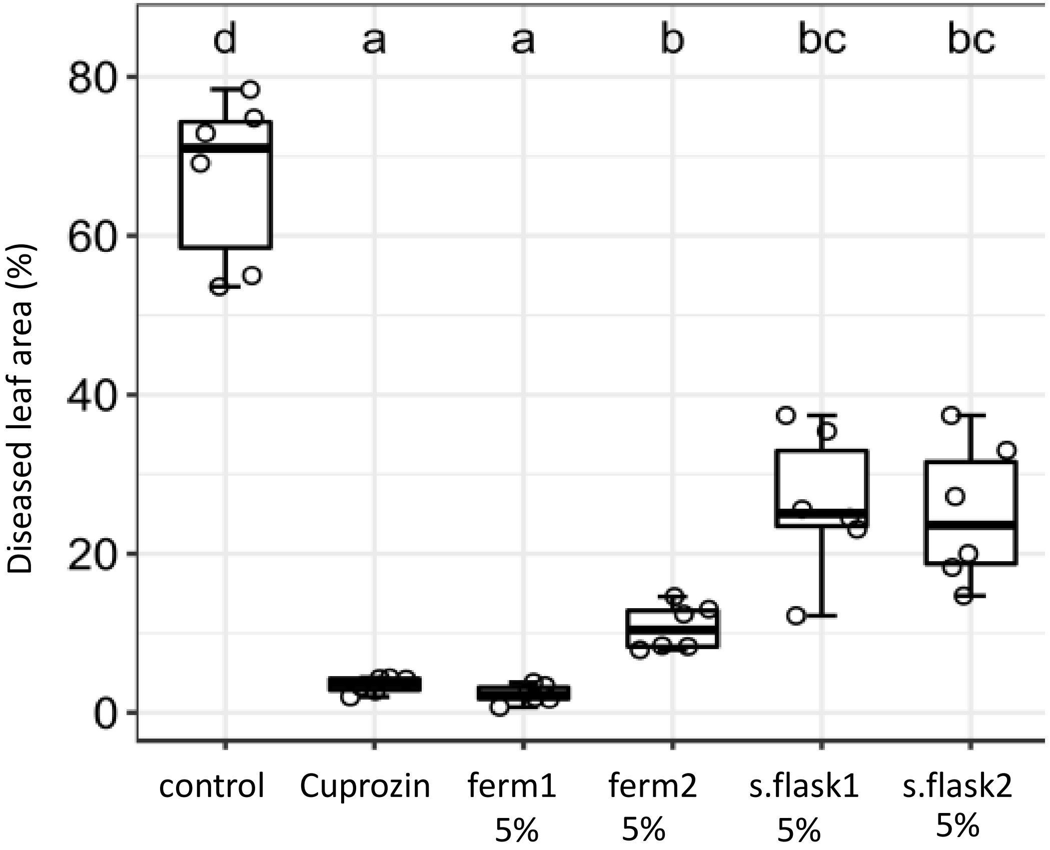
Efficacy comparison of shake flask and fermenter culture against *Phytophthora infestans* on potato



- upscaling in 3l volumes by fermenter culture possible
- no loss of activity
- fermenter cultures tend to be more active than shake flask cultures

n = 6 , Tukey-Test ($p<0.05$)

Production and processing of *L. enzymogenes* LEC

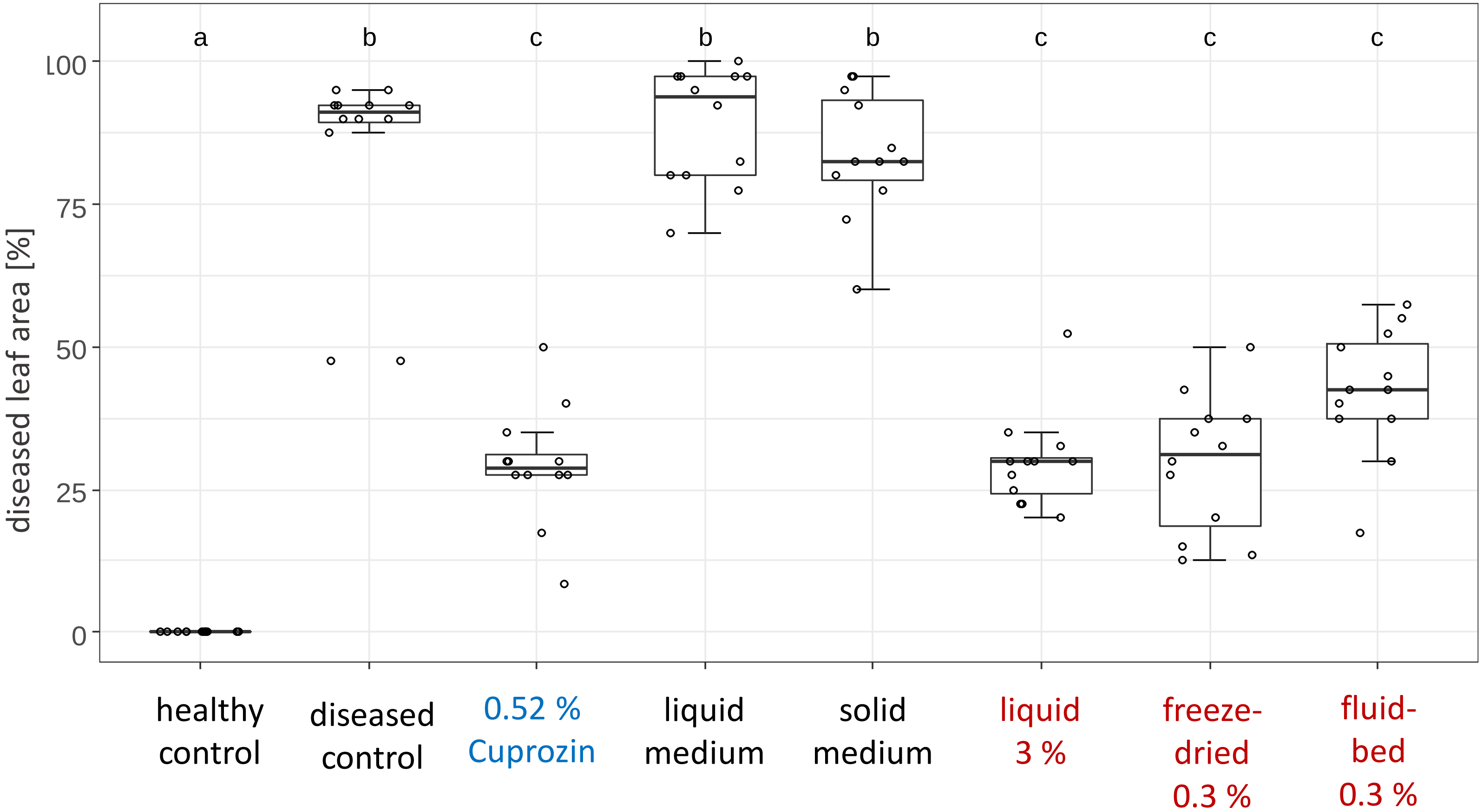
Liquid culture, fermenter

freeze-dried

fluidised bed-dried

Plant trials: indoor and field

Production and processing of *L. enzymogenes* LEC

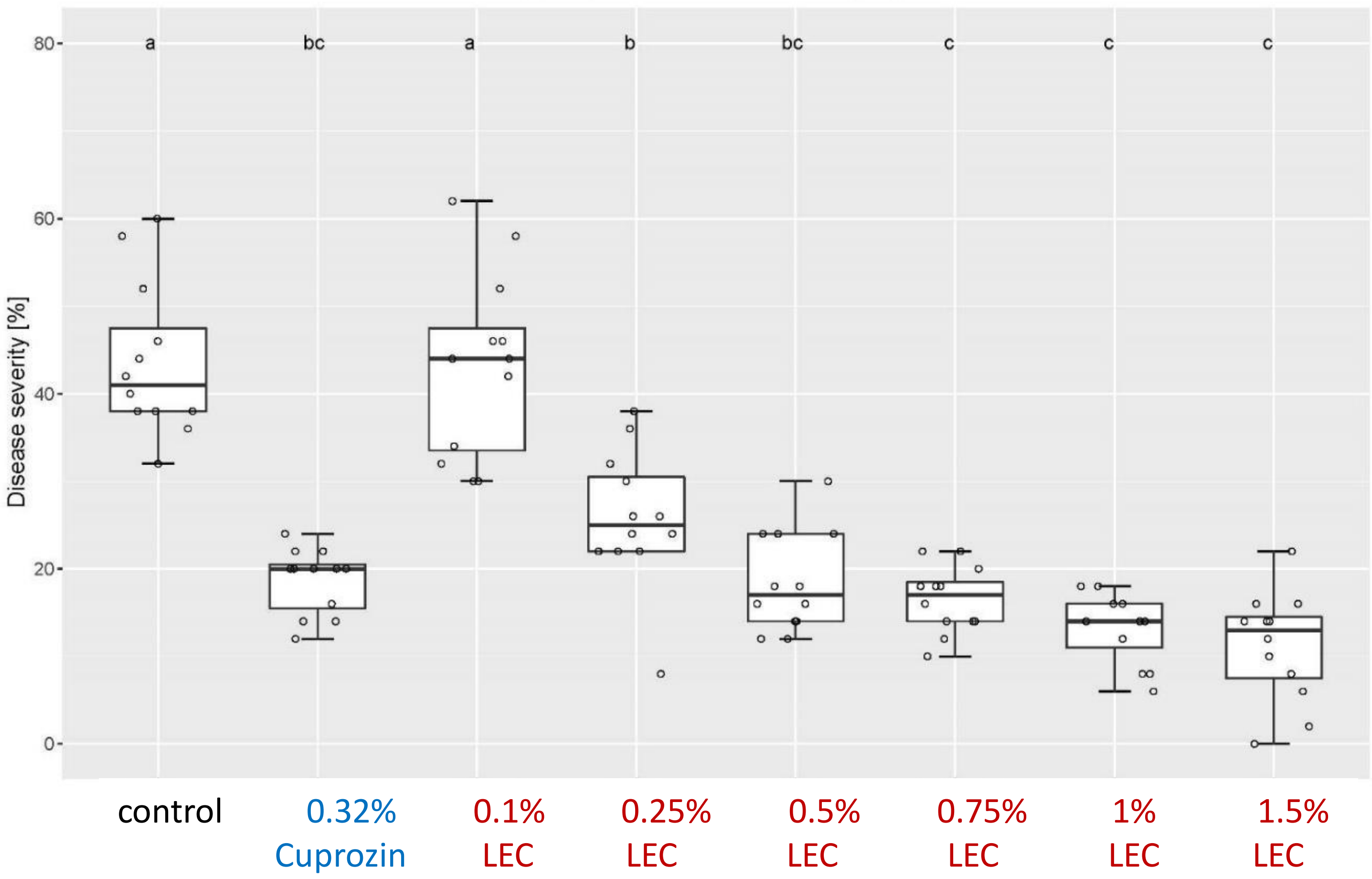





- comparable effectiveness between conservation methods
- shelf life: no activity loss within 6 months storage time

n=12; Tukey-Test ($p<0.05$)

Efficacy against downy mildew on grapevine (freeze-dried culture, protective application)



(GLS-Anova p<0,05)

- dose dependent effect of freeze-dried product observable
- 0.5% *L. enzymogens* (LEC) comparable to chemical control (Cuprozin)

Summary efficacy

		in vitro/ lab	*in vivo*/growth chamber
P. infestans	**potato**	✓	✓
P. infestans	**tomato**		✓
P. viticola	**grape**	✓	✓
P. cubensis	**cucumber**	✓	✓
V. inaequalis	**apple**	✓	✓ ✗

plants

MDPI

Article

Characterization of a Disease-Suppressive Isolate of *Lysobacter enzymogenes* with Broad Antagonistic Activity against Bacterial, Oomycetal and Fungal Pathogens in Different Crops

Christian Drenker [1], Doris El Mazouar [1], Gerrit Bücker [1,2], Sonja Weißhaupt [3], Eveline Wienke [3], Eckhard Koch [1], Stefan Kunz [3], Annette Reineke [2], Yvonne Rondot [2] and Ada Linkies [1,*]

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Efficacy and host spectrum

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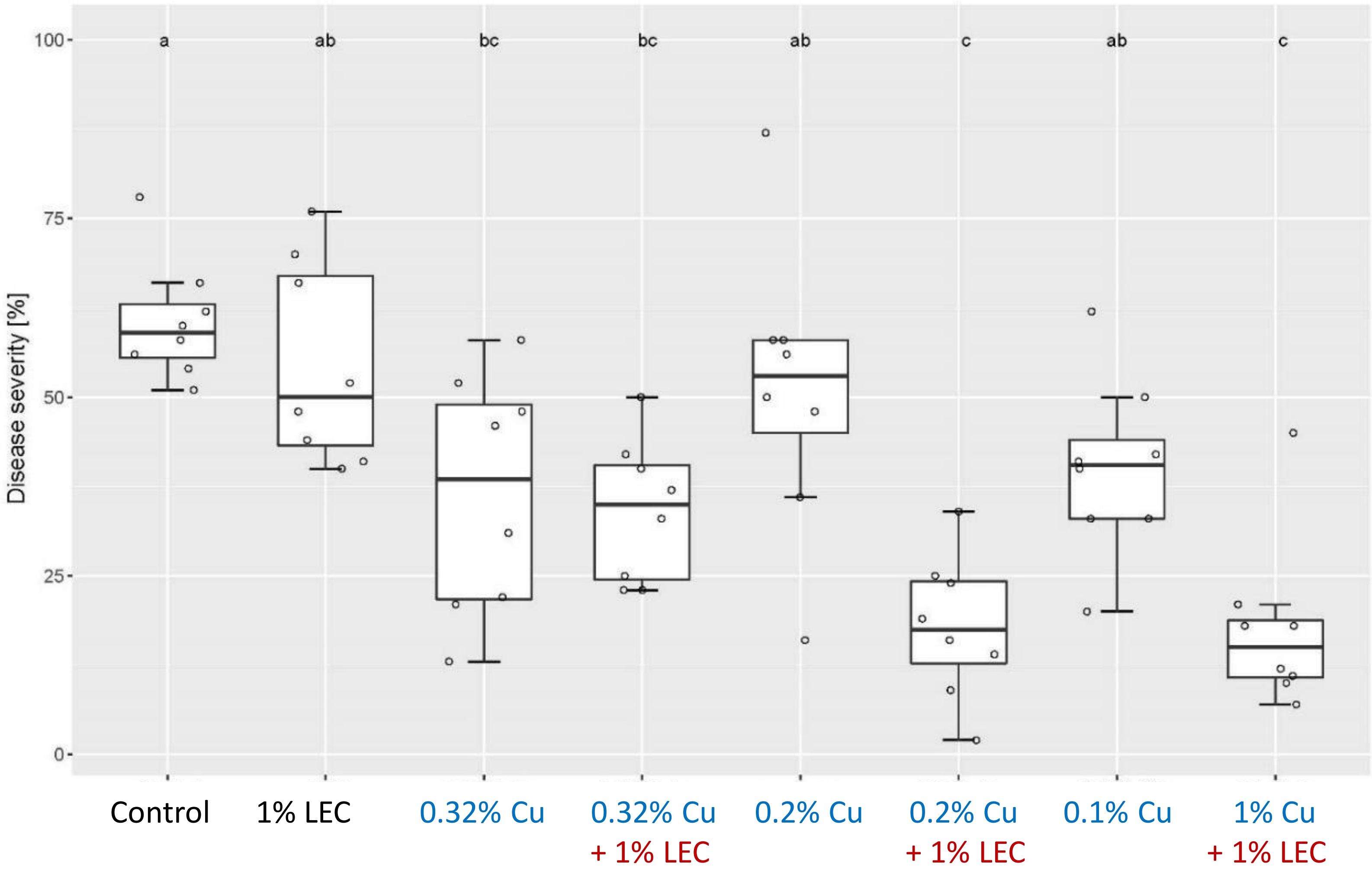
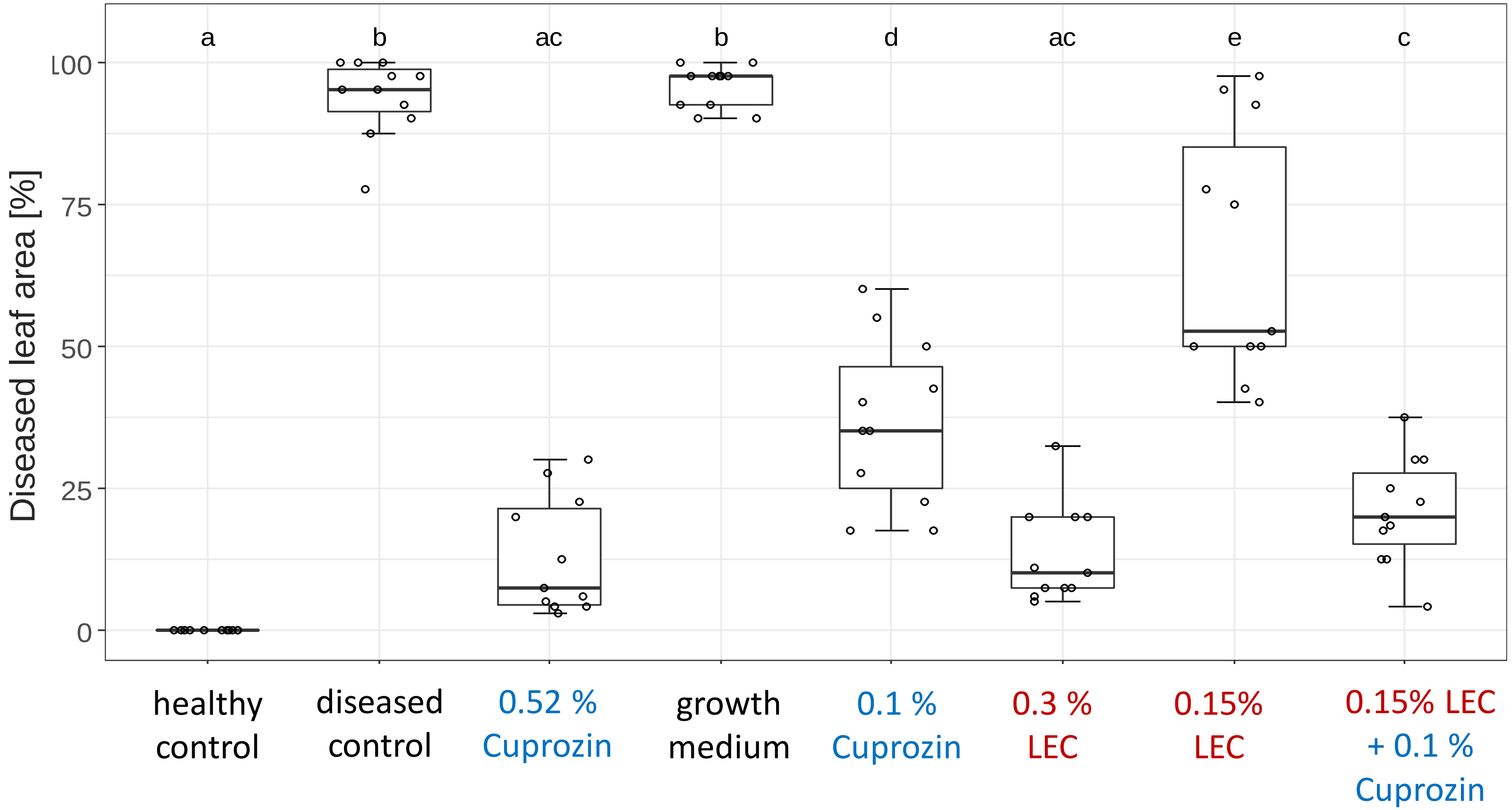
Integration of *LEC* in a copper reduction strategy (downy mildew on grapevine, freeze dried product)

- *L. enzymogenes* (LEC) and copper compatible
- *L. enzymogenes* (LEC) increases effect of reduced copper applicaton



(GLS-Anova p<0,05)

Integration of *LEC* in a copper reduction strategy (downy mildew on cucumber, freeze dried product)



- Reduced LEC enhances effect of reduced copper application
- Integration into copper reduction strategy seems possible

n=11; Tukey-Test ($p<0.05$)

Efficacy of *L. enzymogenes* in open field trials

- → no reproducible suppressive effects of LEC in field trials!
- → loss of efficacy by abiotic factors like light or rain?

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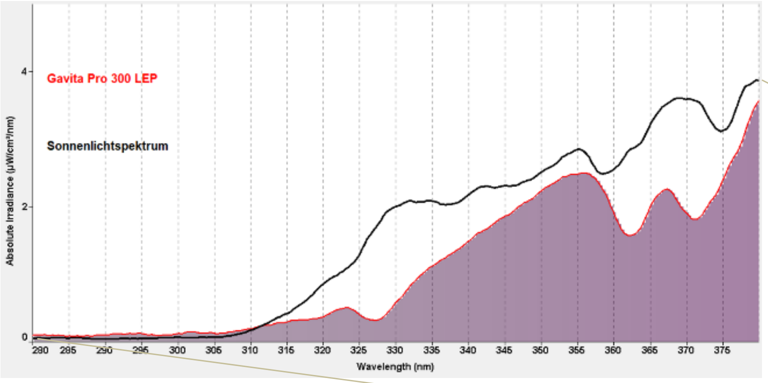
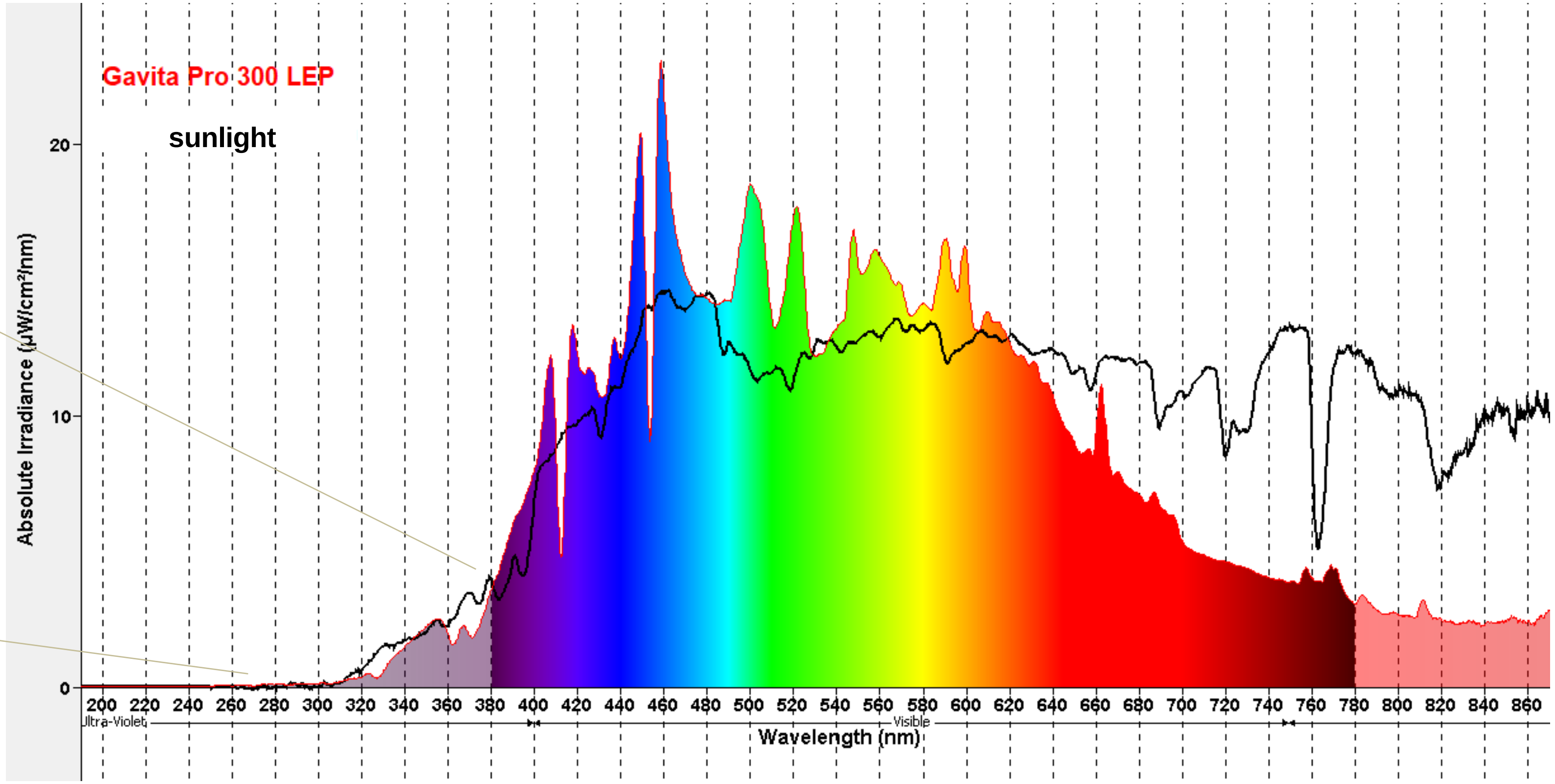
Radiation stability testing of *L. enzymogenes*

Gavita Pro 300 LEP plasma lamp:

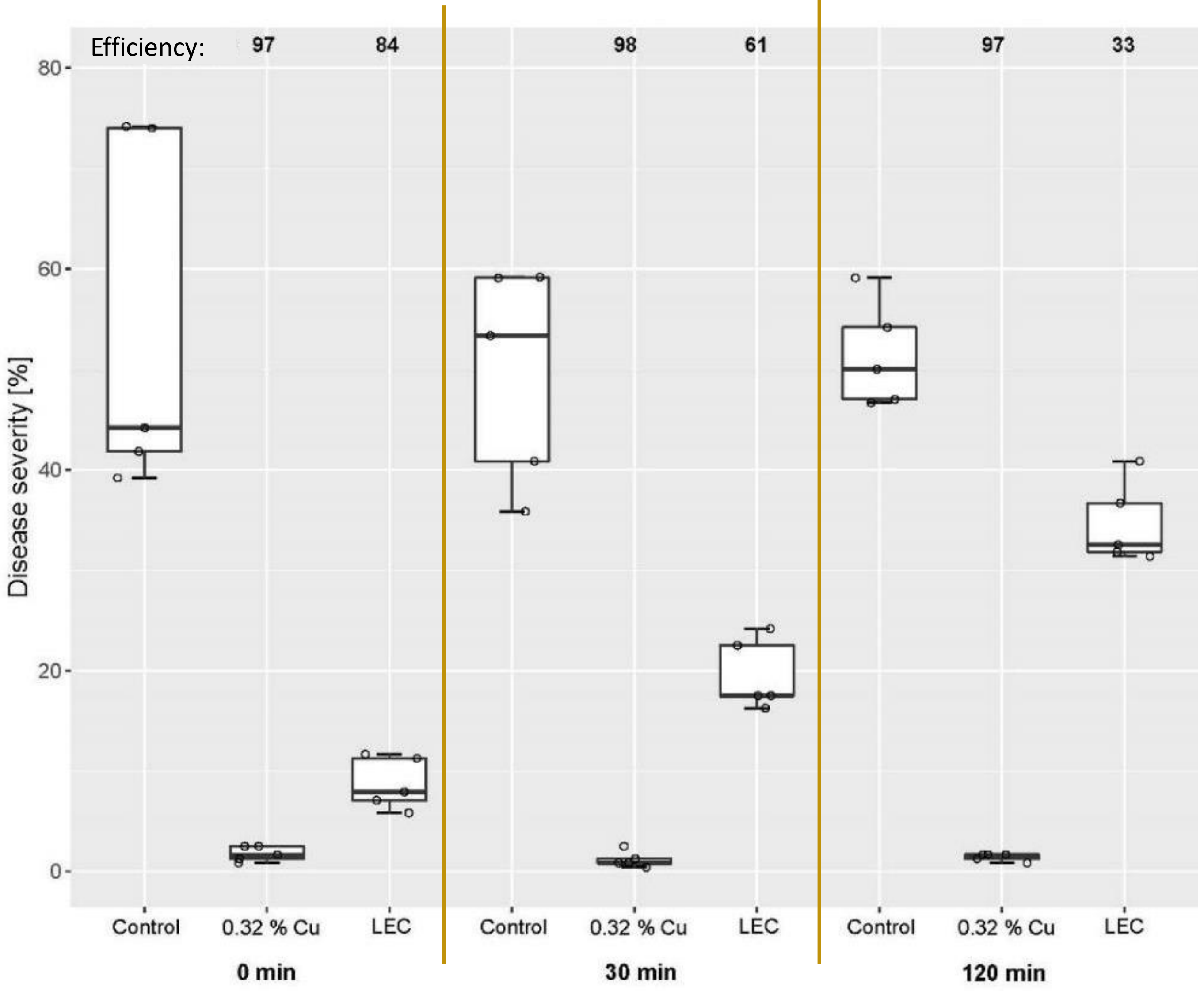
- resembles spectrum of sunlight
- includes UV-A (380–315 nm) and UV-B (315–280 nm) light

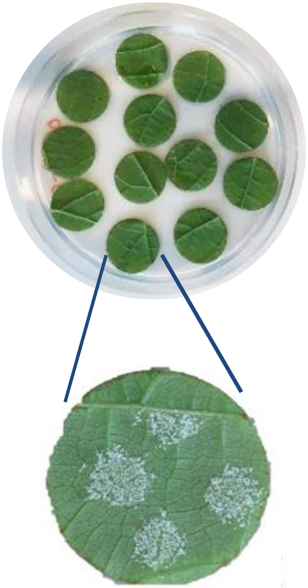


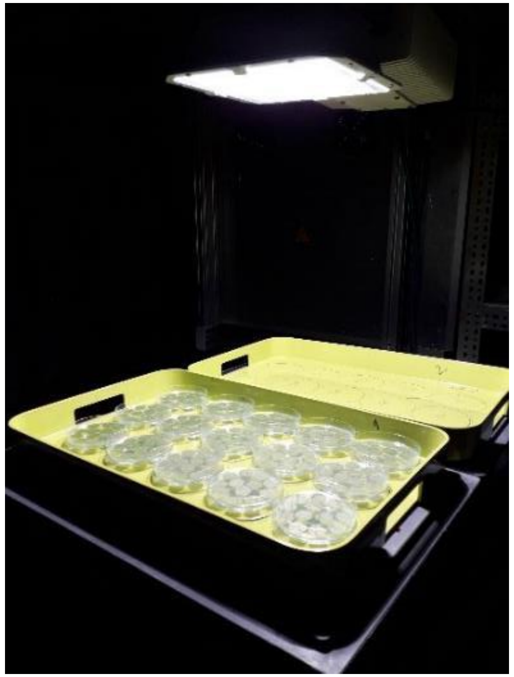
Spectrum comparison lamp and sunlight (170 µmol/m²*s)



Radiation stability testing of *L. enzymogenes* (downy mildew on grapevine, leaf disc assay)

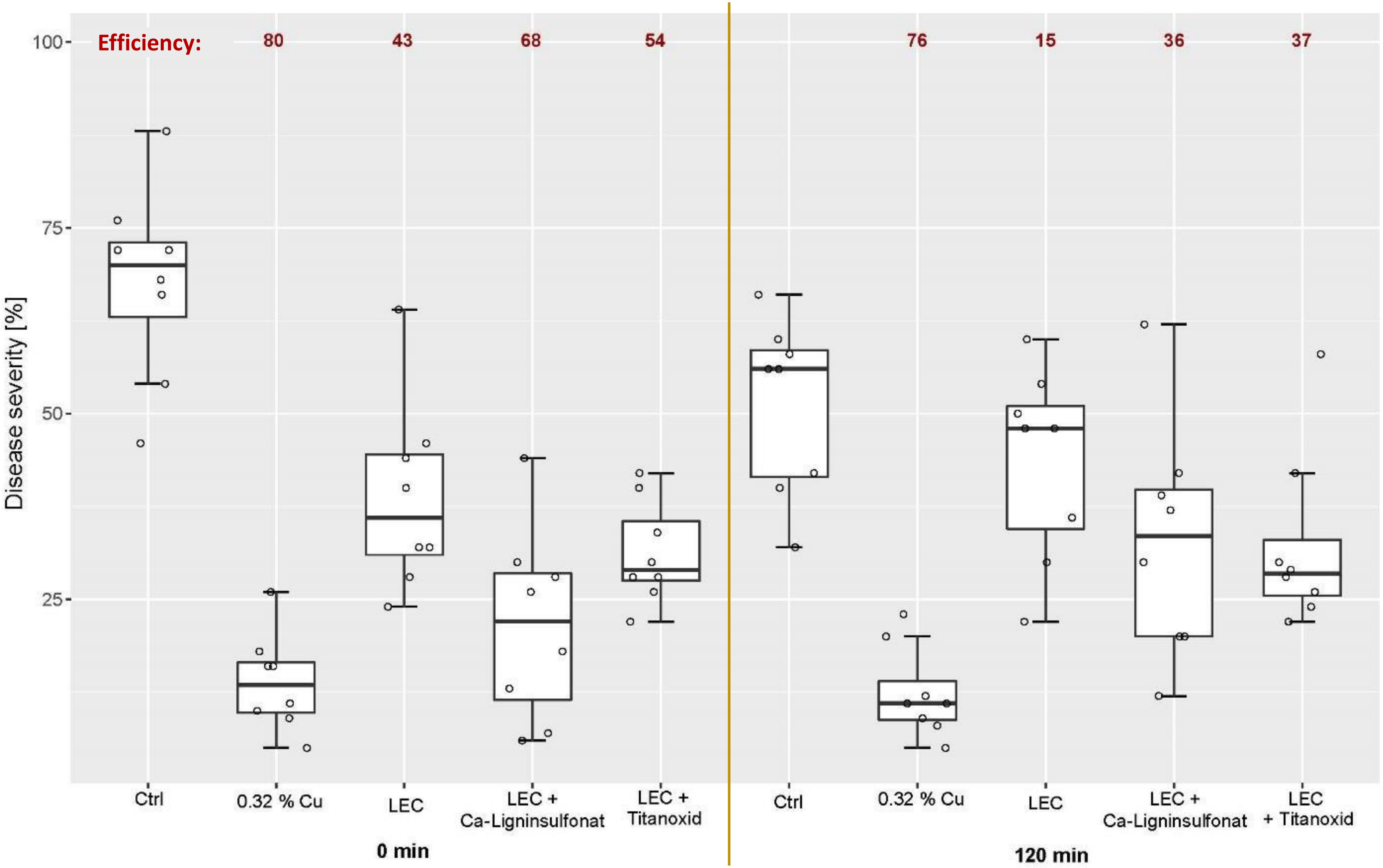






- Reduction of efficiency after 30 min
- 50% reduction after 120 min

Additive testing of *L. enzymogenes* (downy mildew on grapevine)



- Additives support the effect of LEC
- Additives tend to provide protection against the influence of sunlight radiation

Testing systems for radiation

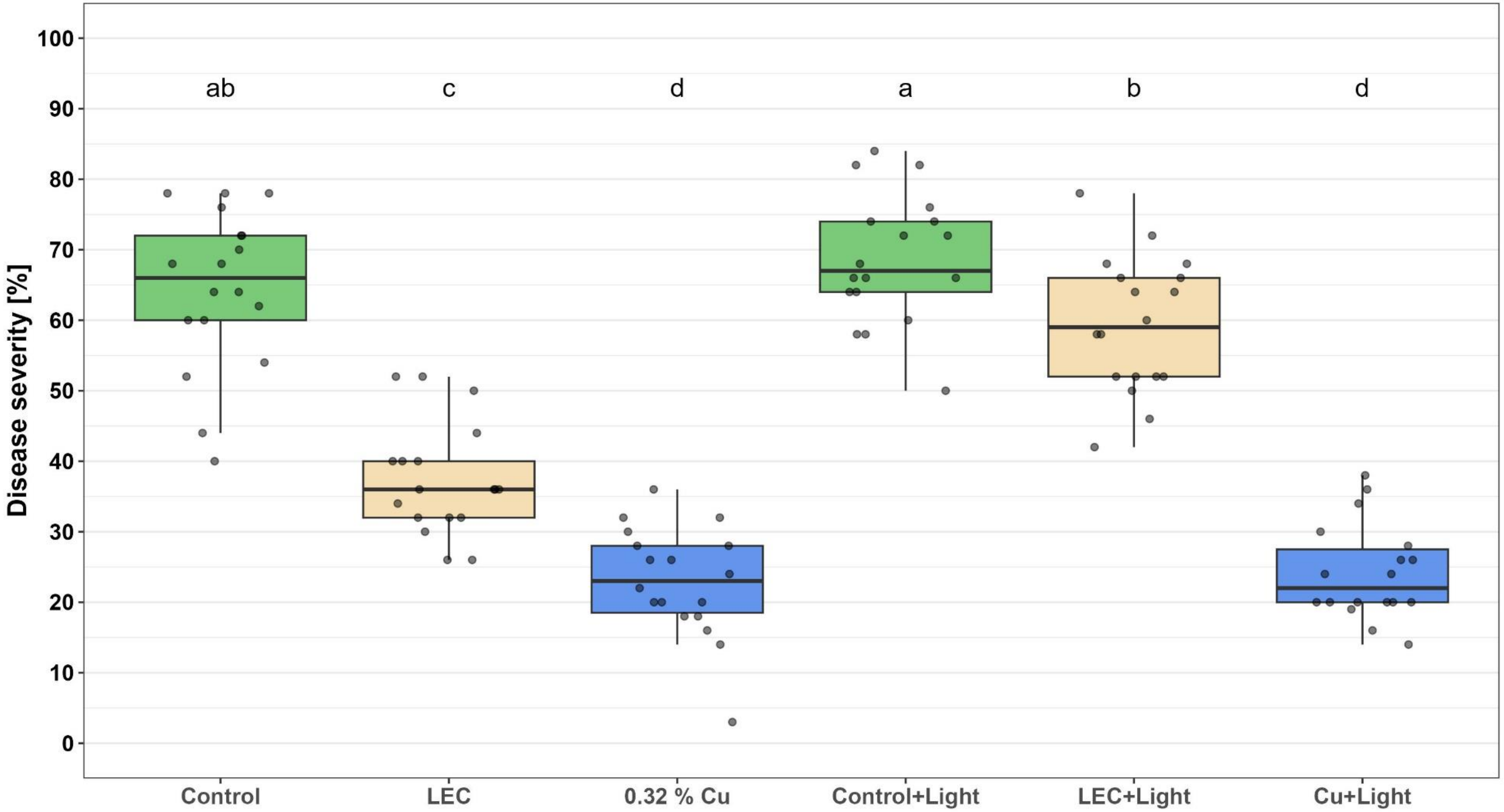
Radiation test system at Hochschule Geisenheim:
LED-KE 400 VSP + LED-MID UV-A

Weathering system at JKI:
UV-Suntest XXL (Atlas)

Testing systems for radiation

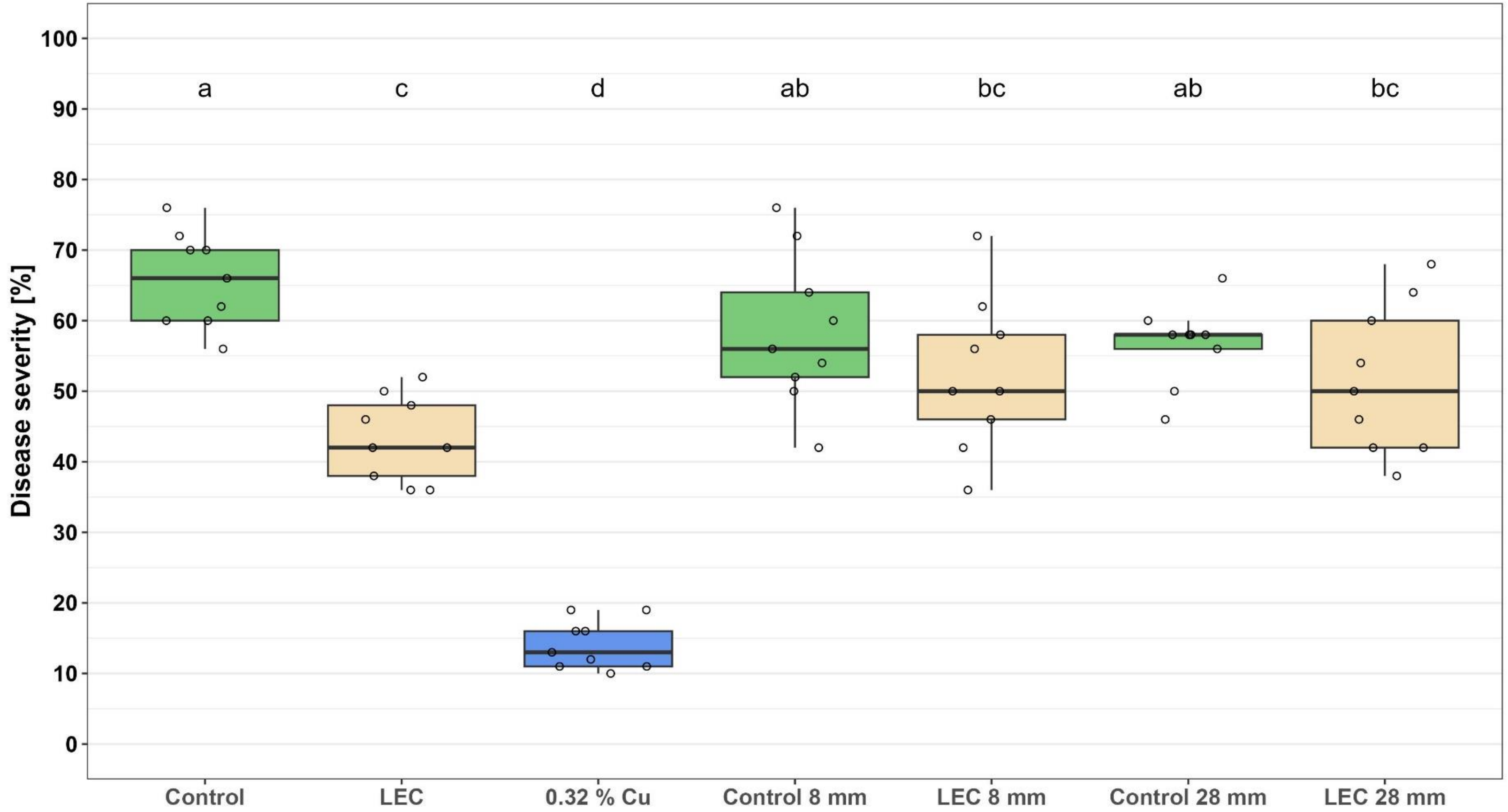
3 hours of lightning

→ Significant reduction in efficacy after 3 hours of lightning



n=18, ANOVA p < 0,05

Testsystems for rainfastness



Slightly reduction in efficacy after 8 or 28 mm of rain

Outlook

2 PhD students

Goals of follow-up project *OptiLyso* :

- Environmental stability (radiation, rain, heat, drought)
- Duration of activity
- Process optimization
- Mode of action
- Influence on plant microbiome

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