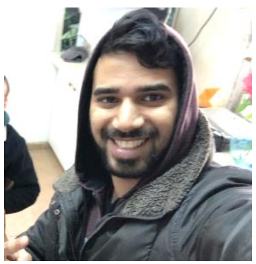
ICPP 2023 LYON

ONE HEALTH
for all plants,
crops and trees

Trichoderma asperellum secreted 6-pentyl-alpha-pyrone

protects maize plants from the late wilt pathogen, *Magnaporthiopsis maydis*

Prof. Soliman Khatib



Asaf Gordani

Paz Becher

Raviv Harris

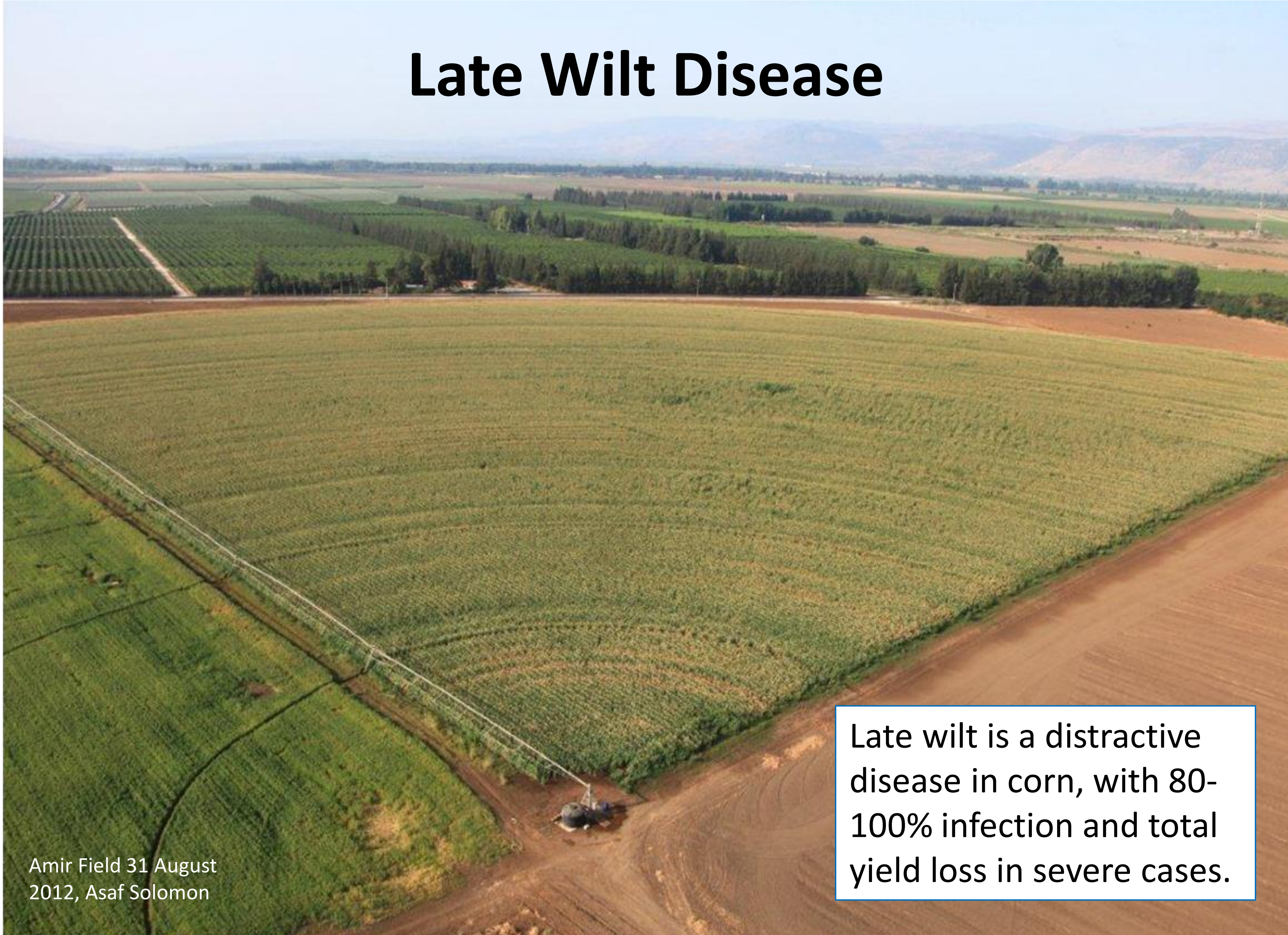
Tel-Hai College

MIGAL Galilee Research Institute

Tel-Hai College

MIGAL research institute and Tel-Hai College are in northern Israel, where LWD was first discovered.

Late Wilt Disease

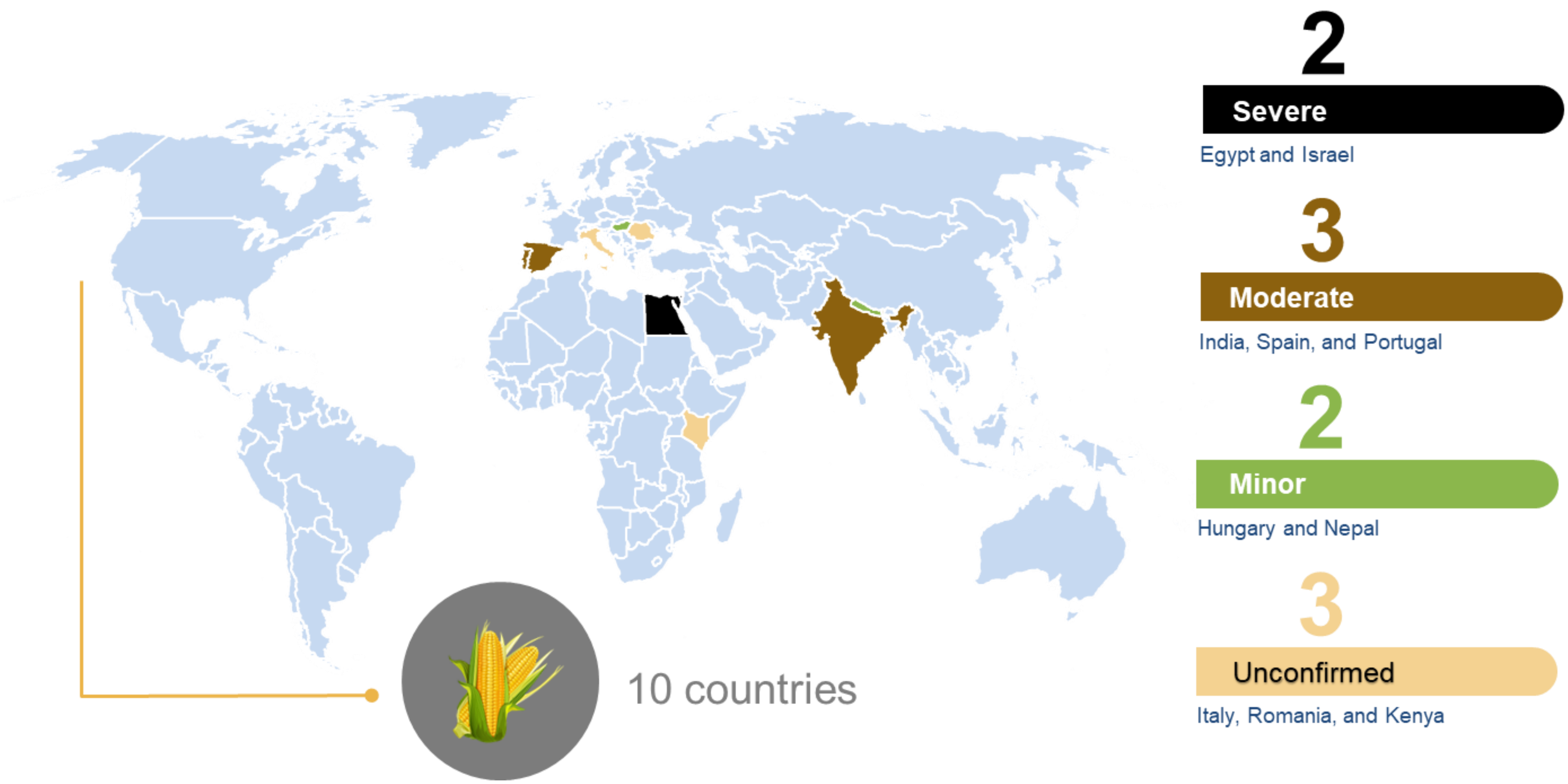


Late wilt is a distractive disease in corn, with 80-100% infection and total yield loss in severe cases.

Degani O. *Journal of Fungi* 2021

World distribution map for late wilt of maize

The pathogen is currently reported in about 10 countries, with Israel and Egypt being considered the most affected areas.



Degani O. *Journal of Fungi* 2021

- The disease is characterized by a rapid wilting of maize plants 70 to 80 days post-seeding.

Pathogenesis

- *M. maydis* is seed-borne and soil-borne and infects seedlings through the roots.

- The pathogen survives for long periods in the soil as sclerotia and spores on corn residuals.

The disease appears as brown patches in the field

Lohamei HaGeta'ot 2017 Colossus cv. maize field

Hulata 2002 Jubilee cv. maize field

Day 50

Day 57

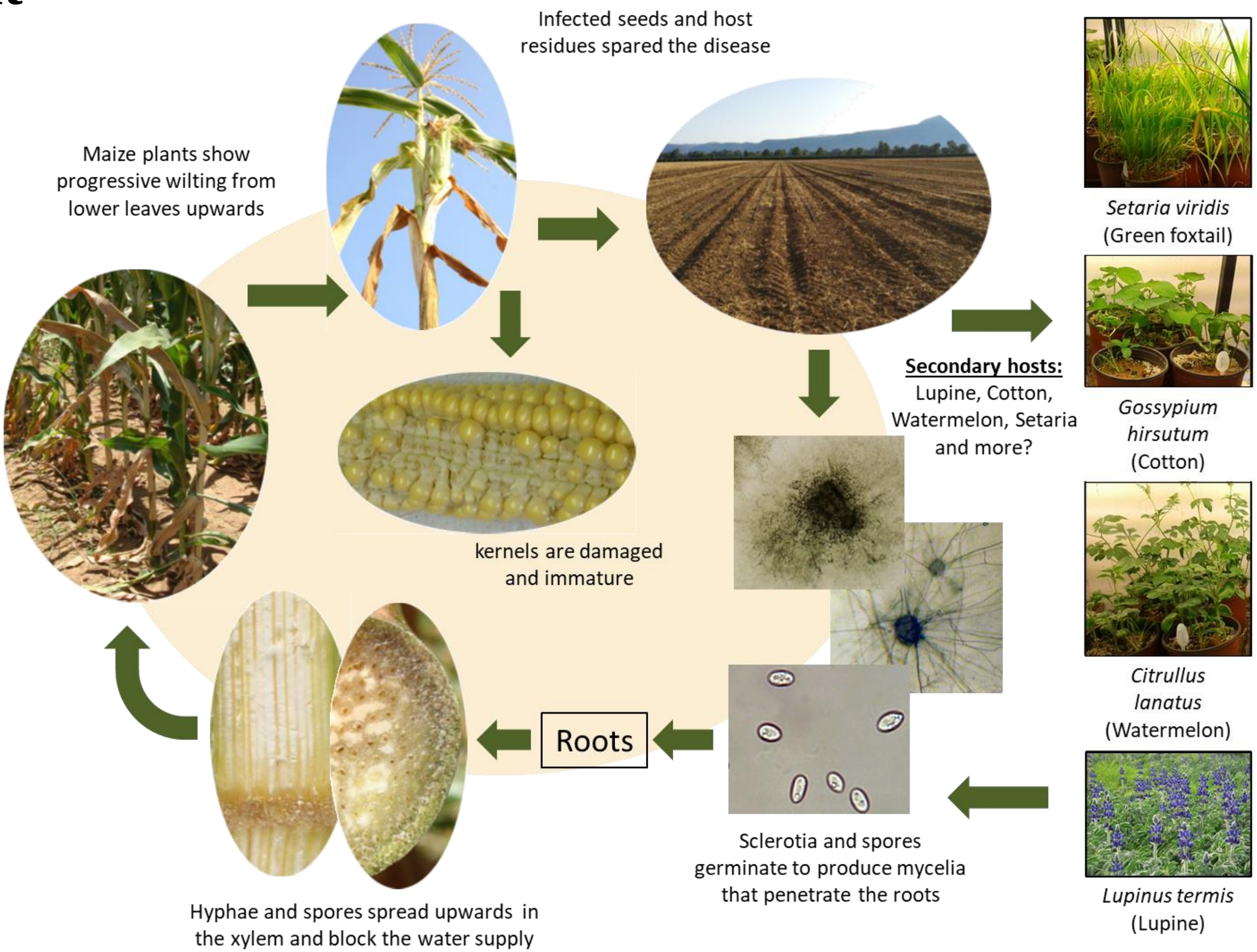
Day 64

Day 72

Pathogenesis in susceptible cultivars with severe dehydration.

Degani O. *Journal of Fungi* 2021

Late wilt disease cycle

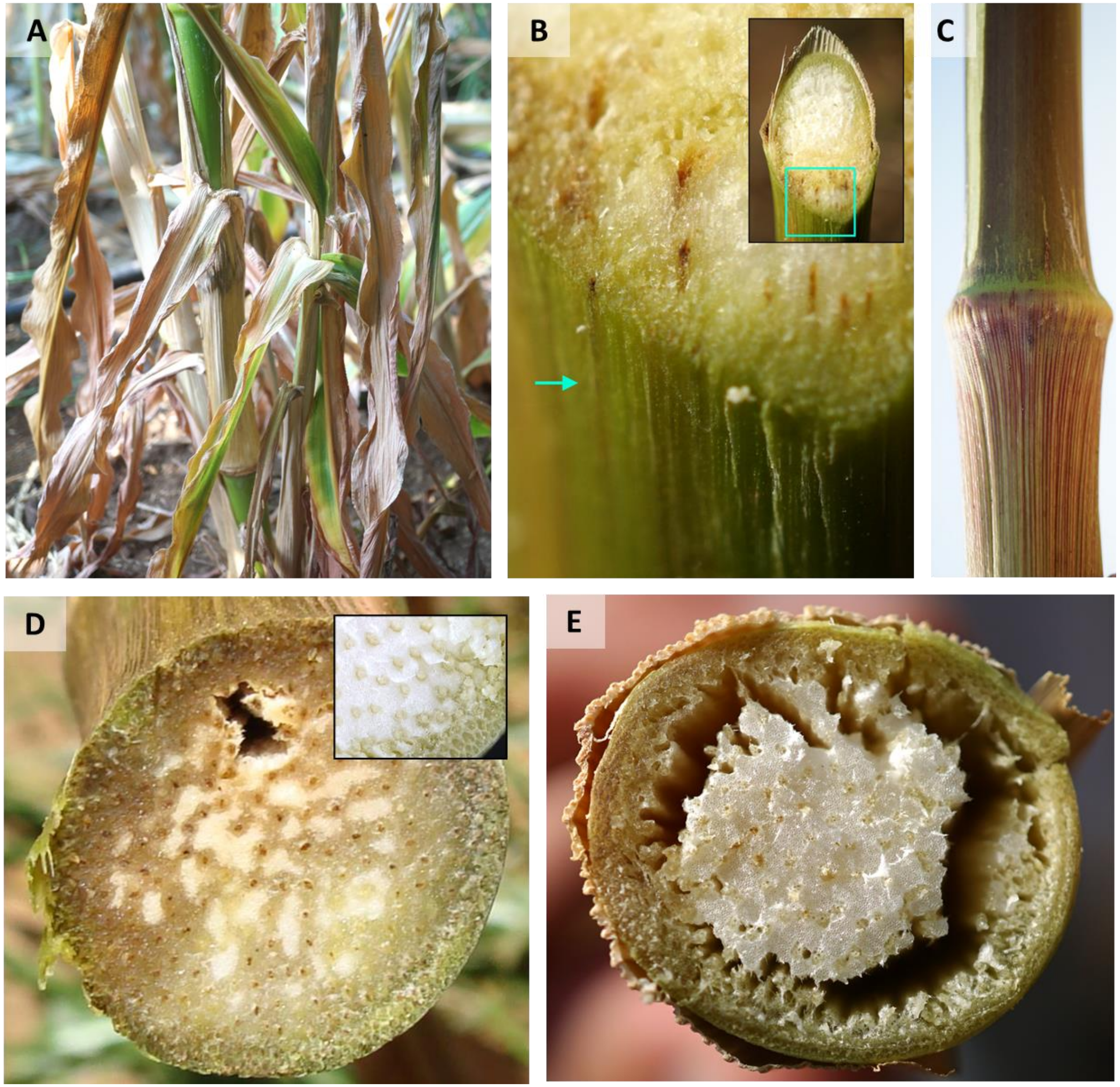


Degani and Cernica, *Adv. Microbiol.*2014

Degani O. *Journal of Fungi* 2021

The pathogen spreads in the xylem, infects seeds, and survives in alternative host plants.

What are the disease symptoms?

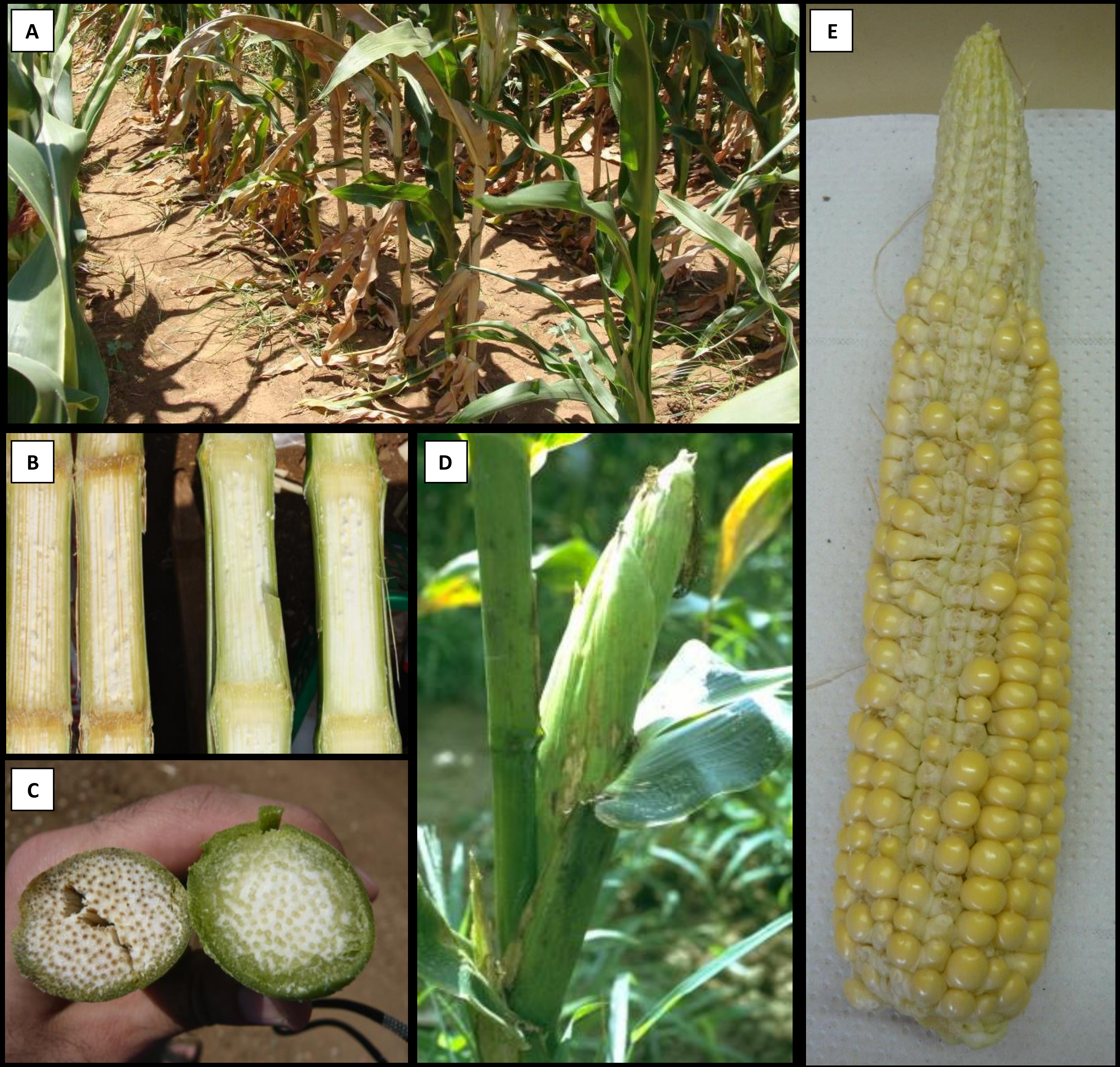


- First symptoms include drying out of the lower leaves.
- Later, symptoms spread upwards.
- The appearance of red-brown stripes on the lower internode.

Degani et al., *Agronomy*, 2019 Degani O. *Pathogens*, 2022

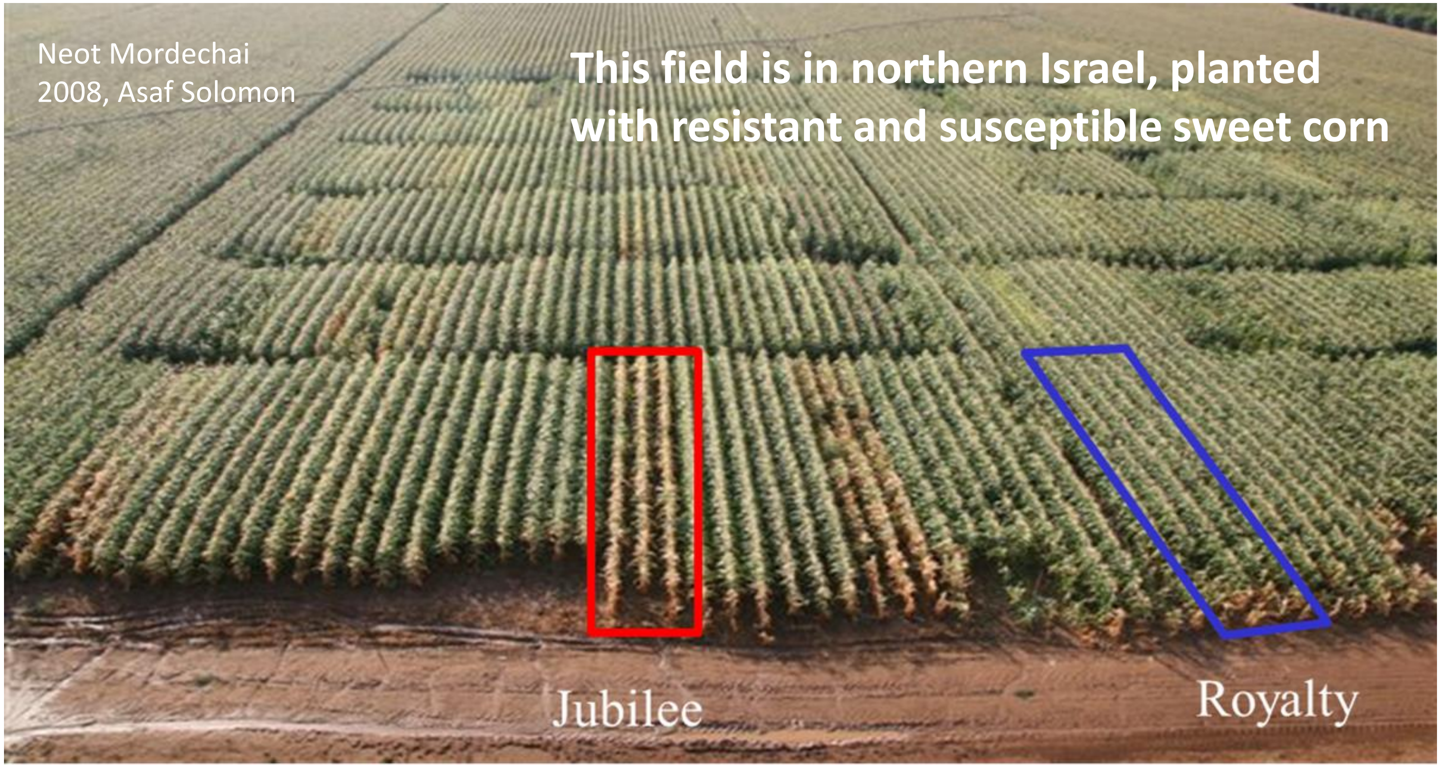

Eventually, the plants will collapse and die
Severe lower stem dehydration

- **Drying Stems have brownish vascular bundles.**
- **If ears are produced, the kernels are poorly developed**



Degani and Cernica,
Adv. Microbiol, 2014

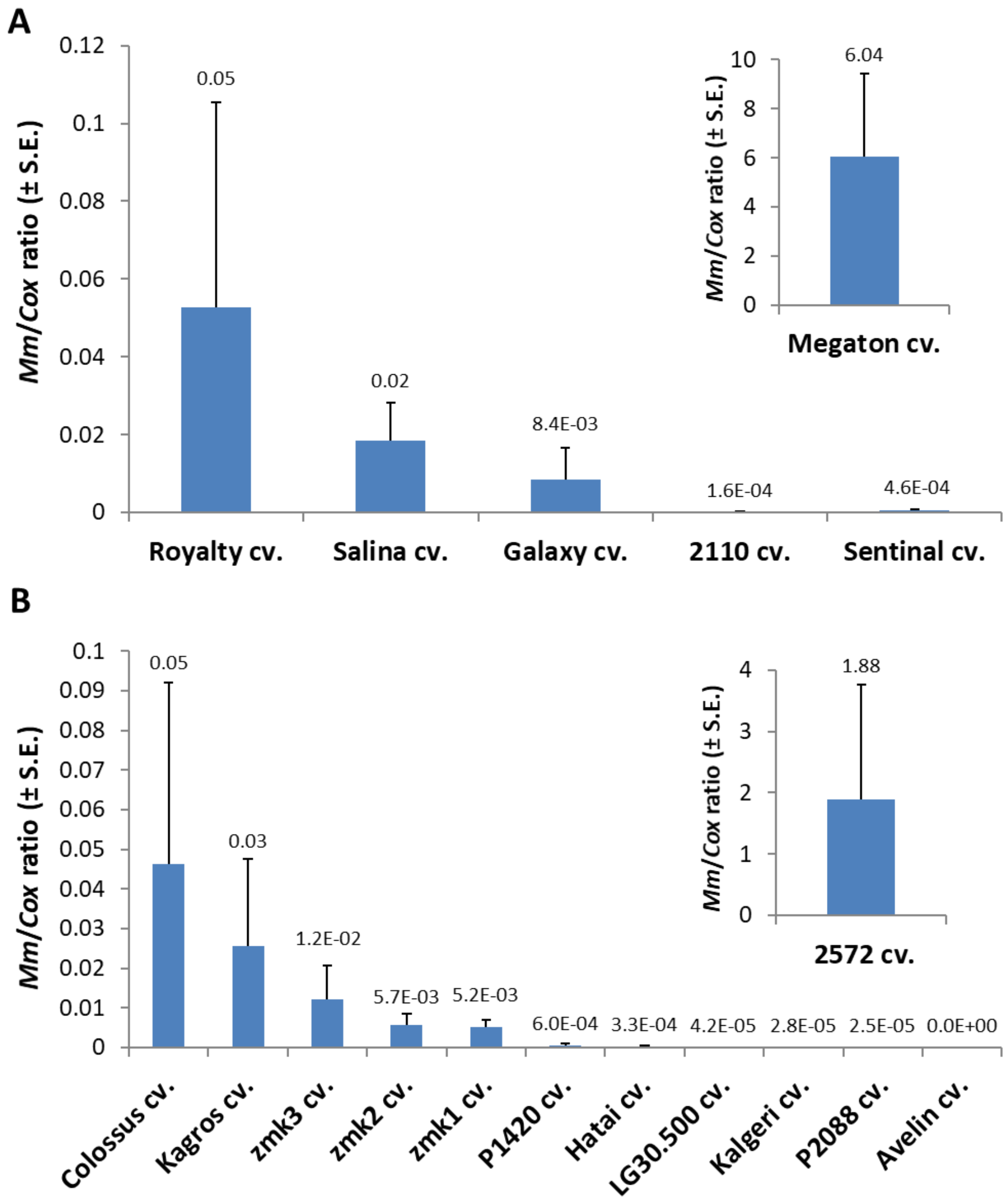
How can we control late wilt?



- Currently, the most effective, economical, and eco-friendly method to control the disease is the use of resistant maize varieties.

Drori et al., *Phytopathologia Mediterranea*, 2013

Degani O. *Pathogens*, 2022

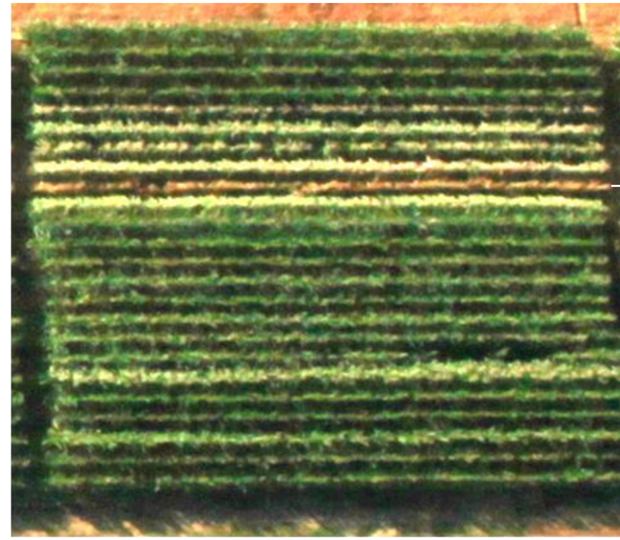


- The fungal *DNA* qPCR detection in the roots can reveal the cultivars' susceptibility degree.
- In some genotypes the DNA is undetectable, while in others, it can be a million times higher.

Degani et al., *Journal of Plant Pathology*, 2022

66 DAS (10 DAF)

Field trial to inspect corn varieties' resistance



- The poorly immune Megaton cv. collapsed at the beginning of the maturation phase, ca. 50 Days post-sowing.

Degani et al., *Journal of Fungi*, 2020

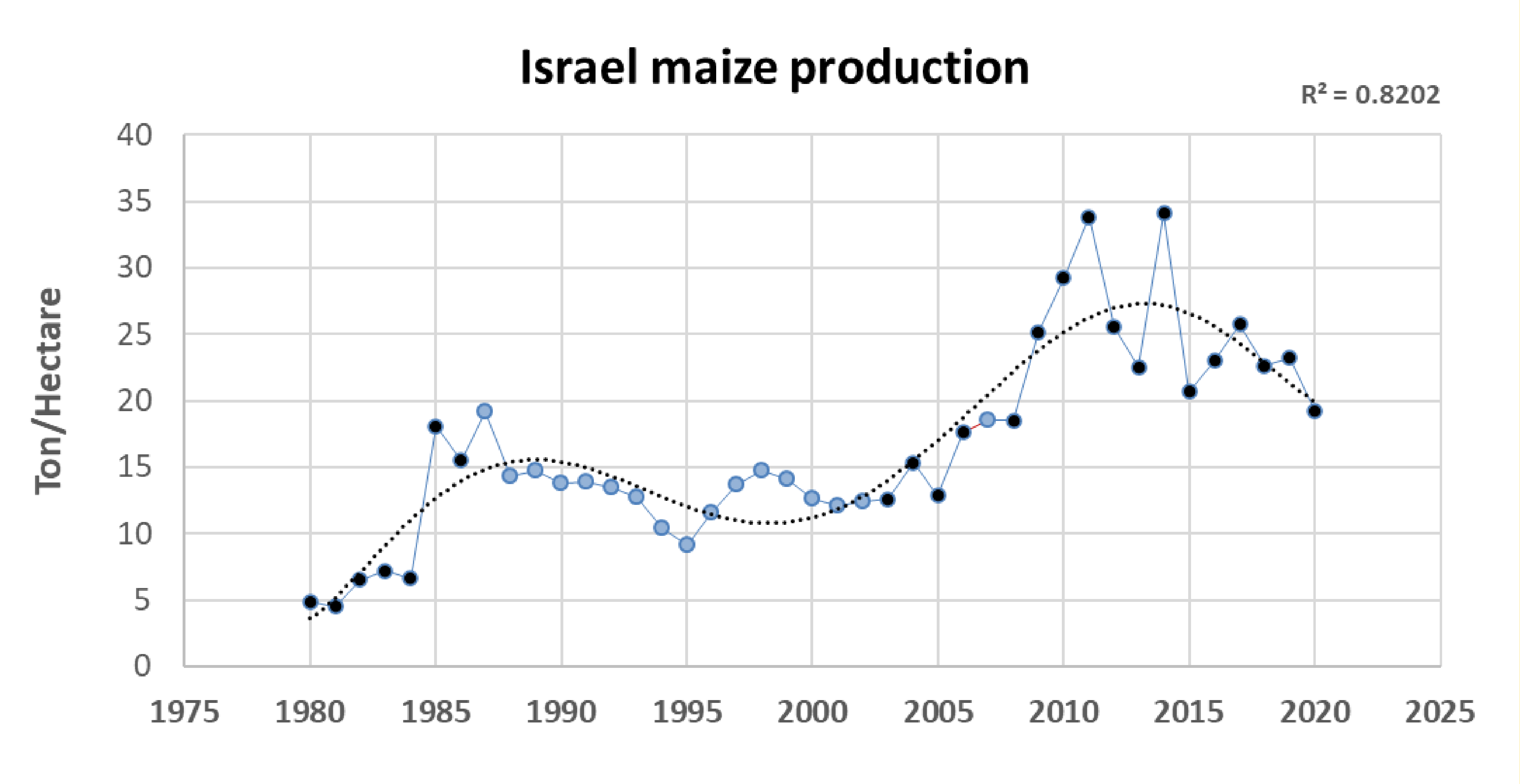
Field observation for testing cultivars

- The highly susceptible genotype appeared as brown strips in the aerial photo.

Degani O. *Pathogens*, 2022
Shofman, et al., *Fungal Biology*, 2022, under review

Israel coastal plain, Yavne 2014

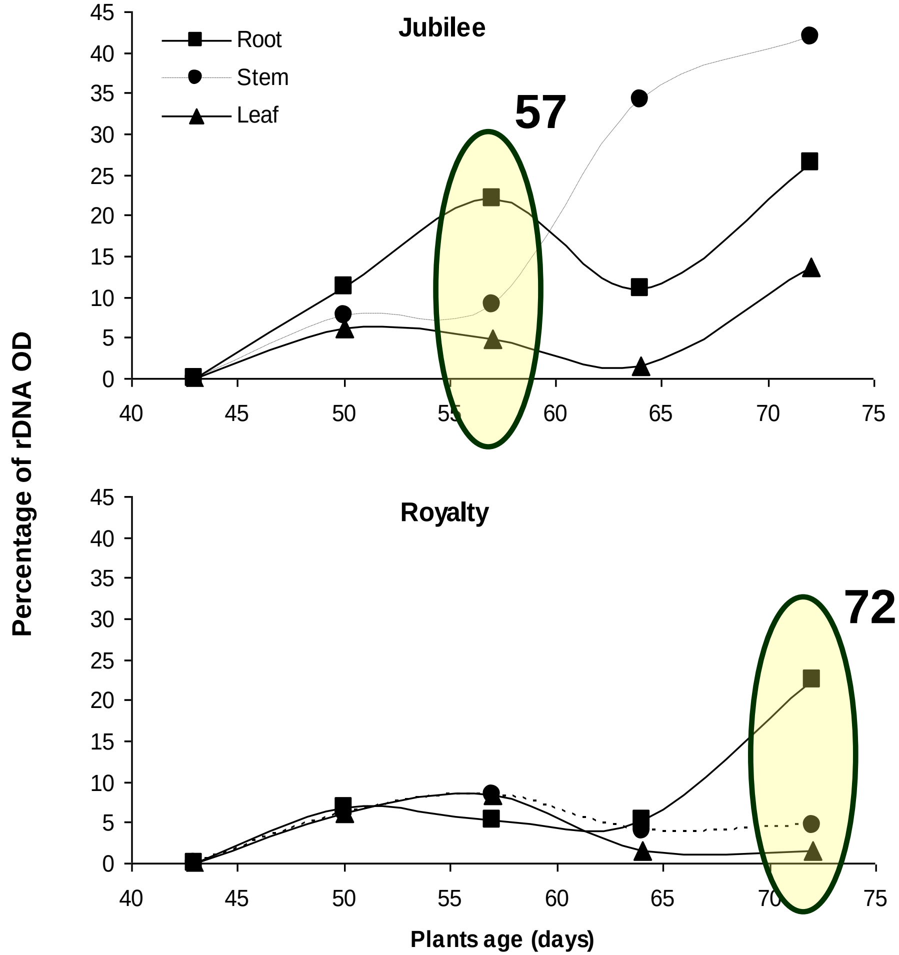
Avoiding susceptible maize cultivars' growth may be a factor in Israel's increased maize crop production over the years.



Degani and Gordani, Agronomy, 2022

Aggressive fungal strains can overcome host resistance

The fungal DNA infection in a resistant maize cultivar is similar to pathogen spreading in the sensitive cultivar
but with two weeks delay.



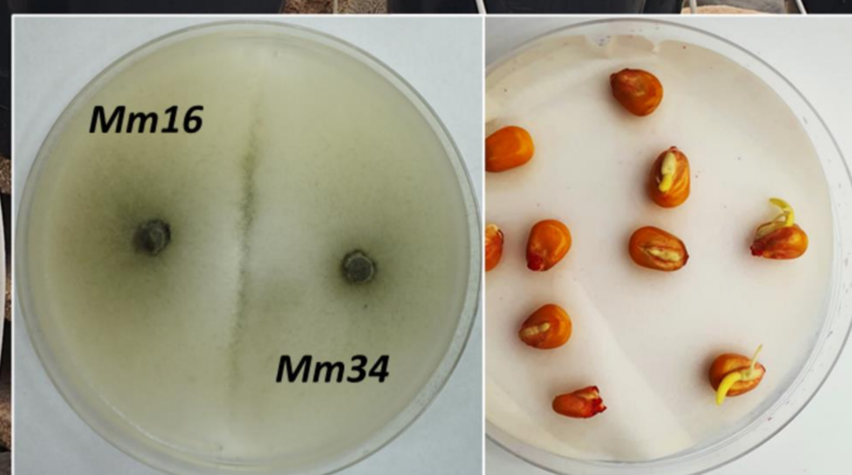
Drori *et al.*, 2013,
Phytopathologia Mediterranea

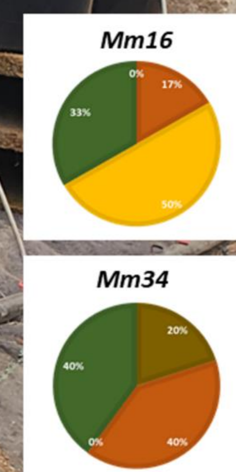
Degani O. *Pathogens*, 2022

Aggressive *M maydis* strains lately reported by us in Israel

<u>See Poster:</u> <u>P4.3-001</u>
Session 1: *21/8-22/8*

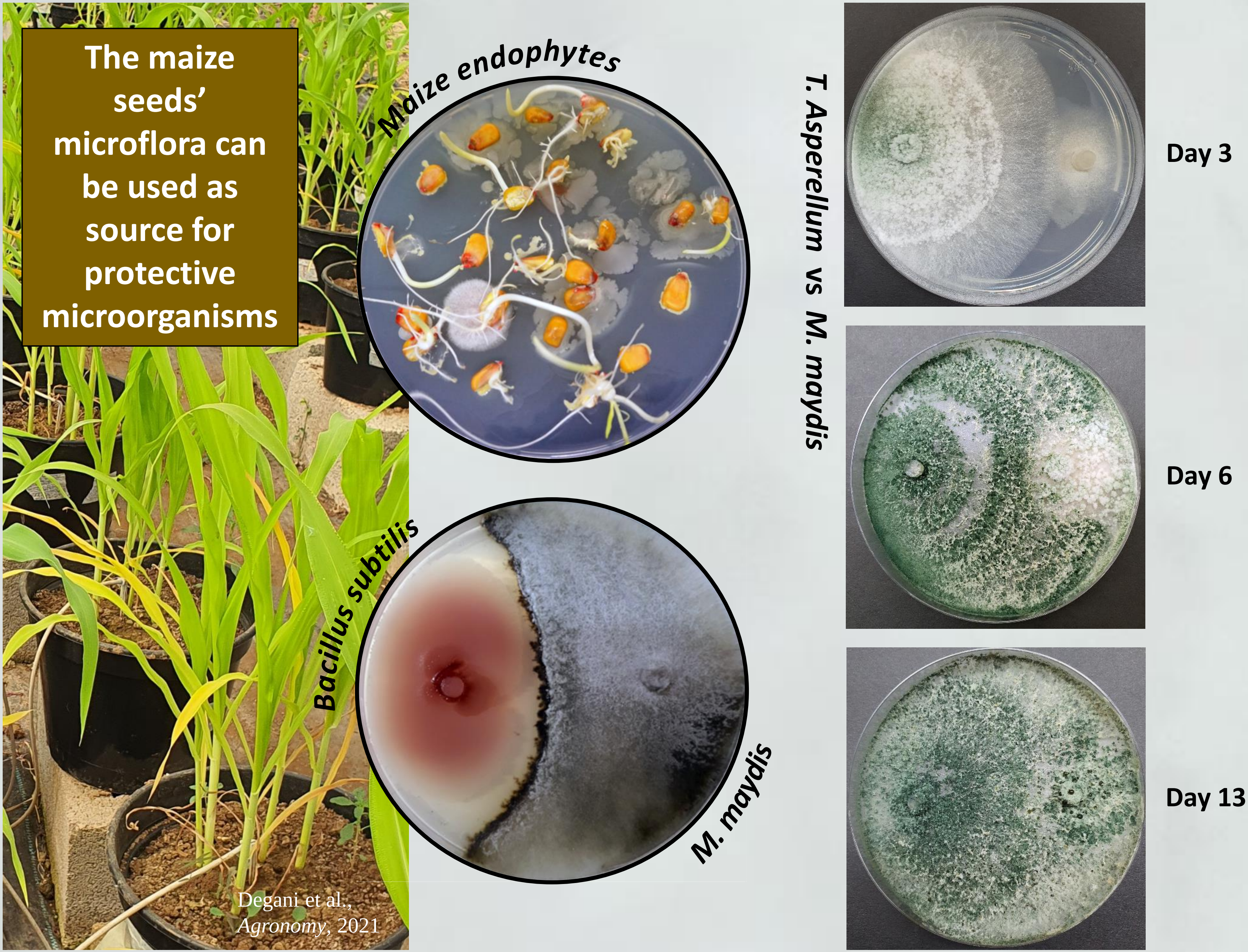
Shofman, et al., *Fungal Biology*, 2022





Results

Developing biological seeds enrichment
for Late wilt eco-friendly control


The maize seeds' microflora can be used as source for protective microorganisms
Maize endophytes
Bacillus subtilis
M. maydis
T. Asperellum vs M. maydis
Day 3
Day 6
Day 13
Degani et al.,
Agronomy, 2021

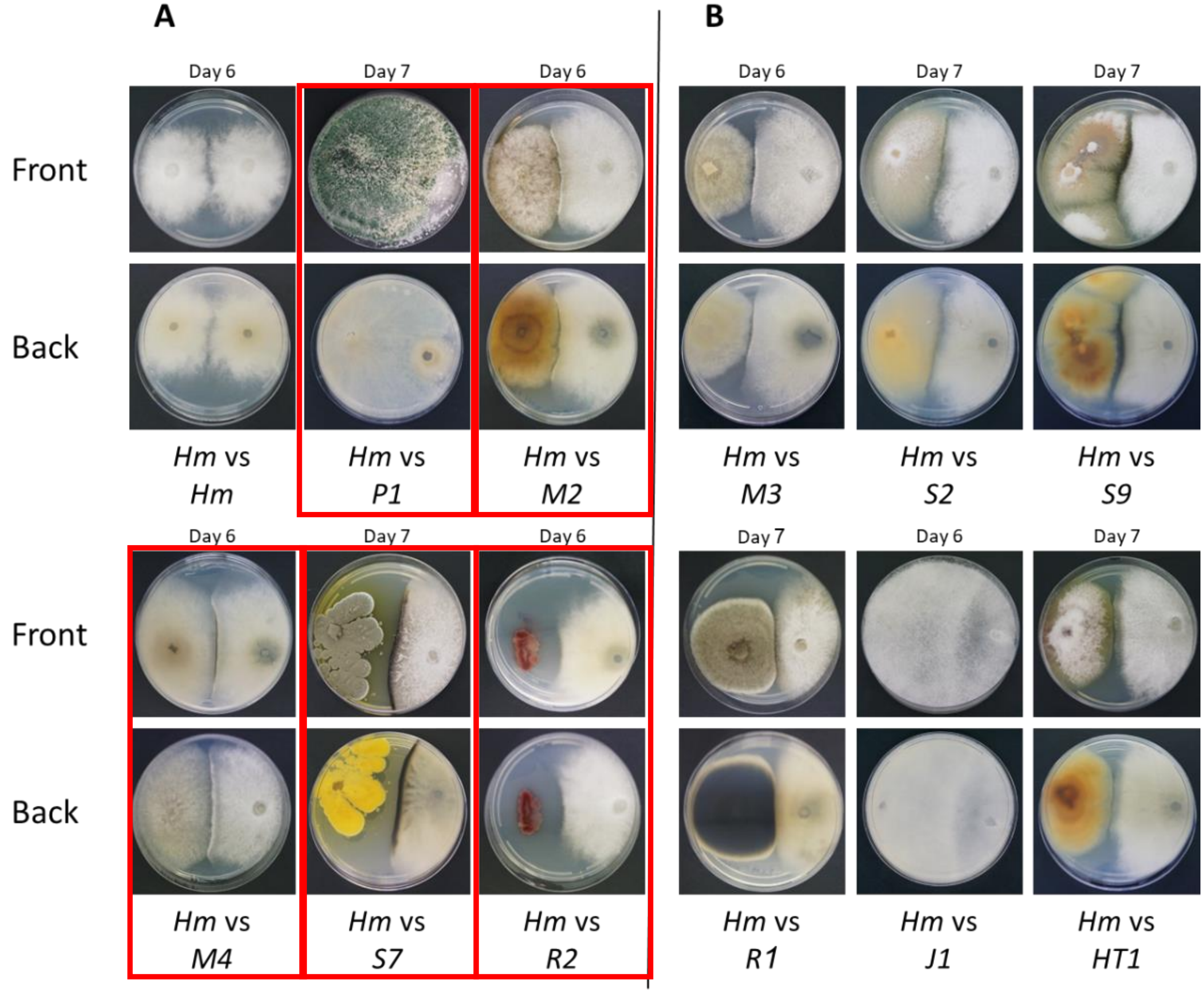
Rationale

These protective species can be used for Biological seeds enrichment

- **C**ost-effective.
- **C**ompatible with any cultivation method.
- **D**ecreased chemical use.
- **I**ntegrated with other pest control methods.
- **M**ay protect plants from other diseases.

Plated confrontation assay

- We isolated and identified several protective endophytes from seeds.



Degani et al., *Agronomy*, 2021

Selected *M. maydis* highly antagonists

P1 –
T. asperellum

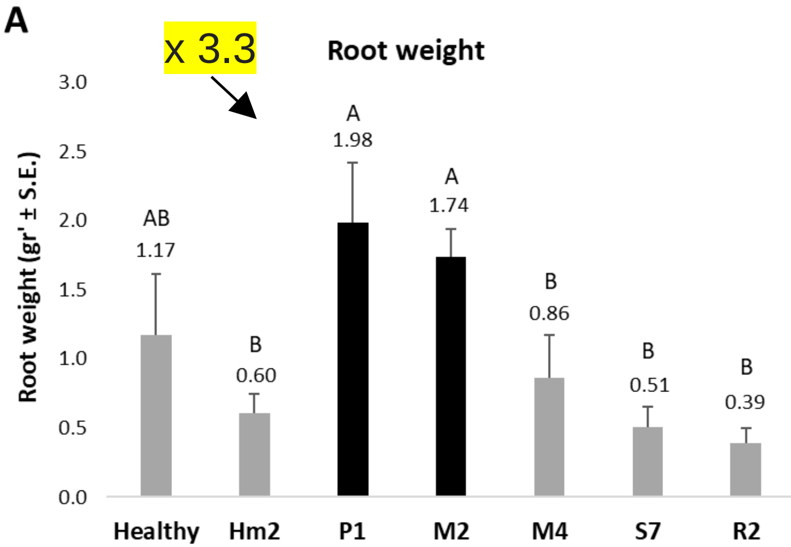
M2 –
Chaetomium subaffine

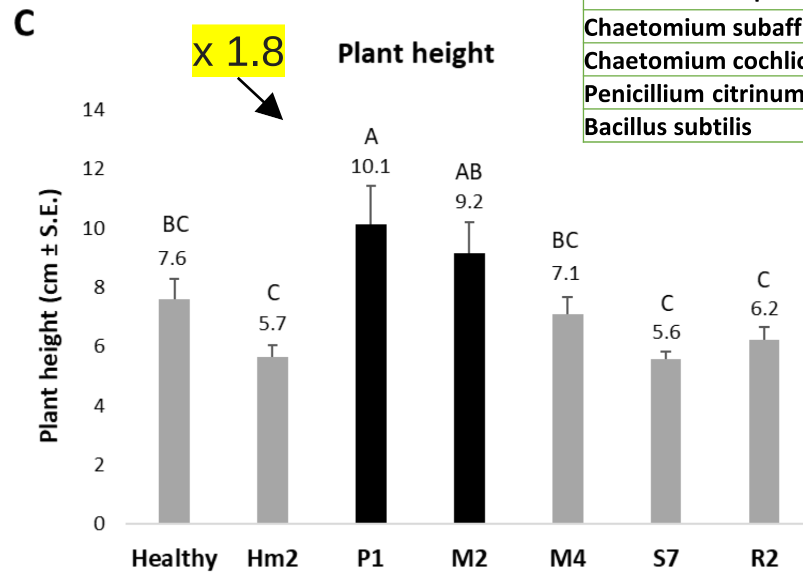
S7 –
Penicillium citrinum

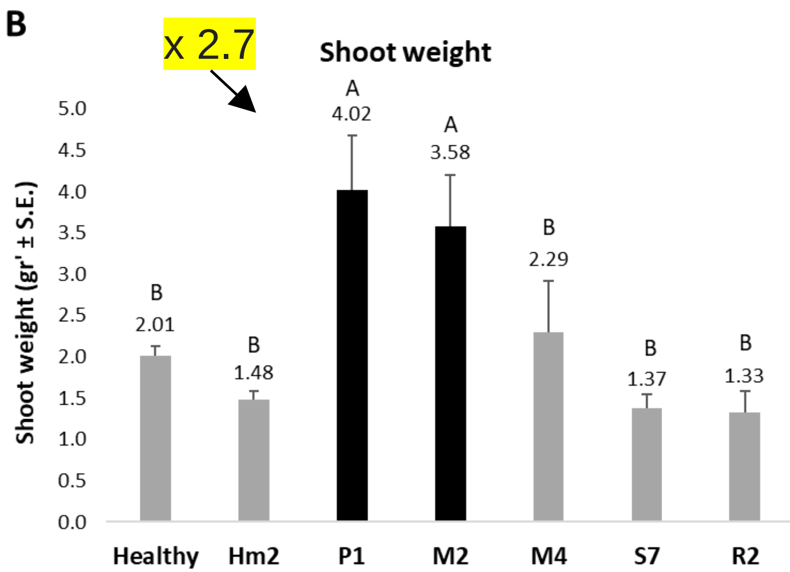
Biological enrichment of seeds (sprouts in a growth room)

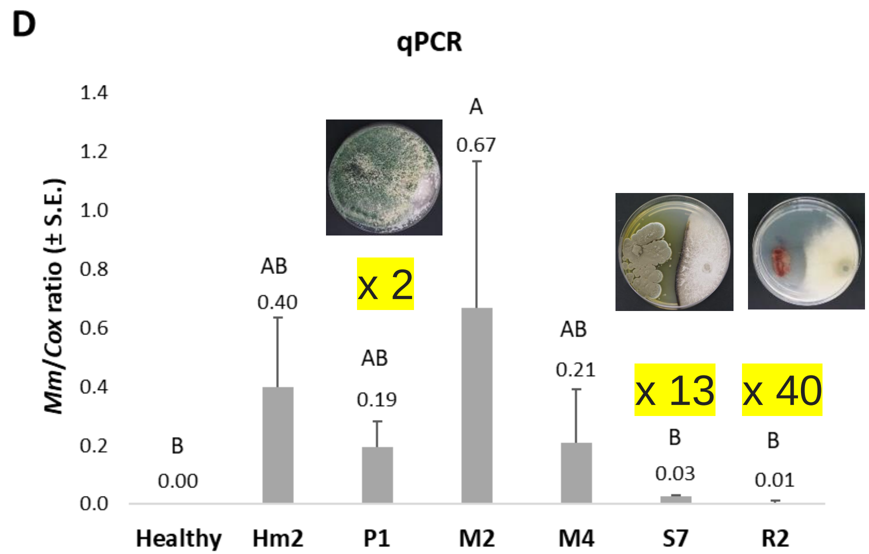
Day 42

Species	Des.
Trichoderma asperellum	P1
Chaetomium subaffine	M2
Chaetomium cochliodes	M4
Penicillium citrinum	S7
Bacillus subtilis	R2



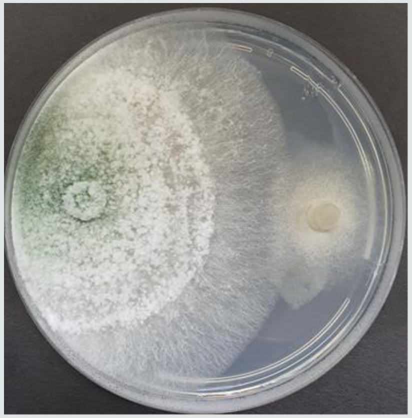






T. asperellum (P1) eco-friendly based LWD control

From the fungal medium, we isolated a powerful (ca. 400 mg/L) A.I. that fully inhibiting *M. maydis*.



Day 3

Day 6

Degani et al., *Agronomy*, 2021

Sprouts' pathogenicity evolution

Growth parameter	Control -		Control + [3]		P1	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Emergence (%) 8 DAS	68.6%	5.3%	64.3%	7.1%	54.3%	5.3%
Root wet weight (g)	1.17	0.44	0.60	0.14	1.98 [2]	0.44
Shoot wet weight (g)	2.01	0.11	1.48	0.10	4.02 [2]	0.66
Total dry weight	0.59	0.19	0.44	0.11	0.98 [2]	0.20
Shoot hight (cm)	7.60	0.68	5.65	0.40	10.13 [2]	1.32
qPCR (*Mm*/*Cox* ratio)	0.006	0.005	0.41	0.24	0.20	0.09

(day 42)

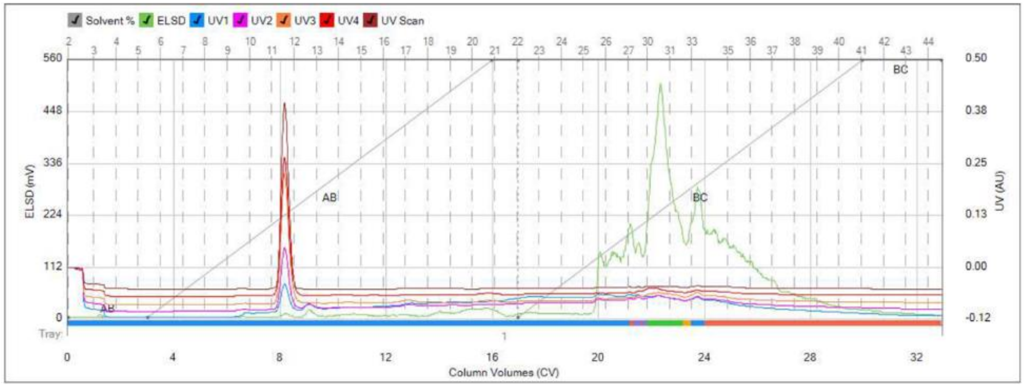
Applying *T. asperellum* to the seeds with the sowing 2-3-fold growth promotion while suppressing the pathogen

Degani and Gordani, *Agronomy*, 2022

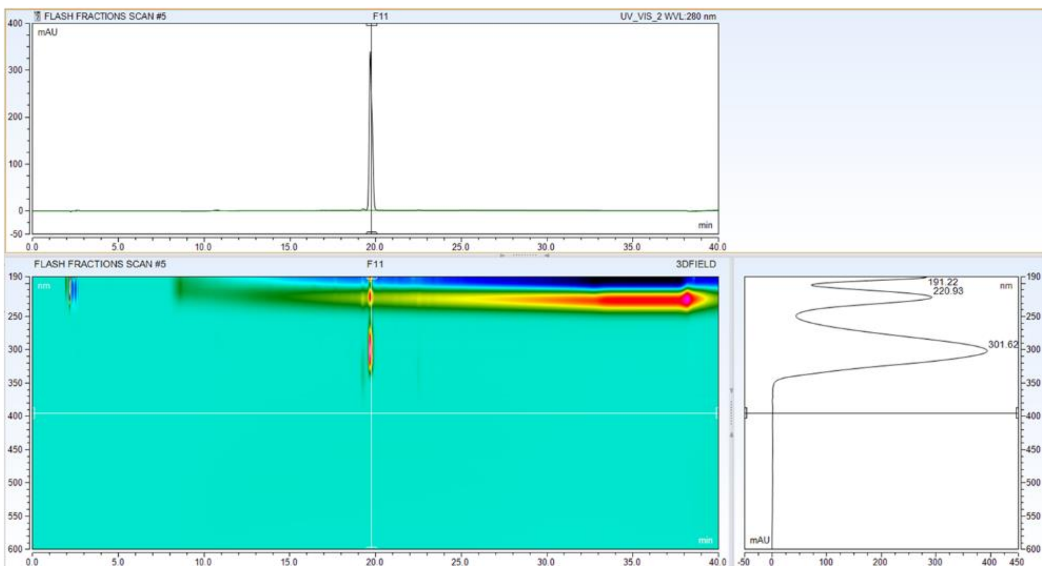
The Isolation of Antifungal components in the *T. asperellum* secretion was led by Asaf and Paz from my lab.

Purification of the P1 secreted A.I. :

A



B



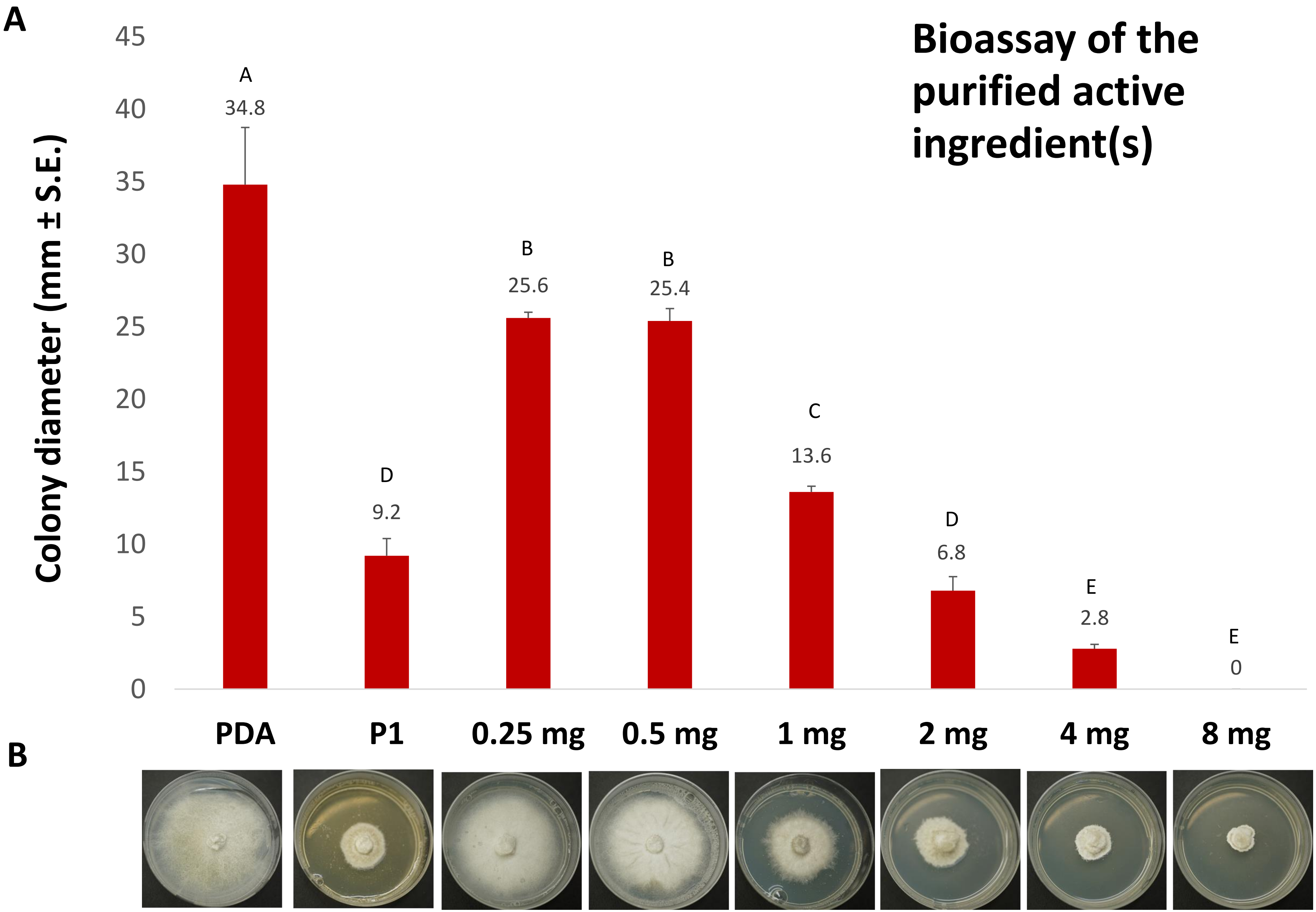
(A) <u>Chloroform extract purification</u> using a flash chromatography system & silica column, diode array, and ELSD detectors.

(B) <u>HPLC chromatogram and UV spectra.</u>

Prof. Soliman Khatib

Degani et al., *Biology*, 2021

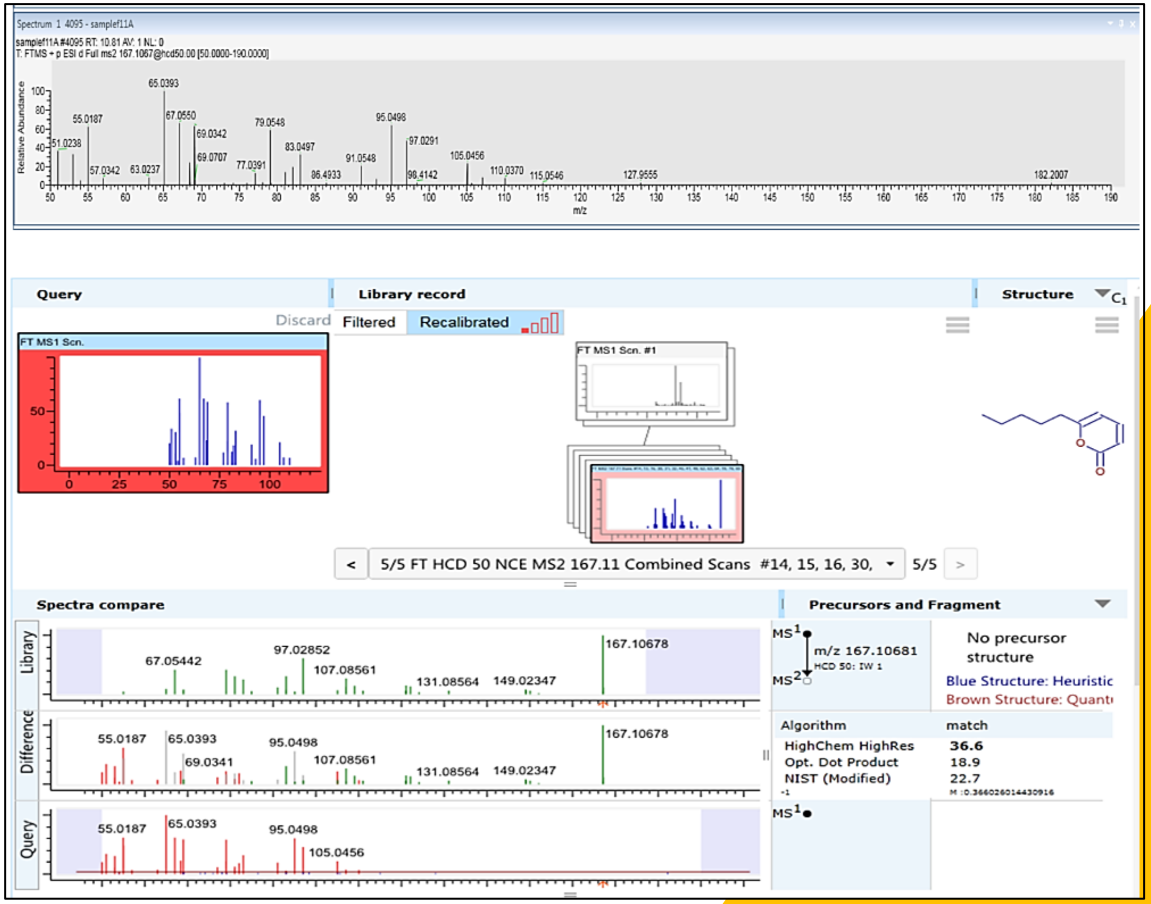
Bioassay of the purified active ingredient(s)



Degani et al., *Biology*, 2021

Identification of active ingredient(s) using LC-MS/MS analysis

An unsaturated lactone with a molecular mass of 166 Da.

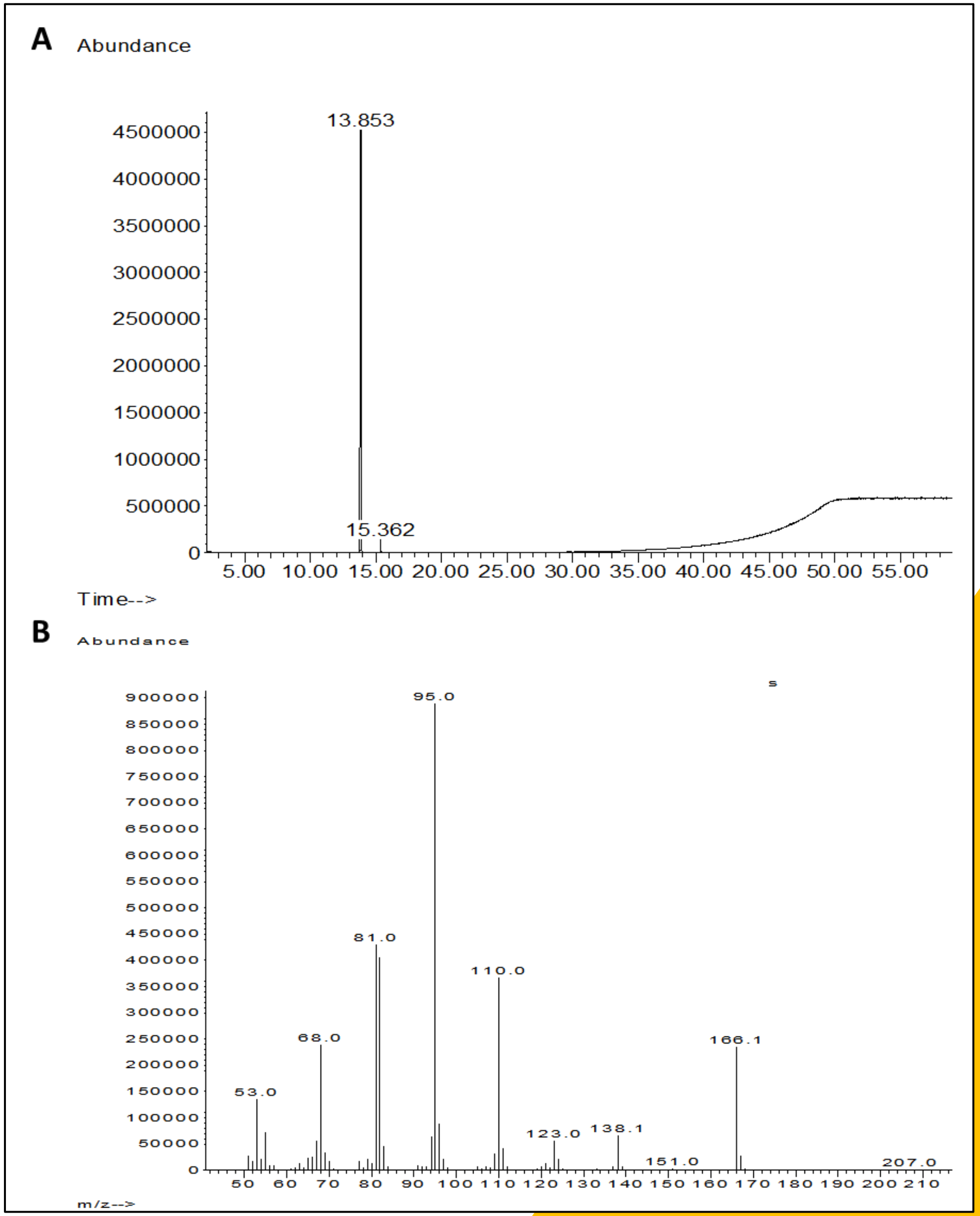


Degani et al., *Biology*, 2021

Identification of active ingredient(s) using GC–MS/MS analysis

(**A**) GC–MS chromatogram

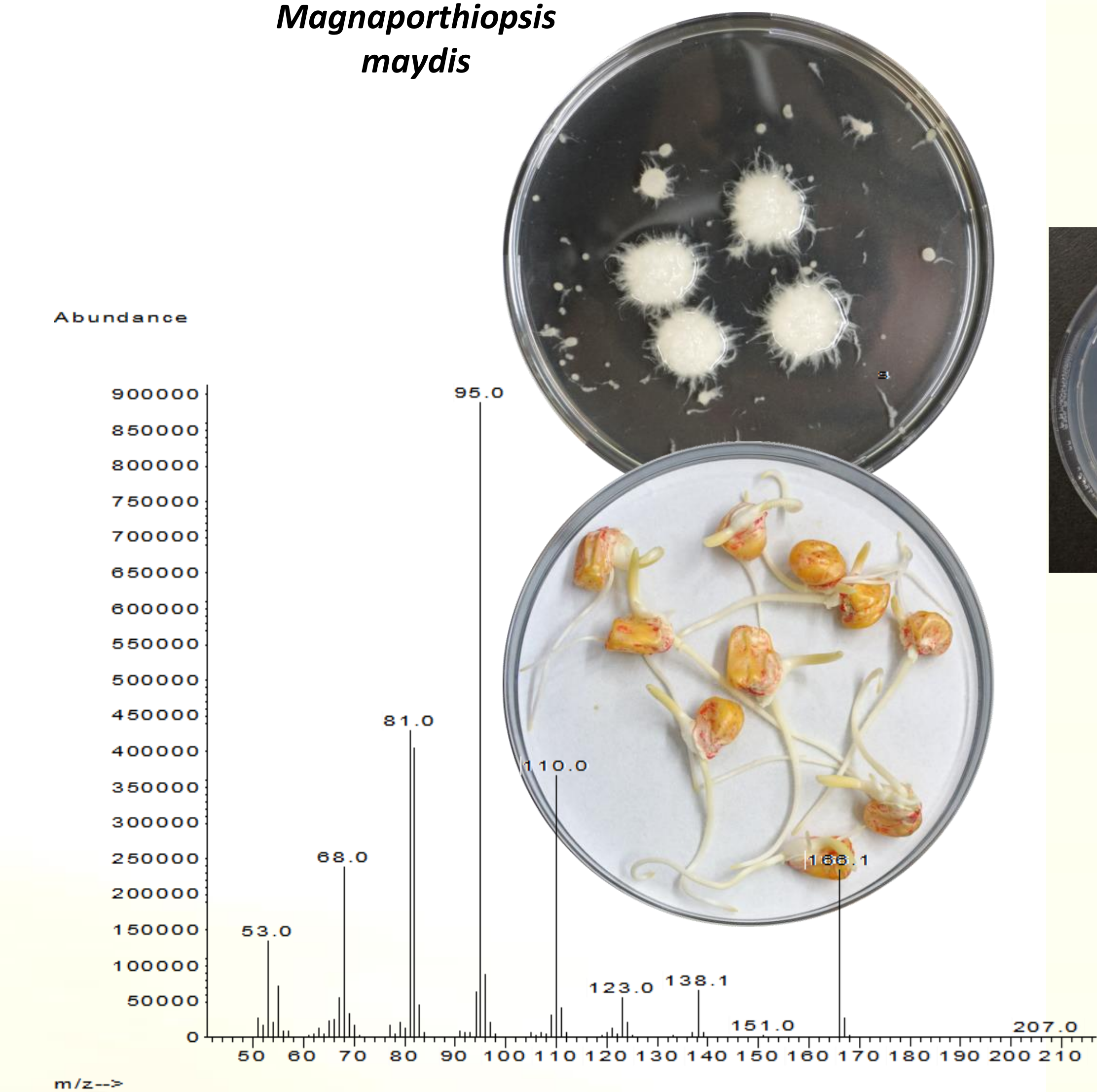
(**B**) MS spectra



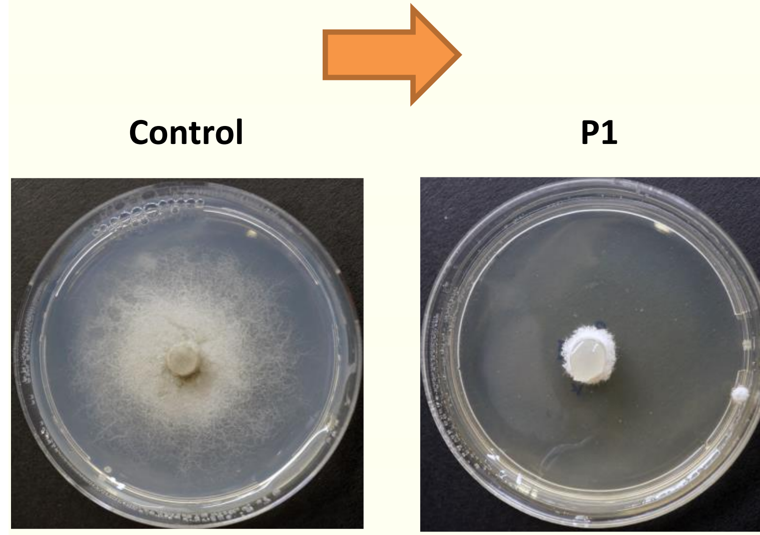
Degani et al., *Biology*, 2021

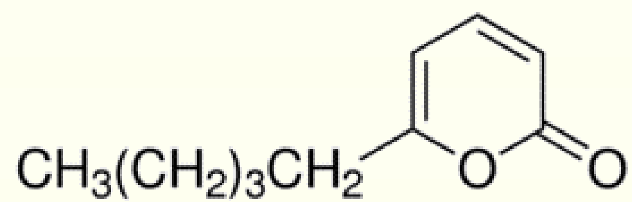
Final confirmation of the metabolite was done using a commercial 6-PP as a standard

Magnaporthiopsis maydis



Trichoderma asperellum





6-pentyl-α-pyrone

Degani et al., *Biology*, 2021

6-PP It is a key bioactive compound of several *Trichoderma* species

It has known antifungal activity against other phytopathogens and can inhibit:

- Mycelium growth
- Spore germination
- Pigmentation
- Mycotoxins production

Ahmed A. Ismaiel and Dalia M.I. Ali

Antimicrobial properties of 6-pentyl-α-pyrone produced by endophytic strains of *Trichoderma koningii* and its effect on aflatoxin B1 production

De Gruyter | 2017

DOI: https://doi.org/10.1515/biolog-2017-0173

Biologia

Volume 72 Issue 12

JOURNAL AND ISSUE

Joo-Hyun Hong, Jaejung Lee, Mihee Min, Seung-mok Ryu, Dongho Lee, Gyu-Hyeok Kim and Jae-Jin Kim

6-Pentyl-α-pyrone as an anti-sapstain compound produced by *Trichoderma gamsii* KUC1747 inhibits the germination of ophiostomatoid fungi

De Gruyter | 2014

DOI: https://doi.org/10.1515/hf-2013-0171

Holzforschung

Volume 68 Issue 7

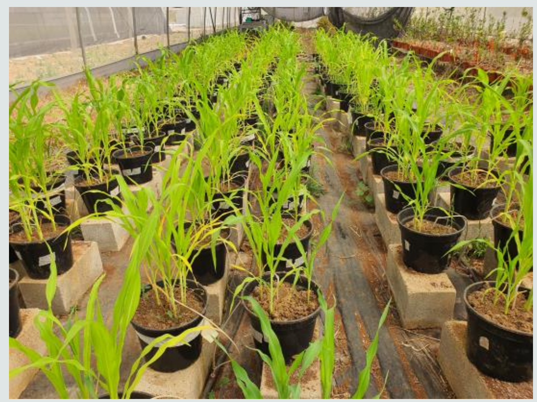
JOURNAL AND ISSUE

Since 6-PP is a food-grade metabolite, **it can be used in post-harvest**.

Performance in the greenhouse over a full period.

Day 0

Day 36

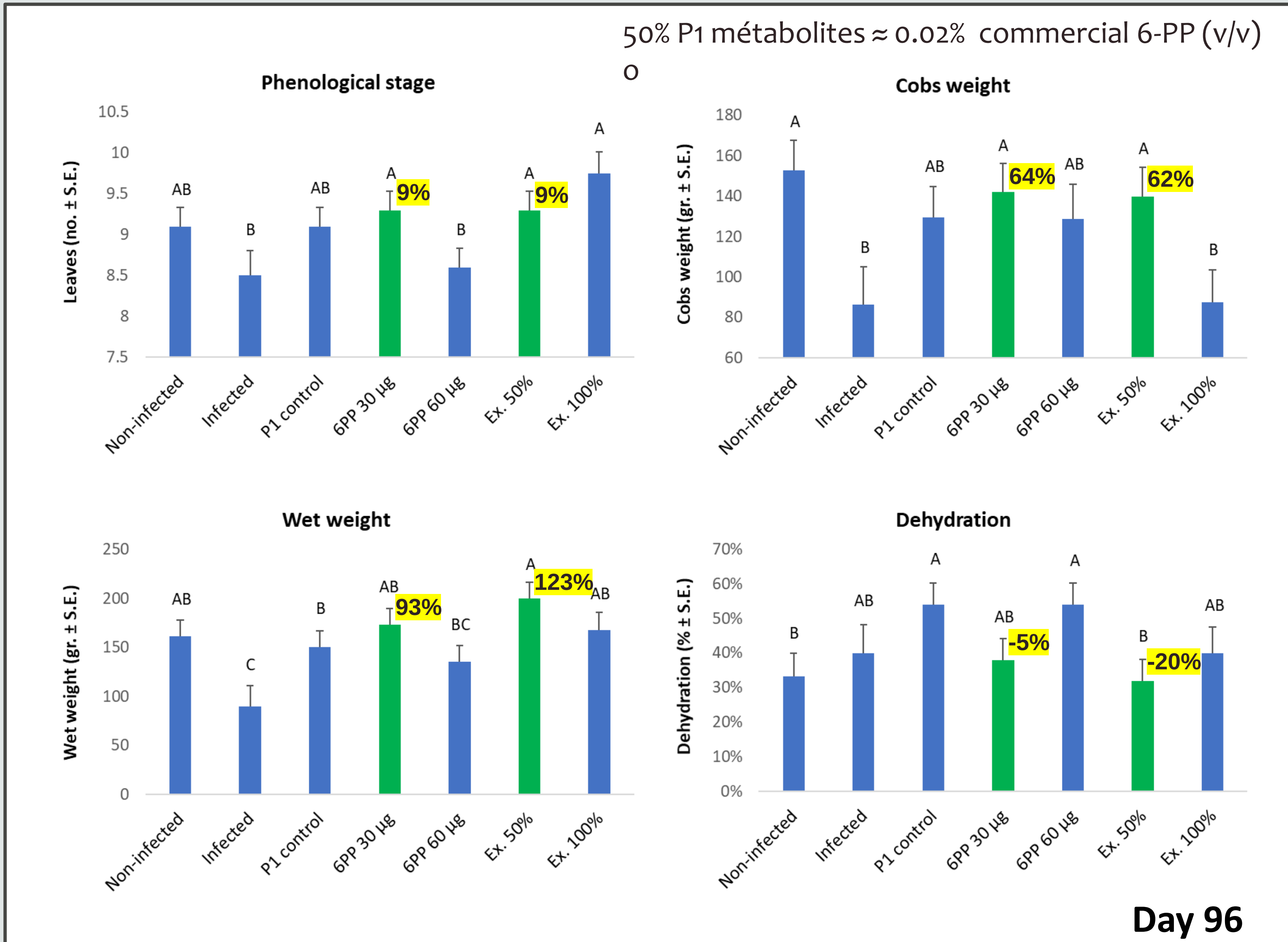


Day 62

Day 83

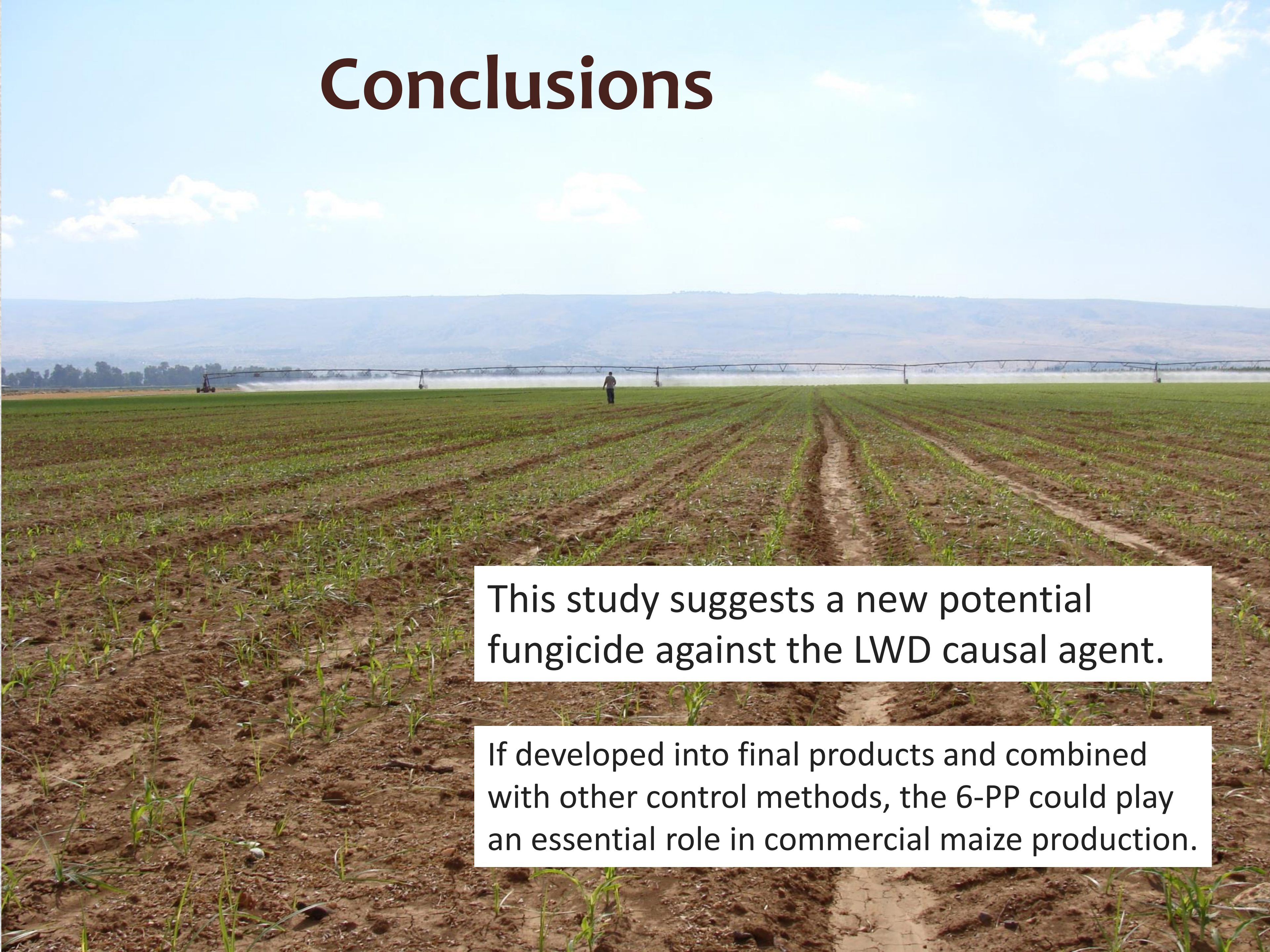
Degani et al., *Unpublished data*

At harvest, the 6-PP metabolites improved plant biomass by 90–120%, cob weight by 60%, and blocked the infection by up to 98%.



Degani and Gordani, *Agronomy*, 2022

Conclusions



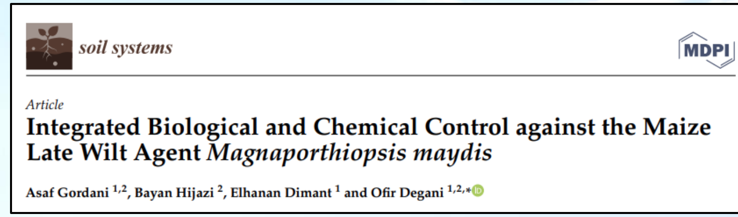
This study suggests a new potential fungicide against the LWD causal agent.

If developed into final products and combined with other control methods, the 6-PP could play an essential role in commercial maize production.

Future Challenges and Opportunities

- **Applied** the chemical-biological interface.
- **Enhance** Trichoderma's potency against *M. maydis* (e.g., via freshwater microalgae).
- **Identify and assess** novel eco-friendly microorganisms and their byproducts.
- **Explore synergistic effects** of multiple bio-protective microorganisms.

Future Challenge


soil systems

MDPI

Article

Integrated Biological and Chemical Control against the Maize Late Wilt Agent *Magnaporthiopsis maydis*

Asaf Gordani [1,2], Bayan Hijazi [2], Elhanan Dimant [1] and Ofir Degani [1,2,*]


אלחנן
דימנט


ביאן
חג'אזי


אסף
גורדני

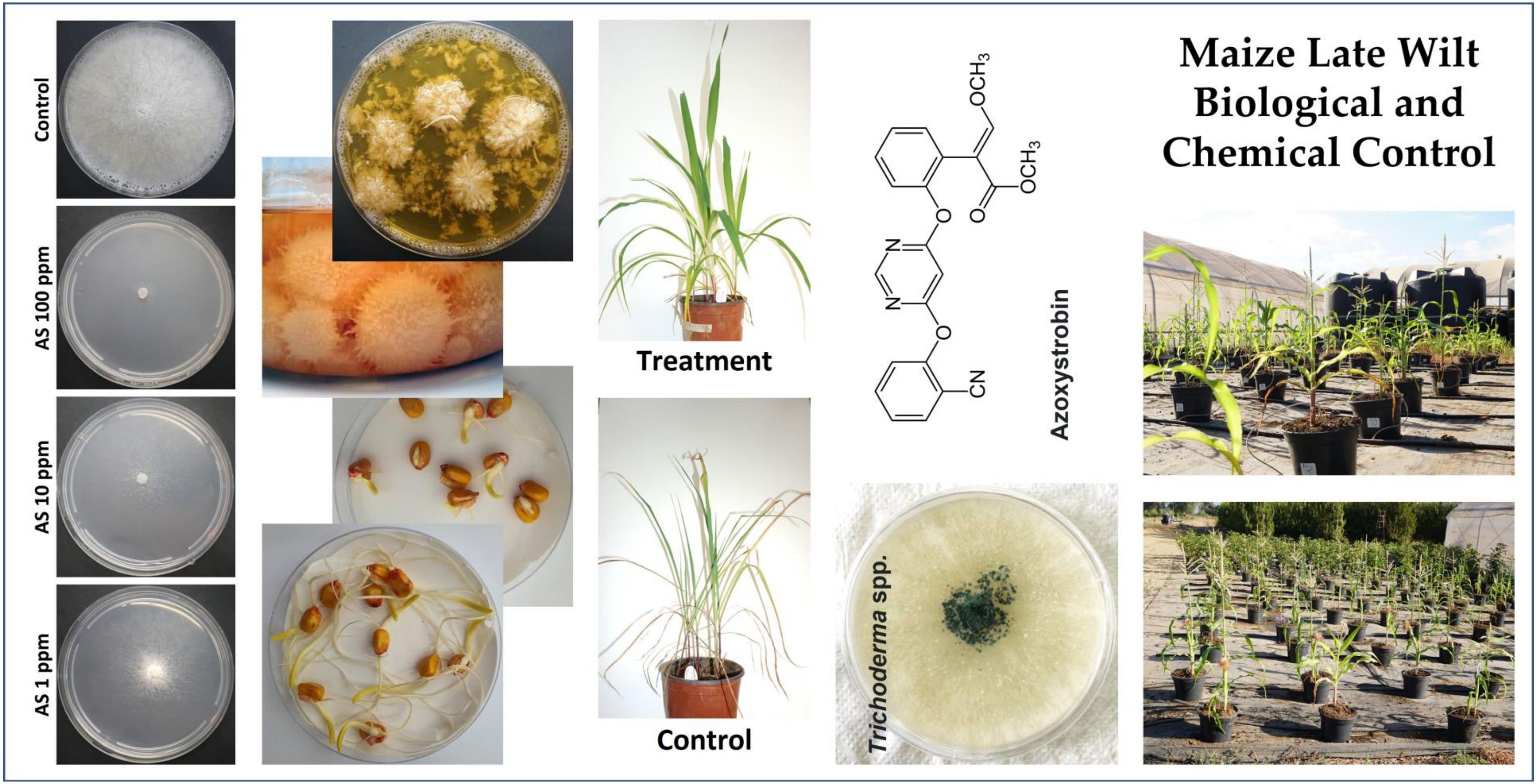
Biological-chemical integrated control

Minimal Chemical Pesticide

Seeds Toxicity

Sprouts Assay

Potted plants in the field



Gordani et al.. *Soil systems* 2023

- **Dekel Abraham** (MIGAL–Galilee Research Institute, Israel)
- **Dar soled** (Tel-Hai College, Israel)
- **Menashe Levi** (MIGAL North R&D, plantation farm, Israel)

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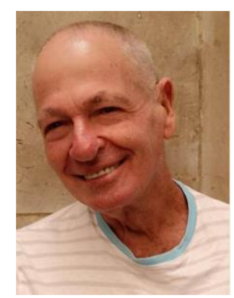

The phytopathology lab

Thank you all for the listening .. :-)