Influence of temperature on *in vitro* and *in vivo* growth of bacteria of genus *Pectobacterium* and *Dickeya*

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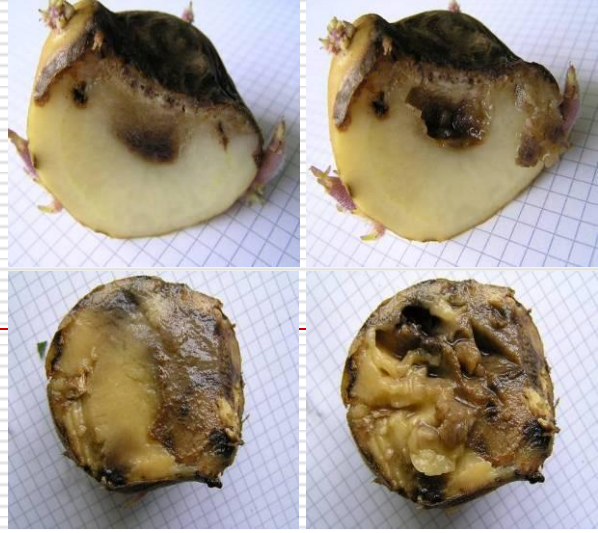
Overview

- Introduction
- optimal temperature of growth of '*Dickeya solani*'
- bacterial growth *in vitro* in different temperatures
- tuber soft rot in different temperatures
- summary

Soft rot of potato tubers - losses



A single rotting tuber contaminates c. 100 kg of potatoes during mechanical grading

(Elphinstone and Perombelon, 2007)

Yield reductions of 20–25% resulting from Dickeya infections - on various potato cultivars, where disease incidence was greater than 15%

(Tsror et al., 2009)

Strict tolerances in the Netherlands have led to increased direct losses of up to €30M annually

(Prins and Breukers, 2008 - after Toth et al. 2002)

Bacteria of great concern in potato production

Pectobacterium atrosepticum

P. carotovorum subsp. *carotovorum*

Dickeya dianthicola

'D. solani'

Dickeya solani Fera, UK Crown Copyright

Potato resistance to *Dickeya* spp.

All major potato cultivars in England were susceptible to ***D. dianthicola***, with some variation in severity of blackleg symptoms
(Toth et al., 2011)

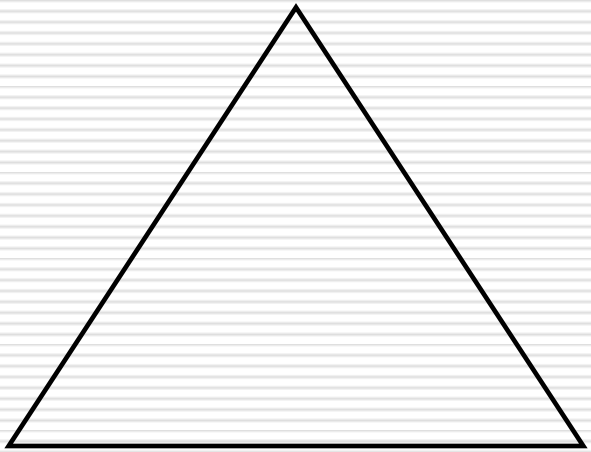
Certain cultivars have also been identified as highly susceptible to ***Dickeya*** spp. under Israeli conditions
(Tsror et al., 2009)

Point inoculation method

- Mean diameter of rotten tissue [mm],
- or weight [g]

Factors inflencing soft rot development

Temperature



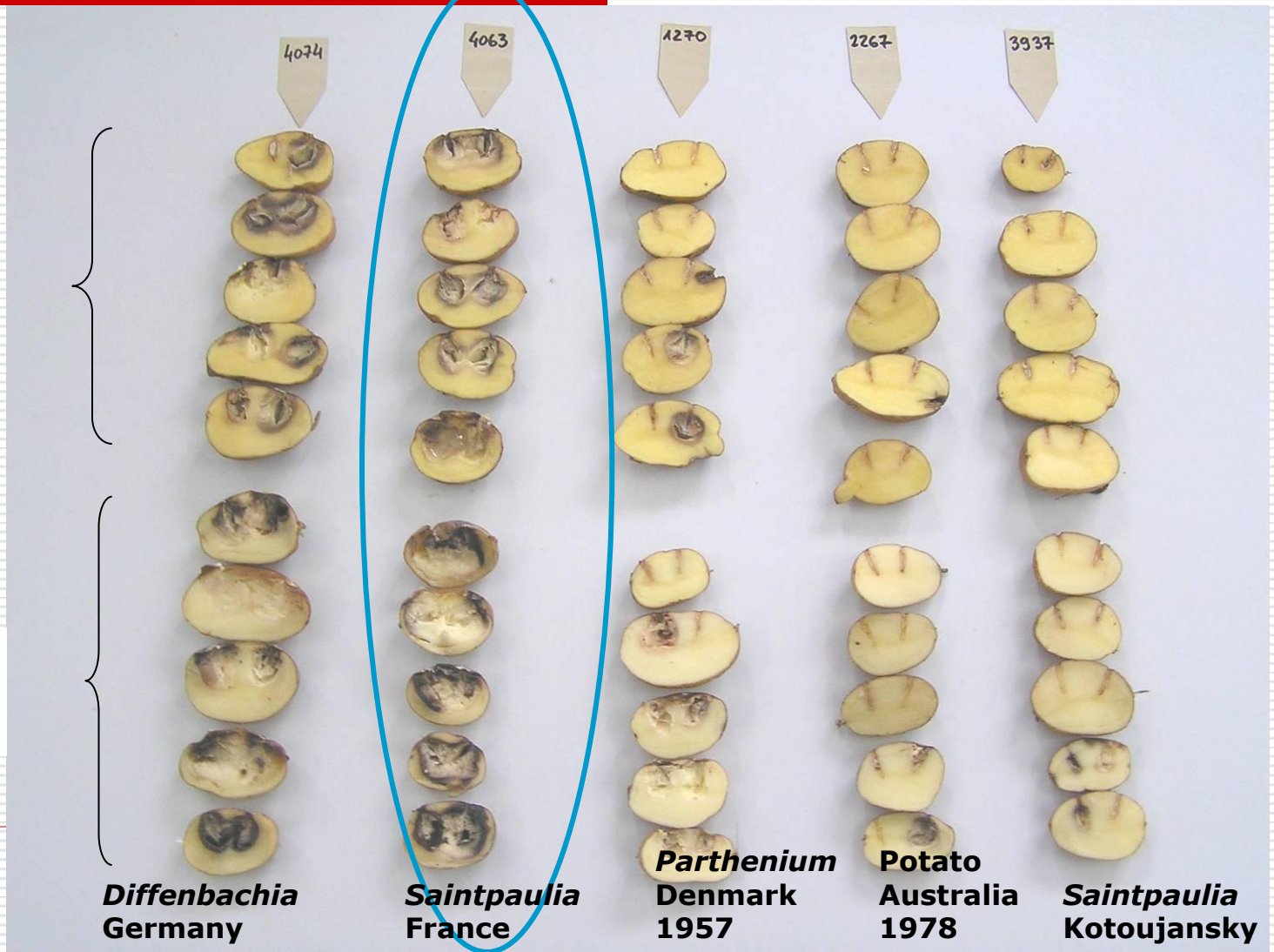
Virulence/
# of bacteria

Hypoxia /
free water

Virulence of bacteria *Dickeya* spp.

Cultivar Tajfun

Cultivar Irga



Hypoxia

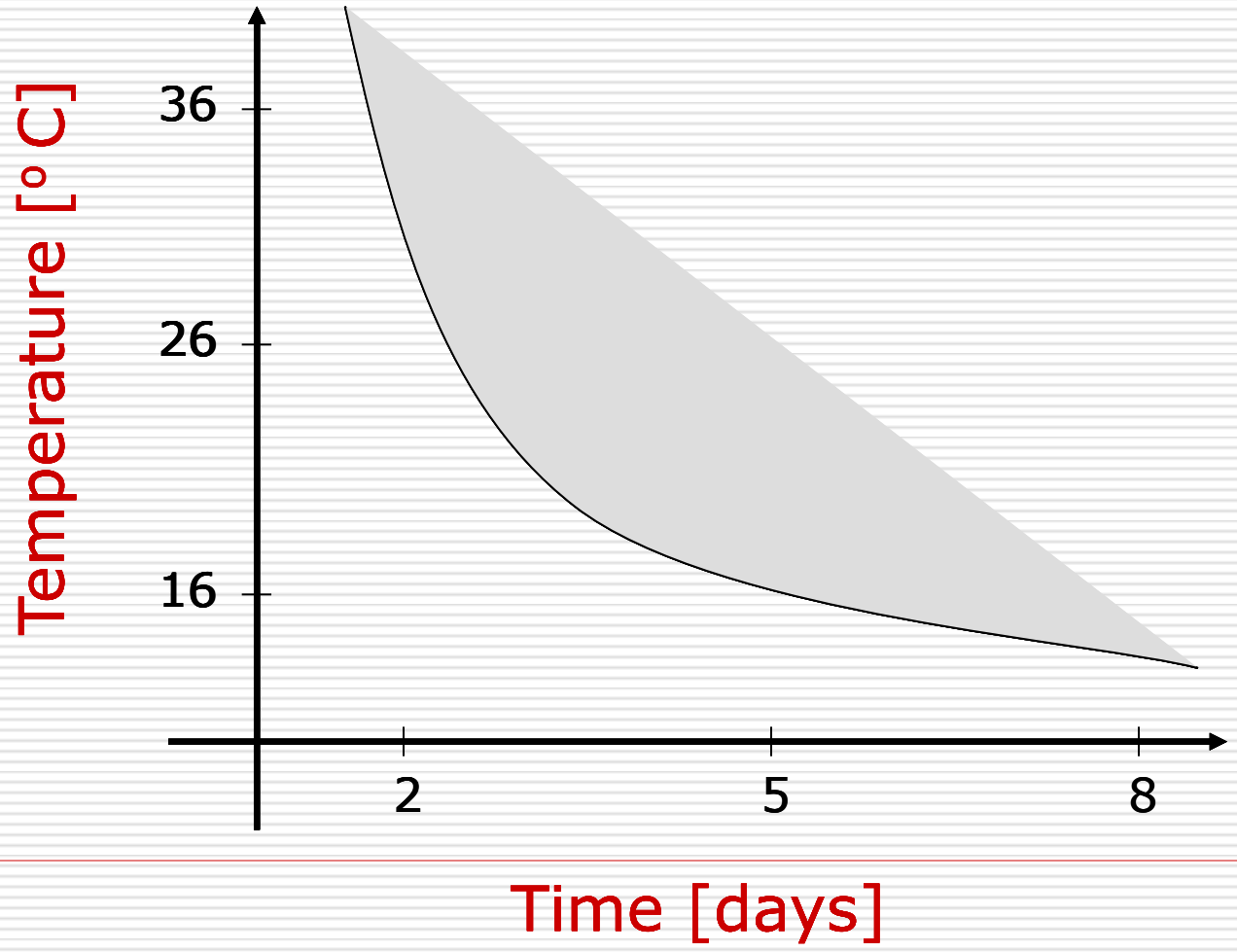
Oxygen depleted due to respiration of tubers – not renewed by diffusion from the air because of the water film. Hypoxia impairs oxygen-dependent host resistance system: phytoalexins, phenolics, free radicals, etc., inhibits cell wall lignification and suberization

(Perombelon, 2002)

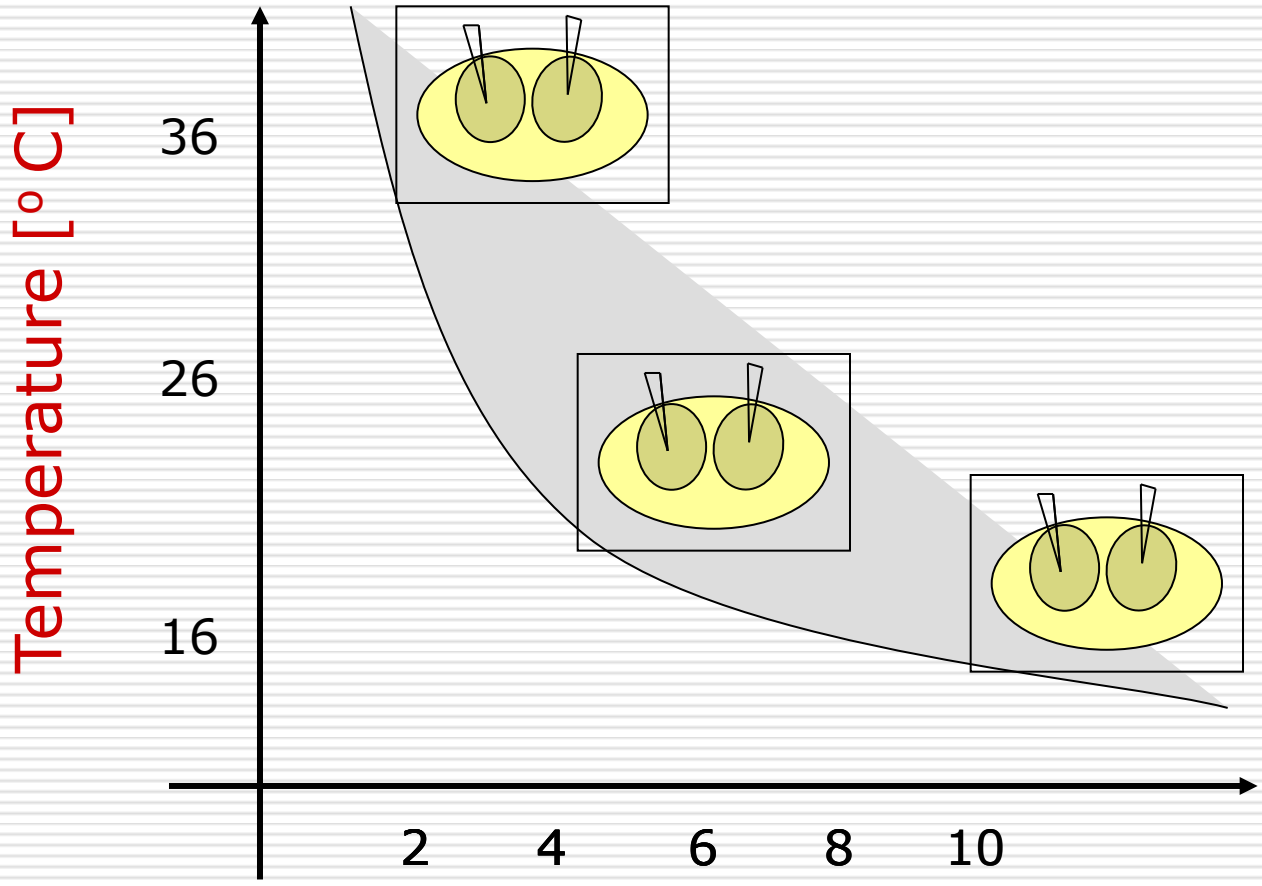
Tubers inoculated after wounding showed **significantly heavier symptoms of infection** in 3 % and 10 % of CO_2 than tubers incubated in 0% of CO_2

(Harper & Cunnington, 2011)

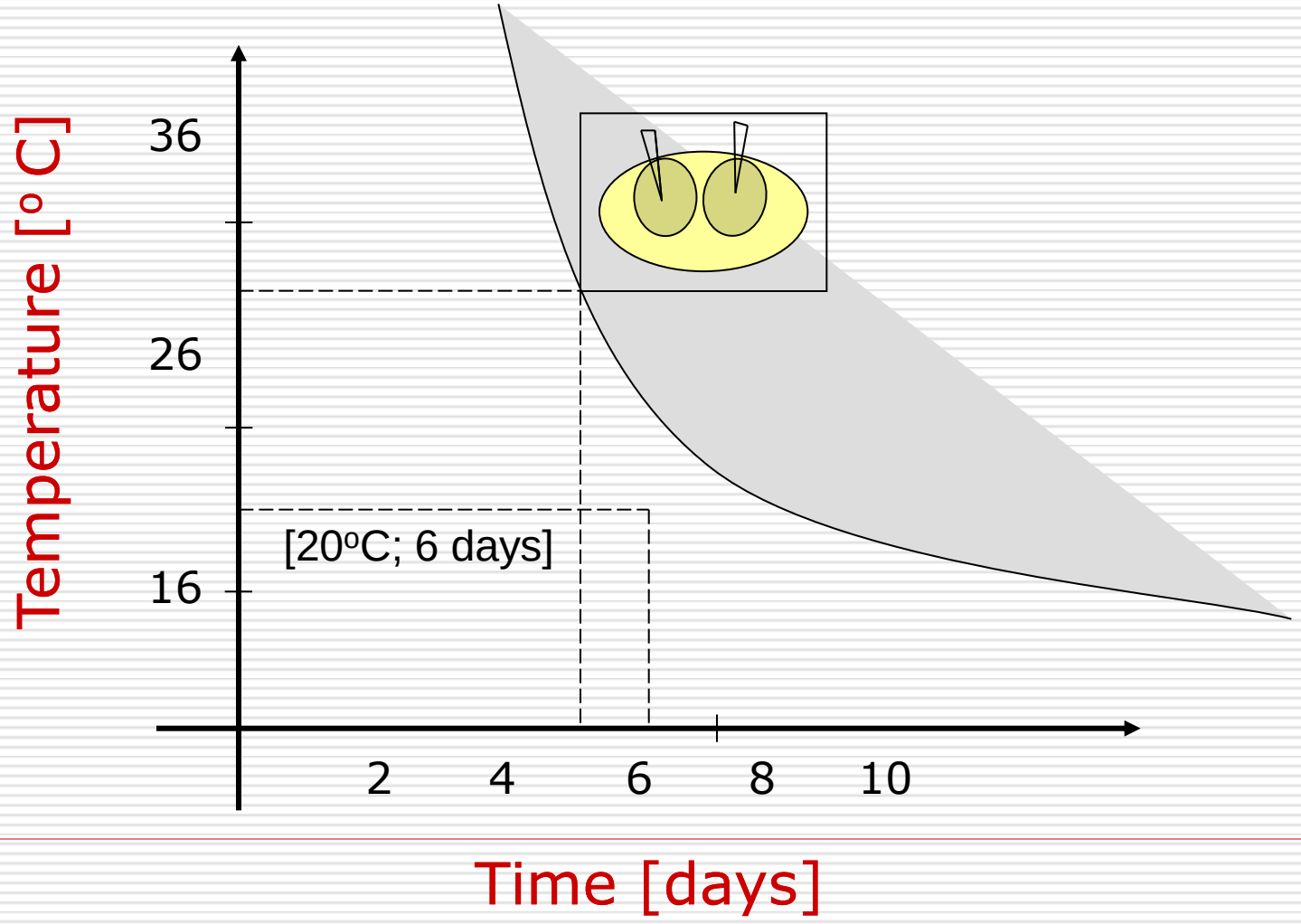
Rotting of tuber tissue as a function of temperature and time of incubation



Rotting of tuber tissue as a function of temperature and time of incubation



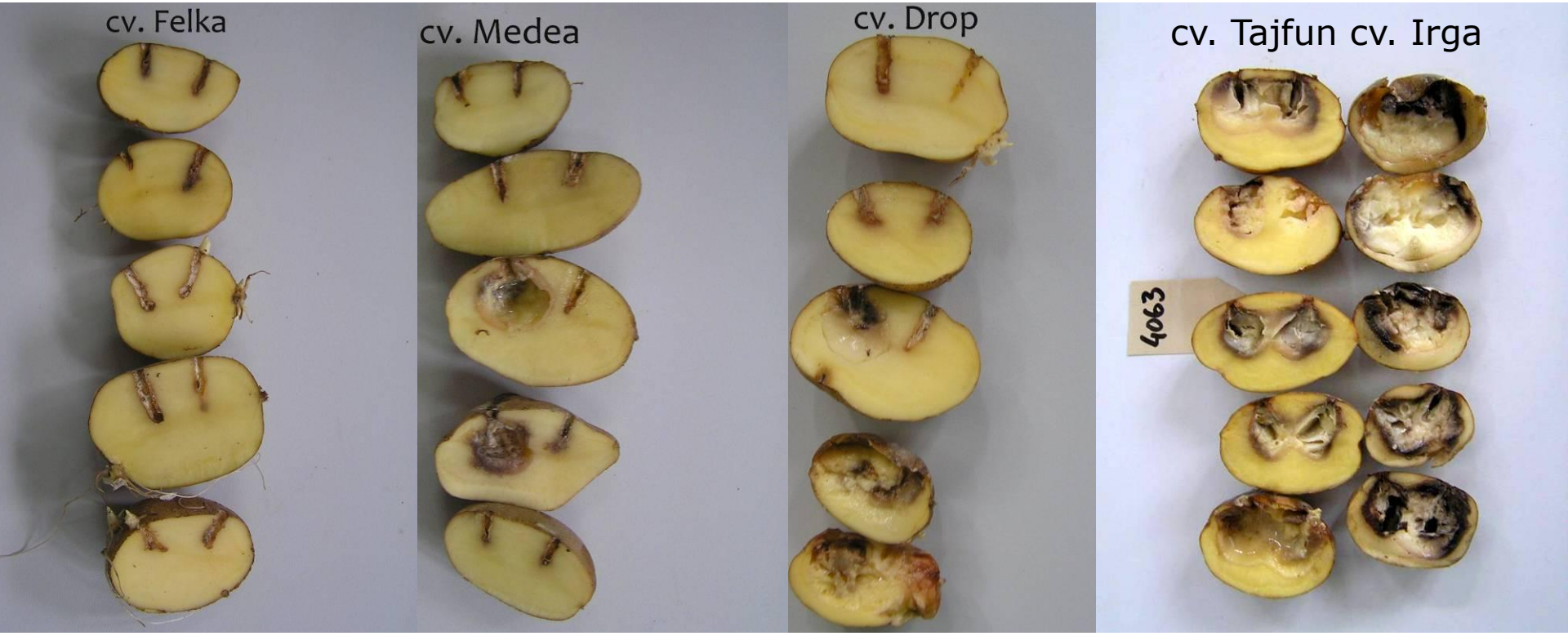
Infection of tuber tissue depend on temperature



Dickeya spp. SCRI 4063

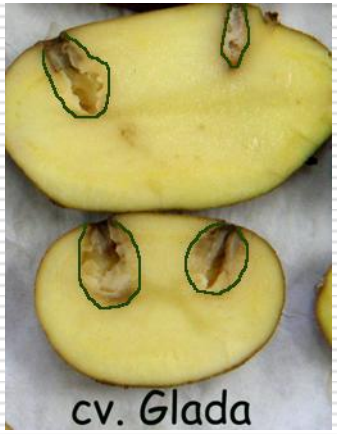
20°C, 6 days

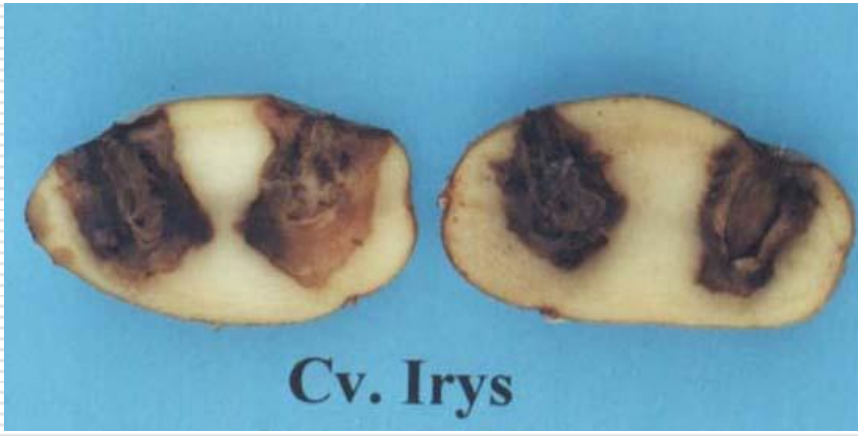
30°C, 5 days



Potato resistance to soft rot is relative

Resistant Medium-resistant Susceptible





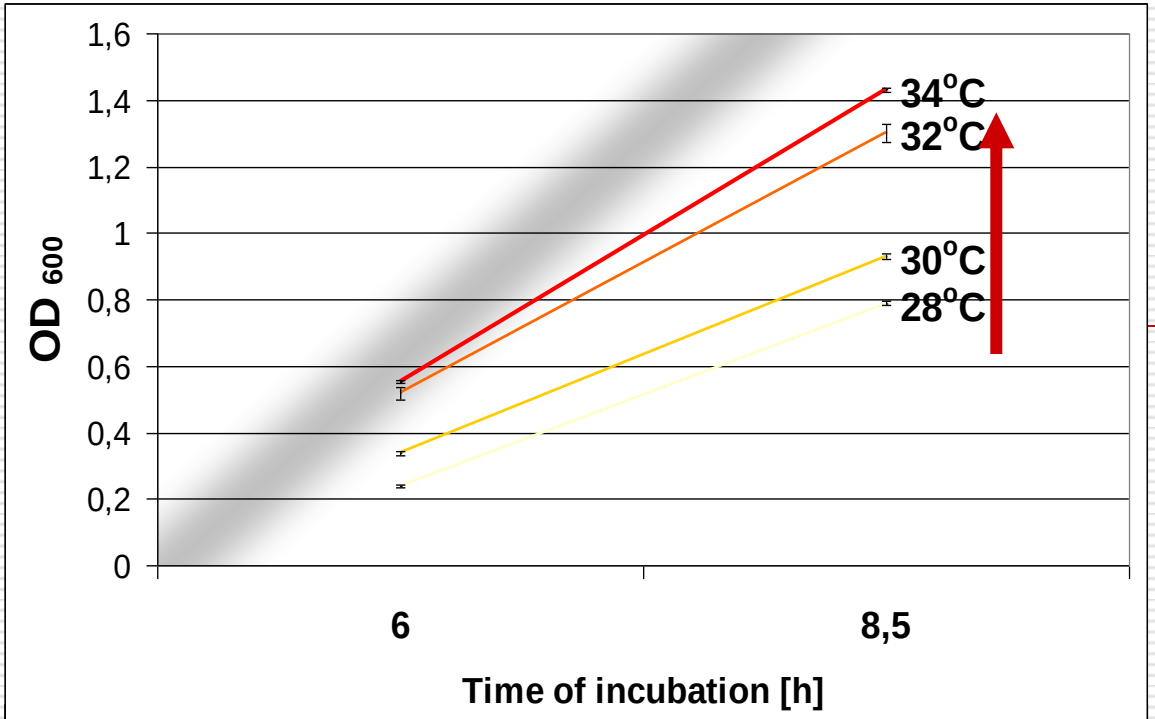
Bacteria cultures

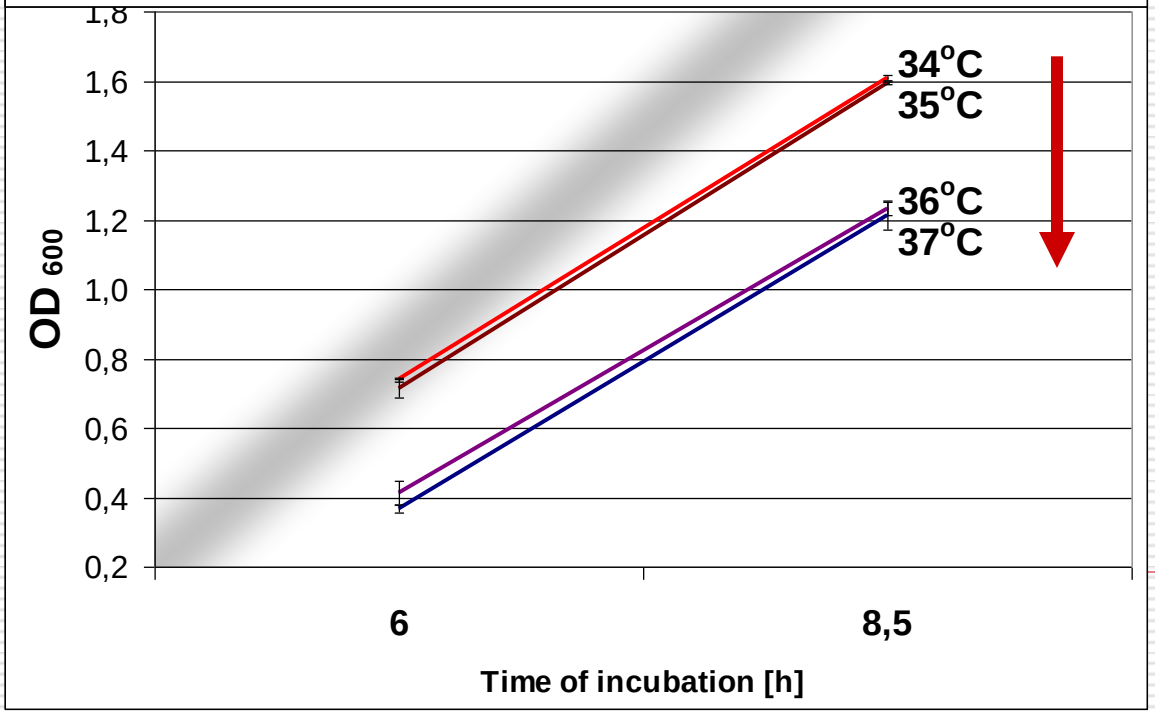
- *P. atrosepticum* 2/95M [**Pa**]
- *P. c.* subsp. *carotovorum* (IFB 5A/5/2012)*, [**Pcc**]
- *D. solani* (IFB 0099)* [**Ds**]
- *D. dianthicola* (IFB0157)* [**Dd**]

*kindly provided by **prof. E. Łojkowska**, Intercollegiate Faculty of Biotechnology, UG-MUG, Gdańsk, Poland

Methods – the optimal temperature of growth of *Dickeya solani*

- Lysogeny Broth (LB),130 rpm min-1 in 250 ml Erlenmayer flasks
- The bacteria were kept in the temperatures: 28, 30, 32, 34, 35, 36, and 37ºC in **three replications**
- For each combination **two measurements** of $OD_{600,}$ after 6 and 8.5 hours of incubation, were performed

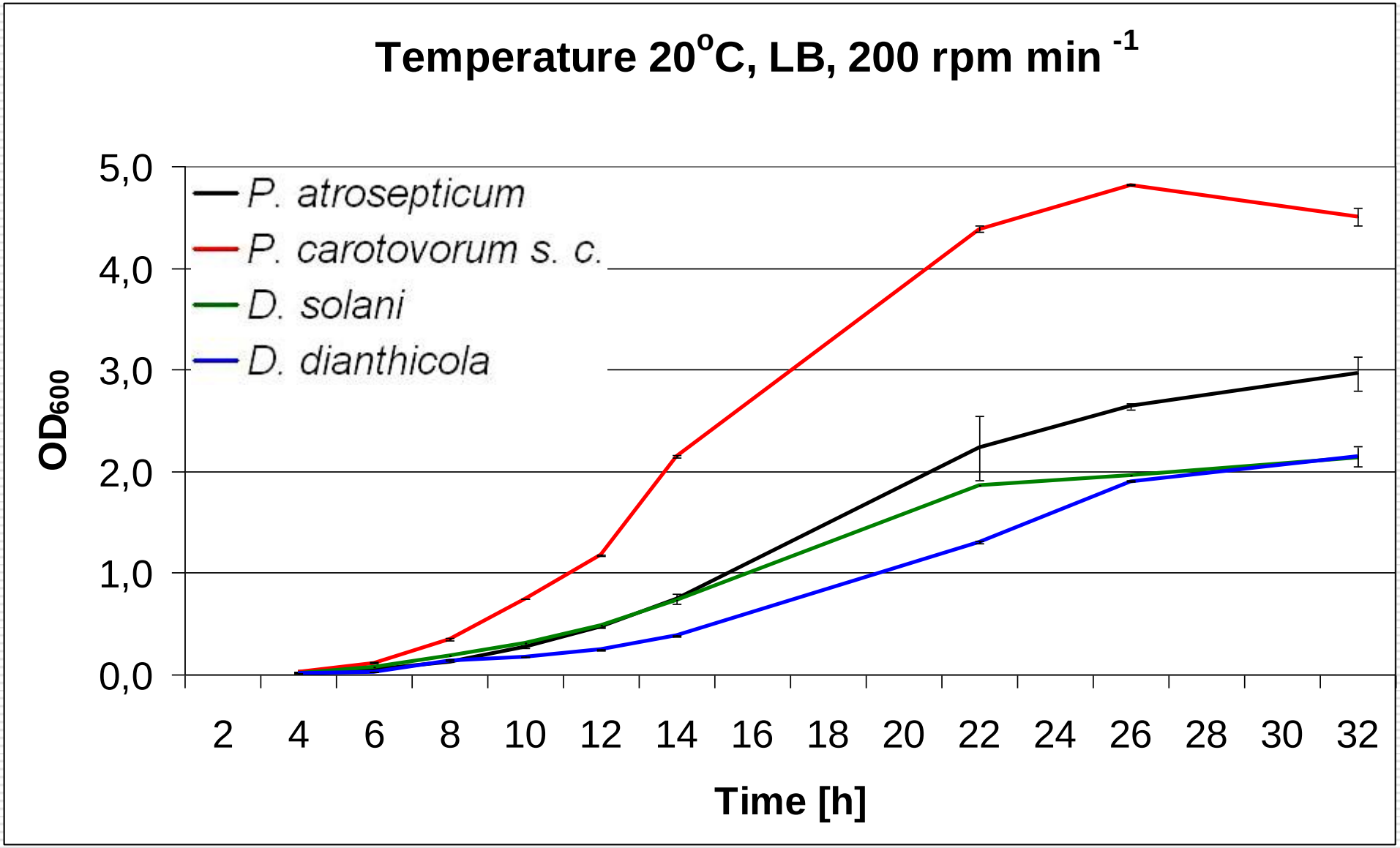


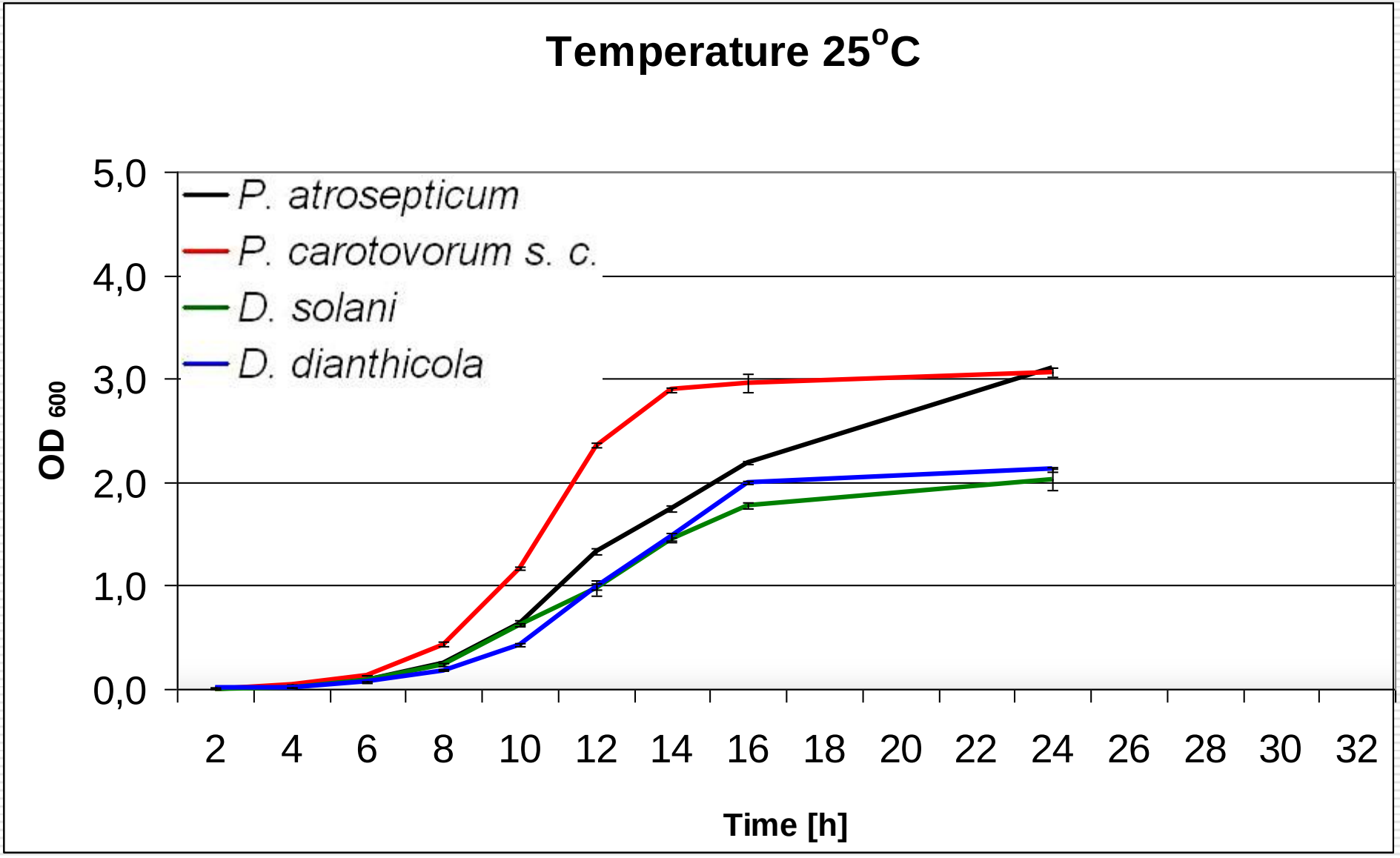


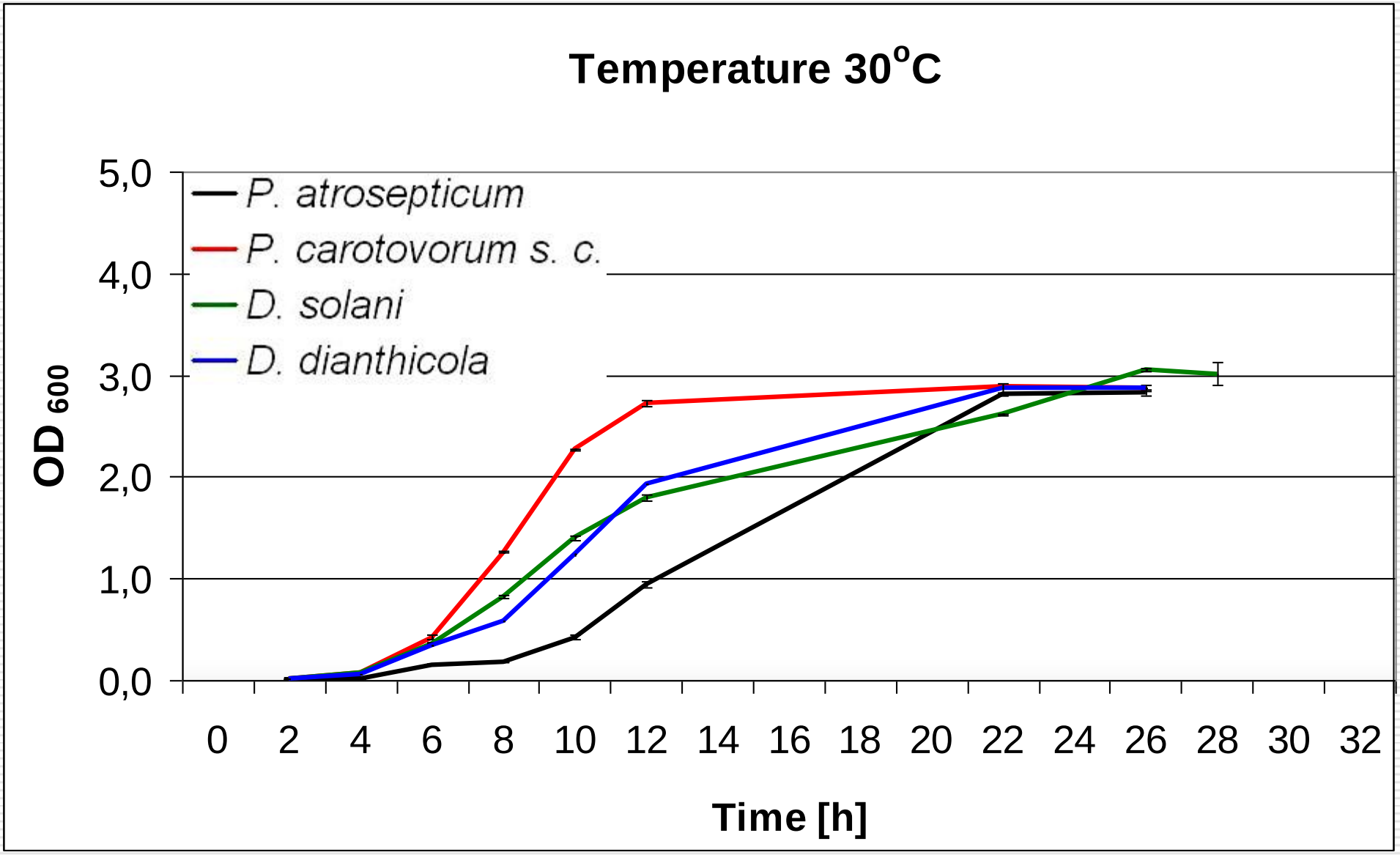
Optimal temperatures of growth of *D. solani* 99 is **34ºC, 35ºC**

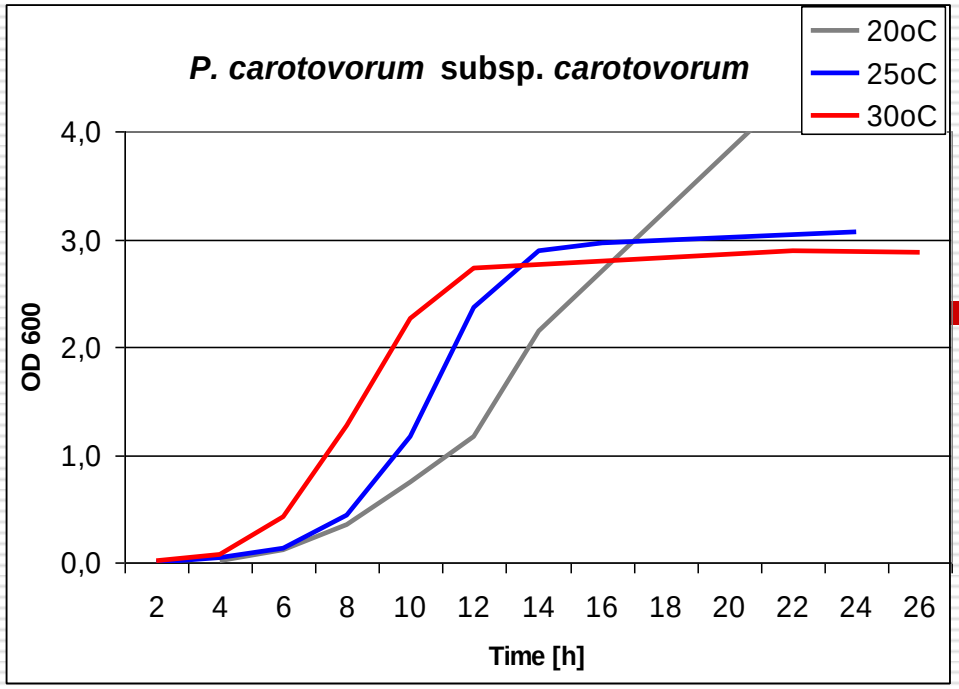
Materials and methods - the growth of bacteria (**Pa, Pcc, Ds, Dd**) in different temperatures

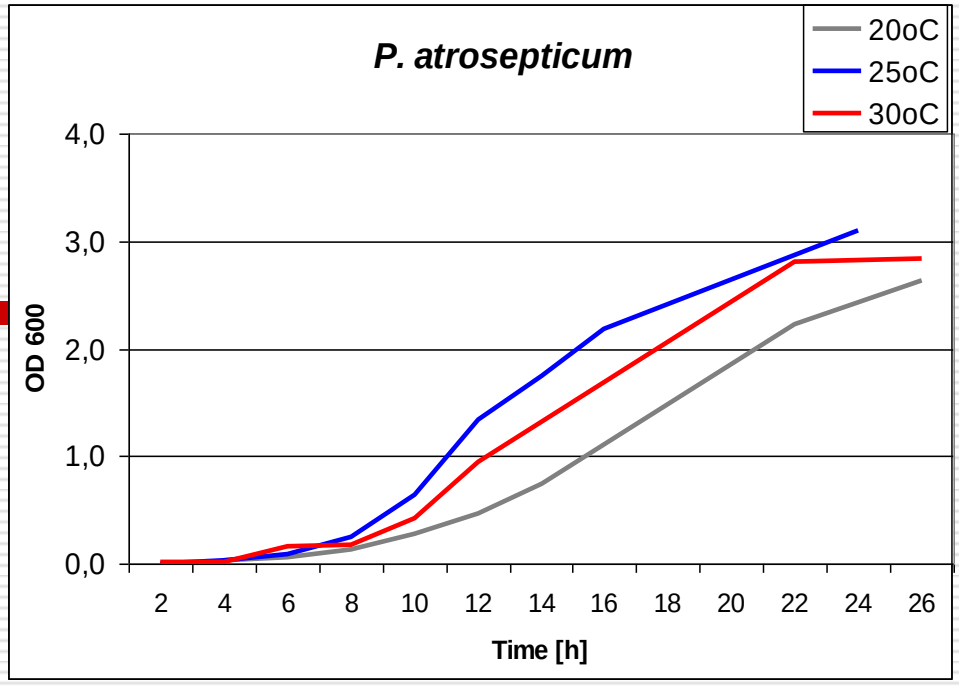
- 1 ml of each of bacterial 24 h cultures [OD_{600} = 0.100 - 0.108] was added to 50 ml of LB
- LB, 130 rpm min-1, in 250 ml Erlenmayer flasks
- temperatures 20 ^{o}C, 25 ^{o}C, and 30 ^{o}C
- For each combination 8 times measurements of OD_{600} were performed within 24 – 32 hours hours of incubation (3 rep.)

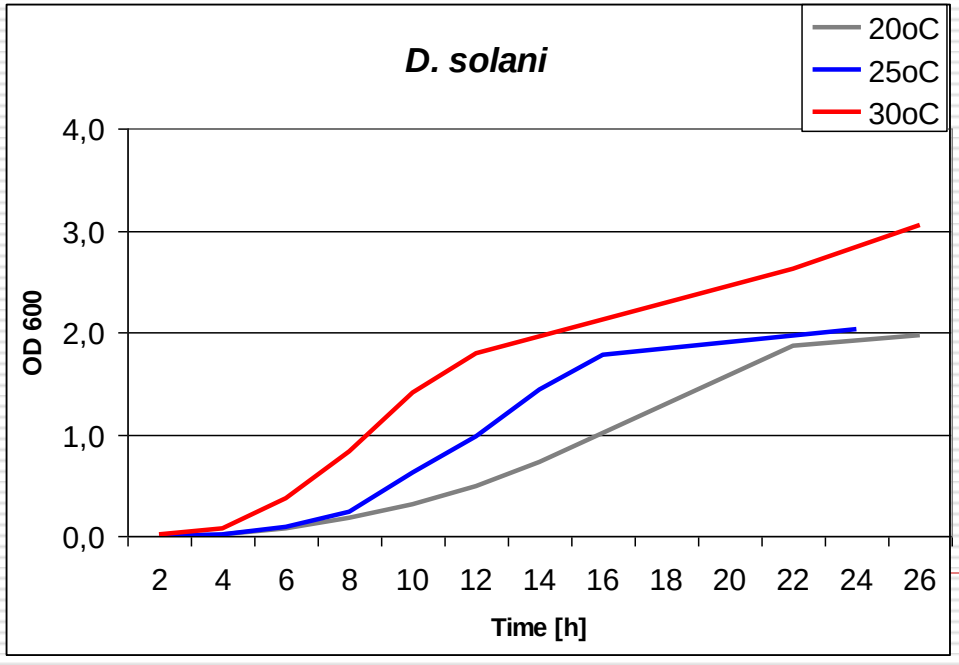

Temperature 20°C, LB, 200 rpm min -1
5,0
4,0
3,0
2,0
1,0
0,0
OD600
P. atrosepticum
P. carotovorum s. c.
D. solani
D. dianthicola
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Time [h]

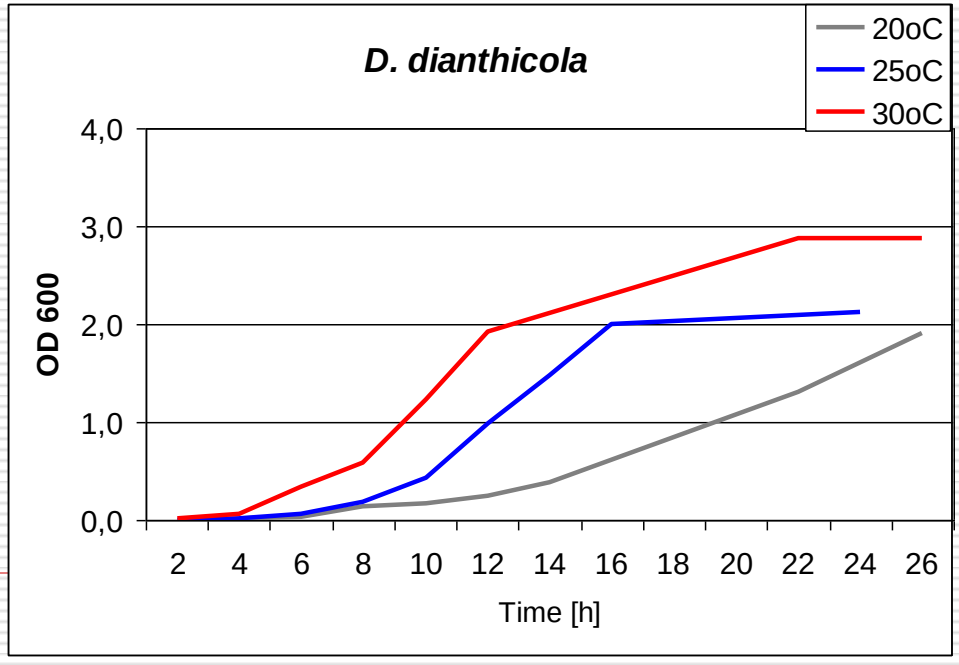

Temperature 25°C
5,0
4,0
3,0
2,0
1,0
0,0
OD 600
P. atrosepticum
P. carotovorum s. c.
D. solani
D. dianthicola
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Time [h]


Temperature 30°C
5,0
4,0
3,0
2,0
1,0
0,0
OD 600
P. atrosepticum
P. carotovorum s. c.
D. solani
D. dianthicola
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Time [h]


P. carotovorum subsp. carotovorum
20oC
25oC
30oC
OD 600
4,0
3,0
2,0
1,0
0,0
2 4 6 8 10 12 14 16 18 20 22 24 26
Time [h]


P. atrosepticum
20oC
25oC
30oC
OD 600
4,0
3,0
2,0
1,0
0,0
2 4 6 8 10 12 14 16 18 20 22 24 26
Time [h]


D. solani
20oC
25oC
30oC
OD 600
4,0
3,0
2,0
1,0
0,0
2 4 6 8 10 12 14 16 18 20 22 24 26
Time [h]


D. dianthicola
20oC
25oC
30oC
OD 600
4,0
3,0
2,0
1,0
0,0
2 4 6 8 10 12 14 16 18 20 22 24 26
Time [h]

Materials and methods – testing tuber soft rot resistance in temperatures 26ºC, 30ºC, 34ºC

Cultivars – susc. Irys, medium res. Sleza

Bacteria – **Pc**, **Ds**

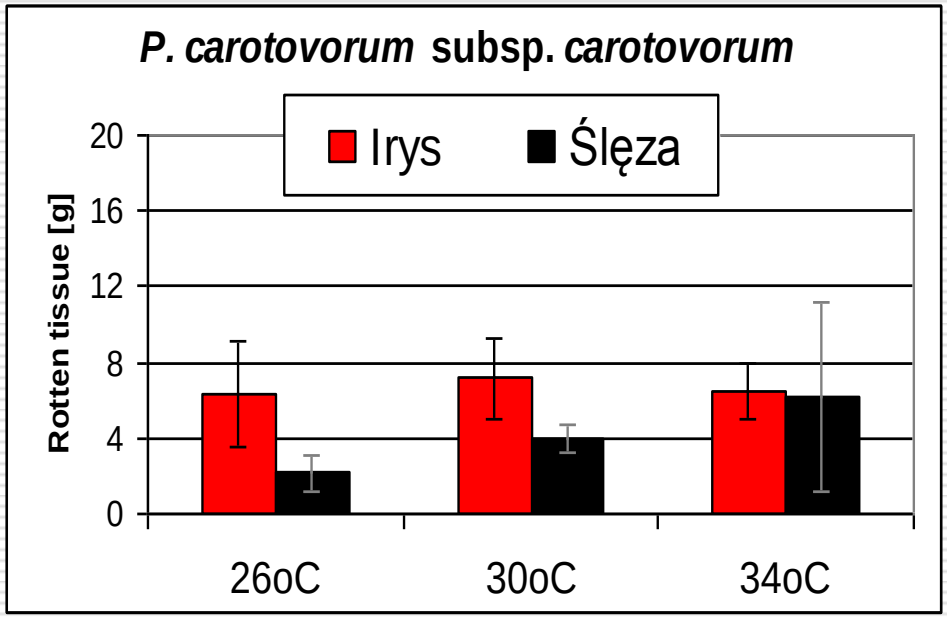
Inoculum – 0.095 OD_{600}

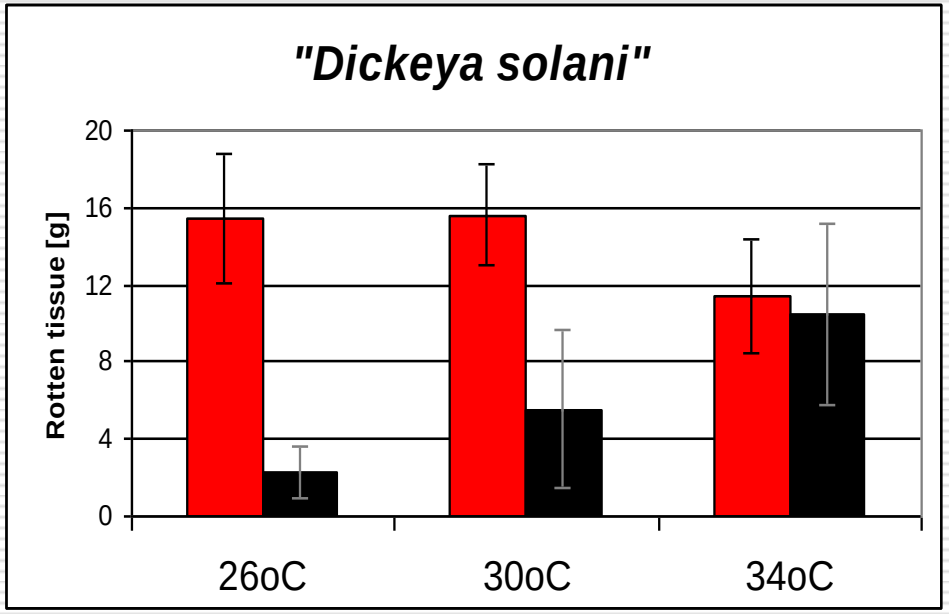
Incubation – 3 days

Weight of rotten tissue [g]

Four tubers, two inoculation sites

Soft rot [g] of susceptible cv. Irys and medium-resistant cv. Ślęza after three days in T 26, 30 and 34°C



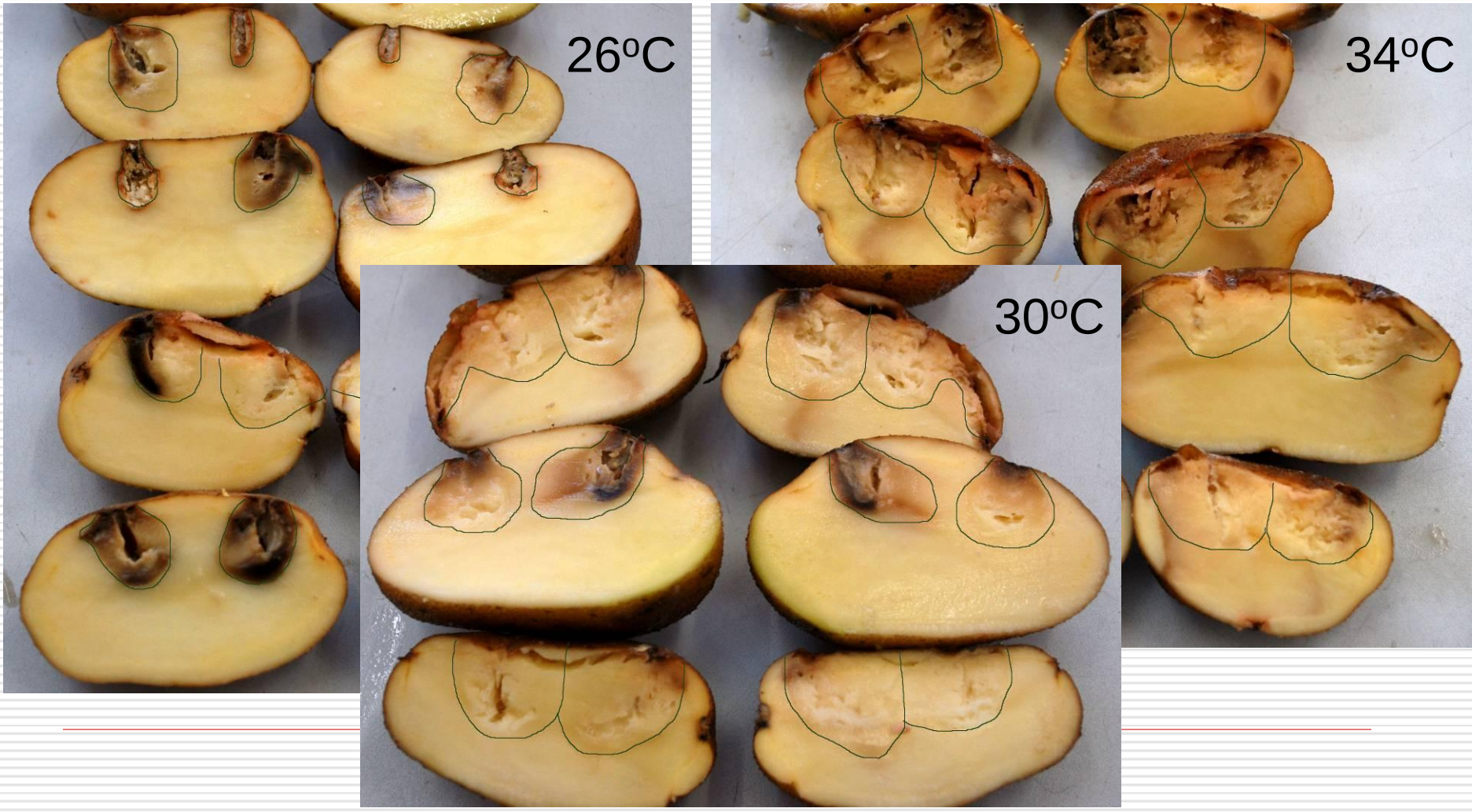


Susceptible cv. Irys and medium resistant cv. Ślęza inoculated with ***D. solani*** after three days in T **26ºC**

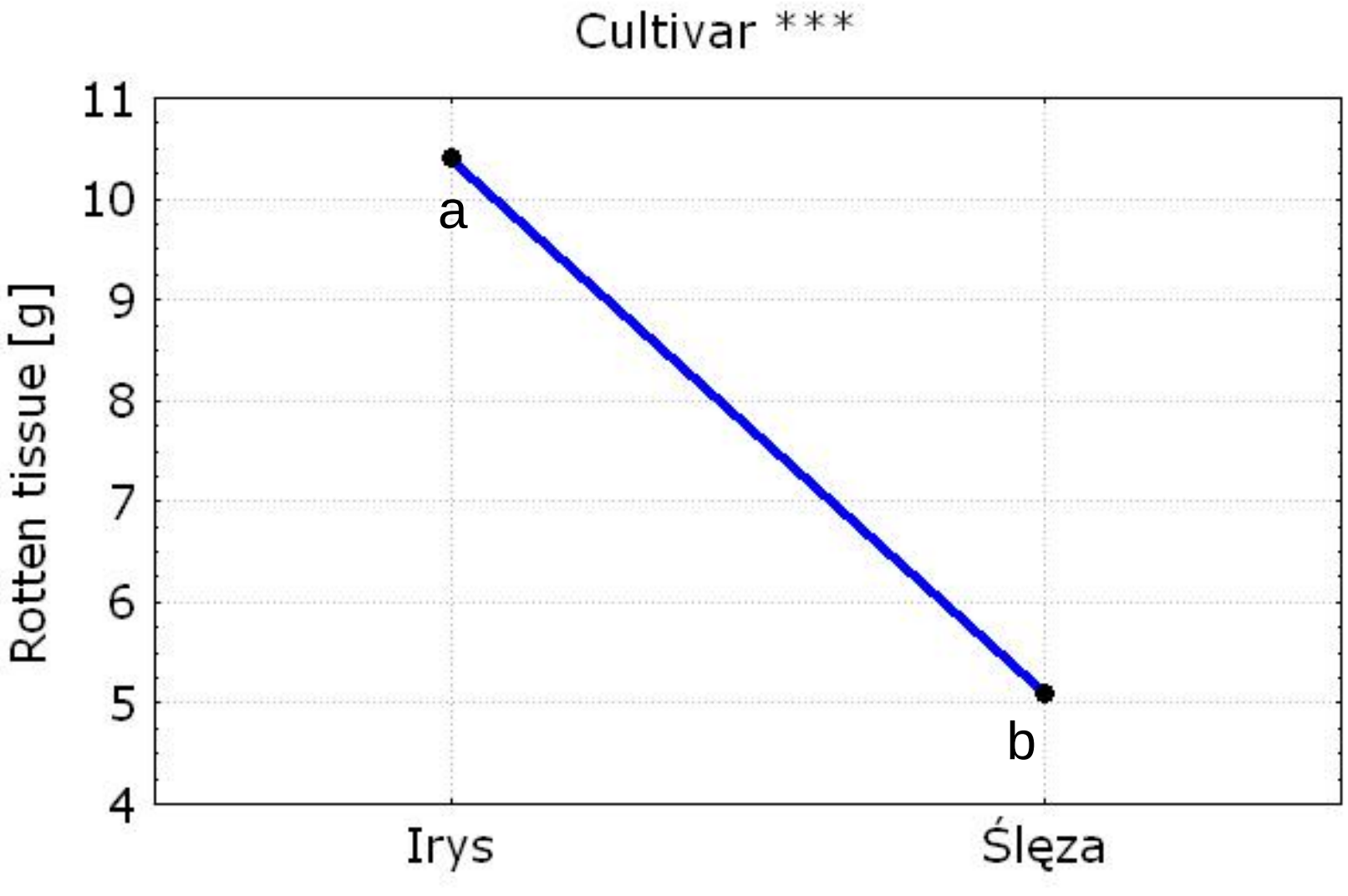
cv. Irys

cv. Ślęza

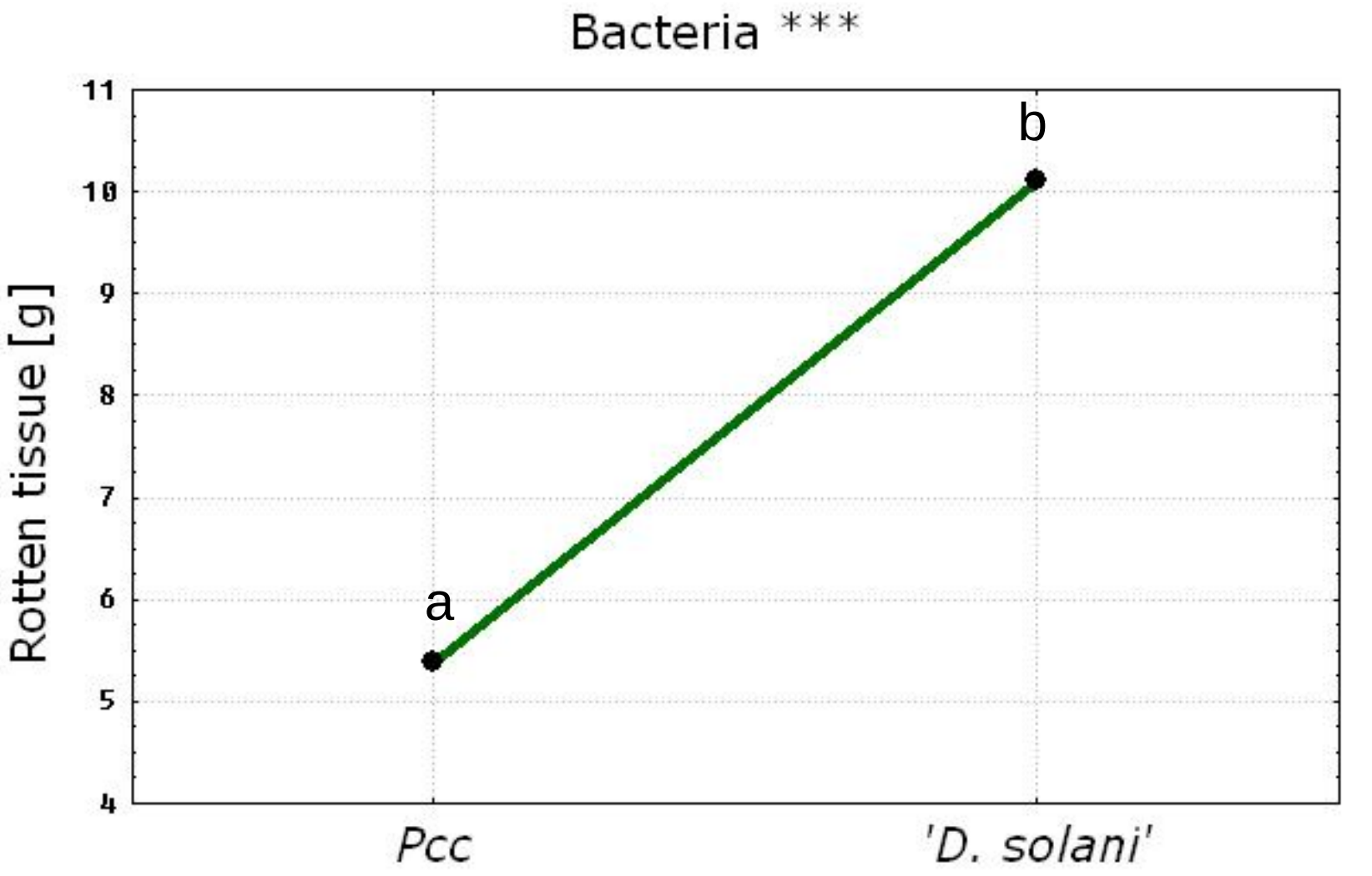
Medium-resistant cv. Ślęza inoculated with *D. solani* after three days in T 26°C and 34°C



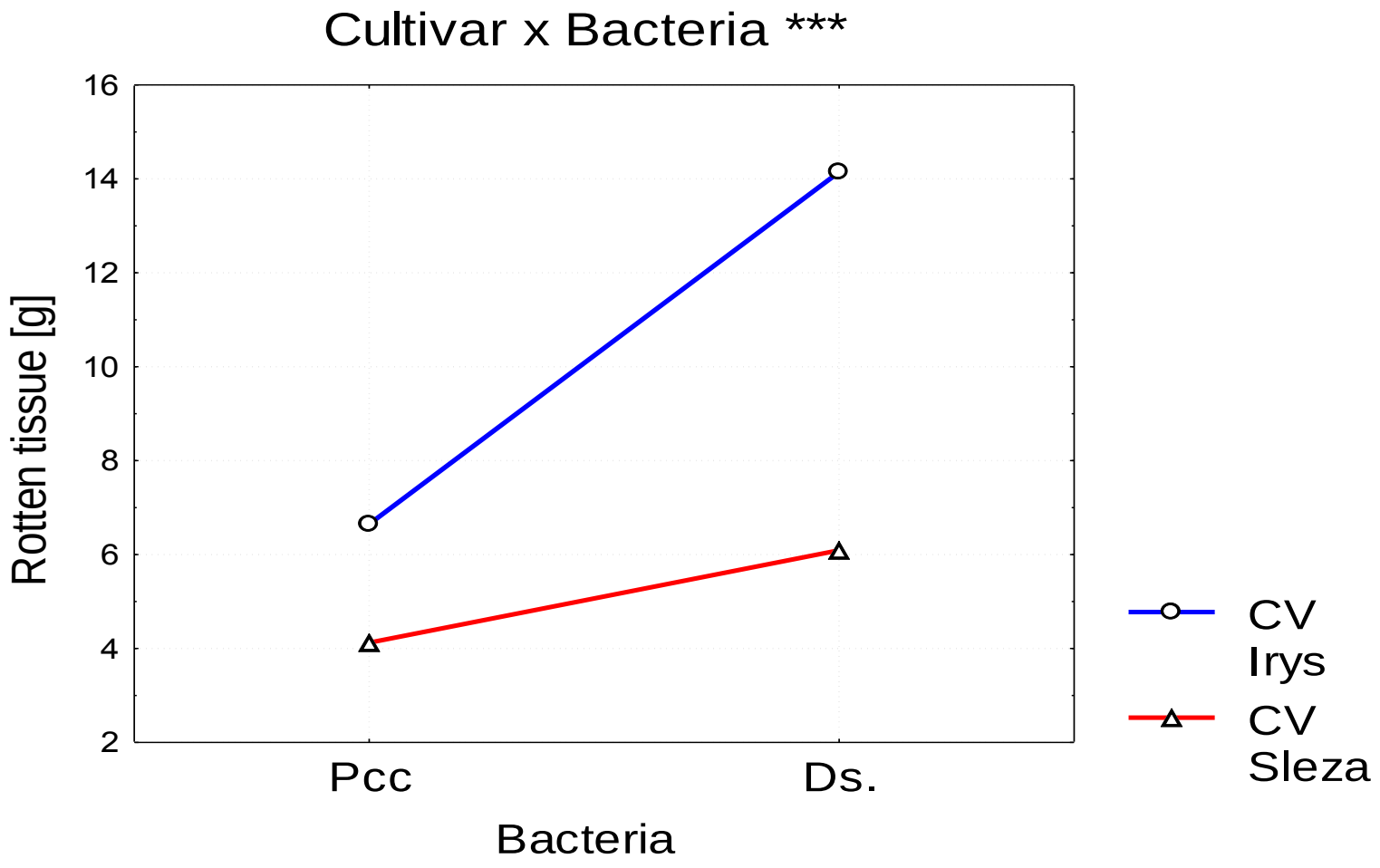
Cultivar - 42% of variance



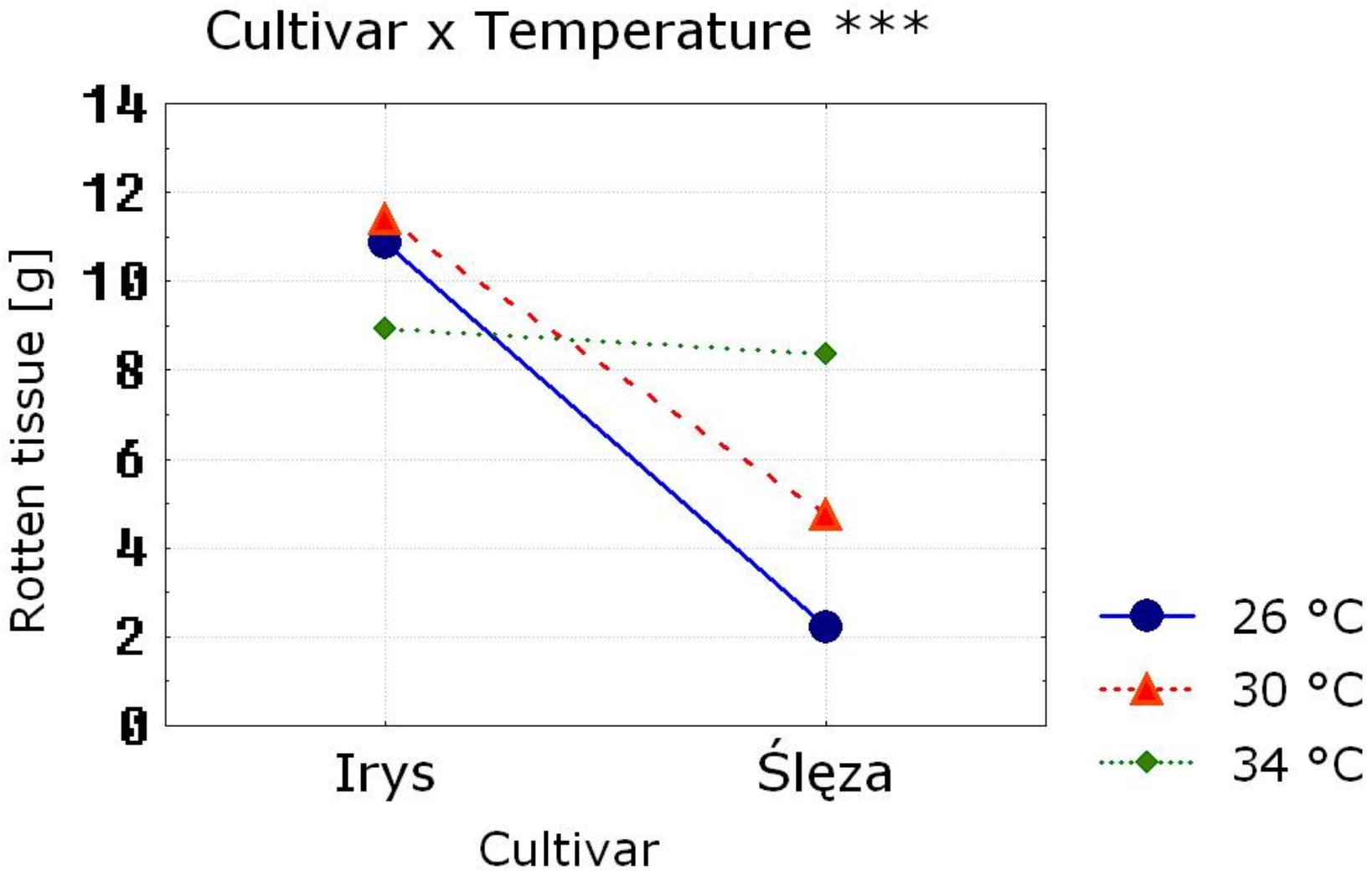
Bacteria - 33% of variance



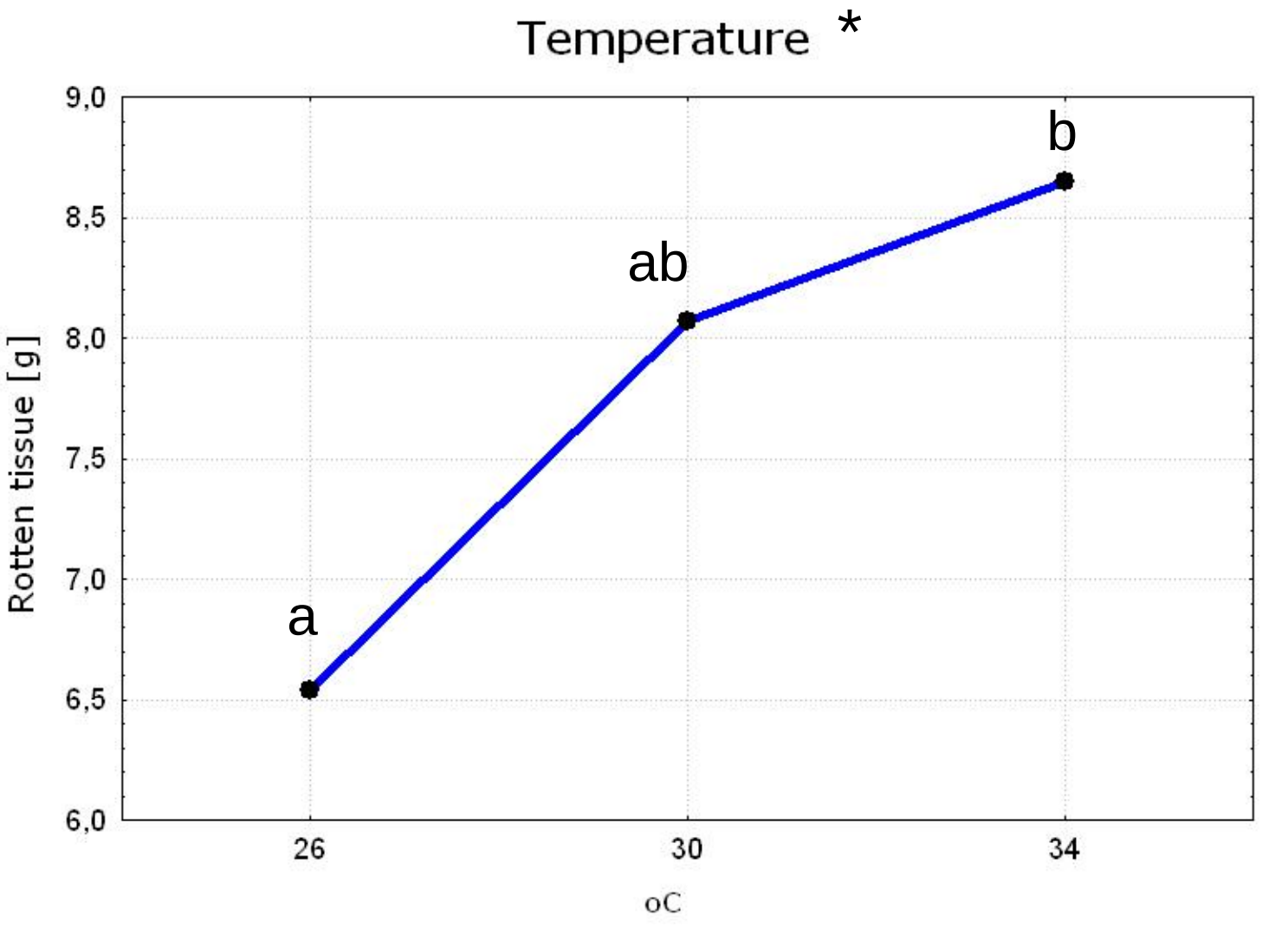
Cultivar x Bacteria 11 %



Cultivar x Temperature - 9% of variance



Temperature - 2% of variance



Summary

- The optimal temperatures of growth for *D. solani* IFB0099 were **34 ºC** and **35 ºC**
- **Pcc**, **Ds**, **Dd** *in vitro* growth increased with temperature in range **20 - 30 ºC**,

Summary

- **Pcc** multiplication was significantly faster than all other bacteria in temperature from **20** to **30 ºC**
- **Ds** was significantly more aggressive to potato tubers than **Pcc** in temperatures from **26 to 34 ºC**

Summary

- Growth of **Pa** was significantly slower than growth of **Ds** and **Dd** at **30 ºC**, **but not** at temperatures **20** and **25 ºC**
- The significant differences in resistance of two cultivars (susceptible and medium res.) were observed in temperatures **26** and **30 ºC but not** in **36 ºC**, for both bacteria **Pcc** and **Ds.**
- The resistance of medium resistant cv. Sleza was not expressed in temperature **34 ºC** after inoculation with **Ds** and **Pcc**