



Austrian Agency for Health and Food Safety
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Rearing and maintaining Austrian PCN populations at AGES

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Occurrence of PCN in Austria

First findings of PCN in Austria



Living PCN collection at AGES

- ☞ start of living PCN collection in 2013
- ☞ cysts which were multiplied in the bioassay (standard procedure for soil testing at AGES until 2011)
- ☞ cysts from infested fields which were collected during re-sampling
 - cyst amount per 250 ml soil: approximately 1 - 5 cysts with living cyst content



Living PCN collection at AGES

rearing/multiplication - maintaining



☞ rearing/multiplication

- as many cysts as possible for inoculum use for experiments
 - only used for cysts which are multiplied in the bioassay

☞ maintaining

- keep the population alive with as less effort as possible
 - only used for extracted cysts from fields (1 - 5 living cysts per 250 ml soil)

Living PCN collection at AGES

rearing/multiplication - maintaining



- ☞ circumstances are the same
- ☞ test procedures for rearing/multiplication and maintaining are the same
 - pot test in greenhouse with susceptible potato varieties
 - controlled climate conditions
 - controlled water supply
- ☞ preparing inoculum density
- ☞ cyst extraction with MEKU soil sample extractor
- ☞ ethanol method
- ☞ investigation of produced cyst amount and cyst content
- ☞ storage
- ☞ difference in pot size (used soil amount) and inoculum density

Living PCN collection at AGES

Rearing/multiplication – material & method



- ☞ greenhouse pot test starts in February
- ☞ susceptible variety "Desiree"/ locally used susceptible variety "Hermes"
- ☞ tuber size 35/45 mm
- ☞ 2 litre pots
- ☞ strong paper liner is used to prevent roots from growing out
- ☞ inoculum density: 5 eggs and larvae/ml soil
- ☞ cyst inoculum on basis of the mean cyst content on a small batch of cysts (cysts from bioassay samples)

Living PCN collection at AGES

rearing/multiplication - material & method



- ☞ pot is filled with 1000 ml soil
- ☞ prepared cysts are stirred in the soil with a glass rod
- ☞ the tuber is planted with one sprout and covered with the remaining 500 ml soil
- ☞ total soil amount: 1500 ml

Living PCN collection at AGES

rearing/multiplication - greenhouse conditions



☞ temperature regime:

- 17,5°C-19,5°C (room temperature)
- temperature measured in pots

☞ light regime: 16 h (D), 8 h (N)

→ climate is monitored continuously

☞ water regime:

- before sprouts emerge water apply is done manually (2-3 x/week)
- after sprouting automatic water supply (2-3x/week)

Living PCN collection at AGES

rearing/multiplication - material & method



☞ temperature regime:

- 17,5°C-19,5°C (room temperature)
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Living PCN collection at AGES

rearing/multiplication - material & method

- ☞ plants are tied to a stick to prevent them from toppling down
- ☞ extra sprouts emerging from tubers are removed
- ☞ only one stem is allowed to grow
- ☞ pest monitoring with yellow and blue sticky card traps
- ☞ pest control with use of beneficial insects
 - *Encarsia formosa* – greenhouse whitefly
 - *Chrysopa carnea* - aphids
 - *Neoseiulus cucumeris* - thrips



Living PCN collection at AGES

rearing/multiplication - material & method



- ☞ after approximately 12 weeks potato plants wilt and die
- ☞ water supply is stopped
- ☞ above ground parts are removed
- ☞ soil dries in pots for at least two weeks (greenhouse)
- ☞ pots are stored in a climate chamber at 8°C,
until elutriation of cysts

Cyst recovery - elutriation of cysts

MEKU – soil sample extractor

- ↪ 1500 ml soil is divided in 6 portions
- ↪ the extracted material is washed in a porcelain bowl
- ↪ floating material is transferred into a funnel with filter paper
- ↪ floating material with cysts dries at room temperature



Cyst recovery - elutriation of cysts

Ethanol method

- ☞ dry floating material with cysts is transferred into a 500 ml Erlenmeyer flask containing a rubber stopper on a wire
- ☞ ethanol is added
- ☞ the ethanol with debris is stirred well and wait for a few minutes
 - cysts float on the surface
- ☞ pour floating material onto a filter paper in a glass funnel
- ☞ remaining organic matter and cysts dry



Cyst recovery - elutriation of cysts

ethanol method

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- ☞ pour floating material onto a filter paper in a glass funnel
- ☞ remaining organic matter and cysts dry



Cyst recovery - elutriation of cysts storage

- ↪ cysts are rolled on a filter paper and counted
- ↪ cysts are stored dry in labelled glass tubes at 4°C in laboratory refrigerator with constant temperature regime



Living PCN collection at AGES

Maintaining on the host plant

- ☞ equal test procedure as for rearing and multiplication
- ☞ only difference:
 - pot size: 500 ml
 - soil amount: 250 ml
 - inoculum density: after extraction of soil samples 1 - 5 cysts with living content
 - but not more than 8 viable cysts per 250 ml soil



Living PCN collection at AGES

Populations and data



- ☞ *G. rostochiensis* populations
- ☞ *G. pallida* populations
- ☞ mixed *G. rostochiensis* and *G. pallida* populations
- ☞ available data: federal state, field locality, field name, size of field, collecting year, collector, year of multiplication and storage
- ☞ populations are different concerning locality and region
- ☞ every five years action is taken to keep the population alive
- ☞ identity is confirmed at the beginning of maintaining, rearing/multiplication



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