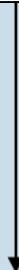


Maintenance of *Globodera* spp. in Mecklenburg Western Pomerania- Requirements and possibilities





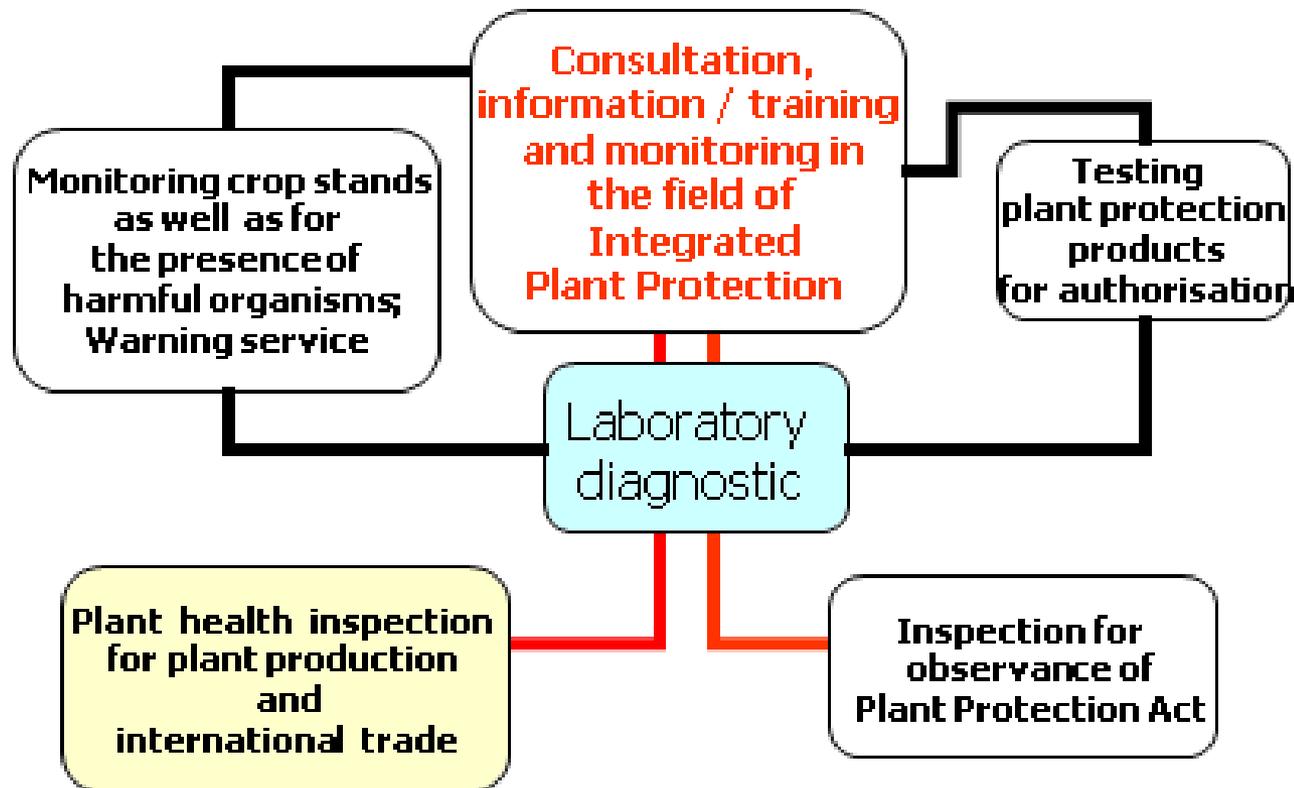
Ministry of Agriculture and Environment
Western Pomerania



State Office for Agriculture, Food Safety and Fishery
Mecklenburg- Western Pomerania
-Plant Protection Service-



Tasks of the Plant Protection Service





Reasons for maintaining PCN

1. Populations for **resistance testing**

- Screening (greenhouse)
- Official preliminary examination (climate chamber)

2. Support during the determination of **virulence**

- Collection of populations with origin in Mecklenburg-Western Pomerania
- Collection of the former G.D.R.



Testing-/reference strains Potato Cyst Nematodes

(Status 05/12/2017)

Designation of the population	Ref.-No.	Virulence	Introduced (recently replaced)	Origin	Place of storage
Groß Lüsewitz	1	Ro1/4 (Ro1)	1995	IfK Groß Lüsewitz	Gülzow/ plot
Hannover	2	Ro1/4 (Ro1)	1995	JKI Münster	fridge/R.No. 114a
Obersteinbach	3	Ro2/3 (Ro2)	1995	JKI Münster	fridge/R.No. 114a
Wageningen	4	Ro2/3 (Ro3)	1995	JKI Münster	fridge/R.No. 114a
Dutch F	5	Ro 1/4 (Ro4)	1995 (2013)	JKI Münster	fridge/R.No. 114a
Harmerz	7	Ro 5 (Ro5)	1995	JKI Münster	fridge/R.No. 114a
Port Glenone	8	Pa 1 (Pa1)	1995	JKI Münster	fridge/R.No. 114a
Kalle	10	Pa2/3 I (Pa2)	1995 (2012)	JKI Münster	fridge/R.No. 114a
Pukekohe	11	Pa2/3 I (Pa2)	1995	JKI Münster	fridge/R.No. 114a
Delmsen	12	Pa2/3 II (Pa3)	1995	JKI Münster	fridge/R.No. 114a
Chavornay	13	Pa2/3 III (Pa3)	1995 (2012)	JKI Münster	fridge/R.No. 114a
PT 77	14	Pa 2/3 I (Pa2)	1995	IfK Groß Lüsewitz	fridge/R.No. 114a
<i>G. tabacum</i> *	15	non-target organism	1995	IfK Groß Lüsewitz	fridge/R.No. 114a

* Maintained on *Solanum nigrum*



Resistance testing- Screening



Greenhouse in Gülzow

- 1-4 replications
- 7 cm clay pots in the greenhouse
- 20°C day, 16°C night
- additional light from November to February

Season 2016/17:

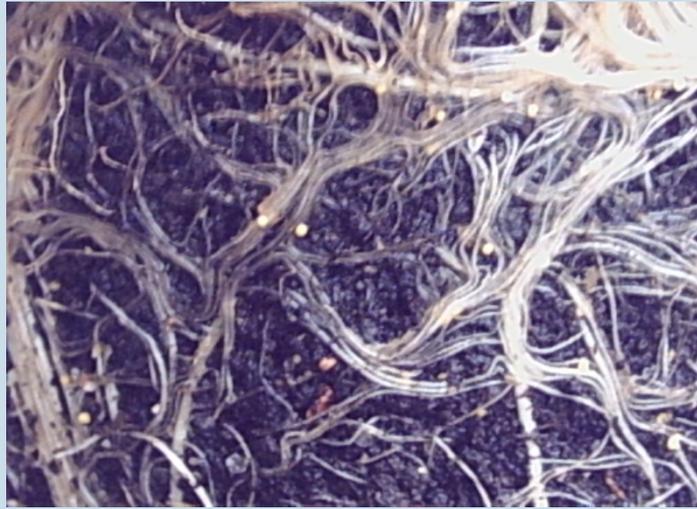
- 5104 pots Ro1* with a regional population (Groß Lüsewitz),
- 4032 pots other virulences than Ro1

-of these: 2319 Chavornay (Pa2/3), 733 Kalle (Pa2/3), 527 Wageningen (Ro2/3), 363 Harmerz (Ro5)

* Following the pathotype scheme by Kort et al.(1977)



Screening- Results



G. rostochiensis on the surface of a pot ball

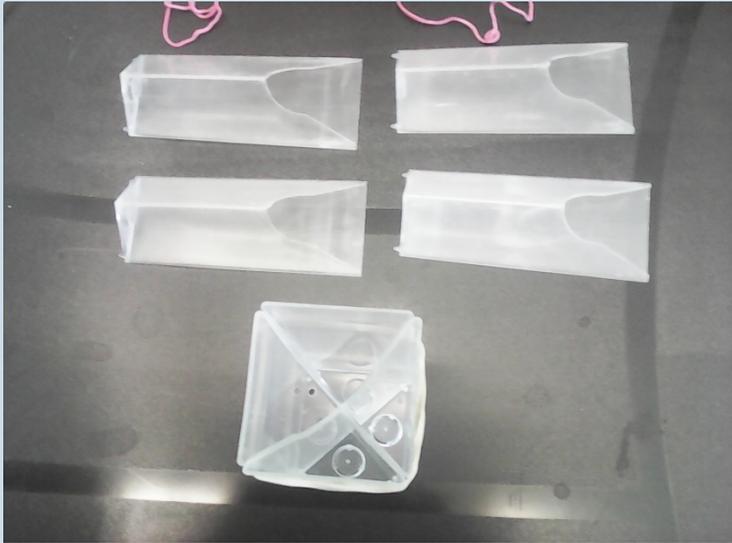
As a control a fully susceptible, a partially (species-) resistant and a (species-) resistant cultivar for every virulence is included. (each with 6 replications)

The test is valid, if at least 30 cysts on average are counted at the roots of the susceptible and not more than 5 cysts on the resistant cultivar.

A breeding strain is compared with these reference varieties



Resistance testing- preliminary examination



4 chamber-containers according to
BEHRINGER

- carried out in a climate chamber
- with 6 replications per cultivar
- all added and new cysts are counted (including Ro1)

The test is valid, if at least 100 cysts on average are counted at the roots of the susceptible and not more than 15 cysts on the resistant cultivar.



Maintenance of cysts for resistance testing- technical details –Screening Ro1- mixture

The regional Ro1 population 'Groß Lüsewitz' is maintained in an open air plot:
10 sq.m, concrete walls 1 m deep, 30 cm high, downwards open, secured against
transmission by animals (flexible fence)



500 l of soil are sieved after a susceptible
potato cultivar every second year



concrete mixer to obtain a contamination
level of about 2000 larvae per 100 ml

[Data sheet](#)



Extraction of single cysts for virulences, higher than $R_{01/4}$ and for the preliminary examination

Populations reared for at least 12 weeks in silica sand culture in a climate chamber,
20°C, 12 hours light





Extraction of single cysts for virulent (more than $R_{o1/4}$) populations and preliminary examination- technical details

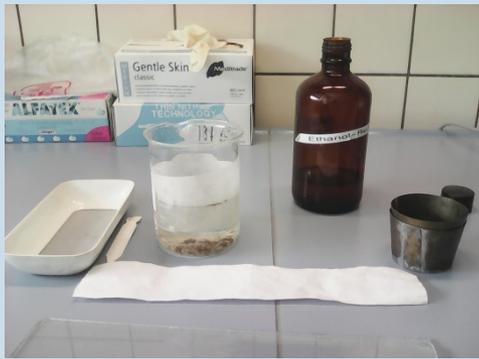


Composition of the substrate for the silica sand culture:

- 90% silica sand (3 parts fine 0.1 – 0.3 mm, 1 part rough 0.7 -1.2 mm)
- 10 % sterilized mole earth
- slow release fertilizer



Extraction of single cysts for high-virulent (more than $Ro1/4$) populations and preliminary examination- technical details



Separation of the cysts :

- Up to 3 pots silica sand culture are rinsed into one sieve by Fenwick can .
- The further separation is carried out by using the paper strip method.
- Storage at 4-8°C for at least 4 months up to 6 years



Determination of an unidentified virulence

Methods of laboratory analysis in Mecklenburg-Western Pomerania:

- PCR, IEF, (morphological) for the determination of the species
- multiplication on a susceptible or a Ro1/4 resistant
- 2-D-electrophoresis, test range of varieties with known resistance

Results:

- the species
- the virulence within the species *Globodera rostochiensis* is Ro 1/4 or not
- the approximate degree of virulence of *Globodera pallida* (test range)
- more details may be obtained by comparison with populations of known virulence → collections



Collections

Cysts of the collections are maintained in the same way as the populations – silca sand culture every second or third year with 1-3 pots.

Collection of populations with origin in Mecklenburg-Western Pomerania:

collected in the last 25 years → 56 samples

- not for every of this populations a content of higher virulence is confirmed
- if the population contains virulence different from Ro 1/4, this proportion is always Ro2/3

Collection of the former G.D.R.:

collected by Dr. Rolf Kuhn in the former Institute for Potato Research of the G.D.R. in Groß Lüsewitz, near Rostock (handed over in 1995)

- contains 58 origins today
- most of them sent from the plant protection services of the districts of East Germany; some of them origin from international collections



Conclusions

Observations:

- fitness of populations maintained exclusively in the climate chamber may decrease
- maintenance of PCN is casual coupled with resistance tests
- biological resistance tests are time consuming and a target for savings

Solutions:

- reference laboratories should have the capacity to replace weakened populations
- reference laboratories or an engaged institution maintains the collection of interesting populations
- miniaturization of resistance tests: in vitro-tests/
molecular detection of resistance genes



Thanks
for your
attention!