

Species composition and resistance to fungicides of Russian potato and tomato early blight pathogens



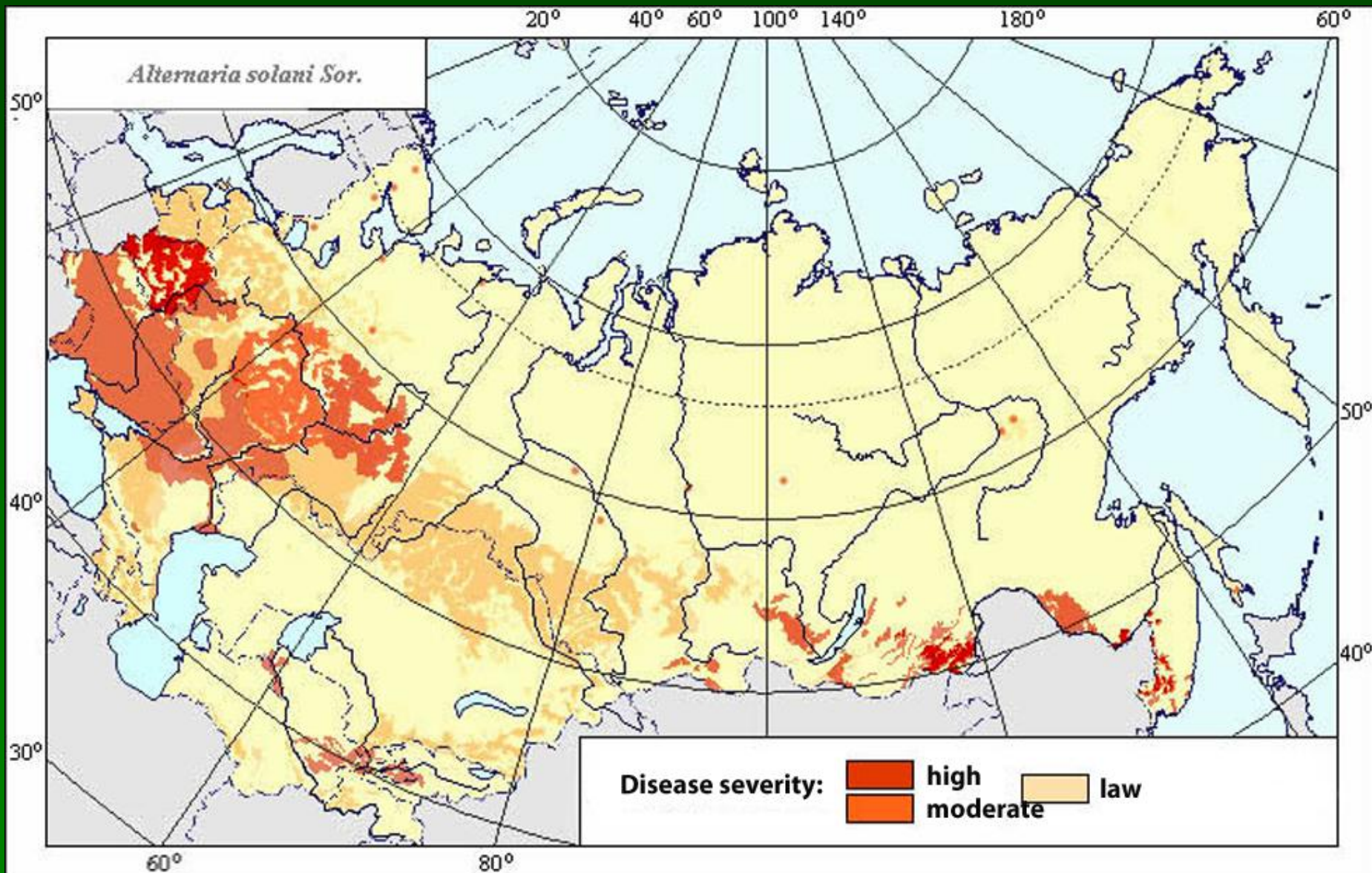
**Lyudmila Kokaeva,
Sergey Elansky**

*Moscow M.V. Lomonosov State University,
Biological faculty*

Alexander Nikolaev

*Agricultural research institute of the
Kostroma region*

Distribution of potato and tomato early blight in Russia

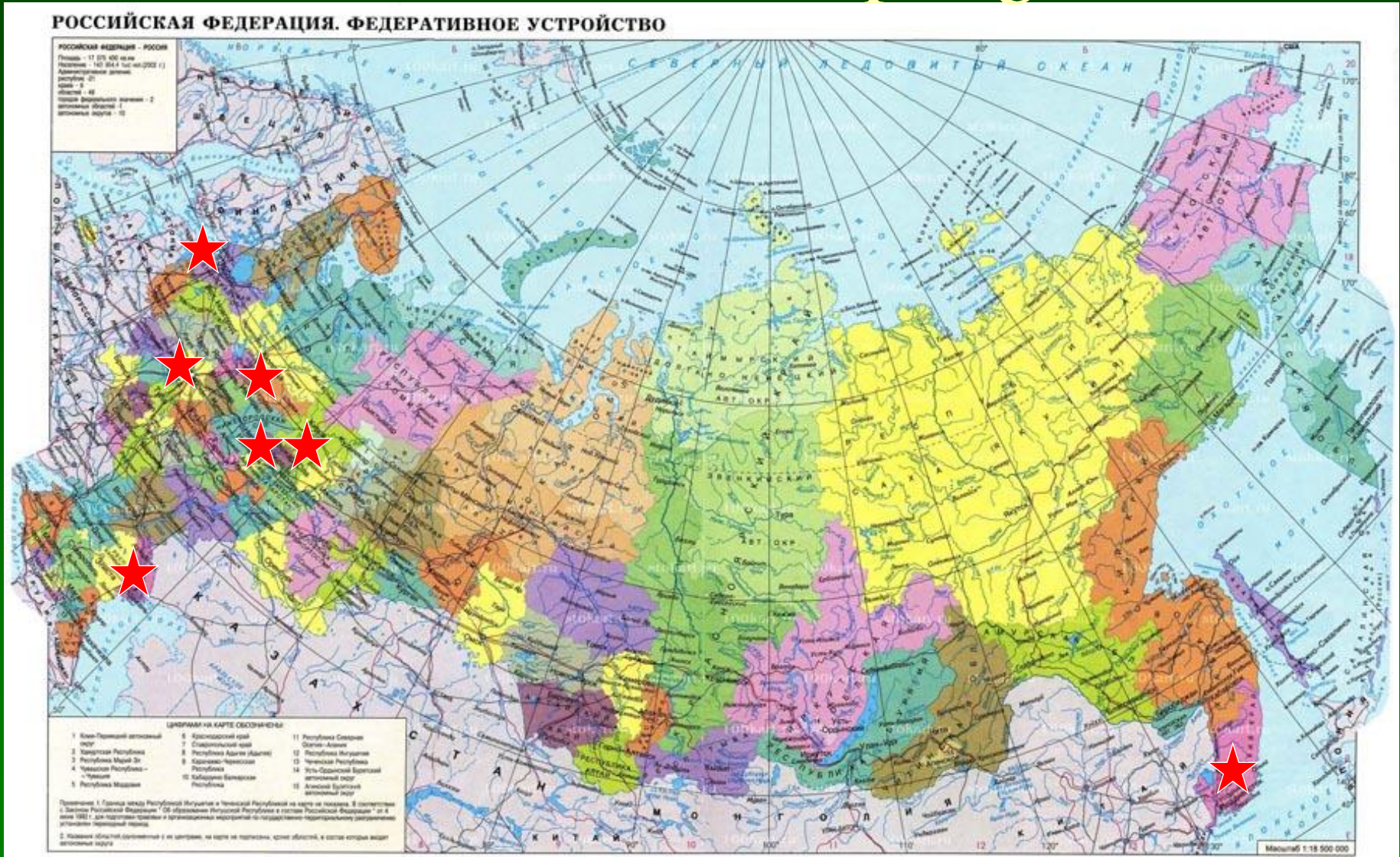


Collecting blighted samples



Samples with typical symptoms were used for isolation

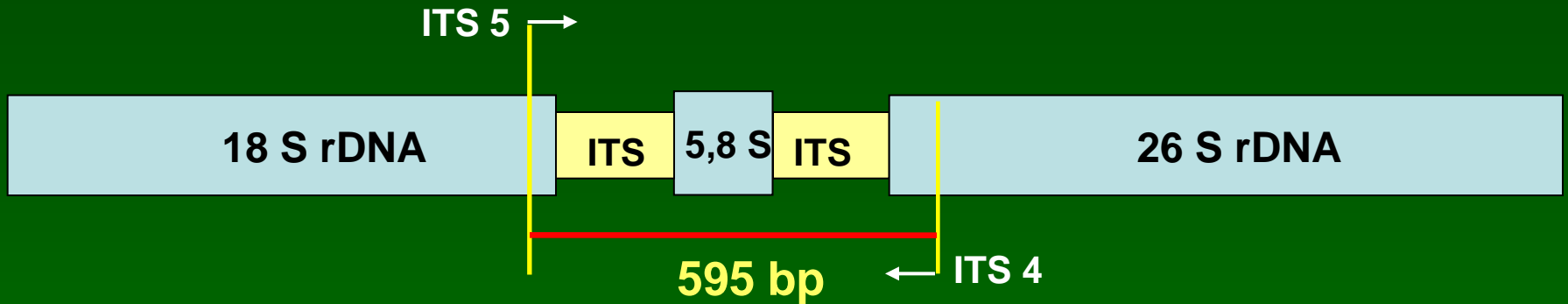
Locations of sampling sites



190 isolates from 7 locations were tested

Composition of species

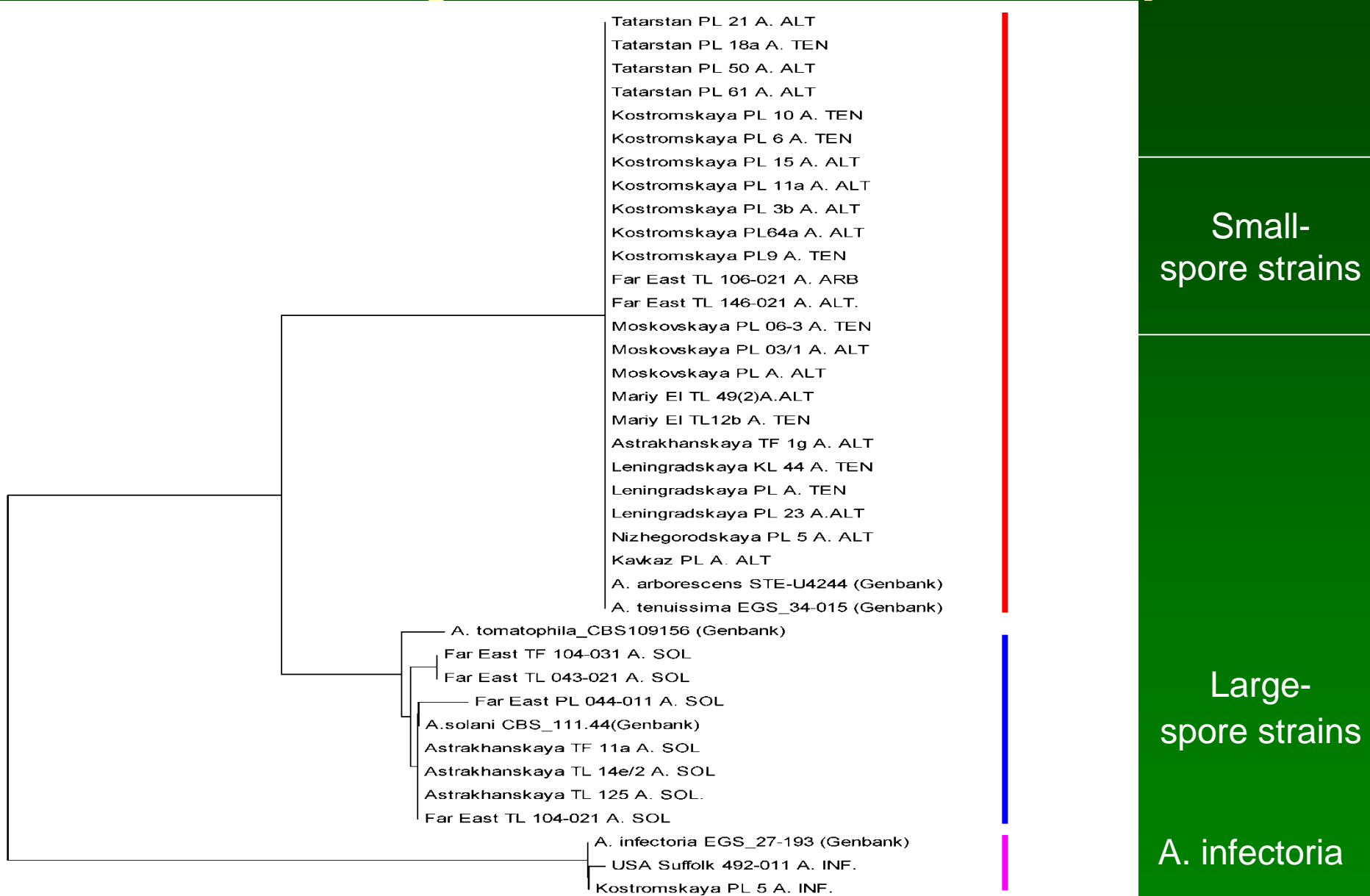
Location of the sequenced region and positions of primers ITS 5 and ITS 4



Name of primer	Sequence
Forward primer ITS 5 (White et al, 1990)	5' – GGAAGTAAAAGTCGTAACAAGG
Reverse primer ITS 4 (White et al, 1990)	5' – TCCTCCGCTTATTGATATGC

Sequence of this region allows to identify the small-spore species, *A. solani*, *A. tomatophila*, *A. infectoria*.

ITS 5 – ITS 4 regions of 33 strains were sequenced



0.01

List of the identified species:

Alternaria solani Sorauer

A. infectoria E.G. Simmons

Group of small – spore species

**After morphological investigation
this group was devoted to 3 species:**

A. alternata (Fr.) Keissl.

A. infectoria E.G. Simmons

A. tenuissima (Kunze) Wiltshire

Comparison of ITS 4 – ITS 5 regions of *A. alternata* and *A. solani*

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ITS A. alternata - A. solani
Query 1  GGAAGTAAAAGTCGTAACAAGGTTCTCCGTAGGTGAACCTGCGGAGGGGATCATTACACAAA 60
          ||||| ITS 5 |||||
Sbjct 2  GGAAGTAAAAGTCGTAACAAGGTTCTCCGTAGGTGAACCTGCGGAGGGGATCATTACACAAA 61

Query 61  TATGAAGGCGGGCTGGAACCTCTCGGGGT-TACAGCCTTGCTGAATTATT-CACCCTTGT 118
          |||||
Sbjct 62  TATGAAGGCGGGCTGGCACCTCCCAGGGGTGGCCAGCCTTGCTGAATTATTCCACCCGTGT 121

Query 119  CTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCACCACCTAGGA-CAA-ACATAAAC 176
          |||||
Sbjct 122  CTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCACCACCAAGGACCAACCCATAAAC 181

Query 177  C-TTTTGTAAATTGCAATCAGCGTCAGTAACAAAT-TAATAA-TTACAACCTTTCAACAACG 233
          |
Sbjct 182  CTTTTGCAATGGCAATCAGCGTCAGTAAC-AATGTAATAATTTACAACCTTTCAACAACG 240

Query 234  GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTG 293
          |||||
Sbjct 241  GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTG 300

Query 294  CACTCTCTATC-AGCA--AAGGTC 353
          ||
Sbjct 301  CACTCTCTATC-AGCA--AAGGTC 360

Query 354  GCGCTCTCT-TCCAGCCCCAAGGTC 413
          |
Sbjct 361  GCGCTCTCT-TCCAGCCCCAAGGTC 420

Query 414  -TCFAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAG 472
          |||||
Sbjct 421  GTCTCCCCTTTCGGGAGACTCGCCTTAAAGTCATTGGCAGCCGGCCTACTGGTTTCGGAG 480

Query 473  CGCAGCACAAAGTCGCACTCTCTATC-AGCA--AAGGTC 529
          ||||| MR |||||
Sbjct 481  CGCAGCACAAAGTCGCGCTCTCT-TCCAGCCCCAAGGTC 539

Query 530  t-CAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAACATATCAATAAGC 588
          ||||| ITS 4 |||||
Sbjct 540  TTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAACATATCAATAAGC 599

Query 589  GGAGGAA 595
          |||||
Sbjct 600  GGAGGAA 606
  
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Comparison of ITS 4 – ITS 5 regions of *A. alternata* and *A. infectoria*

A. alternata - *A. infectoria*

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Query 1  GGAAGTAAAAGTCGTAACAAGG|CTCCGTAGGTGAACCTGCGGAGGGATCATTACACAAA 60
          ||||| ITS 5 |||||
Sbjct 1  GGAAGTAAAAGTCGTAACAAGG|CTCCGTAGGTGAACCTGCGGAGGGATCATTACACAA- 59

Query 61  TATGAAGGCGGGCTGGA-ACCTCTCG---GG----GTTACA----- 93
          || ||||| ||||| ||||| |||||
Sbjct 60  TAACAAGGCGGGCTGGACACCCCCGCTGGGCACTGCTTCACGGCGTGCGCGGCGGGGCC 119

Query 94  -GCCTTGCTGAATTATTCACCCCTTGCTTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTTCG 152
          ||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 120  GGCCCTGCTGAATTATTCACCCGTGCTTTTTGCGTACTTCTTGTTTCCTGGGTGGGCTCG 179

Query 153  CCCACCACTAGGACAAAC-ATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTA 211
          ||| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 180  CCCGCCCTCAGGACCAACCACAAACCTTTTGCAATAGCAATCAGCGTCAGTAACAACGTA 239

Query 212  AT-AATTACAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGA 270
          || ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 240  ATTAATTACAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGA 270

Query 271  AATGCGATAAGTAGTGTGAATTGCAGAA| 300
          ||||| ||||| ||||| ||||| |||||
Sbjct 300  AATGCGATACGTAGTGTGAATTGCAGAA| 300

Query 331  TGCGCCCTTTGGTATTCCAAAGGGCATG| 360
          ||||| ||||| ||||| ||||| |||||
Sbjct 360  TGCGCCCTTTGGTATTCCAAAGGGCATG| 360

Query 391  TGCTTGGTGTTGGGCGTC--TTGTCCTACCTTTGCTGGAGACTCGCCTTAAAGTAATTG 448
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 420  TGCTTGGTGTTGGGCGTCCTTTGTCCTCAG-TTCCTGGAGACTCGCCTTAAAGTCATTG 478

Query 449  GCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCC|CACTCTCTATCAGCAAAGGTT 508
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 479  GCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCC|CGCTCTTTGCCAGCCAAGGTC- 537

Query 509  AGCATCCATTAAGCC-ttttttCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTG 567
          ||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 538  AGCGTCCAGCAAGCCTTTTTTTTCAACCTTTGACCTCGGATCAGGTAGGGATACCCGCTG 597

Query 568  AACTTAACCATATCAA| 598
          |||||
Sbjct 598  AACTTAACCATATCAA| 598
  
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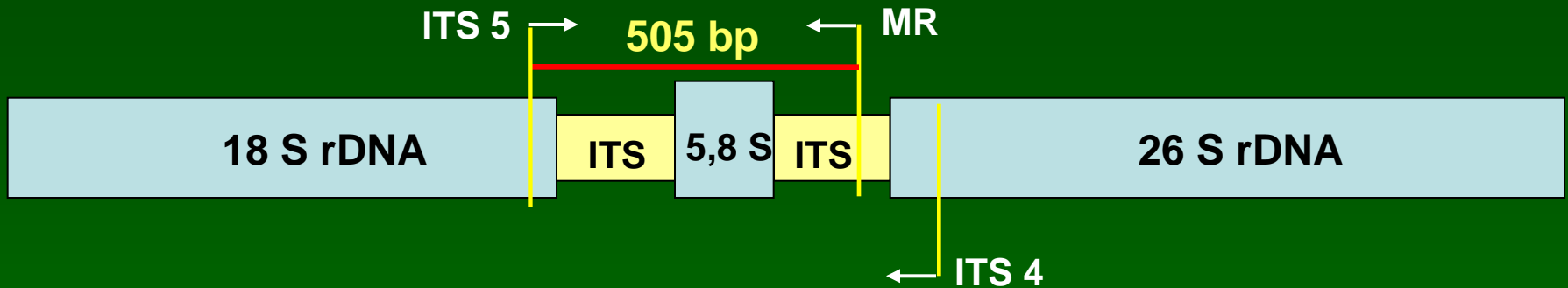
CACTCTCTATCAGCAAAGGTC
 ||||| ||||| ||||| ||||| |||||
 CGCTCTTTGCCAGCCAAGGTC

ITS 5

MR

ITS 4

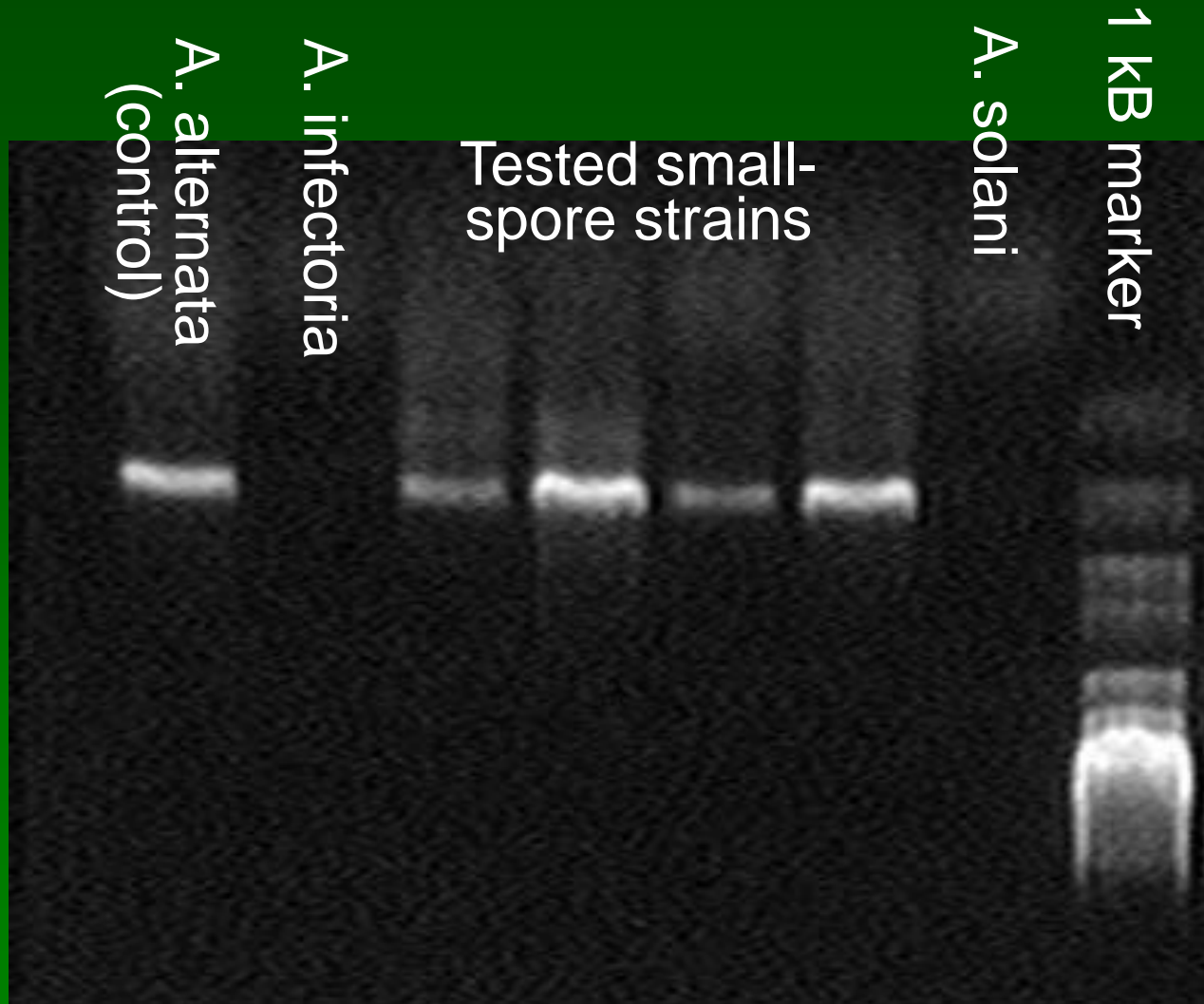
Location of the sequenced region and positions of primers ITS 5 and MR



Name of primer	Sequence
Forward primer ITS 5 (White et al, 1990)	5' – GGAAGTAAAAGTCGTAACAAGG
Reverse primer MR	5' – GACCTTTGCTGATAGAGAGTG

Pare of primers ITS 5 – MR allow to distinguish species with small spores from *A. solani* and *A. infectoria*

Application of primers ITS 5 and MR for *Alternaria* species identification



190 *Alternaria* sp. strains were tested

Identification of species

Isolation in pure culture



Extraction of DNA (CTAB method)



PCR with pair of primers ITS 5 - MR



Small-spore group



Not small-spore group



Morphological identification

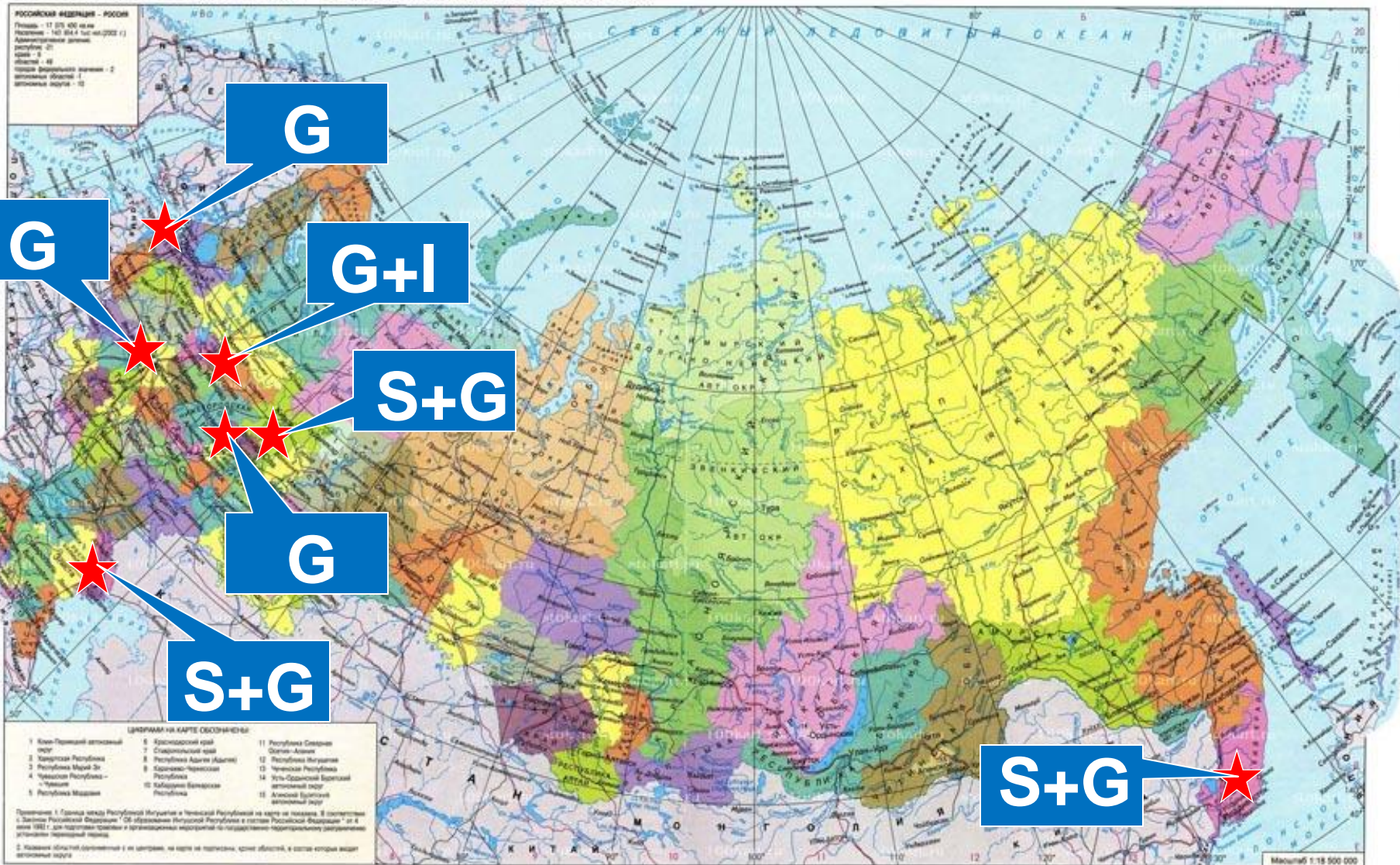


Sequencing

```
CACTCTCTATCAGCAAAGGTC
| | | | | | | | | |
CGCTCTTTGCCAGCCAAGGTC
```


Species of *Alternaria* in different regions

РОССИЙСКАЯ ФЕДЕРАЦИЯ. ФЕДЕРАТИВНОЕ УСТРОЙСТВО

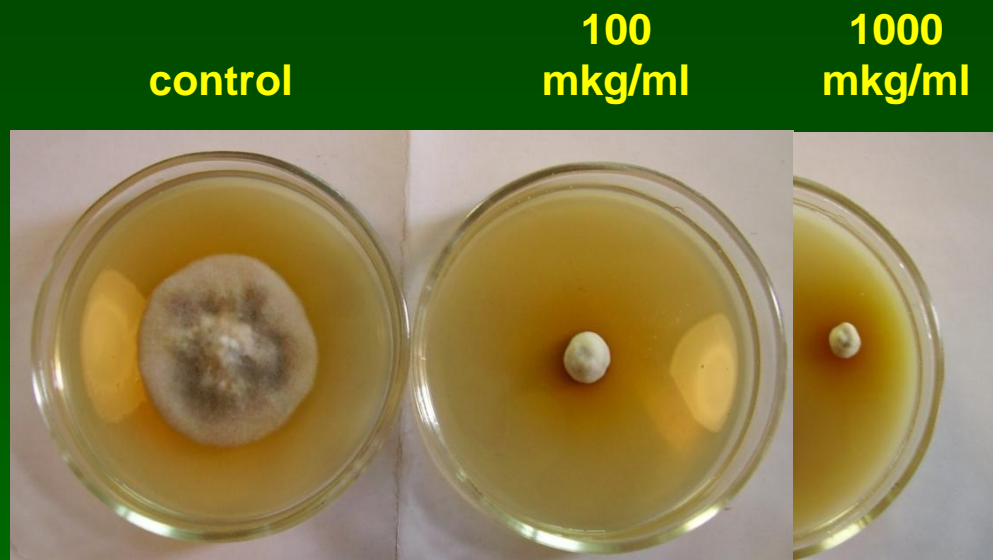


S – *A. solani*, I – *A. infectoria*, G – group of small spore species

Resistance to fungicides

Testing of the resistance to fungicides

Alternaria solani



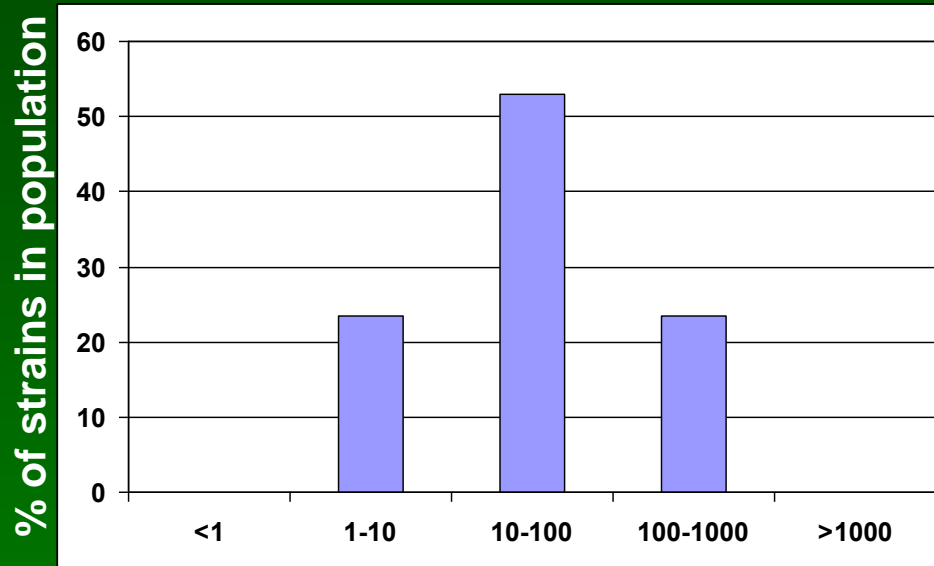
Alternaria alternata



EC₅₀ – concentration of the fungicide in agar media twice decreasing the growth rate of the colony. Concentrations 0,1; 1; 10; 100; 1000 mkg/ml were used for testing.

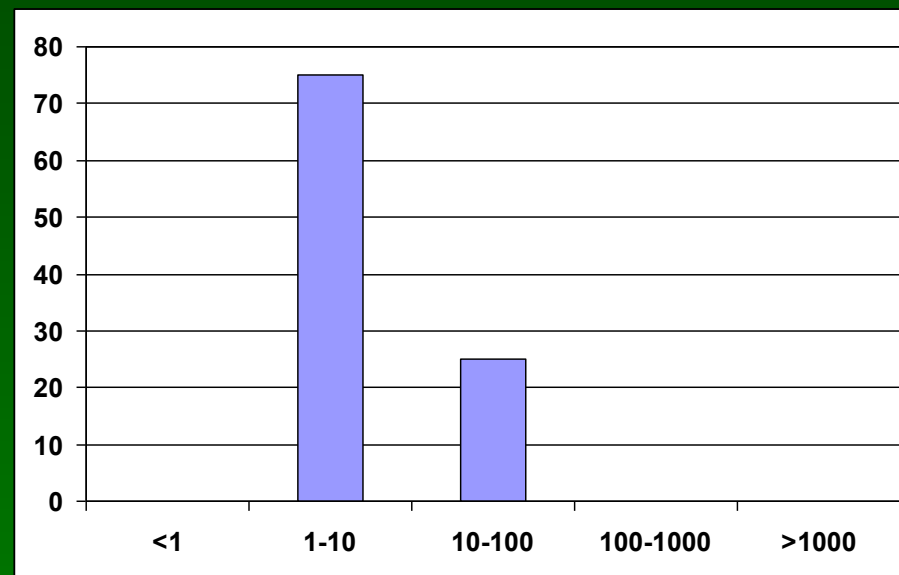
Resistance to mancozeb

Small spore species



EC₅₀ range

A. solani

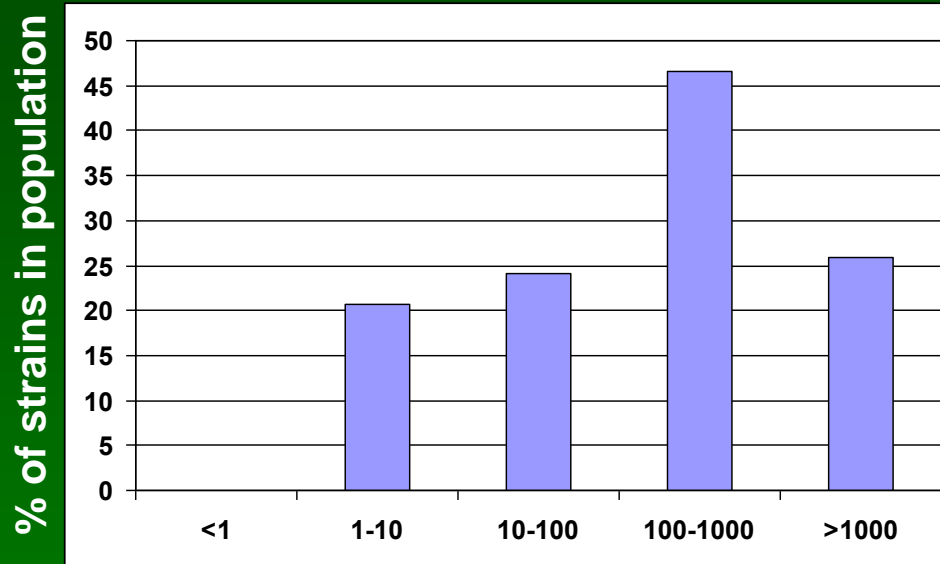


EC₅₀ range

There were no highly resistant *A. solani* strains to mancozeb in all tested field populations.

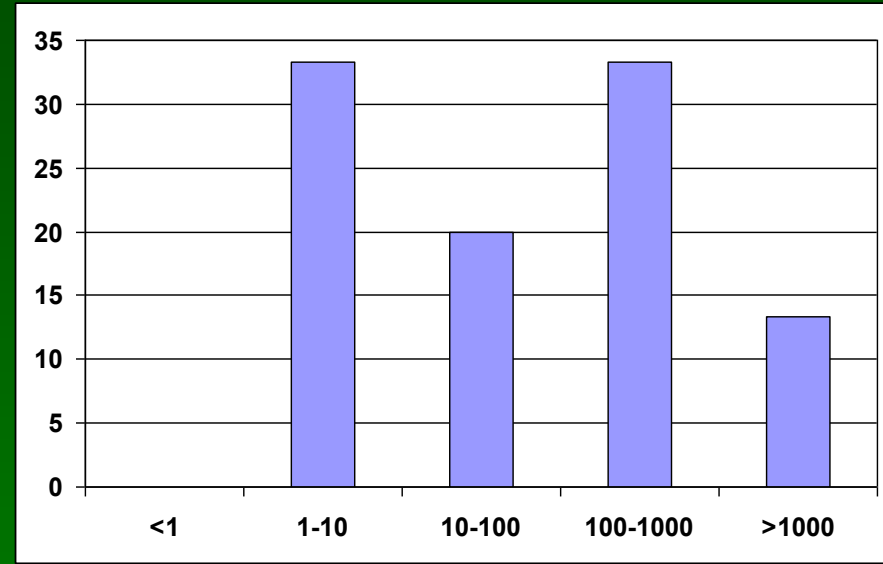
Resistance to chlorothalonil

Small spore species



EC₅₀ range

A. solani

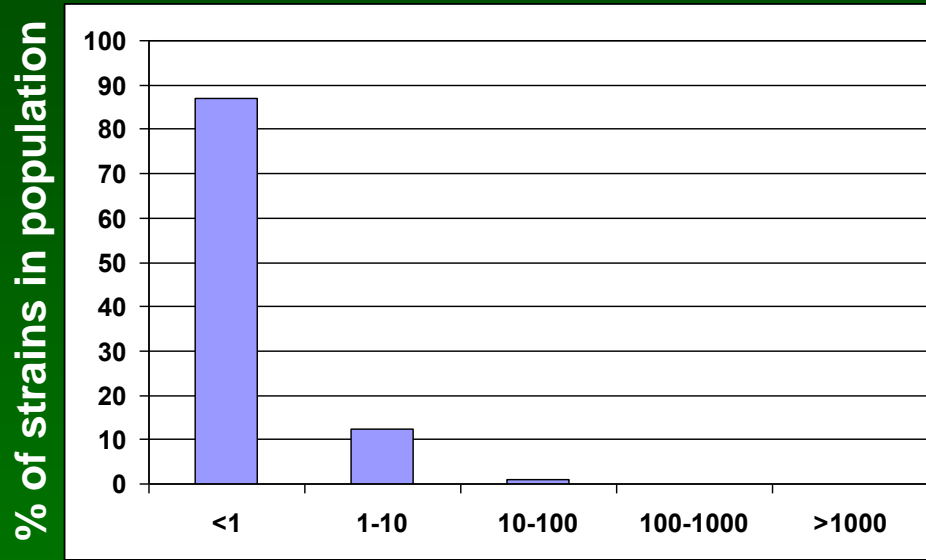


EC₅₀ range

There were no differences in resistance of *A. solani* and small spore species to chlorothalonil. Highly resistant strains were found in all tested field populations.

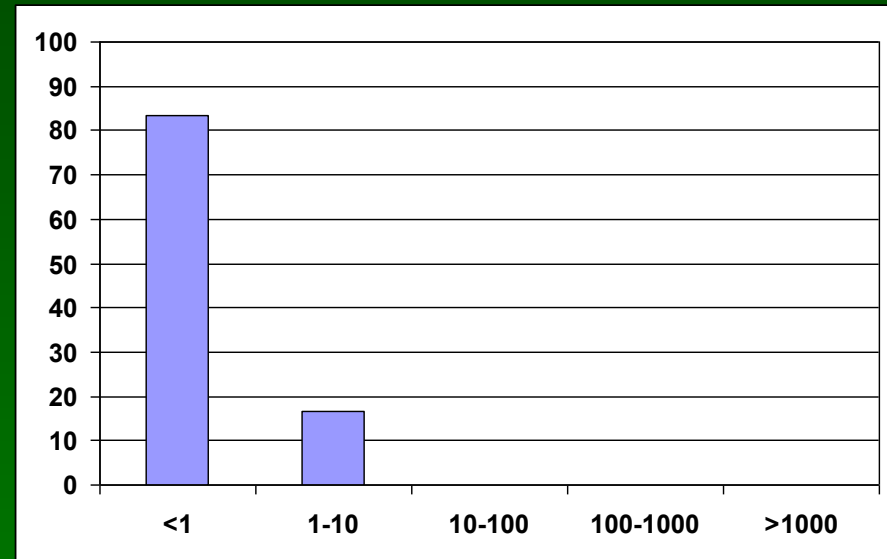
Resistance to fludioxonil

Small spore species



EC₅₀ range

A. solani

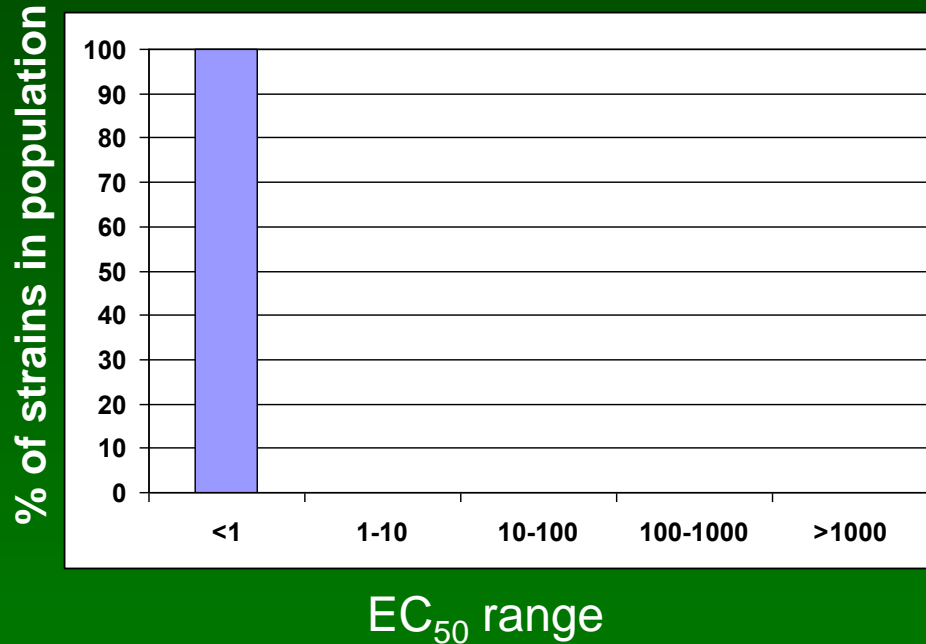


EC₅₀ range

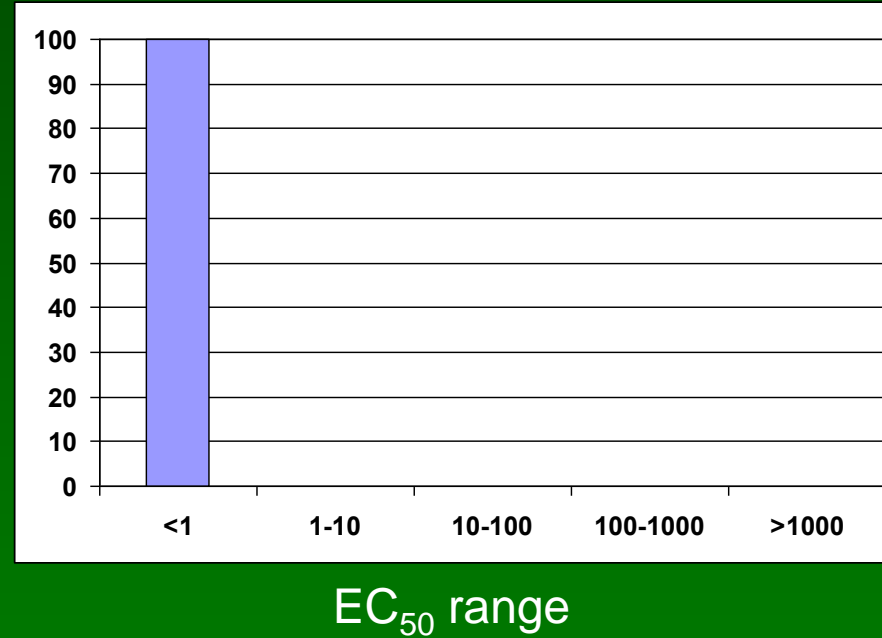
All tested strains were very sensitive to fludioxonil.

Resistance to difenoconazole

Small spore species



A. solani



All tested strains were very sensitive to difenoconazole

Conclusions

Species with small spores (morphologically identified as *A. alternata*, *A. tenuissima*, and *A. arborescens*) were found in all tested populations,

A. solani was found in Astrakhan region, Mari-El, and Far East,

A. infectoria was found only one strain in Kostroma region.

All tested strains were highly sensitive to **fludioxonil** and **difenokonazole**

All tested *A. solani* strains were sensitive to **mankozeb**, but there was many resistant strains between small-spored *Alternaria*.

Majority of strains were resistant to **chlorothalonile**.

*Thank you for
your attention!*