Control of pests and pathogens affecting fruit crops

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Prof. dr hab. Piotr Sobiczewski

- Head of Fruit Plant Protection Department, Research Institute of Horticulture, Skierniewice, Poland
- Head of Section for Plant Bacterial Diseases, member of Board and Head of Warsaw Division of Polish Phytopathological Society;
- Member of Editorial Board of journals *European Journal of Plant Pathology* and *Phytopathologia*;
- Head of Plant Diseases Section of Committee for Plant Protection of Polish Academy of Sciences; member of presidium and secretary of Committee for Horticultural Sciences of Polish Academy of Sciences,
- ; Advisor to the Main Inspector for Plant Protection and Polish Ministry of Agriculture on diagnostic and control of fire blight *(Erwinia amylovora).*
- Member of Scientific Council of Bank of Plant Pathogens at Institute of Plant Protection, Poznan, Poland
- Chairman of Working Group on Fire Blight of International Society for Horticultural Sciences;

Research expertise: diseases of fruit trees and ornamental plants, particularly caused by bacteria: prediction, diagnostics, epidemiology and control, including, taxonomy of causal agents, biological control, genetics of bacteria virulence and plant resistance, apple replant disease.

Experience in international cooperation: Coordinator of joint projects: "Prediction and control of fire blight *(Erwinia amylovora)"* (2001-2002, bilateral Polish-German project). Partner in joint projects "Application of environmentally friendly bioregulators for regulation of fruit crops and control of fire blight in apple orchards" (bilateral, Polish-Hungarian), "The development and structures to promote the efficient use of pesticides" (1995-1997, PHARE: Partnership and Institution Building Programme); "Ensuring the Quality of Innovative Crop Growth Inputs Derived from Biological Raw Materials" (2002-2005, 5th FP project), Partner in 5FP "Centre for Excellence in Sustainable Fruit Production - PomoCentre" (2002-2005, 5th FP project), Member of Management and Executive Committees and co-chair of WG3 (Plant Protection Methods) of COST Action 864 "Combining traditional and advanced strategies for plant protection in pome fruit growing' (2005-2011), coordinator of LIFE+ Project 'Sustainable use of chemical fumigants for the control of soil-borne pathogens (SustUse Fumigants)' LIFE08 ENV/IT/00042 (2010-2012).

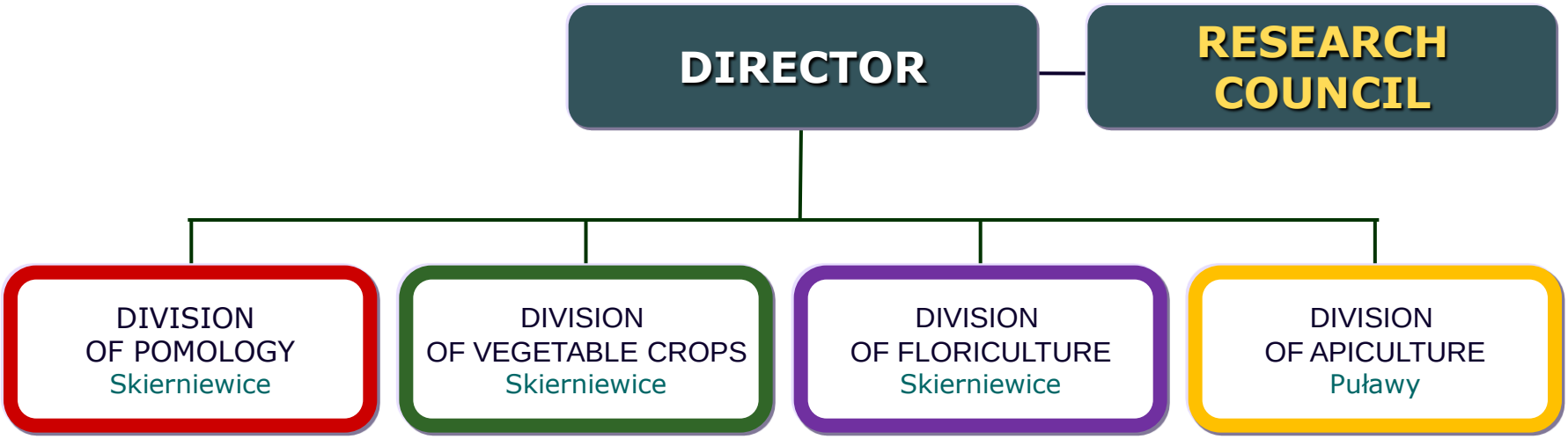
Author of more than 500 papers, including 98 original peer reviewed research papers.

Research Institute of Horticulture

Skierniewice, Konstytucji 3 Maja 1/3, POLAND

Research Institute of Horticulture

ORGANIZATIONAL STRUCTURE



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Research Departments

Laboratories

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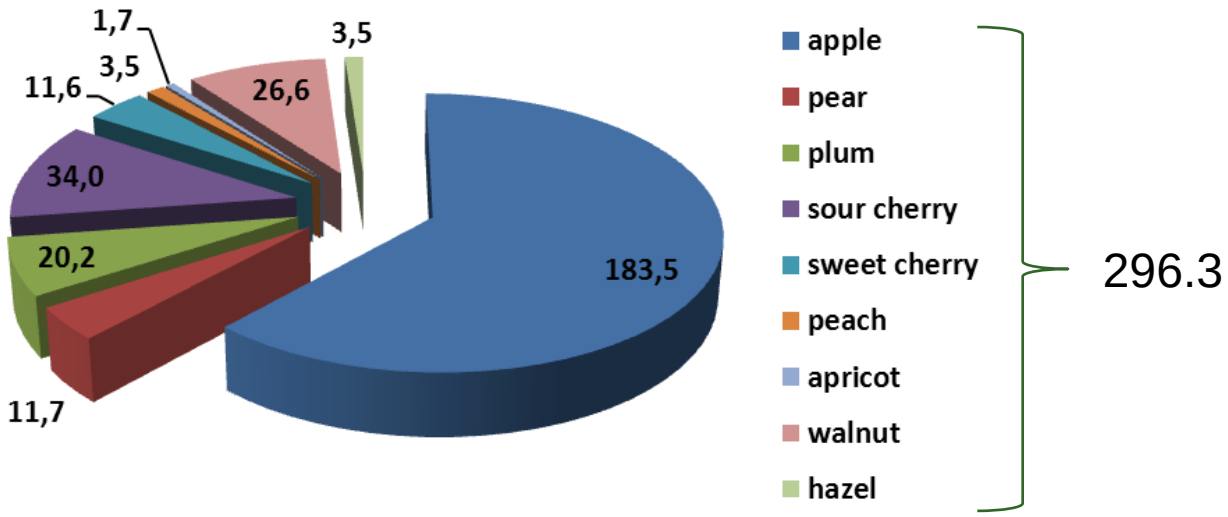
Department of Fruit Plant Protection

Head: Prof. P. Sobiczewski

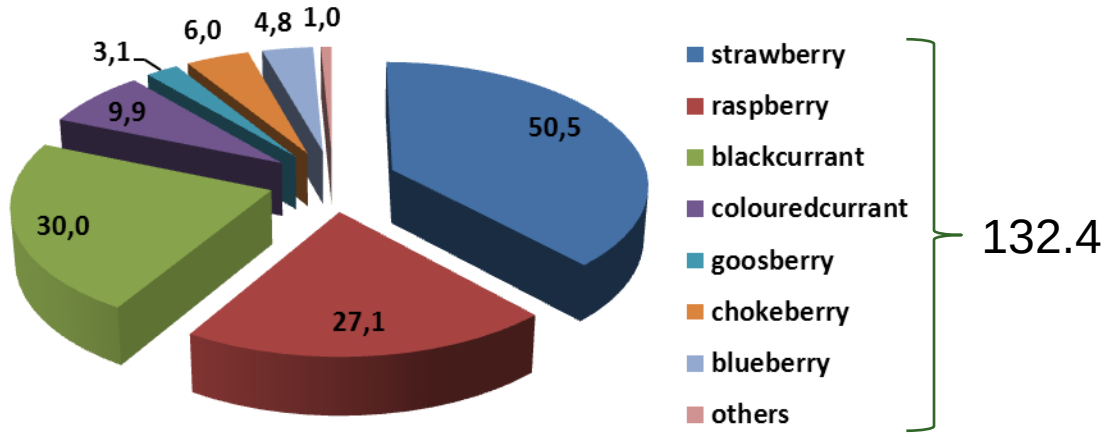
- Laboratory of Applied Entomology - Prof. B. Łabanowska
- Laboratory of Plant Pathology - Prof. P. Sobiczewski
- Laboratory of Virology - Prof. M. Cieślińska




Area of fruit tree production in Poland (in thousands ha)



Area of small fruit production in Poland (in thousands ha)



Laboratory of Plant Quarantine Pathogens

Fot. D. Rasz-Zając

LABORATORY EQUIPMENT

High speed centrifuge

Real time PCR machine

Biohazard laminar chamber

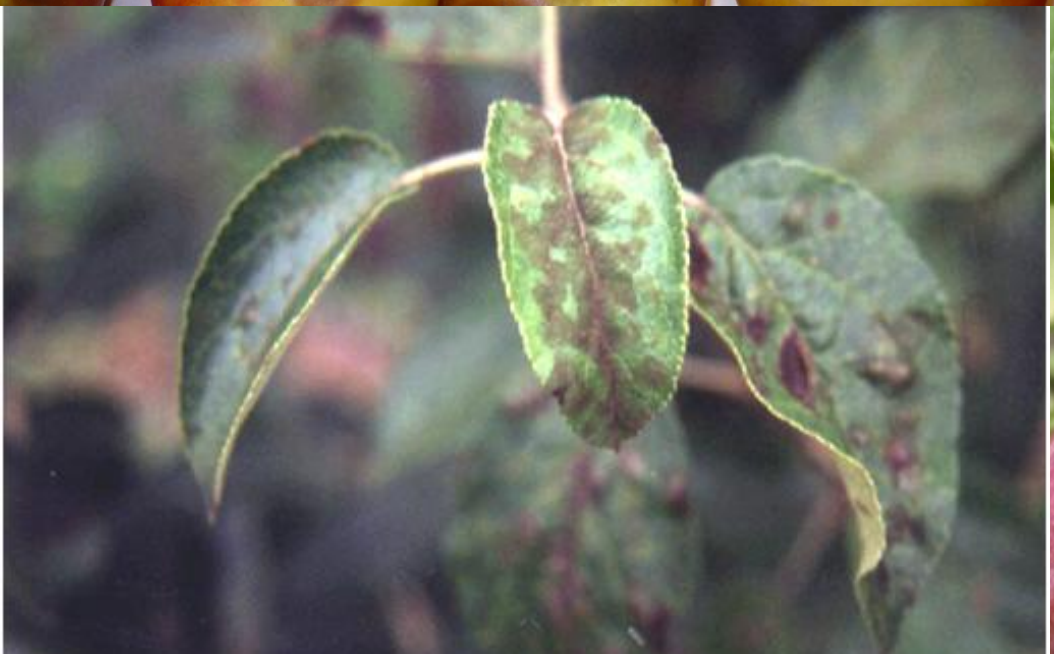
4 x PCR machine

Deep freezer -80C


Modern glasshouse complex ~ 2000 m²

Experiments on apple genotypes susceptibility to fire blight

Plant Pathology Lab.



Main research topics:

- Biology of pathogens, diseases etiology and control,
- Use of molecular techniques in study on pathogens diversity and diseases diagnostics,
- Evaluation of plant resistance to diseases
- Determination of pathogens resistance to fungicides
- Prediction of diseases occurrence
- Biological efficacy of fungicides
- Proecological methods for diseases control
- Discovery and monitoring new pathogens

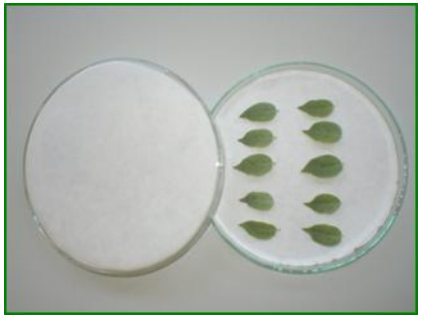
MAIN RESEARCH RESULTS & ACHIEVEMENTS

1. Development of monitoring system for detection of resistant forms of *Venturia inaequalis,* to fungicides, (causal agent of apple scab)

A. RESISTANCE TO STROBILURINS

► Test on detached apple leaves

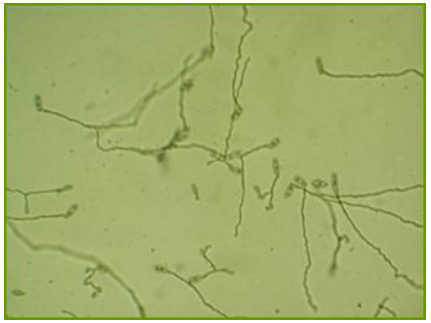





B. RESISTANCE TO DODINE AND ANILINOPYRIMDINES

► Conidia germination test on PDA



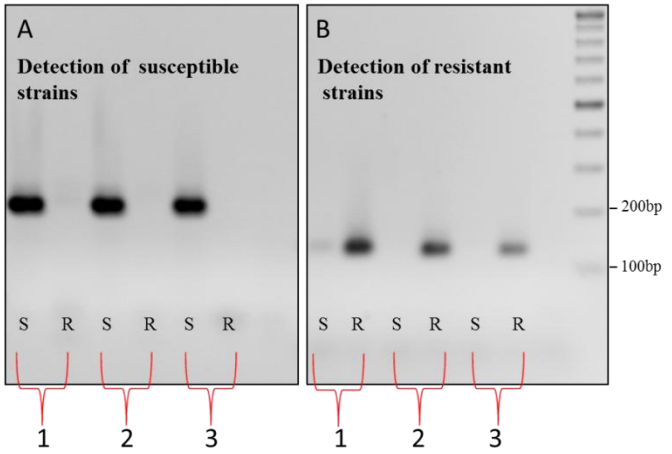


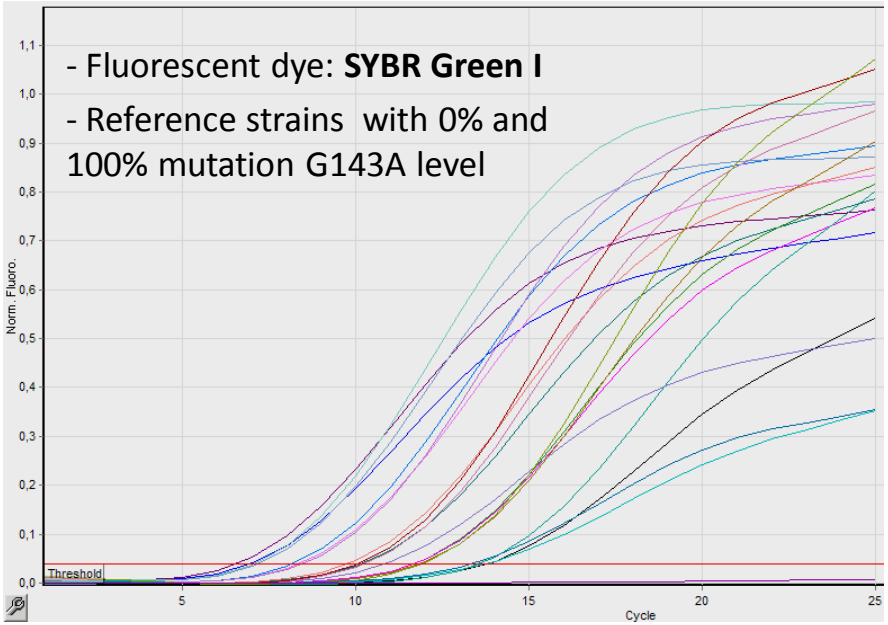
C. Detection of G143A mutation in *Venturia inaequalis* populations using real-time PCR assay

- The **G143A (C -> G)** mutation in *cytochrome b* of ***Venturia inaequalis*** was detected in field samples, derived from Polish orchards with QoI (strobilurins) program applied in which low efficacy is observed.

- **The real-time PCR assay, enabling for the detection of G143A mutation level in *Venturia inaequalis* field samples is proposed**

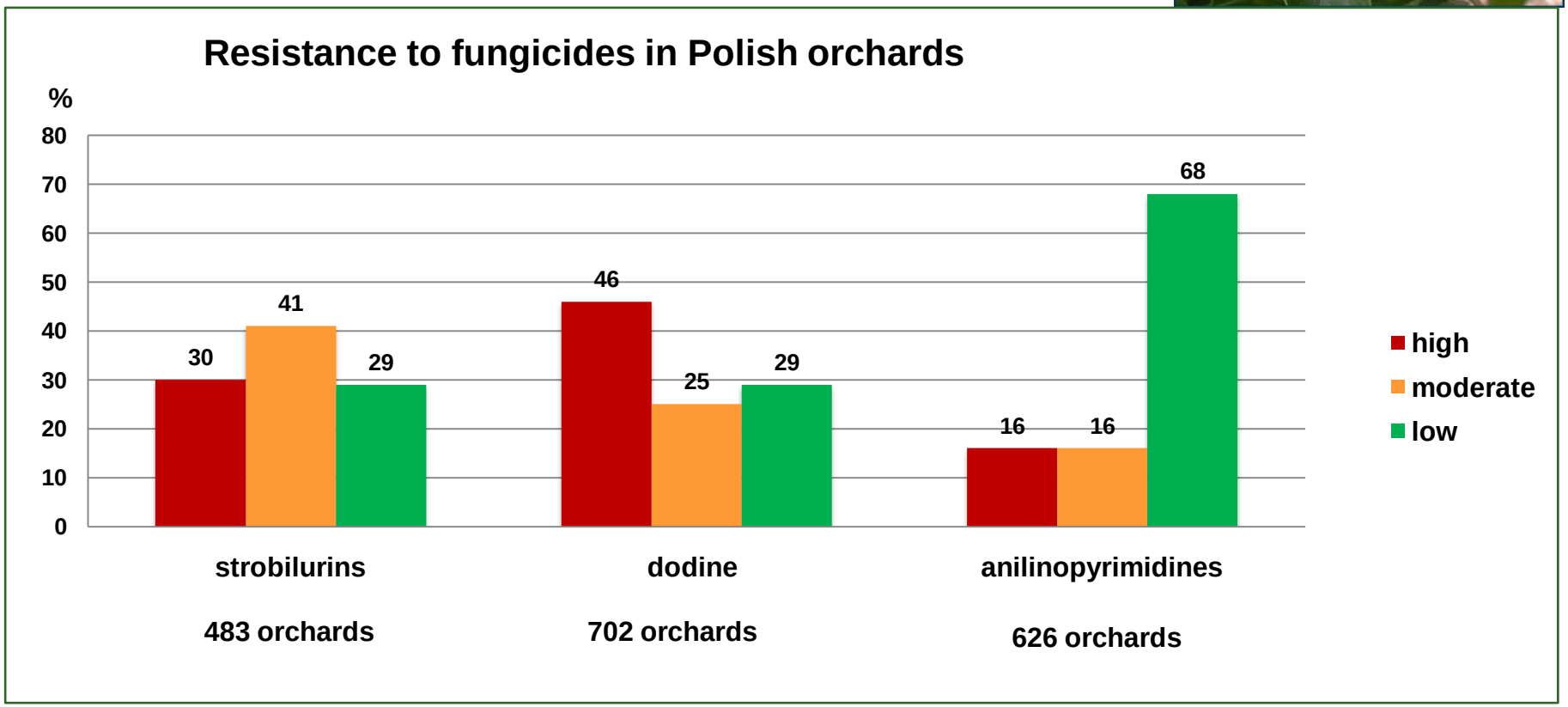
=> Useful when traditional methods fail....





RESULTS: monitoring of *Venturia inaequalis* resistance to:

- strobilurin (2004-2012)
- dodine (2004-2012)
- anilinopyrimdines (2008-2012)

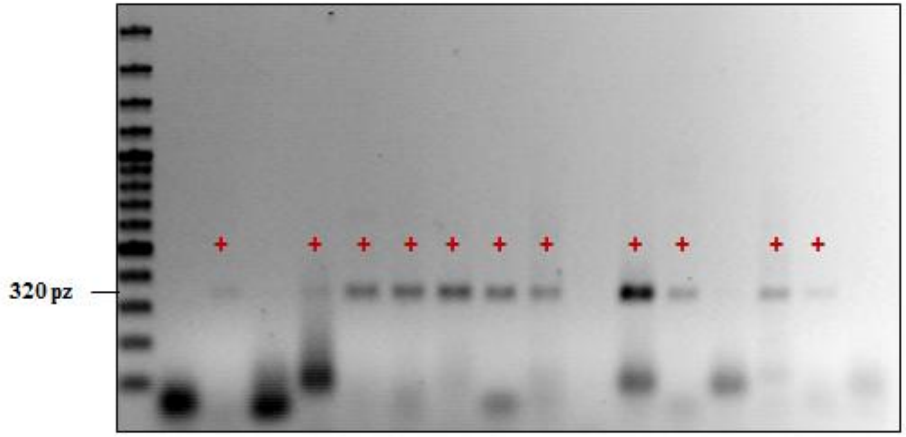


2. Detection of *Verticillium dahliae* in soil samples using nested-PCR assay

• Optimization of DNA isolation from soil according to the protocol of Garcia-Pedrajas et al. (1999)

• Amplification with the primers specific to *V. dahliae* in nested-PCR (Kuchta et al. 2008)

• Application of nested-PCR assay allows for quick (2-3 days) and sensitive detection of small quantity (1 propagation unit/g) of pathogen in examined soil samples (vs traditional method – 4 weeks).



3. Monitoring of the occurence and spreading of two new pathogens *Monilinia fructicola* and *Monilia polystroma* causing brown rot of fruit trees

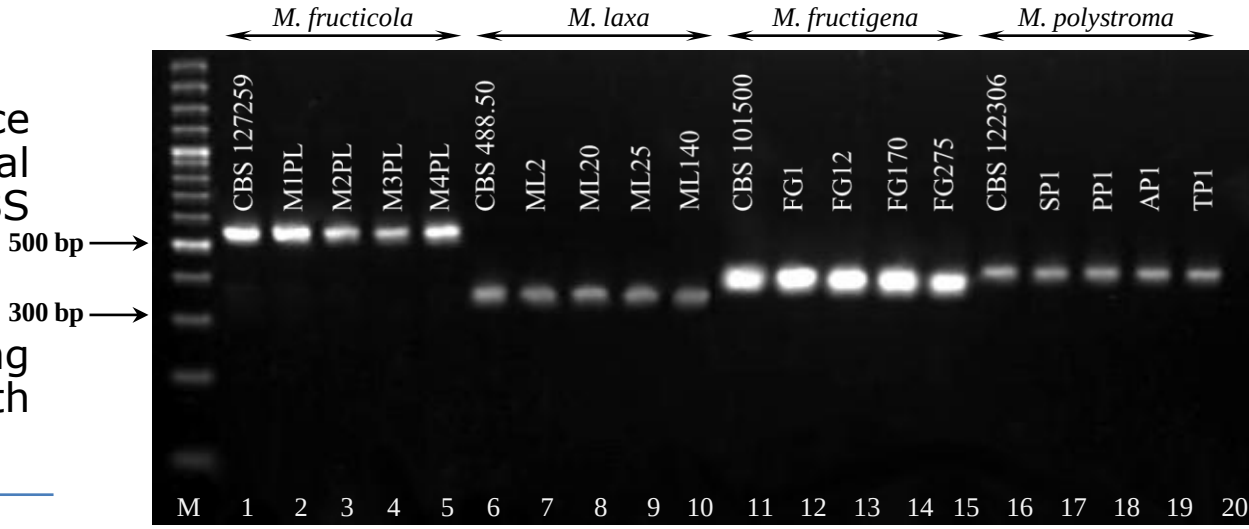







Molecular identification:

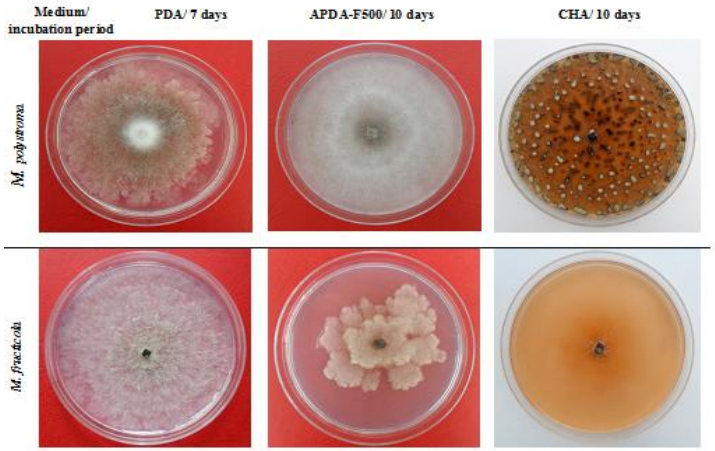
- phylogenetic and sequence analyses of the internal transcribed spacer (ITS1/5.8S rDNA/ ITS2) region of ribosomal DNA
- on the base of non–coding region of *Monilinia* spp. with unknown function.



Specific products obtained in multiplex PCR amplification with MO368-5, MO368-8R, MO368-10R and Laxa-R2 primers and with DNA isolated from *M. fructicola*, *M. laxa*, *M. fructigena* and *M. polystroma* pure cultures. Lines 1, 6, 11 and 16 correspond to standard *Monilinia* isolates: *M. fructicola* CBS 127259, *M. laxa* CBS 488.50, *M. fructigena* CBS 101500 and *M. polystroma* CBS 122306 respectively. In the remaining lines are products obtained for selected examined isolates: no. 2 – 5 -*M. fructicola*, 7 – 10 - *M. laxa*, 12 – 15 - *M. fructigena* and 17 – 20 *M. polystroma*. NC - is a negative control (NC), containing the reaction mixture with double deionized water instead of DNA. M – molecular DNA ladder (O'Gene Ruler™ 100 bp DNA Ladder Plus, Fermentas, Lithuania).

Morphological characteristic

of *Monilinia* spp. isolates on standard PDA medium and two selective media APDA-F500 and CHA

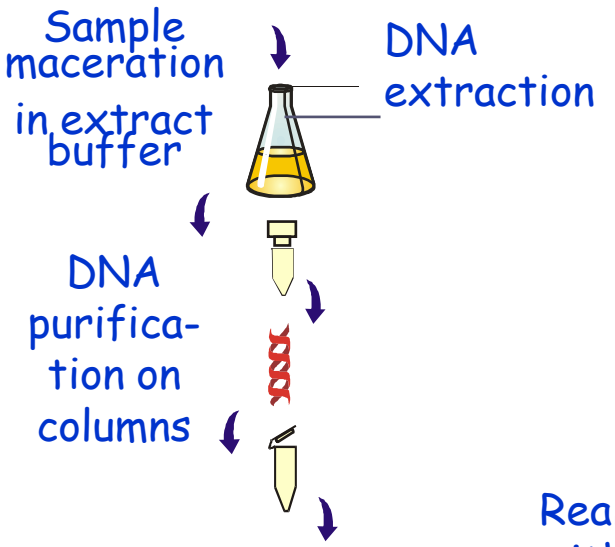


Morphology of four *Monilinia* spp. on tree artificial media: PDA, APDA-F500 and CHA.

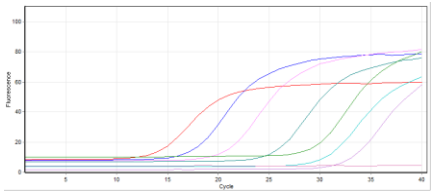
Pathogenicity test of *Monilinia* spp. isolates under greenhouse conditions

Experiments studying brown rot blossom blight of peach

4. Development and implementation of the modern method of fire blight *(Erwinia amylovora)* diagnostics and control



Real – time PCR with primers complementary to pEA29



Detection level: a few cells

Plant protection:

-Selection of effective bacterial strains for biocontrol

-Selection of effectictive protective products of natural origin

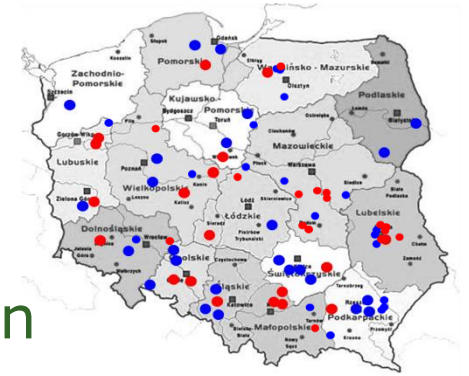
-Selection of apple cultivars resistant to fire blight

-Application of resistance inducers

-Implementaion of disease prediction systems

-Development of integrated plant protection system

5. Biodiversity of bacterial plant pathogens



- monitoring
- isolation, identification
- characterization of pathogens:
  - phenotypic features
  - genetic diversity:
    - DNA fingerprinting methods
    - Sequence analysis of housekeeping genes
    - etc

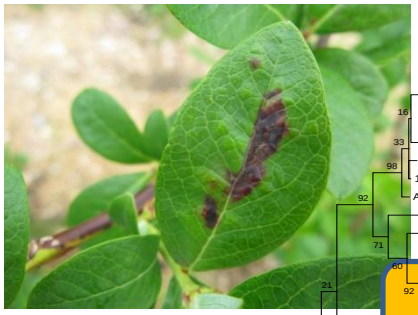
New pathogens discovered

Agrobacterium/Rhizobium

Rhizobium skierniewicense (Puławska et al., 2012)

Rhizobium nepotum sp. nov. (Puławska et al. 2012)

Pseudomonas



Pseudomonas sp. under study

Applied Entomology Lab.

Main research topics

- Biology, ecology and control of pests
- Mechanisms of plant resistance against pests
- Monitoring and prediction of pests occurrence
- Biological efficacy of pesticides
- Side effect of pesticides – selectivity
- Evaluation of some biological agents as „tools” of insect pest control, e.g. control of spider mites with predatory mites
- Investigations on pest resistance to some group of insecticides using molecular and biochemical methods

MAIN RESULTS AND ACHIEVEMENTS

1. Control of codling moth (*Cydia pomonella*)

- ✓Biocontrol
- ✓Mating desrupition (desintegration of males)
- ✓‚Attract and kill'

Codling moth (moth and caterpillar) and damaged fruits

A. Biological products tested

- ✓ Spinosad (product of *Sacharopolyspora spinosa* bacteria fermentation) – VERY HIGH EFFICACY
- ✓ Granulosis virus (Cp Gv)
- ✓ Bacterial preparation (*Bacillus thuringiensis*)
- ✓ Azadirachtin – extract from *Azadirachta indica*

EFFICACY
16,5% to 75,7%

B. Mating disruption method

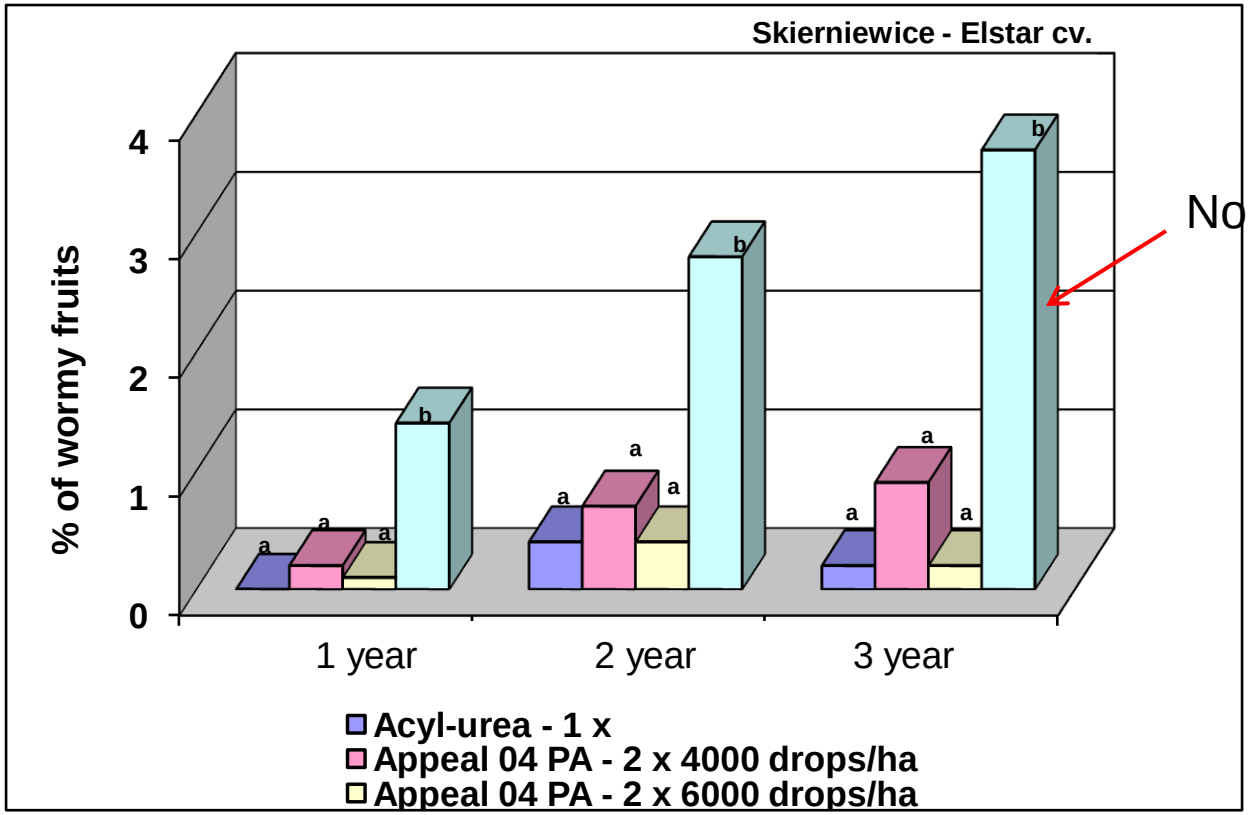
- Ecodian CP
- Isomate CTT

Reduction the level

of fruit damage: 43,7 – 95,8%

C. „Attract and kill” – alternative tactics in the control of codling moth (Appeal 04 PA, Last Call™ CM)

Efficacy of Appeal 04 PA in controling codling moth

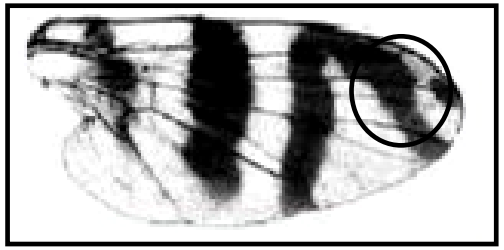


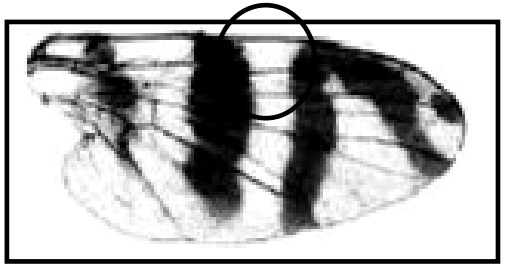
2. *Rhagoletis cingulata*

– new pest discovered on fruits of sweet and sour cherry in Poland

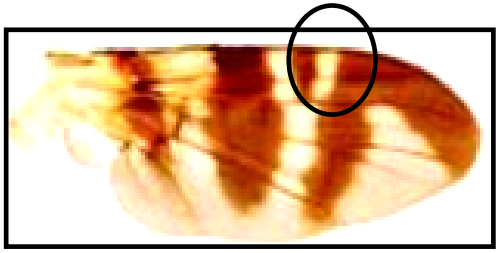
First noted in Polish orchards in 2009; gradually spreading.

Rhagoletis cingulata

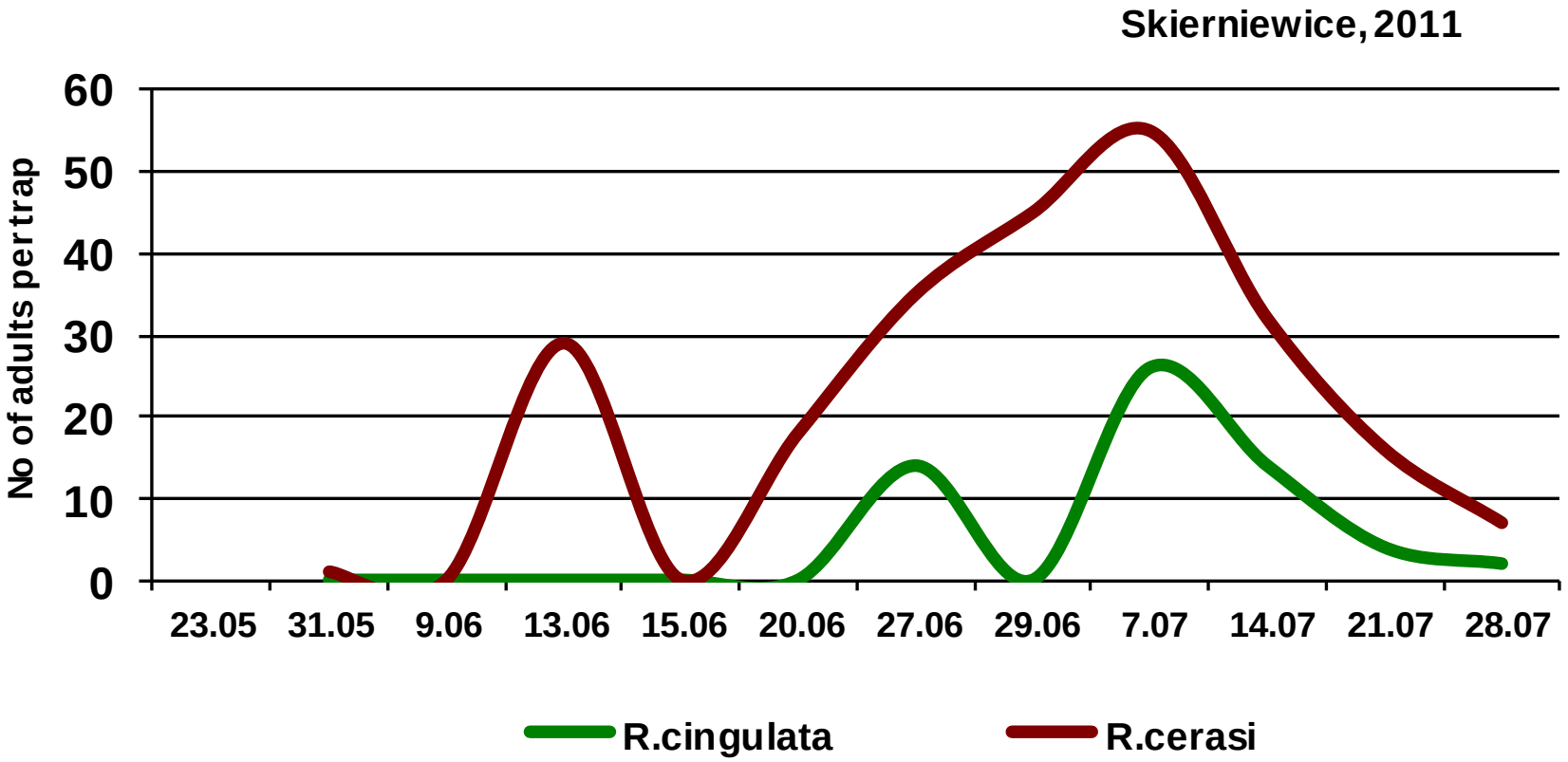




Rhagoletis cerasi



Dynamic of the fly of *Rageletis cerasi* and *Ragoletis cingulata* on sour cherry 'Schattenmorelle'



Very important: optimization of control term and the determination of insecticides safe for humans and environment.

2/ Selection of black current cultivars and clones for high level of resistance to big bud mite (*Cecidophyopsis ribis*)

Polish cultivars and clones:
'Polares', PC -1/4 and PC – 7/9 - ***fully resistant***
'Ores' - ***resistant***,
'Tisel' - *moderately resistant*,
'Ruben', 'Tines' and 'Tiben' - *moderately susceptible*

Big bud mite – damaged buds

Reversion on blackcurrant, damaged leaves and flowers

3. Development of method for large scale introduction into orchards of *Typhlodromus pyri* against phytophagous mites

Laboratory rearing of *T. pyri* using bean plants infested with two-spotted spider mite *Tetranychus urticae*

Plants of bean with phytoseiid mites were introduced inside the canopies of trees

Cloth belts with overwintering predatory females (collected from apple orchards with phytoseiids in the autumn) were placed on trunks in spring.

Efficacy of 80%

4. Pest resistance to insecticides

Colding moth (*Cydia pomonella*)

Green apple aphid (*Aphis pomi*)

Two-spotted spider mite (Tetranychus urticae)

Methods:

A. Preliminary screening (spraying at laboratory and survival check)

B. Enzymatic test (enzymatic activity of multi-function oxidase (MFO) responsible for the detoxification of the insect body.

C. Molecular test – Mutations sodium channel gene (*kdr, Ache*) of *Cydia pomonella* correlated with fosfororganic insecticides resistance.

A. Tab 1.Green apple aphid survival 48 h after spraying with insecticides

active substance	orchard number							
	wild control	1	2	3	4	5	6	7
pirimikarb	0	0	0	0	0	0	+	0
acetamipryd	0	+	0	++	0	++	++	++
tiachlopryd	0	+	0	+	0	+	+	0
tiametoksam	0	+	0	+	0	+	+	+
flonikamid	0	++	++	++	++	++	++	++

„0" - 100% dead, „+" - less than 10 % survived, „++" - more than 10 % survived

Tab 2.Spider mite survival 72 h after spraying with acaricides

active substance	orchard number								
	Positive control	wild control	1	2	3	4	5	6	7
pirydaben	+	0	+	0	0	0	+	0	0
fenpyroximate	+	0	+	0	0	0	+	0	0
fenazaquin	+	0	+	0	0	0	+	0	0

B. Tab 3. Mfo analysis results of *Aphis pomi individuals.*

	orchard number						
	1	2	3	4	5	6	7
Increased level of enzyme (MFO) relative to control	+	++	++	+	+	+	++

„+" - less than 10 % individuals, „++" - more than 10 % individuals

C.

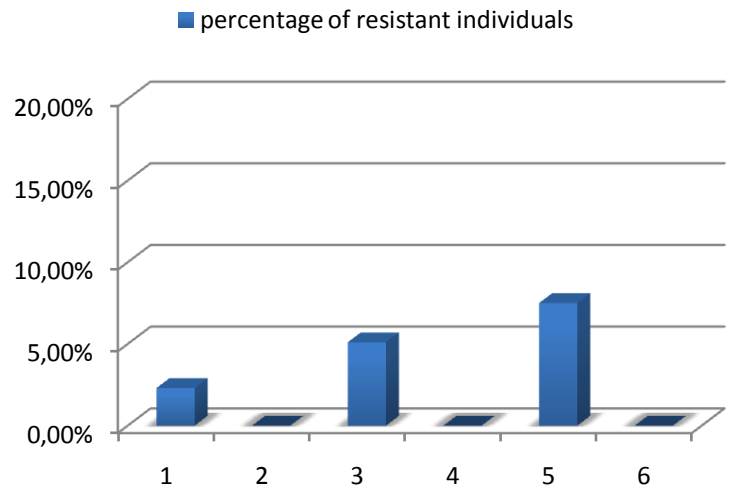


Fig 1. (kdr) resistance.

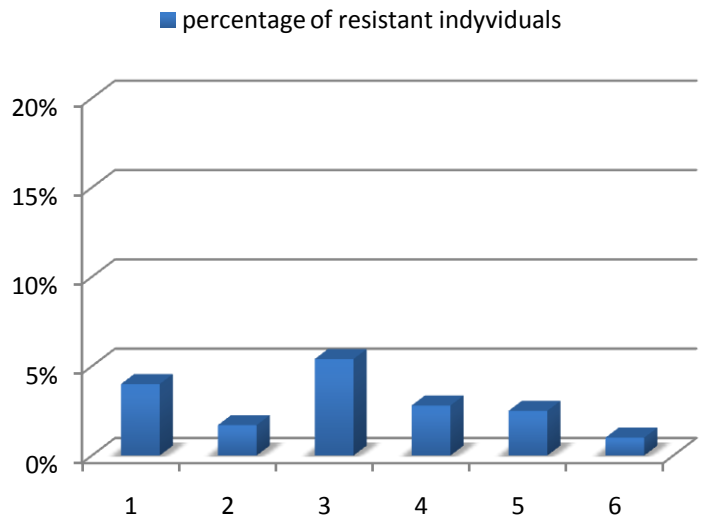


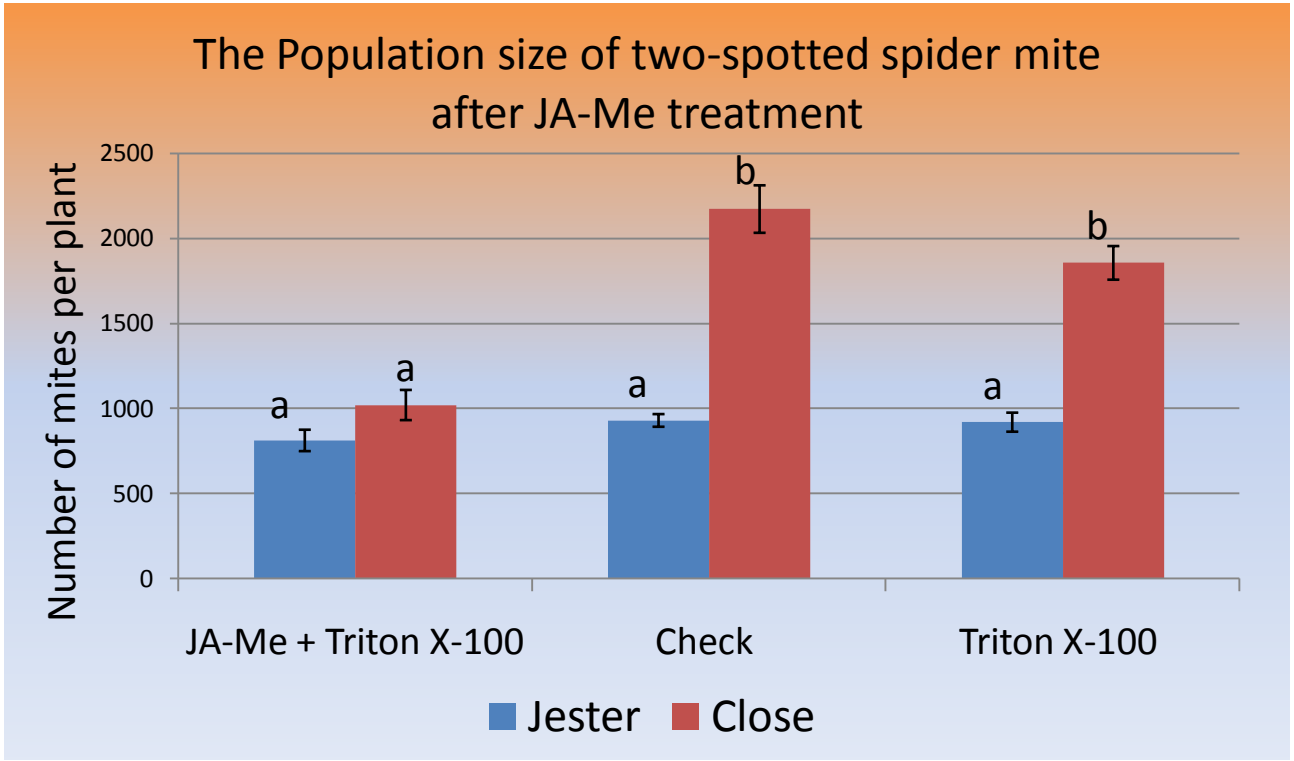
Fig 2. (AchE) resistance

5. The influence of JA-Me treatment of apple trees on population size of two-spotted spider mite

Greenhouse

Environmental chamber

Two-spotted spider mite



Induced direct defense

The level of selected phenolic compounds in leaves of Close and Jester cultivar

Treatment	Chlorogenic acid [µg/g]		Epicatechin [µg/g]		Phloridzin [µg/g]	
	Close	Jester	Close	Jester	Close	Jester
JA-Me + Triton X-100	121 b	83 b	78 b	211 b	3471 b	3092 b
Triton X-100	70 a	65 a	34 a	113 a	2364 a	2311 a
Check	53 a	63 a	34 a	90 a	2366 a	2213 a

Projects of Fruit Plant Protection Department

Last 5 years

- 23 finansed by Polish Ministry of Science
- 1 Special project ordered by Polish Ministry of Science
- 3 projects in 6th and 7th FP
- 1 Life and Environment EU Project

Project :

„Biological efficacy of chemical products against diseases and pests of fruit crops” (cooperation with fitopharmaceutical industry) , Registration of about 50 pesticides

International coopeartion

- **Cost Action 864:**
  "Combining traditional and advanced strategies for plant protection in pome fruit growing (2006-2011)", : (MC, EC)

- **Cost Action 873:**
  „Bacterial diseases of stone fruits and nuts" (2008-2013) (MC)"

- **Cost Action 924:** *„Enhancement and preservation of quality and Heath promotion components in fresh fruits and vegetables (2004-2008)".*

- **Cost Action FA0807:** *„Integrated managment of phytoplasma epidemics in different crop systems (2009-2013)", (MC).*

- **COST Action FA1104**: ,,*Sustainable production of high-quality cherries for the European market'' (2012-2016)*

International cooperation

- **INRA Angers, France:**
  „Genetic diversity of *Venturia inaequalis* populations”

- **Istituto Sperimentale per la Frutticola, Roma, Italy**
  „Characterization and genetic diversity of *Pseudomonas syringae* from stone fruits and hazelnut using rep-PCR and MLST”

- **Project Vinquest:**
  „*Venturia inaequalis* pathotypes”
  (Coordination: ACW Wadenswil, Switzerland, 18 countries)

Our main resources and research expertise

- PomoDiseaseas and Pests Image Gallery
- Fruit Pathogen Bank;
- Collection of benefical bacteria for biocontrol

- Diagnostic of fruit diseases and pests
- Evaluation of biological efficacy of PPR for registration purposes
- Detection and identification of unknown fruit pests and pathogens; discovery of new taxons
- Molecular and conventional detection of fruit pathogens and pests resistant to pesticides
- Breeding for resistance

Proposed topics for international cooperation

- Development of integrated strategies to prevent fruit crops against quarantine and new invasive pathogens and pests improving fruit quality and safety.

- Development of sustainable cultivation technologies and the use of valuable fruit cultivars, for the production of high-quality fruits under climate changes.

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Thank you for your kind attention