Development of a New Clubroot Differential Set

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Background

- **Multiple strains of *P. brassicae* are known to exist**
  - **Differ in their ability to infect different host species, lines or cultivars**
  - **‘Physiologic specialization’ = the occurrence of multiple races or pathotypes**

- **Breeding efforts must be guided by a good understanding of pathogenic diversity in *P. brassicae* populations!**

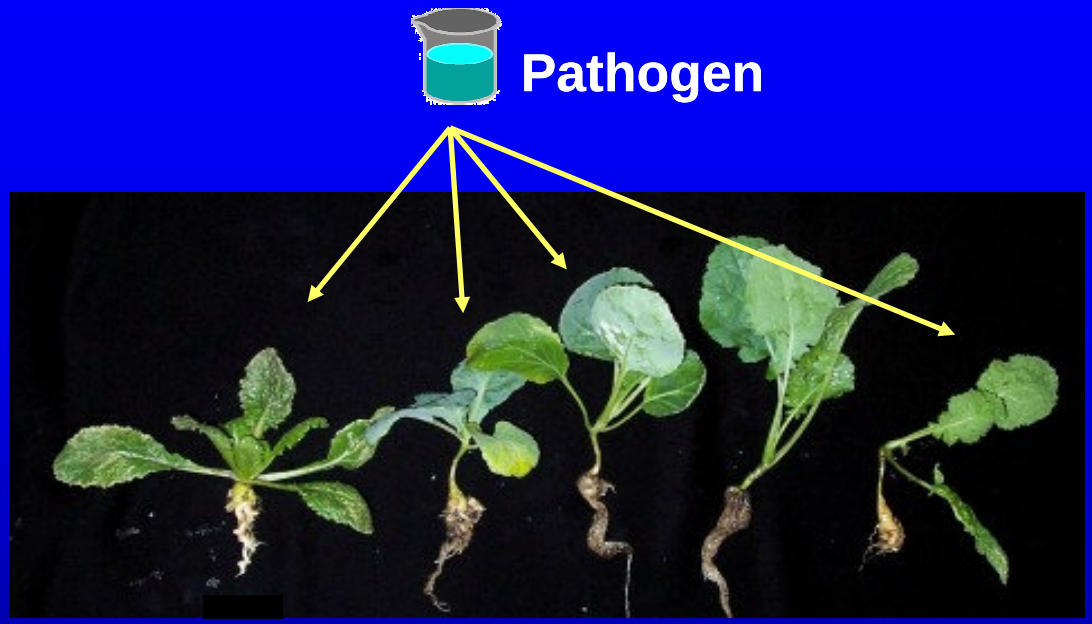
Assessments of Pathogenic Diversity

- **Strains of a pathogen are identified by their virulence on a *host differential set***

Differential Set

= A group of host plants that serve to distinguish between various strains of a pathogen based on disease symptoms

(Definition modified from APS*net*)



Pathogen isolates are grouped into strains based on the symptoms they cause on a defined group of hosts

Clubroot Differential Sets

- Numerous differential sets have been proposed to identify clubroot strains
- Three differential sets are most commonly used:
  - Williams (1966)
  - European Clubroot Differential Set (1975)
  - Differentials of Somé et al. (1996)
- Each has its advantages & disadvantages

Williams' Differential Set

- Developed by P.H. Williams (1966)
- Differential set consists of two rutabagas and two cabbage cultivars
- **Advantage:** Straight-forward and consists of a small set of hosts
- **Disadvantage:** Developed to identify pathogen strains from cabbage and rutabaga

European Clubroot Differential (ECD) Set

- **Developed by Buczacki et al. (1975) as an 'international system' for strain identification**
- **Differential set consists of three subsets:**
  - ***B. rapa* subset (5 hosts)**
  - ***B. napus* subset (5 hosts)**
  - ***B. oleracea* subset (5 hosts)**
- **Advantages: Information on multiple species, enables comparisons**
- **Disadvantages: Lots of hosts, not all hosts differential; complicated strain nomenclature**

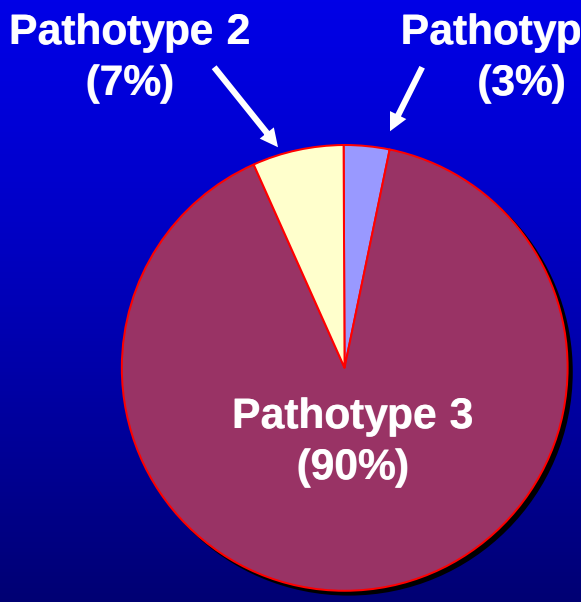
Differential Set of Somé et al.

- Developed by Somé et al. (1996) to identify pathogen strains from France
- Consists of three *B. napus* hosts
- **Advantages:** Straight-forward and consists of a small set of hosts; based on reaction of *B. napus*
- **Disadvantages:** Low differentiating capacity (we can miss strains)

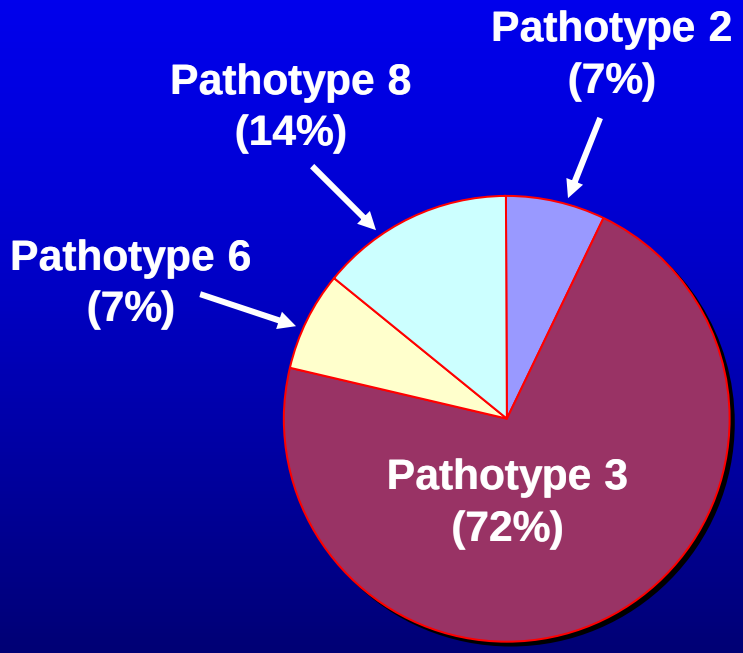
Situation in Canada

- Since the identification of clubroot on canola, we have used all three systems to enable comparisons
- Has been effective in identifying predominant strains, but not a perfect system
- Challenges:
  - Involves a large group of differential hosts
  - Some pathotype distinctions relevant for canola, others are not
  - May not effectively identify all relevant strains

Strains of *P. brassicae* in Alberta



"Field Populations"



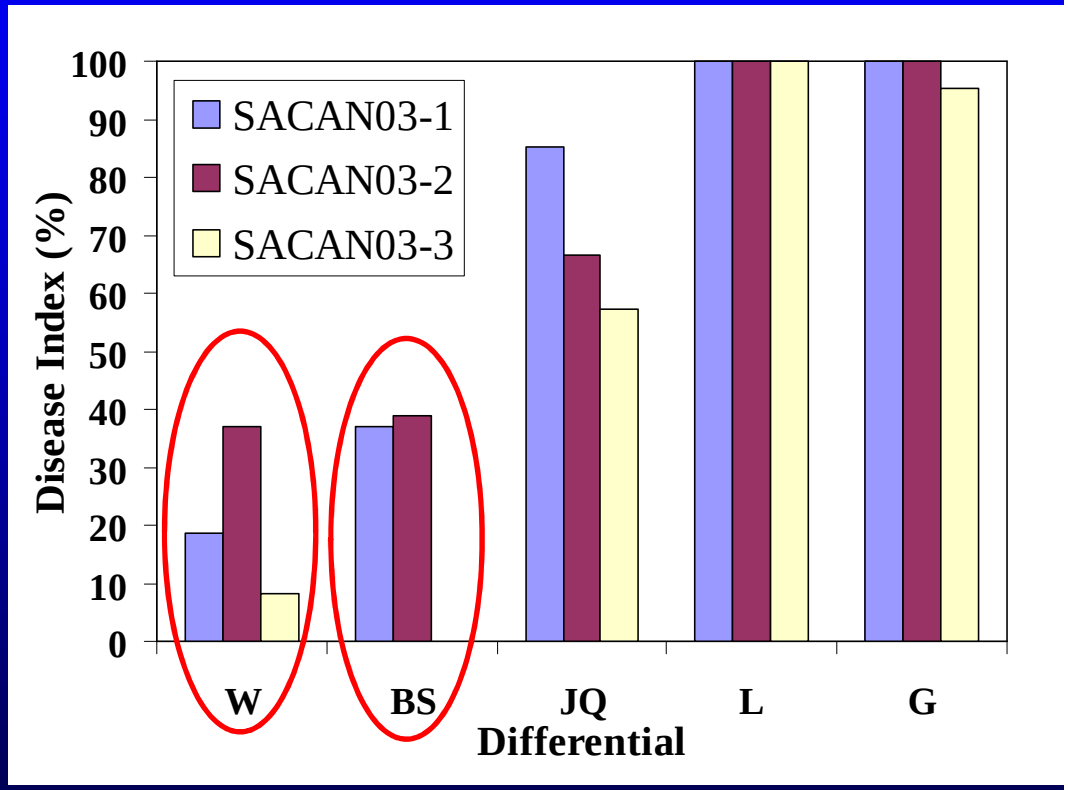
Single-Spore Isolates

Classification on the differentials of Williams (1966)
Pathotype 3 ≈ ECD 16/15/12 or P_2 (Some et al. 1996)

Howard et al. 2010

Strain Identification

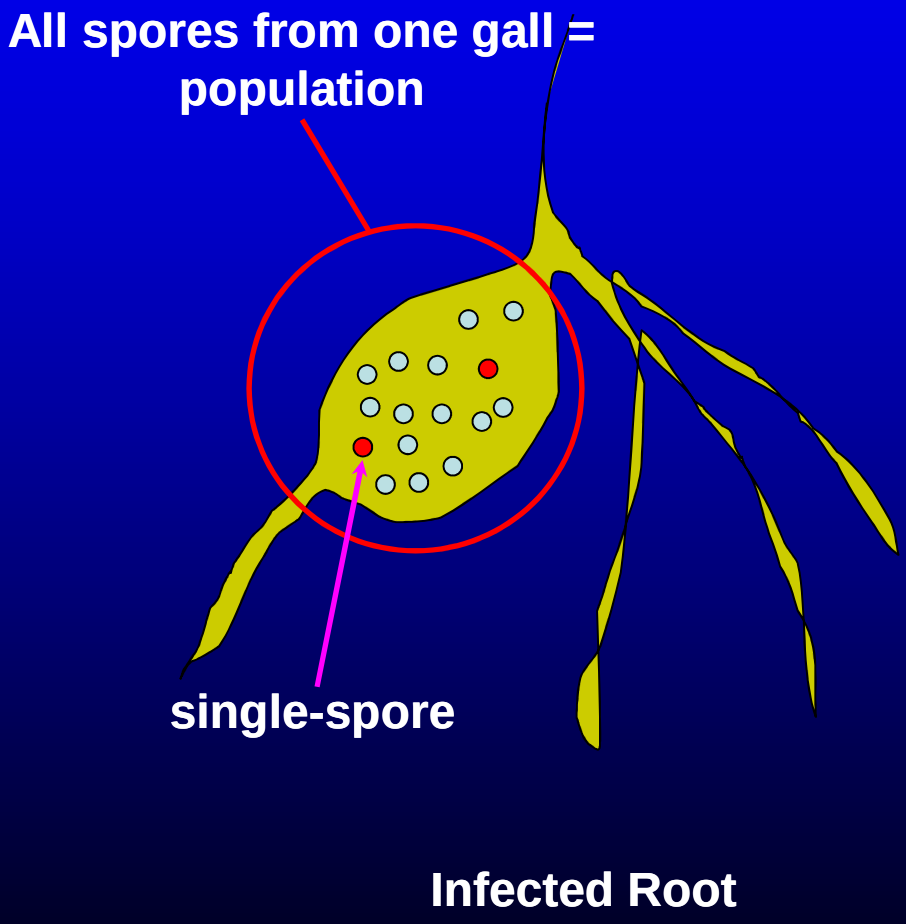
- **Another challenge:**
  - **Some differentials give intermediate and fluctuating disease reactions**
  - **What's a resistant reaction and what's not?**
  - **LeBoldus et al. (2012): host considered resistant if index of disease was <50% and the 95% CI did not overlap 50%**



Adapted from Strelkov et al. (2006)

Fluctuating Reactions

- Largely result of heterogeneity
- In pathogen:
  - Can be addressed by using single-spore isolates instead of populations
- In host:
  - Can be addressed by selecting differentials that give clean reactions



Pathotypes or Races?

- **Largely because of these issues, we refer to clubroot 'pathotypes' instead of 'races'**
- **Terms are largely synonymous <u>but</u>:**
  - **'Pathotype' is a looser term**
  - **More appropriate because neither the differential hosts nor pathogen populations possess genetic uniformity necessary to apply concept of races to the clubroot pathosystem**

A New Differential Set?

- **Given the amount of clubroot work being conducted in Canada and the limitations of existing differentials, a new differential set would be beneficial to identify pathogen strains from canola**

Criteria Required of a New Differential Set (According to Strelkov!)

- **A new set of differentials would have to meet four criteria:**

(1) Good differential capacity

(2) Relevance to canola production

(3) Consistent & clear results

(4) Seeds of differentials must be available

Development of a New Differential Set

- **Using a phased procedure to develop a differential set for *P. brassicae* from canola**
  - **Consultation of literature & previous studies**
  - **Screening of *Brassica* genotypes with representative single-spore isolates & populations from Canada**
  - **Identify subset of putative differentials for screening with wider set of isolates**

Considerations

- **Existing differential sets as a starting point**
  - **Retention of key effective differentials would allow comparisons with literature and international colleagues**
- **Focus on *B. napus* genotypes with good differentiating capacity, but also include some key *B. rapa* genotypes (exclude *B. oleracea*)**
- **Include hosts with IDs < 20% or > 80%**
  - **Avoid hosts with IDs between 20-80% ('indistinct reactions' – Toxopeus et al. 1986)**

Brassica napus

- **Greatest differentiating capacity observed in *B. napus* genotypes (both in our tests & in an international analysis)**
  - **Some can distinguish *between* existing pathotype designations (e.g., pathotype 3 vs. pathotypes 5 & 6)**
  - **Some can differentiate *within* existing pathotypes (e.g., pathotype 6 isolates from BC & ON)**

Differential Host	Original Pathotype Designation (Differentials of Williams)				
	3	5 (AB)	5 (MB)	6 (BC)	6 (ON)
ECD 06	+	+	+	-	-
ECD 07	+	-	+	+	-
ECD 08	+	+	+	-	-
ECD 09	+	-	+	-	-
ECD 10	-	-	-	-	-
'Brutor'	+	+	+	+	-

Strelkov, *unpublished*

MB 'pathotype 5' = AB 'pathotype 3'
ON pathotype 6 ≠ BC pathotype 6 (ON strain attacks only cabbage)

Brassica napus

- **Could also include 'Mendel'**
- **Some commercial Canadian canola cultivars?**
  - **Two cultivars seem to distinguish pathotype 6 from ON & BC**
  - **Cultivar/germplasm resistant to pathotype 3**
- ***B. napus* susceptible check to replace Chinese cabbage ECD 05?**

Brassica rapa

- ***B. rapa*** **(Polish rape) hosts ECD 01 – 04 closely related**
  - **All are resistant to isolates tested from Canada**
  - **Also did not contribute to differentiation in an international analysis (Toxopeus et al. 1986)**
    - **Equally well-represented by ECD 03 alone**
- **Worth keeping ECD 02 as resistant check**
  - **Prefer ECD 02 to 03 because of clearer reactions in our tests**
- **Chinese cabbage (ECD 05) as a susceptible check?**

Putative Canadian Clubroot Differentials for Further Testing

Common name	Scientific name	Cultivar or line	ECD No.
Polish rape	*Brassica rapa* var. *rapifera*	Line AAbbCC	02
Chinese cabbage	*B. rapa* var. *pekinensis*	'Granaat'	05
Fodder rape	*B. napus* var. *napus*	'Nevin'	06
Fodder rape	*B. napus* var. *napus*	'Giant Rape'	07
Fodder rape	*B. napus* var. *napus*	Giant Rape Selection	08
Fodder rape	*B. napus* var. *napus*	New Zealand Resistant Rape	09
Rutabaga	*B. napus* var. *napobrassica*	'Wilhemsburger'	10
Spring oilseed rape	*B. napus* var. *napus*	'Brutor'	n/a
Winter oilseed rape	*B. napus* var. *napus*	'Mendel'	n/a
Spring canola	*B. napus* var. *napus*	'Westar'	n/a
Spring canola	*B. napus* var. *napus*	Commercial cv. (R)	n/a
Spring canola	*B. napus* var. *napus*	Commercial cv. (S)	n/a

Advantages of 'Canadian Clubroot Differential' (CCD)

- **Less differential hosts involved**
- **Clearer reactions**
  - **If used with single-spore isolates, perhaps could move to a race nomenclature system**
- **Better suited to detect variation in pathogenicity on *B. napus* as opposed to cabbage or other hosts**
- **Can compare results obtained with CCD with those obtained with *B. napus* subset of ECD and differentials of Somé et al. (1996)**
  - **Facilitate international collaboration & comparisons with historical record**

Next Steps

- **Receive your input!**
- **Inoculate putative differentials with selected single-spore isolates and populations**
- **Finalize list of differentials**
- **Determine race numbering scheme**

Acknowledgements

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