Associations of glyphosate with *Fusarium* diseases and development of cereal crops on the Canadian Prairies

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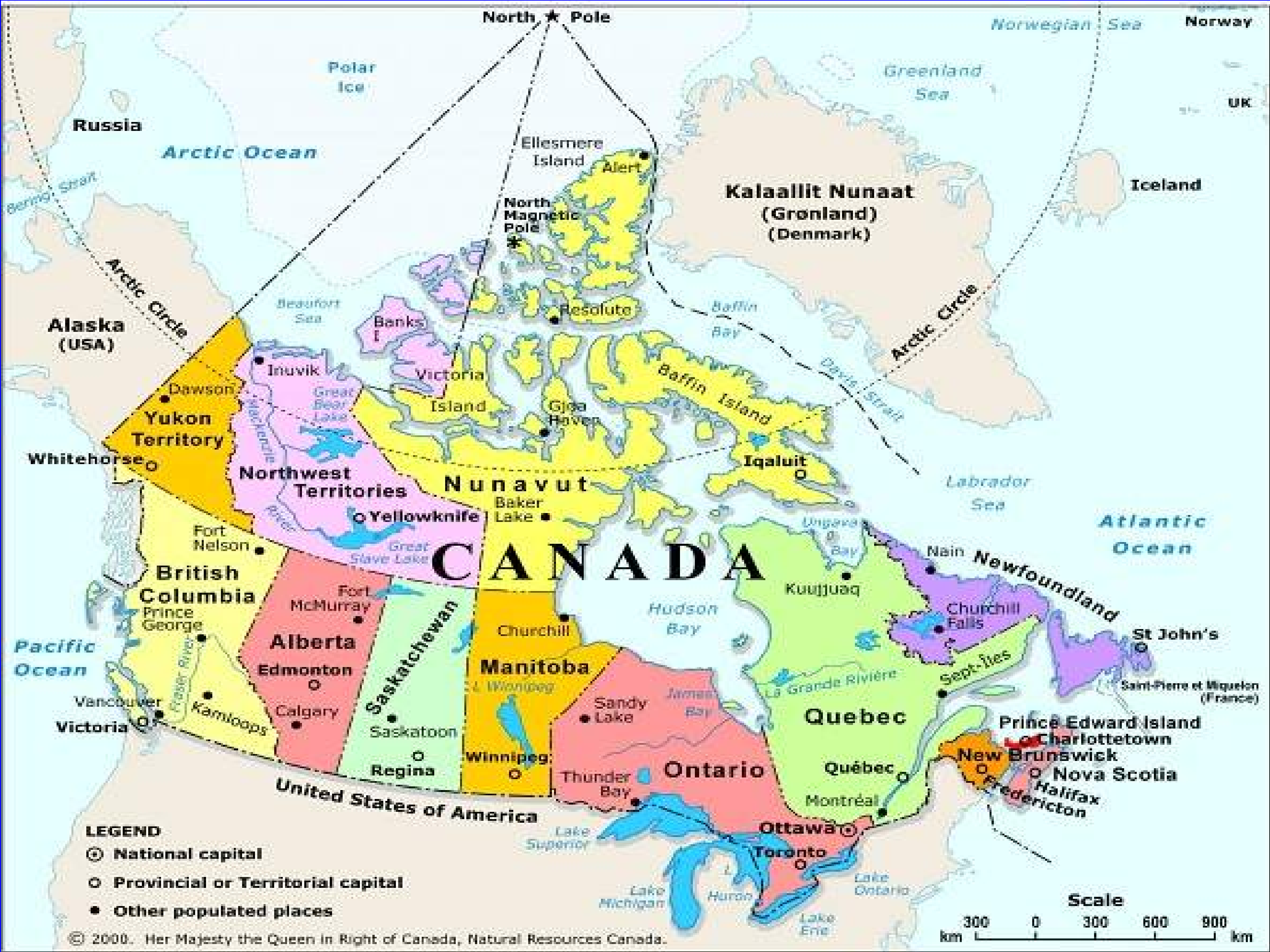

North Pole
Polar Ice
Norwegian Sea
Norway
Greenland Sea
UK
Russia
Arctic Ocean
Ellesmere Island
Alert
Bering Strait
North Magnetic Pole
Kalaallit Nunaat (Grønland) (Denmark)
Iceland
Arctic Circle
Beaufort Sea
Resolute
Baffin Bay
Alaska (USA)
Banks I
Arctic Circle
Inuvik
Victoria Island
Baffin Island
Davis Strait
Dawson
Great Bear Lake
Gjoa Haven
Yukon Territory
Mackenzie River
Whitehorse
Northwest Territories
Iqaluit
Nunavut
Labrador Sea
Baker Lake
Yellowknife
Fort Nelson
Great Slave Lake
CANADA
Ungava Bay
Atlantic Ocean
Nain
Newfoundland
British Columbia
Fort McMurray
Kuujjuaq
Prince George
Saskatchewan
Hudson Bay
Churchill Falls
Churchill
Pacific Ocean
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Calgary
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Prince Edward Island
Charlottetown
New Brunswick
Nova Scotia
Regina
Winnipeg
Ontario
Québec
Thunder Bay
Halifax
Fredericton
United States of America
Montréal
Ottawa
Lake Superior
Toronto
LEGEND
National capital
Provincial or Territorial capital
Other populated places
Lake Ontario
Lake Michigan
Huron
Lake Erie
Scale
300 0 300 600 900
km km
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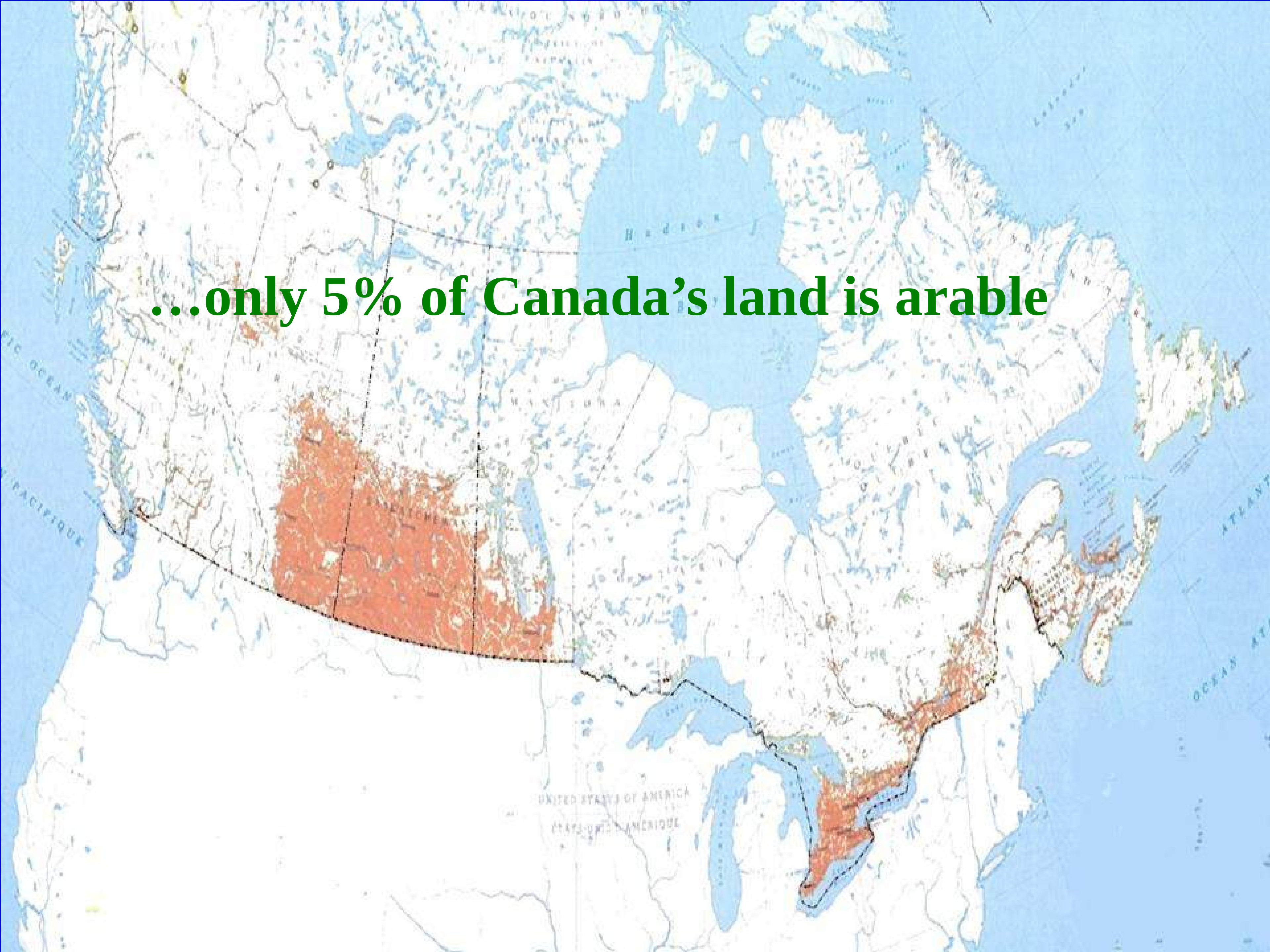
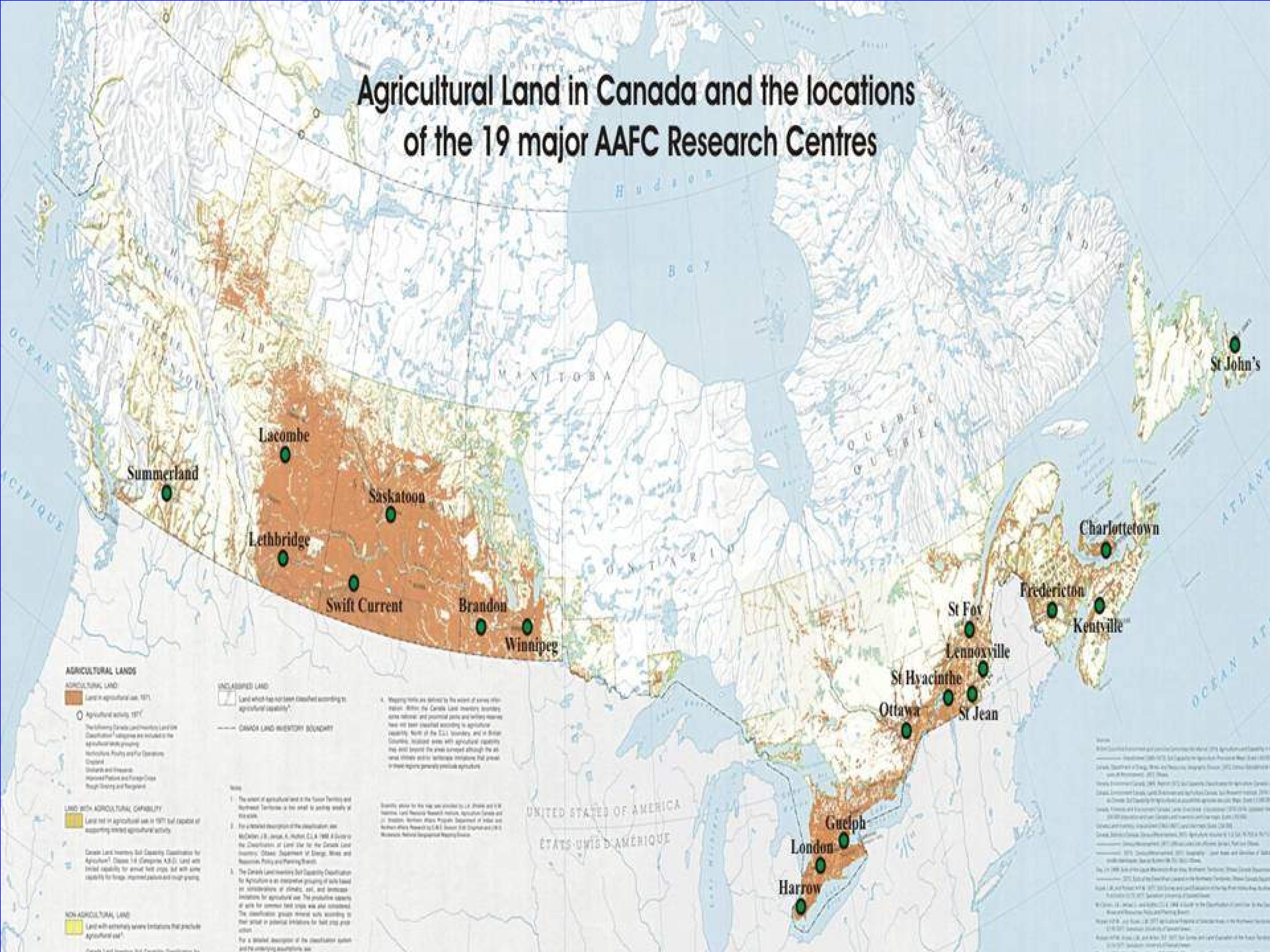
…only 5% of Canada’s land is arable



Agricultural Land in Canada and the locations of the 19 major AAFC Research Centres

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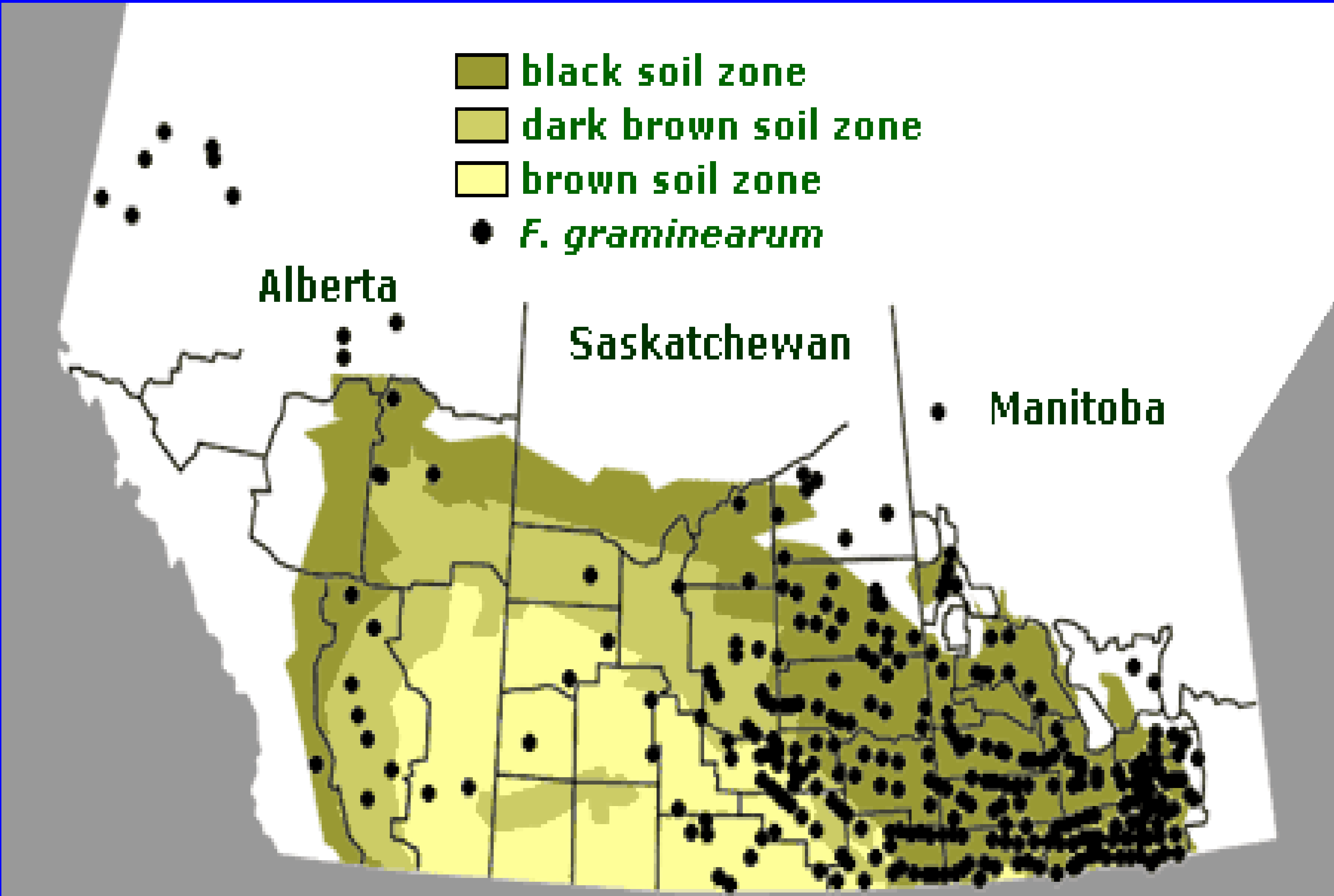
Fusarium head blight causes:

- ✓ reduction in grain yield
- ✓ reduction in quality – low tolerance for *Fusarium*-damaged kernels in top grades
- ✓ accumulation of mycotoxins
- ✓ reduced germination and seedling vigour

- ✓ *main source of fungal inoculum for FHB:*

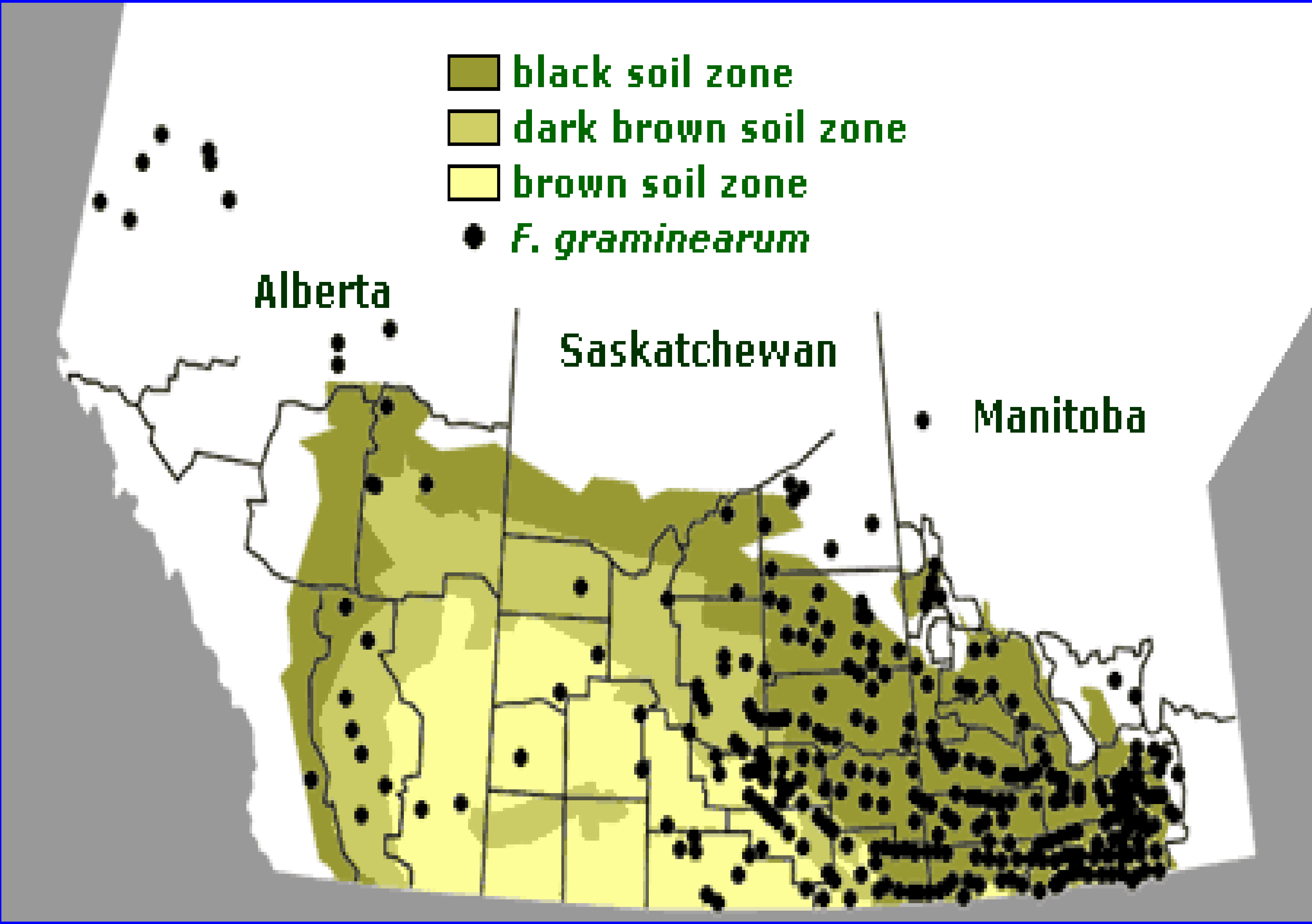
  cereal residues from previous season(s)…



black soil zone
dark brown soil zone
brown soil zone
F. graminearum
Alberta
Saskatchewan
Manitoba

Need to find management practices that:

- *will reduce the damage caused by FHB in areas where it is already well established*
- *prevent its further spread to western regions of the Prairies*


black soil zone
dark brown soil zone
brown soil zone
F. graminearum
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Saskatchewan
Manitoba

Studies on *Fusarium* diseases were conducted in eastern Saskatchewan from 1999 to 2002…

- ✓ 851 commercial cereal fields were sampled
- ✓ cereal spikes were analyzed for incidence and severity of FHB; grain was analyzed for % *Fusarium*-damaged kernels
- ✓ roots/crowns and crop residues were collected from the same fields and fungi analyzed
- ✓ information was obtained from producers regarding agronomic practices in previous 3 years

Several *Fusarium* spp. were found to cause FHB in the wheat and barley crops sampled:

- *F. graminearum*
- *F. avenaceum*
- *F. culmorum*
- *F. poae*
- *F. sporotrichioides*

<u>some were also found in roots and crowns of the cereal and noncereal crops sampled</u>

Effects of agronomic factors on the **FHB index** (caused mostly by *F. graminearum*) of **spring wheat**:

	1999	2000	2001	2002
susceptibility	ns	***	*	ns
previous crop	ns	*	ns	**
tillage system	ns	*	**	ns
glyphosate use	*	**	**	*

*, **, ***: significant at P<0.10, P<0.05 and P<0.01, respectively; ns=not significant

*Effect of glyphosate applications in the previous 18 months on the FHB index (%) in **spring wheat**:*

	none	at least 1 application	*P* value
1999	**0.1** **(n=29)**	**0.2** **(n=60)**	*
2000	**1.7** **(n=48)**	**3.2** **(n=81)**	**
2001	**5.8** **(n=46)**	**9.2** **(n=143)**	**
2002	**0.3** **(n=76)**	**0.5** **(n=137)**	*

*, **: significant at P<0.10 and P<0.05, respectively.

Wheat crops under <u>minimum-till</u>:

Effect of glyphosate applications in the previous 18 months on the FHB index (%):

	none	at least 1 application	*P* value
2000	**1.9** (n=25)	**4.2** (n=40)	**
2001	**5.1** (n=35)	**11.4** (n=79)	***
2002	**0.3** (n=65)	**0.6** (n=68)	*

*, **, ***: significant at P<0.10, P<0.05 and P<0.01, respectively.

Average <u>increases</u> in FHB index in **wheat** crops grown in glyphosate-treated fields in relation to those grown in glyphosate-free fields (2000 and 2001):

- ✓ *75% for all crops*
- ✓ *122% for crops under minimum-till*

spring wheat *– 2001*

# glyphosate applications in previous 3 years	FHB index (%)
none	**4.2**
1 to 2	**6.4**
3 to 6	**12.4**
	*******

- ✓ *environment* was the most important factor in FHB development in eastern Saskatchewan, from 1999 to 2002

- ✓ *application of glyphosate formulations* was the most important agronomic factor associated with higher FHB levels in spring wheat

- ✓ positive association of glyphosate with FHB was *not affected by environmental conditions* as much as that of other agronomic factors…

(Fernandez et al. 2005, *Crop Sci. 45: 1908-1916*)

Effect of glyphosate use (previous 18 mo) on total FHB index, FHB-*Fav*, FHB-*Fg*, FHB-*Fp*, FHB-*Fspo* of barley crops within each tillage system, 1999-2002.

Tillage	Gly	#	FHB-total	FHB-*Fav*[1]	FHB-*Fg*	FHB-*Fp*	FHB-*Fspo*
			---------------------------- Mean % (SE)				----------------------------
CT[2]	No	14	0.8 (0.3)	0.4 (0.2)	0.1 (0.0)	0.0 (0.0)	0.4 (0.2)
CT	Yes	7	2.8 (0.7)	0.4 (0.2)	0.4 (0.2)	0.6 (0.3)	1.5 (0.4)
MT	No	47	1.4 (0.3)	0.1 (0.0)	0.2 (0.1)	0.2 (0.0)	0.7 (0.3)
MT	Yes	76	1.7 (0.3)	0.3 (0.1)	0.4 (0.2)	0.2 (0.1)	0.7 (0.1)
ZT	No	7	0.5 (0.3)	0.3 (0.3)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)
ZT	Yes	36	1.3 (0.3)	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)	0.7 (0.2)

[1]*Fav*: *F. avenaceum*, *Fg*: *F. graminearum*, *Fp*: *F. poae*, *Fspo*: *F. sporotrichioides*.
[2]CT: conventional-till; MT: minimum-till; ZT: zero-till.

Correlation between # of glyphosate applications in previous 18 months and FHB-*Fav* and FHB-*Fg* for barley cultivars under minimum-till, 2000-2002

		R (*P* value)	
Reaction to FHB	# crops	FHB-*Fav*[1]	FHB-*Fg*
susceptible	47	0.115 (0.456)[1]	0.163 (0.289)
intermediate	62	0.439 (0.000)	0.347 (0.005)

[1]*Fav*: *F. avenaceum*, *Fg*: *F. graminearum*

Wheat and barley crops with highest FHB:

- ✓ **susceptible** cultivars
- ✓ under **minimum-till** management
- ✓ grown in fields where **glyphosate formulations** have been used in the previous 18 mo/3 yr
- ✓ crops in rotation with canola crops **(high N and glyphosate use…)**

(Fernandez et al. 2005, *Crop Sci. 45: 1908-1916*; Fernandez et al. 2007, *Crop Sci. 47: 1574-1584*)

- **root rot in barley and wheat…**

(caused mostly by *Cochliobolus sativus* and *Fusarium* spp.)

Effect of glyphosate use (previous 18 mo) on the percentage isolation of fungi from subcrown internodes of barley within each tillage system, 1999-2001

Tillage	Gly	#	Cs[1]	Total *Fusarium*	*Fav*	*Fc*	*Fg*
			------------------------------- Mean % (SE)				---------------------------
CT[2]	No	9	59.6 (6.1)	16.2 (4.7)	4.0 (1.9)	4.5 (3.4)	0.0 (0.0)
CT	Yes	7	51.5 (4.0)	24.4 (4.5)	5.4 (1.7)	5.2 (2.9)	0.0 (0.0)
MT	No	26	56.3 (3.0)	15.5 (2.3)	3.4 (0.9)	1.5 (0.5)	0.9 (0.4)
MT	Yes	55	46.2 (2.6)	23.0 (2.3)	5.1 (0.9)	4.6 (1.3)	2.7 (0.8)
ZT	No	2	61.0 (8.2)	26.8 (8.0)	4.1 (0.1)	0.0 (0.0)	2.1 (1.6)
ZT	Yes	19	43.8 (3.5)	25.9 (2.8)	7.9 (1.5)	2.6 (2.3)	2.1 (1.1)

[1] *Cs,Cochliobolus sativus*; *Fav, F.avenaceum*; *Fc, F.culmorum*; *Fg,F.graminearum.*
[2] CT, conventional-till; MT, minimum-till; ZT, zero-till.

barley and wheat roots…

✓ **change in fungal communities** in roots associated with previous use of glyphosate:

lower levels of *C. sativus* and higher levels of *Fusarium* pathogens in crops grown in fields where glyphosate had been sprayed

(Fernandez et al. 2007, *Crop Sci. 47: 1585-1595;*
Fernandez et al. 2007, *Can. J. Plant Sci. 'in press'*)

- ✓ ***similar results in crop residues sampled from the same fields…***

(Fernandez et al. 2008)

Previous results agree with those from another wheat trial in Saskatchewan…

Three input management systems:

- **High**
- **Reduced**
- **Organic**

Results from 6 years of root rot evaluation:

- ✓ **more *F. avenaceum* and *F. culmorum* (pathogens) in the reduced input system,** and
- ✓ more *F. equiseti* (saprophyte) in the organic system

(Fernandez et al., 2008)

Effects of glyphosate application(s)…

- ✓ previous studies have reported a stimulatory effect of glyphosate on plant diseases and/or fungal communities…

- **no** previous reports on effect of glyphosate on FHB in cereals, or on *F. graminearum*…

- however, there are previous studies on the effect of glyphosate on:

  - *F. avenaceum*
  - other *Fusarium* spp.
  - diseases caused by *Fusarium* spp. in other crops/weeds

- ✓ *Fusarium* spp. shown to act synergistically in causing death of glyphosate-treated plants

- ✓ glyphosate-induced root colonization by *Fusarium* spp.

Johal and Rahe (1984)

Levesque et al. (1987)

Rahe et al. (1990)

Kremer (2003, 2005)

Sanogo et al. (2000, 2003)

Glyphosate effects on *F. avenaceum*:

- ✓ increased root colonization of weeds
- ✓ increased density of propagules in soil

Levesque et al. (1987)

- *due to the nature of our field studies, we were not able to completely separate the effects of **glyphosate** from those of **tillage intensity** and **crop rotation**…*

- it is necessary to determine if increases in cereal head and root diseases caused by *Fusarium* spp. are due to ***direct or indirect effects on the pathogen(s)****…*

- and/or ***direct or indirect effects on the crop****…*

Inconclusive results or discrepancies among published studies on glyphosate:

- ✓ studies conducted in different environments (soil type, weather, etc.)
- ✓ confounding effects of agronomic factors… (i.e. conventional-till/no glyphosate vs. zero-till/glyphosate)
- ✓ different crop species
- ✓ in-crop (RR crops) versus burn-off / pre-harvest / post-harvest (conventional crops) applications

- ✓ sampling done at different stages of plant development, and/or at different times during the growing season and after glyphosate application

- ✓ examined effect of glyphosate applications under field or controlled-environment conditions, **in the absence of weeds or with unknown weed density**

- ✓ studies conducted in lab or greenhouse versus field…

Main objectives of new field trials on the Canadian Prairies:

- to determine a causal effect of glyphosate on diseases caused by *Fusarium* spp., and mechanism(s) responsible for it
- to separate effects of glyphosate from those of tillage and crop rotation on plant diseases, and microbial diversity
- to compare the nutritional status of crops grown in fields treated with glyphosate with those grown in untreated fields

Locations and soil descriptions for study sites:

Site	Soil zone	Texture				Organic	
		Class	Sand	Silt	Clay	matter	pH
			--------------	%	--------------		
Swift Current	Brown	Silt loam	28	49	23	3.0	7.3
Scott	Dark brown	Silty clay loam	31	42	27	4.0	6.0
Brandon	Black	Clay loam	34	32	34	6.7	7.5

Pea-durum wheat trial at Swift Current:

rotation – *tillage* – *glyphosate* (4 reps, split-plot)

Main plots (20 m x 48 m each):

- Rotation: (1) continuous durum wheat, and (2) durum-field pea rotation

Sub-plots (20 m x 12 m each):

- Tillage: (1) zero-till, and (2) minimum-till

- Glyphosate treatments (recommended rate, 0.13 L):
  (1) burn-off with Weathermax before seeding,
  (2) no-glyphosate plots treated only with a non-systemic herbicide (Liberty) before seeding.

Trials at Scott (central-west Saskatchewan) and Brandon (south-west Manitoba):
(glyphosate-free for more than 10 yr)

RCBD, 4 reps

- continuous common wheat under zero-till

- glyphosate treatments:
  - no glyphosate (only Liberty),
  - burn-off applications of Weathermax:
    - (1) 0.13 L
    - (2) 0.57 L
    - (3) 2.19 L

- *at all three locations, high weed populations were simulated by planting winter wheat in the spring for about 3 weeks before the actual trials began…*

Measurements in all field trials:

- ***seedling emergence and plant growth throughout season***
- ***soil and plant tissue analyzed***

- ✓PRS soil probes in first 4 weeks of trials
- ✓size of wheat and pea roots
- ✓root and crown diseases; pathogen identification and quantification
- ✓microbial communities in soil and rhizosphere
- ✓shikimate analysis by Neumann - U. of Hohenheim

Measurements in all field trials:

- ✓seedling emergence and plant growth throughout season
- ✓soil and plant tissue analyzed

- ➢***PRS soil probes in first 4 weeks of trials (2 sets, with a 2-week burial for each set)***

- ✓size of wheat and pea roots
- ✓root and crown diseases; pathogen identification and quantification
- ✓microbial communities in soil and rhizosphere
- ✓shikimate analysis by Neumann - U. of Hohenheim

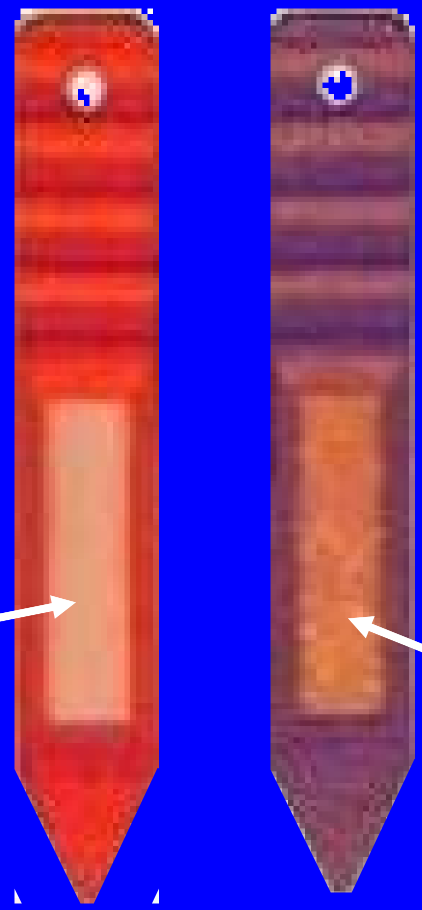
Plant Root Simulator (PRS)™-Probes

Anion PRS™

adsorbs:

NO_3^-, PO_4^-, SO_4^- micros etc.

Anion Resin
quaternary $R\text{-}NH_4^+$



Cation PRS™

adsorbs:

NH_4^+, K^+, Ca^{2+}, Mg^{2+} etc.

Cation Resin
sulfonic acid $R\text{-}SO_3^-$

Developed at the University of Saskatchewan in 1992.

PRS™-Probes *in situ*

- Based on ***Donnan Exchange Principles***
- Act as ion sinks
- Adsorption influenced by:

soil moisture
temperature
buffer capacity and diffusion
mineralization
time of contact with soil

} Plant root environment

Advantages of the PRS^{TM}-probes

- ***Mechanistically similar*** to a plant root
- Continuously adsorbs nutrients in soil solution and those slowly supplied (i.e., dissolution, and mineralization)
- Integrates ***all of the edaphic factors*** affecting nutrient availability
- Adsorbs ***all ions*** simultaneously

- ***initial <u>partial</u> results and <u>preliminary</u> analyses and interpretation…***

Summer of 2007 was much drier and hotter than normal at Swift Current…

Results… wheat and pea in glyphosate treatments:

- *two PRS probe sets in first 4 weeks:*

  there was more N available to emerging plants

- ✓ plants were more lush, greener, had higher %N and %P (and %C?) in leaf tissue
- ✓ lower leaves senesced later
- ✓ plants were taller
- ✓ increased time to heading and physiological maturity

PRS™-probe supply rate (μg/10cm^2/burial length)- Swift Current

FIRST PROBE (1st and 2nd week)

Effect		Total N	Fe	Zn	B	S (x5)
Rotation	Pea	146.3 a				12.9 a
	Wheat(pea)	135.1 ab				8.9 b
	Cont. wheat	108.6 b				8.5 b
	P	***0.042***				***0.069***
Glyphosate	**No**	**107.4 b**	**4.9 b**			**11.7 a**
	Yes	**151.5 a**	**6.7 a**			**8.8 b**
	P	***0.001***	***0.083***			***0.085***

SECOND PROBE (3rd and 4th week)

Effect		Total N	Fe	Zn	B	S (x5)
Rotation	Pea	76.7 ab				1.7 a
	Wheat(pea)	84.0 a				1.4 b
	Cont. wheat	60.2 b				1.4 b
	P	***0.038***				***0.000***
Glyphosate	**No**	**65.1 b**				
	Yes	**82.2 a**				
	P	***0.028***				
rotation X glyphosate				**0.051**	**0.037**	
tillage X glyphosate			**0.095**			

Swift Current trial - 2007:

Treatment	Soil probe N μg/10cm²/burial length
1st and 2nd week	
NO glyphosate	107.4
YES glyphosate	151.5
P	*0.001*
3rd and 4th week	
NO glyphsate	65.1
YES glyphosate	82.2
P	*0.028*

Results… wheat and pea in glyphosate treatments:

- ✓ there was more N in the soil available to plants (two PRS probes sets in first 4 weeks)

- ➢ ***plants were more lush, greener, had higher %N and %P (and %C?) in leaf tissue***

- ✓ lower leaves senesced later
- ✓ plants were taller
- ✓ increased time to heading and physiological maturity


5

Leaf tissue analysis (early collection):

Glyphosate treatments	Total N	N (Kjeldahl)	P (Kjeldahl)	Total C
	------------------------- % ----------------------			
Swift Current				
NO	**5.6 b**	**4.8 b**	**0.31b**	43.0
YES	**5.8 a**	**5.0 a**	**0.33a**	43.3
P	***0.033***	***0.040***	***0.054***	*0.201*
Brandon				
control (NO)	4.6	**4.3 c**	0.35	41.5
0.13 L	5.1	**4.7 a**	0.35	42.4
0.57 *L*	4.8	**4.4 bc**	0.36	41.1
2.19 L	4.9	**4.7 ab**	0.34	42.0
P	*0.270*	***0.035***	*0.398*	*0.382*

Results… wheat and pea in glyphosate treatments:

- ✓ there was more N in the soil available to plants (two PRS probes sets in first 4 weeks)
- ✓ plants were more lush, greener, had higher %N and %P (and %C?) in leaf tissue

- ➢ **lower leaves senesced later**
- ➢ **plants were taller**
- ➢ **increased time to heading and physiological maturity**

Growth of durum wheat in Swift Current trial:

	number of days to:	
	heading	**maturity**
Glyphosate use		
NO	46.8 b	79.1 b
YES	48.3 a	80.6 a
P	***0.000***	***0.001***

Growth measurements of durum wheat - Swift Current:

… *some significant interactions*

Treatment	Effect	Height (cm)
Wheat (pea) (MT & ZT)	*Glyphosate*	
	NO	41.9 b
	YES	44.6 a
	P	*0.075*

Ongoing measurements in all field trials:

✓ seedling emergence and plant growth throughout season
✓ soil and plant tissue analyzed
✓ PRS soil probes in first 4 weeks of trials

- micronutrient analysis of soil and plant tissue
- wheat and pea root growth
- evaluation of root and crown disease severity; pathogen identification and quantification
- microbial communities in soil and rhizosphere
- shikamate analysis of leaf and root tissue by Neumann at U. of Hohenheim

So far…

✓ most significant and consistent difference between glyphosate and glyphosate-free treatments has been in **N (soil and plant tissue)…**

…agrees with previous studies that showed **increased N (and C) mineralization** caused by glyphosate (Haney et al., 2000; 2002)

- ✓ this impact of glyphosate depends on background N and/or mineralization rate…

…*impact most pronounced under very dry/hot conditions, and soils with low organic matter (Swift Current)*

<u>Preliminary results obtained in 2007 explain</u>:

- higher grain yields of cereal crops grown in fields where glyphosate was previously applied…

(highest yields in cereal crops grown after canola – most of which was RR)

- they also explain why glyphosate was the only significant factor affecting FHB development under dry conditions in the surveys conducted in eastern Saskatchewan

(Fernandez et al., 2005, 2007b)

…greater impact of glyphosate on wheat occurs in soils with low organic matter and/or dry conditions because of low mineralization rates???

- our results would also explain higher severity of diseases caused by *Fusarium* pathogens (previous studies showed that *Fusarium* diseases increased with N)…

Most important questions that remain to be answered for the Canadian Prairies:

- mechanism responsible for increased N mineralization???
- long-term impact of increased mineralization with repeated glyphosate applications???
- impact of glyphosate on micronutrient levels?

- whether increases in *Fusarium* diseases associated with glyphosate are of a similar magnitude as those caused by N addition?

- main mechanism responsible for increases in crop diseases caused by *Fusarium* spp. – indirect or direct effects???

Studies on glyphosate effects on plant growth and diseases:

- observations affected by multiple factors, many of which are not yet well-understood…
- difficulty in predicting outcome due to complexity of soil and plant systems, and because many of the results appear to be soil- and environment-specific…

Collaborators:

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