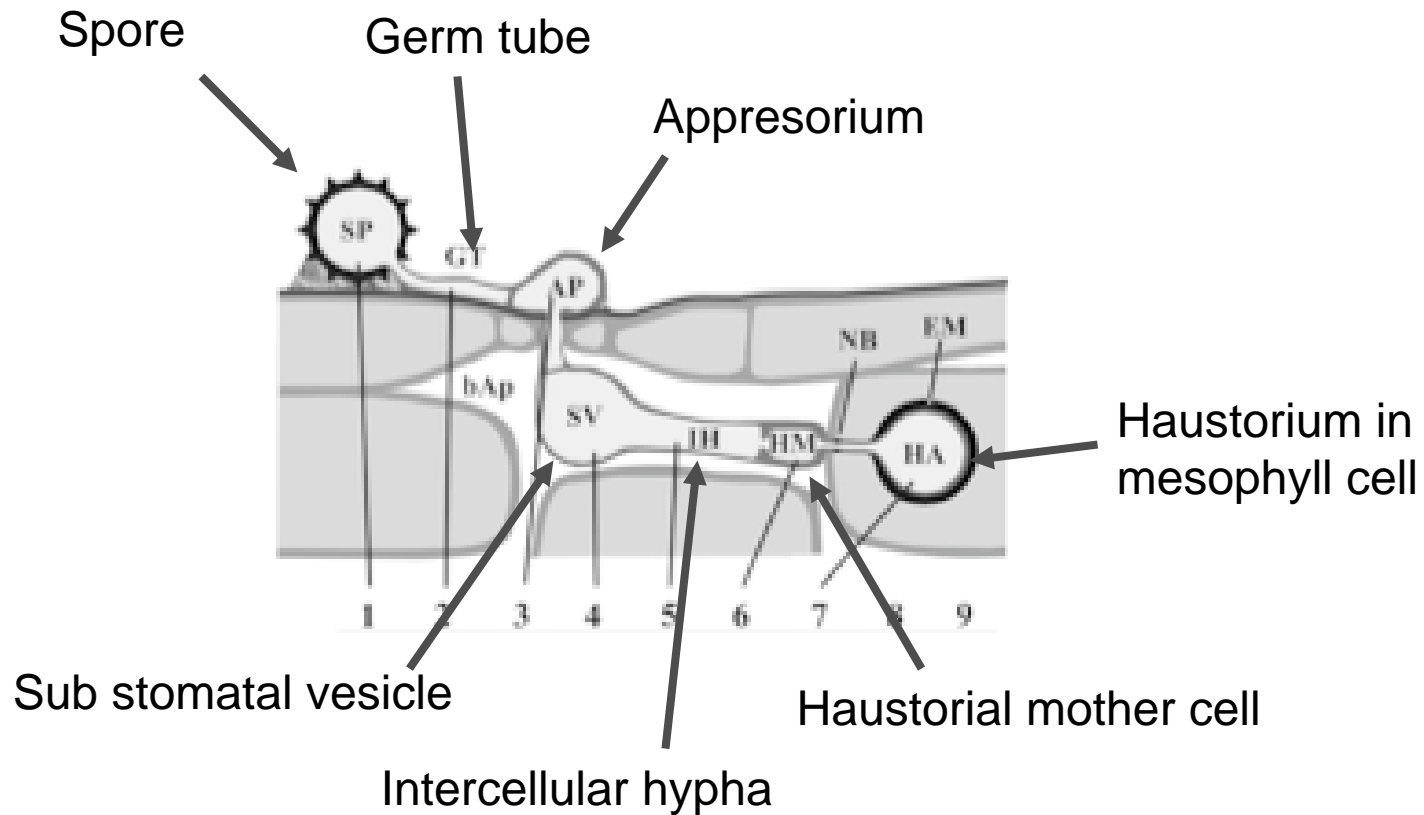


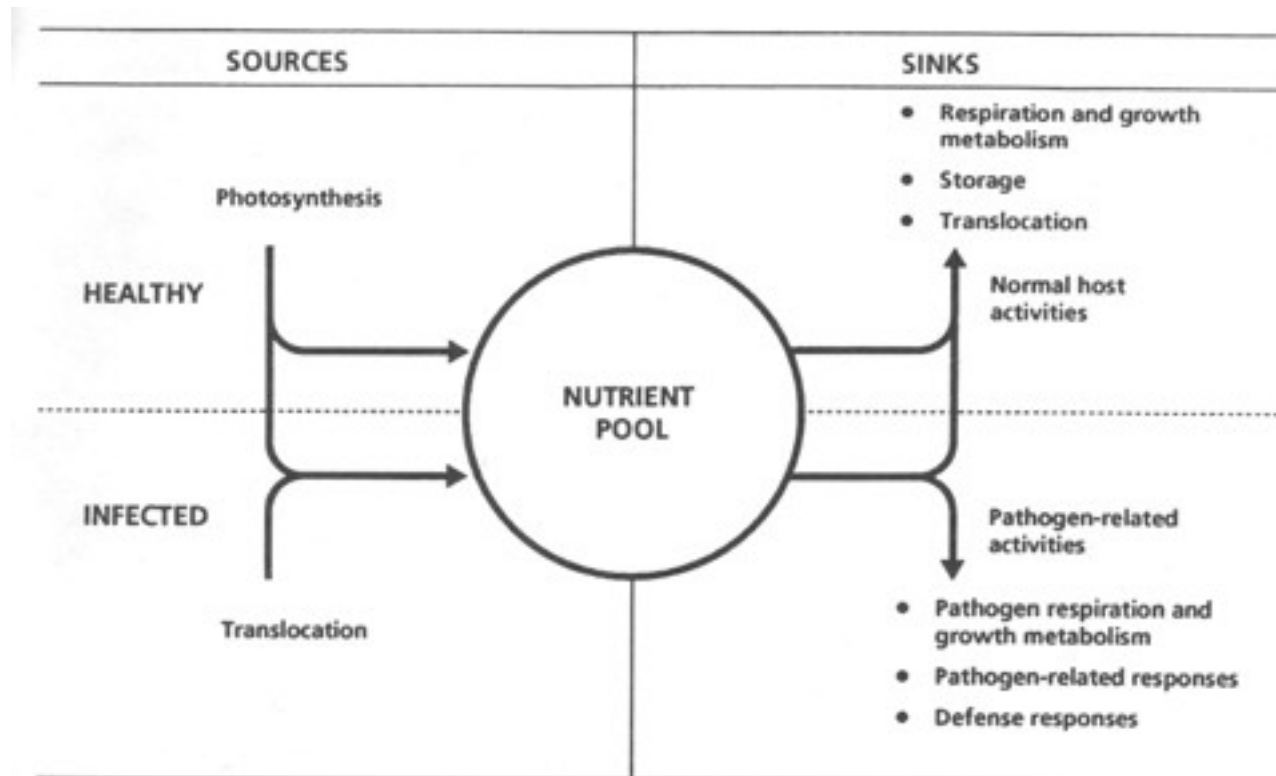
Physiology of Yield Loss: Examples from Other Rusts

Gregory Shaner
Purdue University

Infection structures



Sources and Sinks



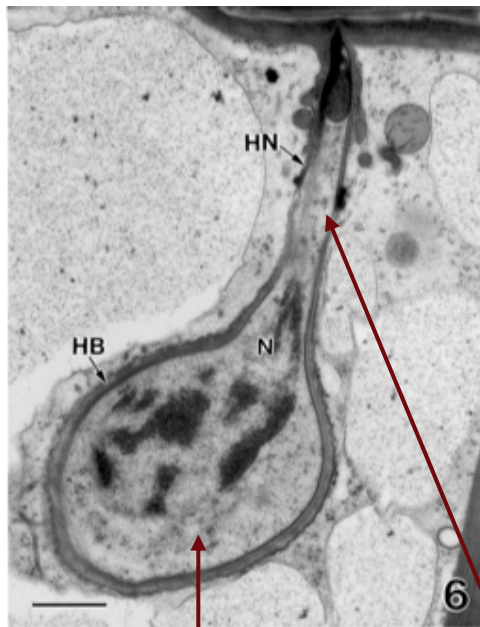
Ayres et al. 1996. P 482 in Photoassimilate Distribution in Plants and Crops

How rusts get nutrients

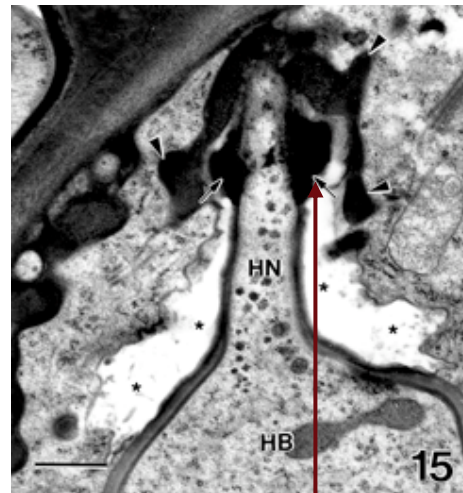
- Haustoria
 - Uptake of nutrients
 - Regulation of host-parasite interaction
 - Haustorium formation in the same host species may differ from one rust species to another
- Intercellular hyphae
 - Direct uptake from apoplast?
 - *Cladosporium fulvum*; downy mildews
 - >80% of colony surface area is apoplastic hyphae in *P. hordei*

Ultrastructure of the haustorium

Frommeëla mexicana var. *indicae* on *Duchesnea indica*

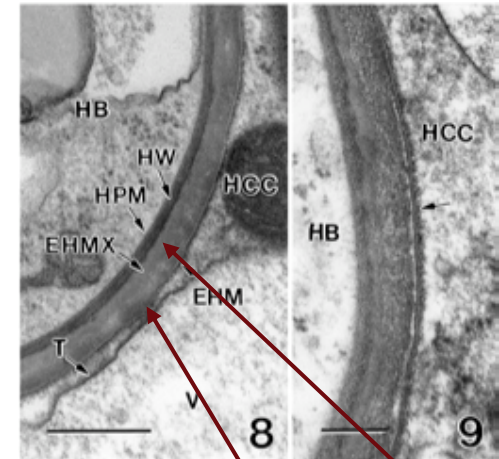


Haustorial body



Haustorial neck

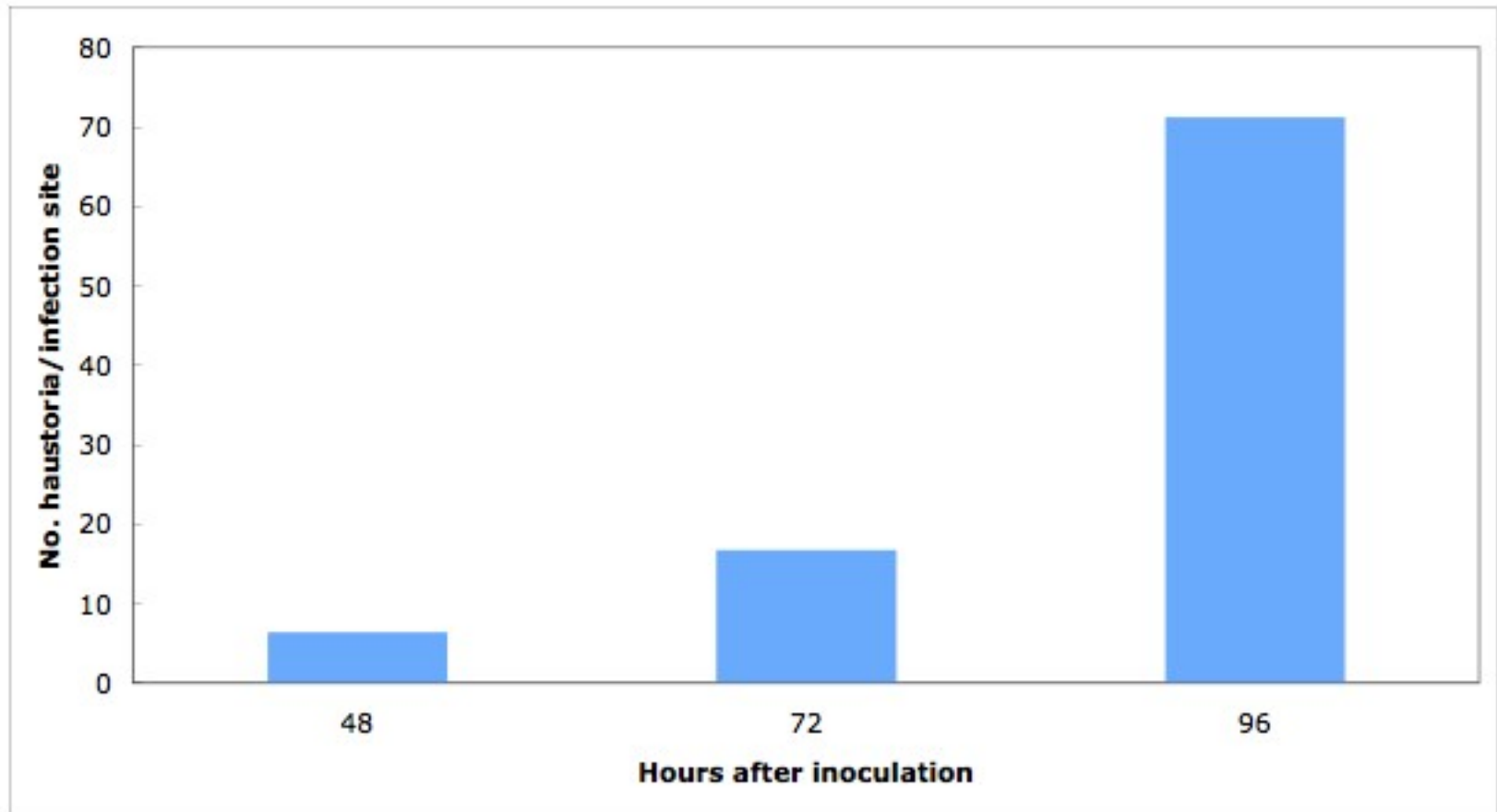
Host wall material associated with neck



Extrahaustorial membrane and matrix

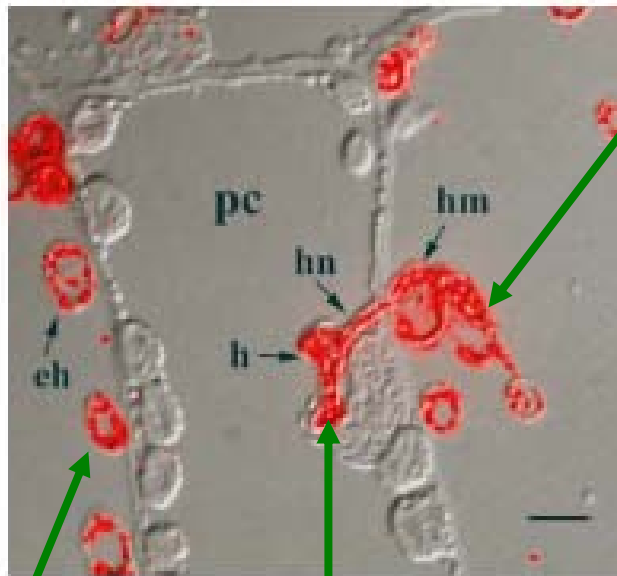
(Mims et al. 2001. Can J Bot 79:49-57)

Number of haustoria per infection site increases rapidly between 2 and 4 days after infection



Data for *Puccinia triticina* on susceptible wheat. Lee. 1985, PhD Diss, Purdue

β -glucosidase is present where *BGL1* is expressed



Haustorial mother cell

- Activity of protein in extracellular space indicates it may aid breakdown of host cell wall for penetration by haustorium.
- Derived hexoses could provide substrate for transporter.

Haustorium

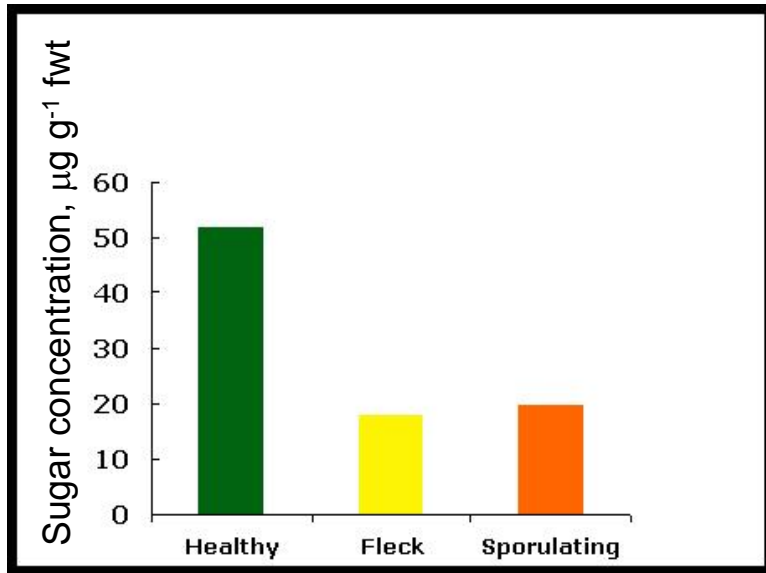
Extracellular hypha

Haerter & Voegelé, 2004. *Curr Genet* 41:96-103.
(*Uromyces fabae*)

Nutrients for rust fungi

- No special nutritional needs
- Sucrose--major translocated photoassimilate in plants
- Can rusts take up sucrose, or must they break it down into hexoses first?
 - Circumstantial evidence suggests glucose is taken up by rust fungi
- Powdery mildew mycelium can take up sucrose, but takes up glucose preferentially (Clark and Hall 1998)

Less soluble sugar in apoplast of rusted barley leaves



- A fungal invertase may break down sugar.
- Phloem cells lack hexose transporters.

Sucrose and glucose are at much lower concentration in infected leaves compared to healthy; fructose about the same.

Nutrient uptake from host

- Not a passive process
- Energy is required to take up nutrients
- Energy comes from host

Membrane-bound transporter in Uromyces fabae

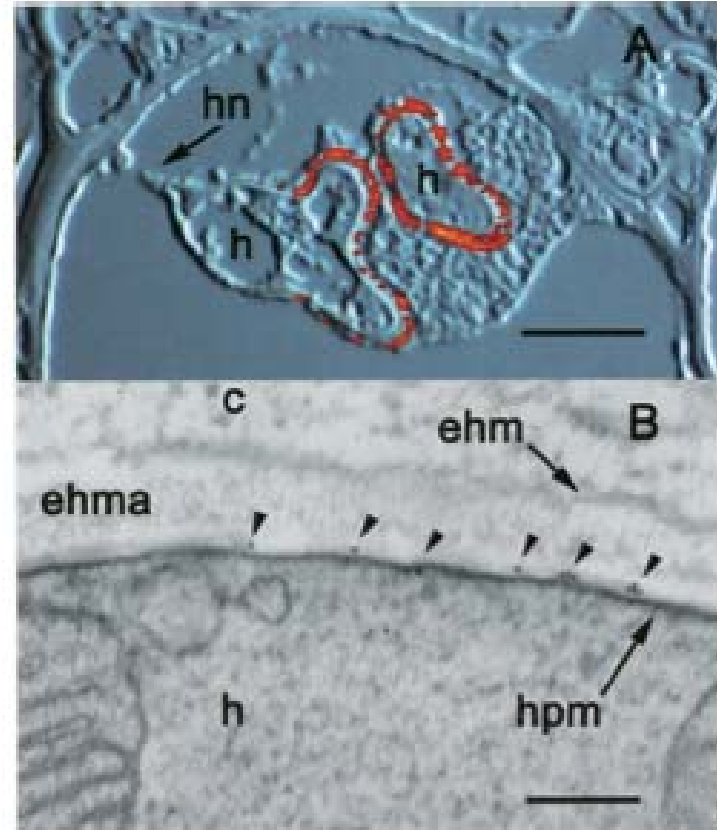
- 30 genes preferentially expressed in haustoria
- *HXT1g* is a single-copy gene expressed only in haustoria
- HXT1p appears to be a hexose transporter
- Accounts for 1.2% of mRNA in haustorium
- HXT1p acquires glucose by a proton symport mechanism (movement into the cell)
- Energy met by ATPase in plasma membrane of haustorium

Voegelé et al. 2001. PNAS 98:8133-8138

HXT1p is present only on membrane of haustorium

Immunofluorescence microscopy reveals expression of *HXT1* exclusively in distal region of haustoria

Gold labeling reveals activity only on the surface of the haustorial membrane



Role of ATPase

- A membrane H⁺-ATPase creates a proton gradient across the haustorial membrane
- This allows a proton symport mechanism for uptake of glucose, fructose, and amino acids
- This gradient is maintained because the extrahaustorial membrane is isolated
 - Extrahaustorial membrane
 - Haustorial membrane
 - Neck band

Uf-PMA1

- Single-copy gene present in *Uromyces fabae*
- Codes for a membrane-bound ATPase
 - Gene has greater homology to ATPases in higher plants than in Ascomycetes
- ATPase levels are much higher in haustoria than in spores or mycelium
 - A consequence of post-translational regulation

Nutrient uptake by haustoria

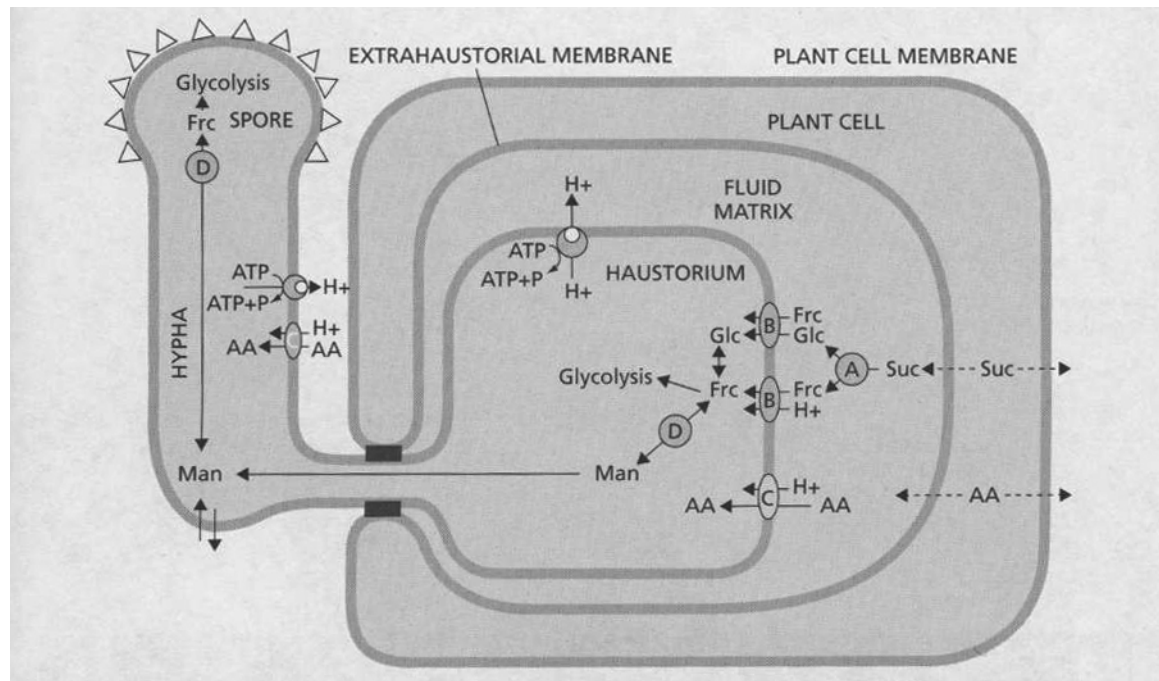
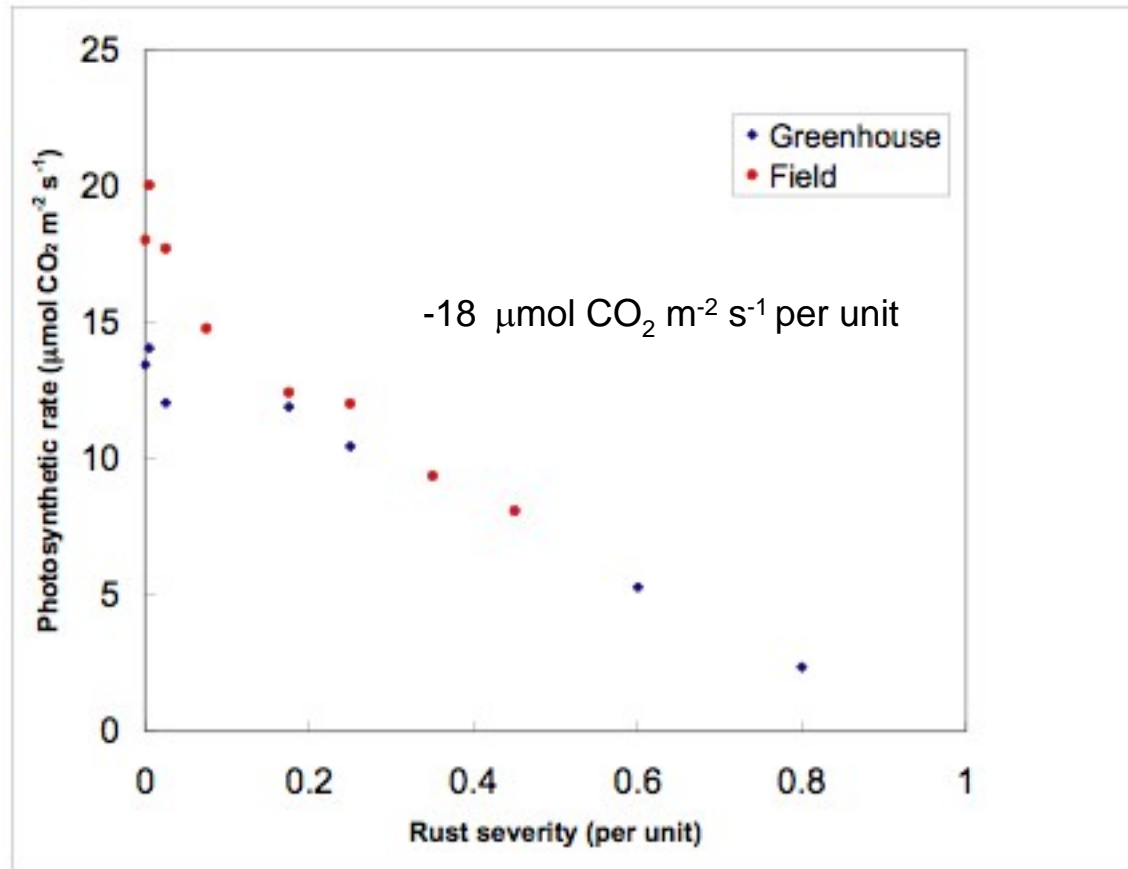


Fig. 14.21 in Deacon. Fungal Biology, 4th edition;
Taken from Voegelé & Mendgen, 2003. New Phytologist 159:93-100.

Rust derives its nutrients from host, but also compromises ability of host to capture carbon through photosynthesis

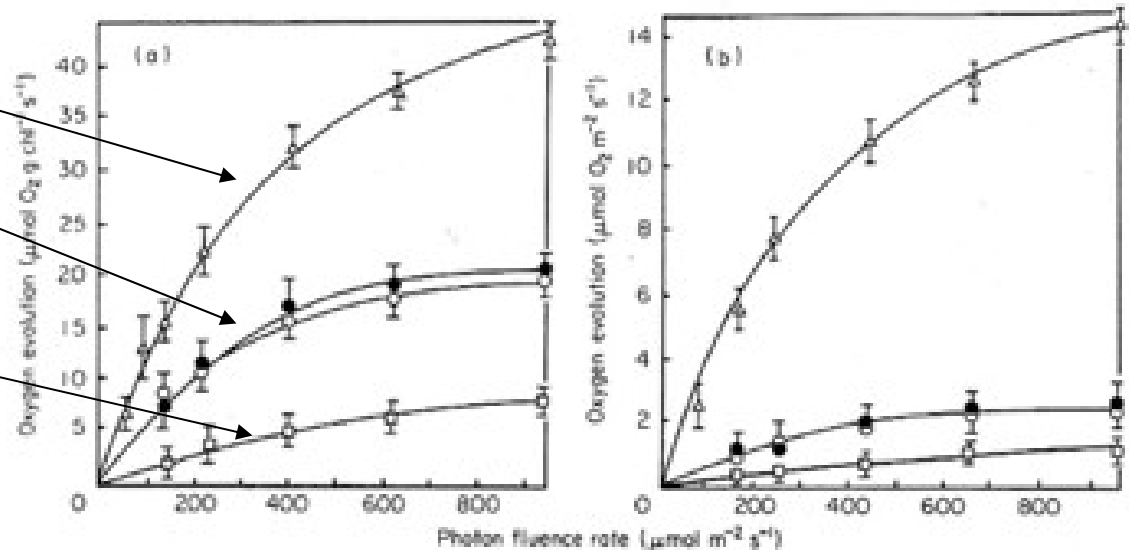
Rust infection reduces photosynthetic rate

Uromyces appendiculatus on bean



Uromyces muscari reduces rate of photosynthesis in *Hyacinthoides nonscripta*

Healthy
Non-sporulating
and green island
Sporulating



Puccinia allii reduces rate of photosynthesis in infected areas in leek

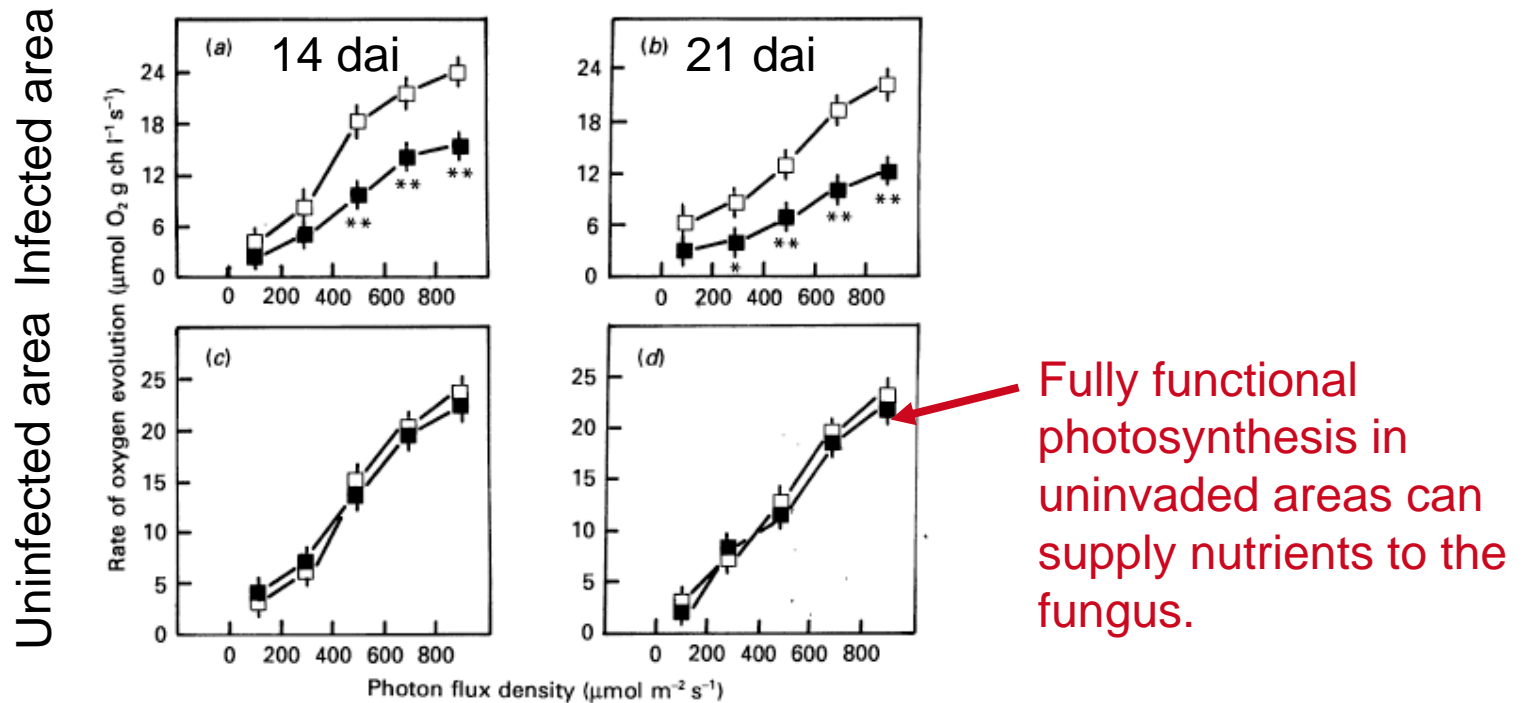
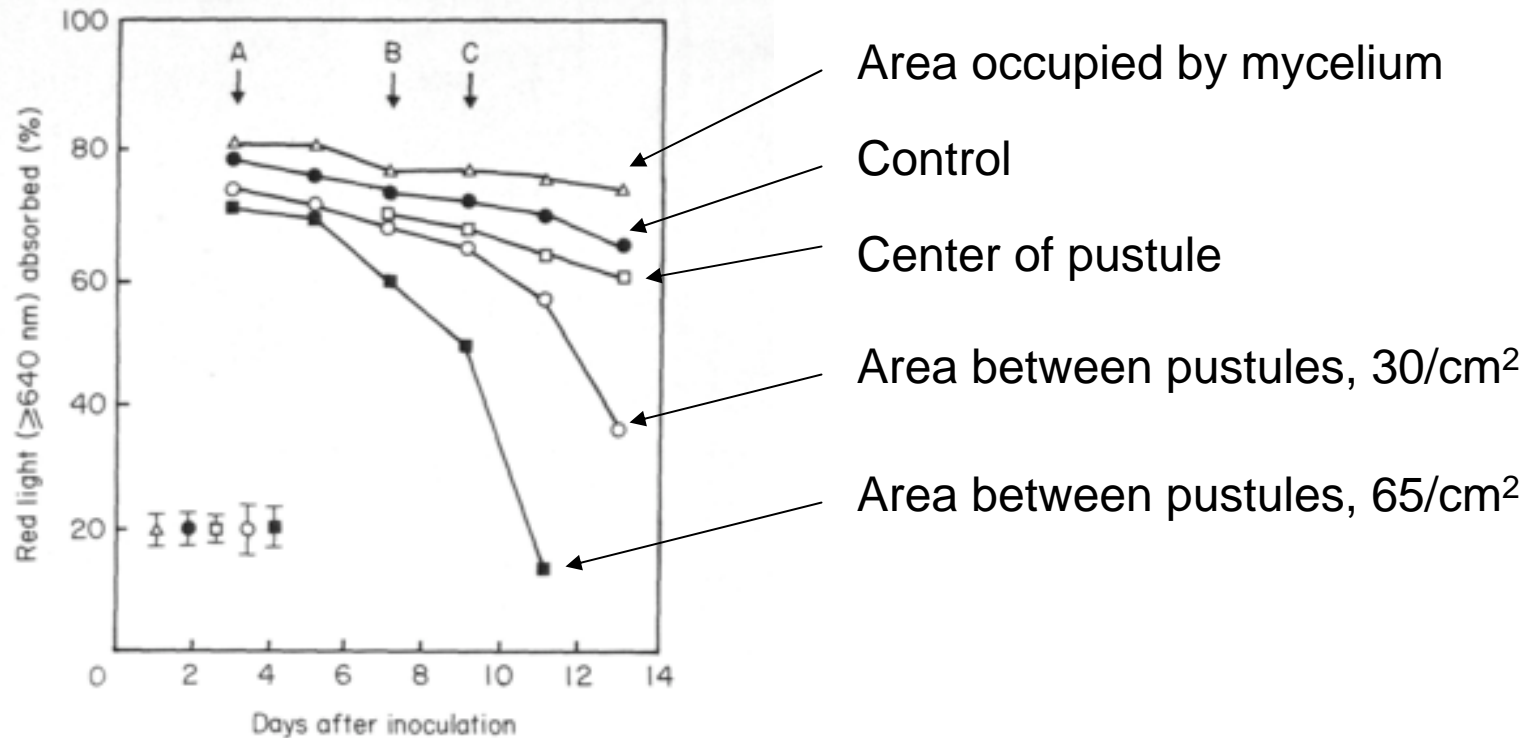
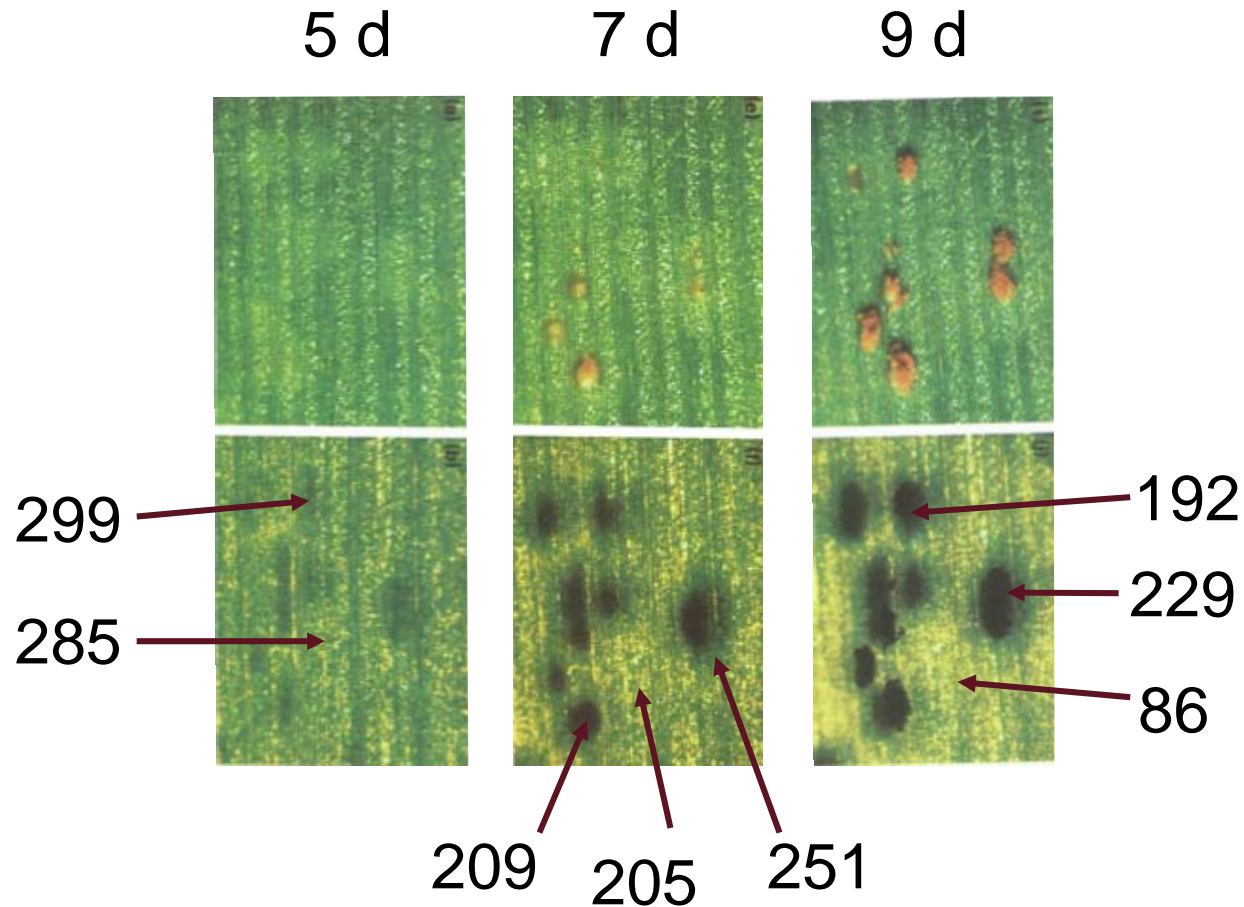


Figure 1. Change in the rate of net photosynthesis ($\mu\text{mol O}_2 \text{ g chlorophyll}^{-1} \text{ s}^{-1}$) as a function of photon flux density for controls (\square), and rusted leaves of leek (\blacksquare). (a,b) Infected regions 14 and 21 d after inoculation respectively, (c,d) uninfected regions 14 and 21 d after inoculation respectively. Standard errors of the means of four replicates are given. Significant differences are shown at $*P = 0.05$; $**P = 0.01$.

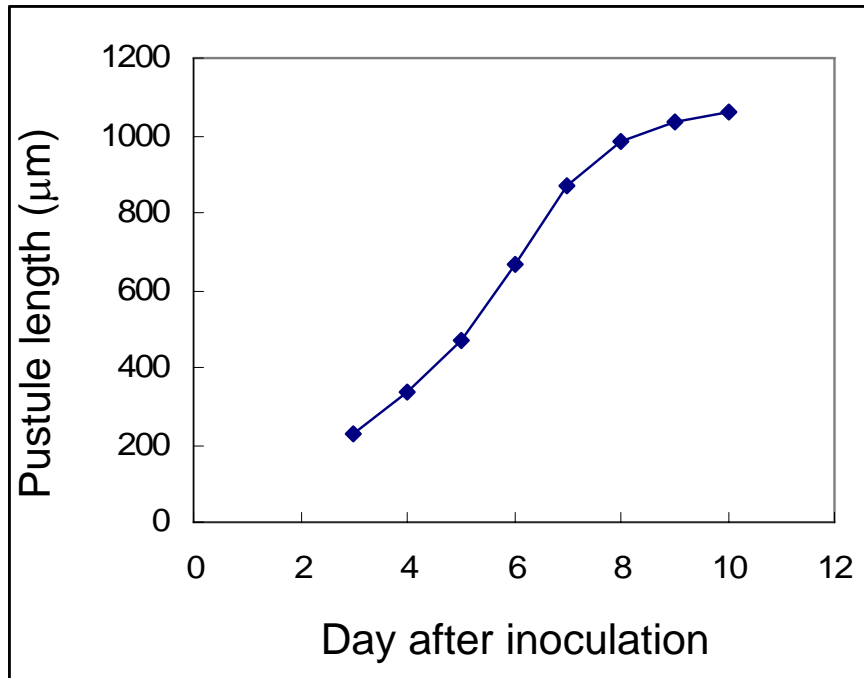
Chlorophyll loss within and between pustules of barley leaf rust



Chlorophyll content in and around barley leaf rust pustules (mg m⁻²)



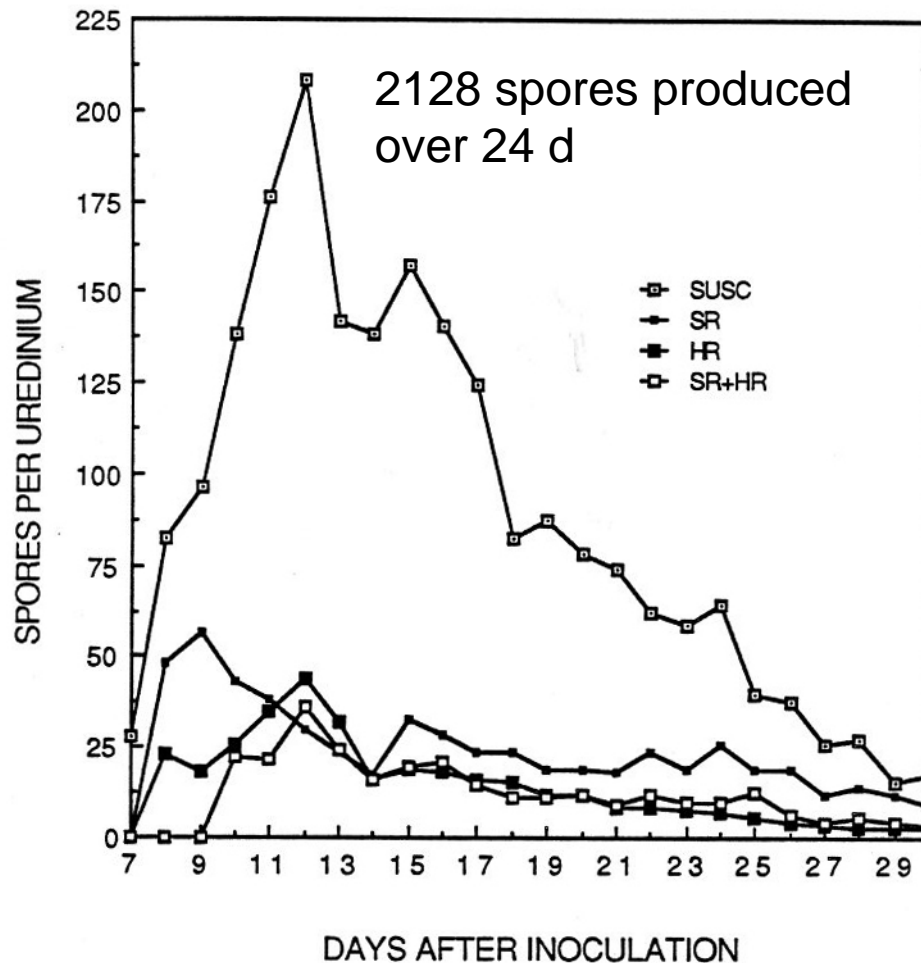
Rate of hyphal growth-barley leaf rust



Sporulating
Puccinia triticina
may account for
50% of dry weight
of leaf.

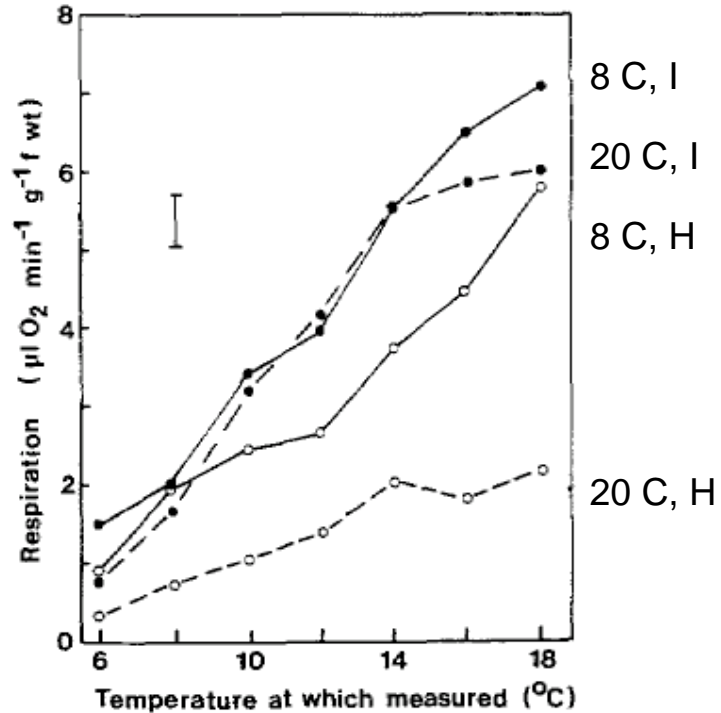
Hyphal growth requires energy. Once sporulation begins, energy demand would presumably increase substantially.

Daily production of spores by *Puccinia triticina* on triticale



How much energy is required to produce one spore? However much is required, it all comes from the host.

Respiration



- **Healthy plants: respiration greater at 8 C than at 20 C.**

- **Disease increases respiration rate.**

Jennings & Ayres. 1990. J Phytopathology 130:245-240.

Senecio vulgaris, *Puccinia lagenophorae*

Respiration in leeks

Table 1. Rates of dark respiration of control leaves, infected regions and uninfected regions of rusted leek leaves

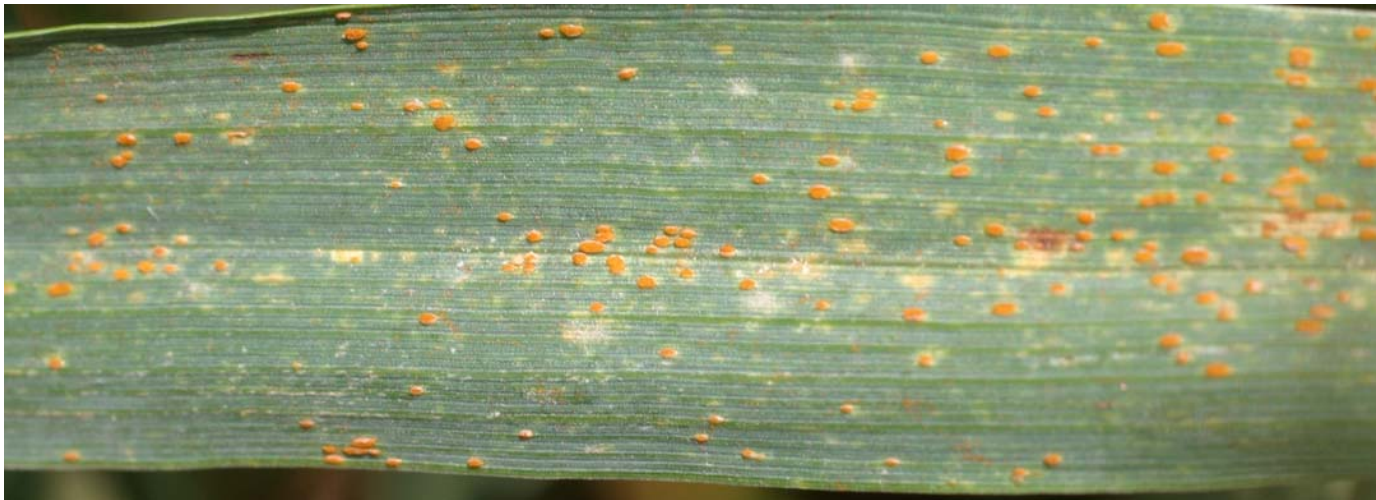
Tissue	Respiration rate ($\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$) days after inoculation			
			Control (%)	
	14	21	14	21
Control	0.48 ± 0.005	0.40 ± 0.003	—	—
Infected regions	1.59 ± 0.07	1.57 ± 0.09	331	395
Uninfected regions	0.31 ± 0.006	0.34 ± 0.005	64	85

Values are the mean of four measurements with standard error.

Roberts and Walters. 1988. *New Phytol* 110:371-376.

Water loss

- Less root mass per unit area of leaf
- No effect on leaf osmotic potential
- Stomates no longer control leaf diffusive resistance after rust sporulates



Rusted bean leaves cannot control water loss

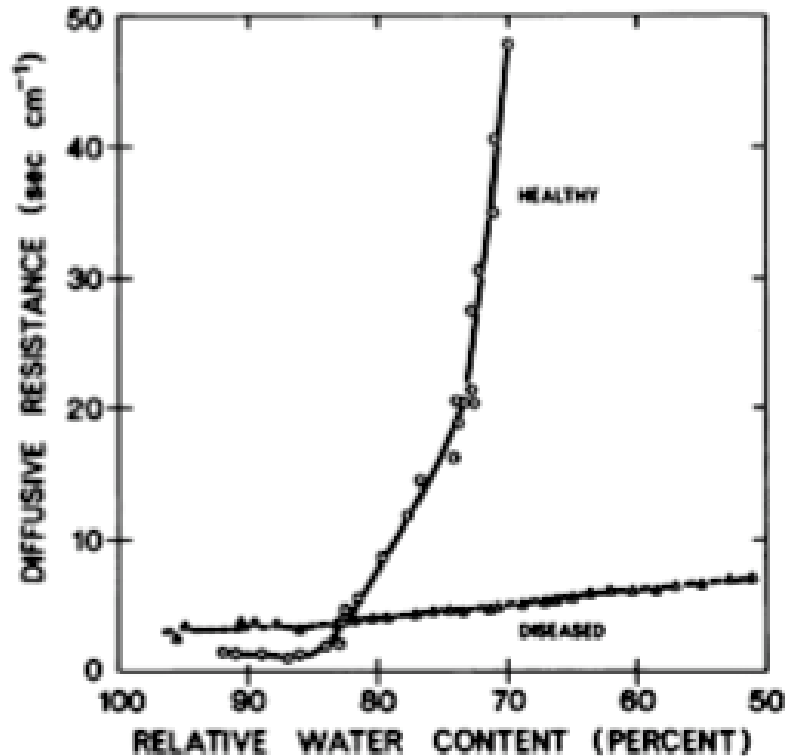


FIG. 3. Diffusive resistance of healthy and diseased leaves plotted as a function of decreasing relative water content. The measurements on healthy and diseased leaves required 10 and 5 hr, respectively.

- Diseased leaf had 30 pustules cm⁻².
- In a healthy plant, stomatal closure controls diffusive resistance.
- Stomates on rusted plants close, but water escapes through the ruptured cuticle.

Future work

- Calculate biomass of fungus per infection site
- Calculate energy required to produce this biomass
- All of this energy comes from host
- In modeling, need to consider disease intensity from the time of infection, not from the time of lesion appearance (visual severity)