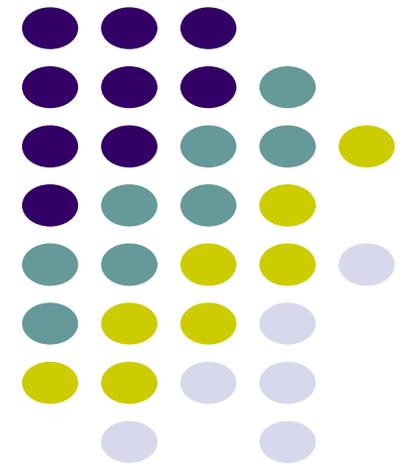
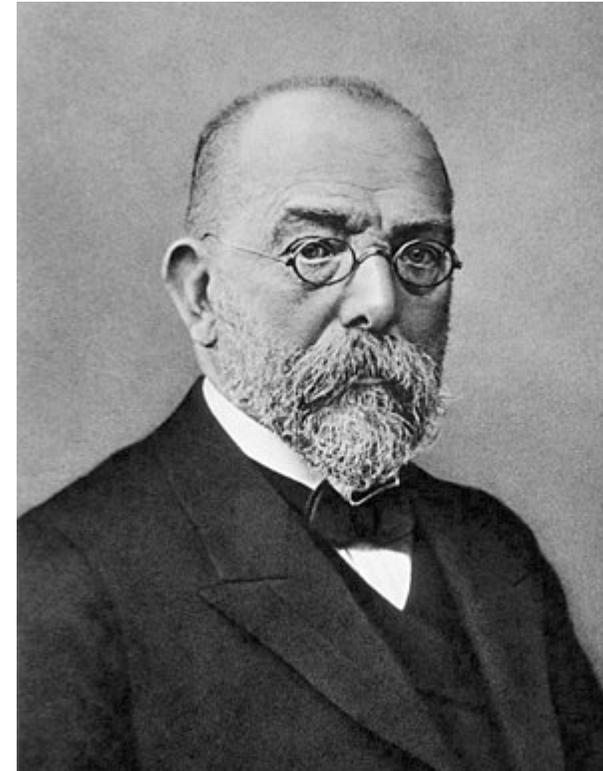


Koch's postulates for biotrophic and necrotrophic pathogens

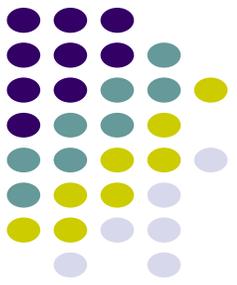


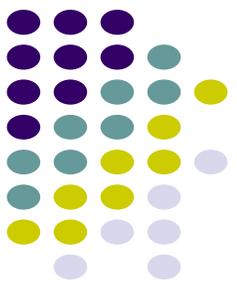
Koch's postulates

- **Koch's postulates** are four criteria designed to establish a causative relationship between a microbe and a disease. The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884, based on earlier concepts described by Jacob Henle, and refined and published by Koch in 1890. Koch applied the postulates to describe the etiology of cholera and tuberculosis, but they have been generalized to other diseases.



Robert Hermann Koch (11 December 1843 – 27 May 1910) was a German physician

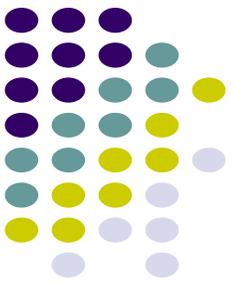




Four steps of Koch's postulates

- The microorganism must be identified in all individuals plants affected by the disease, but not in healthy plants.
- The microorganism should be isolated from the diseased plant and grown in culture.
- When introduced into a healthy plant, the cultured microorganism should cause disease.
- The microorganism must then be re-isolated from the experimental host, and found to be identical to the original microorganism.

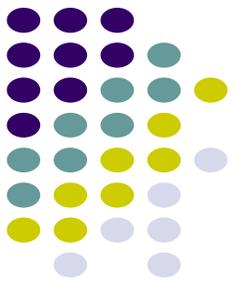
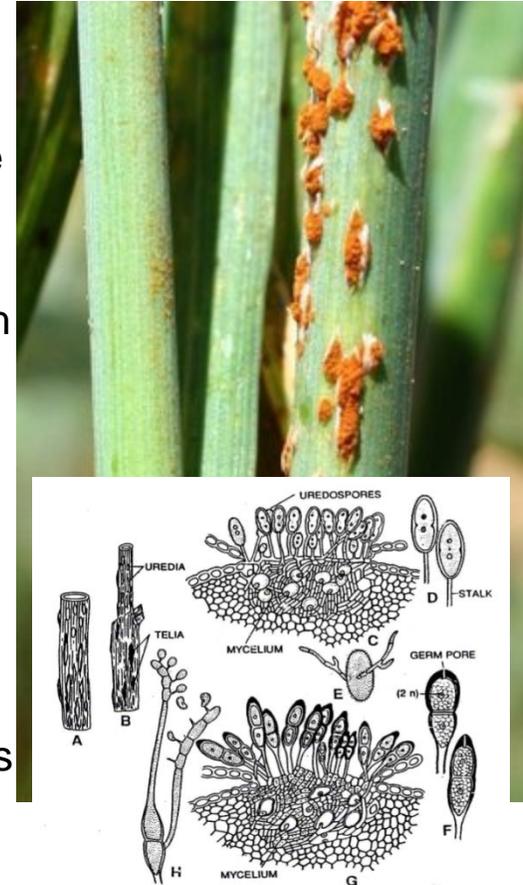
Exceptions



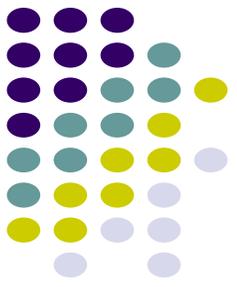
- Viruses were not yet able to be cultured during the 1800's. Thus, while it appeared that an infectious agent was responsible for certain diseases, the lack of available techniques to isolate and culture viruses meant that not all Koch's Postulates could be met.
- The third postulate stipulates that the experimental host “**should**” exhibit disease, not “**must**”. This is because:
 - asymptomatic carriers,
 - immunity, and
 - genetic resistance are possible.

Koch postulate of Biotrophs: Stem rust of wheat

- Plant seeds of a susceptible wheat cultivar into pots
- Grow seedlings until the first leaves fully emerged.
- During inoculation, leaves should be rubbed gently between moistened fingers to remove the waxy layer from the surface which hinders the penetration of the germ tube of the pathogen spores.
- Suspend bulk spores from each sample in distilled water with a drop of Tween 20 and spray until runoff using an atomizer.
- The seedlings be incubated at relative humidity of about 100% and temperature of about 22 °C in a plastic chamber for 24 hours.
- Transfer the seedlings to a greenhouse bench having temperature of 18-27 °C and keep for 11-14 days for development of symptoms.
- Samples with viable spores be selected to multiply inoculums for generation of monopustule isolates.



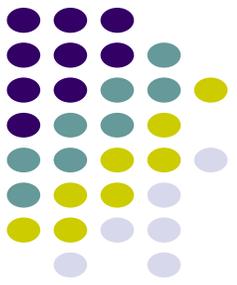
How to select monopustule isolate for identification of races?



- Leaves with monopustule infection should be identified prior to sporulation and then isolated.
- In case where the pustules are aggregated and no isolated pustule occurs, inoculation is repeated on the susceptible cultivar until separate pustules develop and monopustule isolates are generated.
- The generated monopustule isolates are then further multiplied on a susceptible host until sufficient urediospores are collected for differential host test.

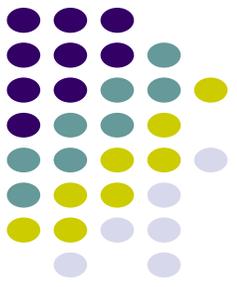
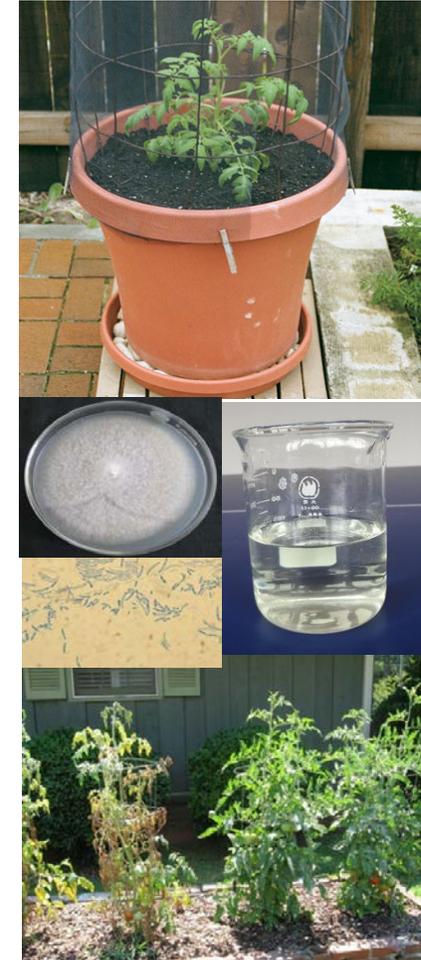
Necrotroph: *Alternaria* spp. (Foliar spray)

- Isolate the pathogen from an infected plant part and obtain pure culture on a suitable agar medium
- Harvest the spores of the pathogen by flooding the culture and gently vortex
- Decant the spore suspension into a beaker
- Determine the spore concentration and prepare a spore suspension after diluting to the desired concentration
- Fill a hand atomizer with the spore suspension and spray on the healthy plants till water drips off from the leaves
- Cover the plants with a plastic bag for 24 hours
- Allow the plants to grow in a glashouse/nethouse till the symptoms develop
- Follow all the four steps of Koch's postulates to confirm the disease causing agent



Soil borne Necrotroph: *Fusarium* spp. (soil drenching)

- Isolate the pathogen from an infected plant part and obtain pure culture on a suitable agar medium
- Harvest the spores of the pathogen by flooding the culture and gently vortex
- Decant the spore suspension into a beaker
- Determine the spore concentration and prepare a spore suspension after diluting to the desired concentration
- Take 30-50 mL of desired concentration of spore suspension in a beaker
- Pour it in the top soil in the pots
- Allow the plants to grow in a glashouse/nethouse till the symptoms develop
- Follow all the four steps of Koch's postulates to confirm the disease causing agent



Sclerotium rolfsii (mycelia and sclerotia application)



- Isolate the pathogen from an infected plant part (Stem, collar) and obtain pure culture on a suitable agar medium
- Inoculate fresh mycelial disc on cereal grains priorly autoclaved, and packed in a polyethylene bag
- Allow the pathogen mycelium to grow and develop sclerotia
- Take out cereal grains (5-10 g) fully colonized by the pathogen mycelia and sclerotia
- Care should be taken that adequate soil moisture is already prevailed
- Inoculate into the top soil in pots and mix with the top soil
- Allow the plants to grow till symptoms are developed