Updated transmission methods from proposal

Question: Are Mfc an effective vector of BYDV to winter wheat?

Importance: BYDV impacts both spring and winter wheat, but spring wheat is able to outgrow virus. Because winter wheat overwinters, when infected in the fall, virus causes more damage. BYDV causes yellowed, stunted plants and yield loss.

Colonies: To determine the vector capacity of Mfc, we will work with one or more laboratory colonies of Mfc, as deemed necessary based on the work carried out in our field surveys. Mfc colonies will be maintained on barley var. Sprinter and spring wheat var. Kelse.

1. Make sure we have BYDV (+) and (-) wheat in greenhouses
   1. Take samples (need to get liquid nitrogen)
   2. Do extractions
   3. Run qPCR

Transmission: 2 day-old Mfc nymphs will be fed on BYDV-infected (BYDV-PAV or BYDV-SGV) spring wheat var. X. After a 48-hour acquisition access period (AAP), the Mfc will be transferred (5 per plant) to 2 week-old winter wheat test plants (var. X) (n = 30 per BYDV strain). 2 temporal blocks of experiment will be done.

* Plant inoculation
  + 48 clean 2 week old plants (sprint wheat or weed [fescue])- 24 for BYDV-PAV, 24 BYDV-SGV
  + 480 BYDV (+) R. padi (adult)- 240 BYDV-PAV (+), 240 BYDV-SGV (+)
  + Add R. padi, 10 per clip cage, leave on for 48-72h IAP
  + After IAP, kill aphids
  + Let virus incubate for 21-28 days
* Aphid inoculation
  + On 40 inoculated plants, add 10 Mfc per plant in clip cages (400 Mfc)
    - Look for clip cages in lab- how many? Need to support leaf, maybe too much work. Can use dialysis tube instead, easier to seal with foam/cork. OR could use entire plant per cage, BUT need to remove all aphids. Can try multiple methods see what works best.
  + Leave on for 48-72h AAP
* Final transmission (Multiple aphid whole plant tests):
  + 75 2 week old clean wheat plants- 15-20 winter wheat with BYDV-PAV (+) aphids, 15-20 winter wheat with BYDV-SGV (+) aphids, 15-20 winter wheat with BYDV (-) aphids, 15 plants with no aphids, 15 plants with R. padi (BYDV +) as positive control (to show transmission success)
  + >375 aphids- 150 BYDV-PAV (+), 150 BYDV-SGV (+), 75 BYDV (-)
  + Transfer 5 adult? aphids per plant
  + Let feed for a 48-72h inoculation access period (IAP)
  + Remove aphids (by hand, soap solution, insecticide)
  + Let virus incubate in growth chamber (18:6 [L:D] h, 22+-2C, and 50+-5% RH)for 21-28 days
  + Take leaf samples
  + Do PCR
* Final transmission (Single aphid clip cages):
  + 75 2 week old clean wheat plants- 30 winter wheat with BYDV-PAV (+) aphids, 30 winter wheat with BYDV-SGV (+) aphids, 15 winter wheat with BYDV (-) aphids, X plants with no aphids?, X plants with R. padi (BYDV +) as positive control?
  + >75 aphids- 30 BYDV-PAV (+), 30 BYDV-SGV (+), 15 BYDV (-)
  + Transfer 1 adult? aphid per plant in clip cage
  + Let feed for a 48-72h inoculation access period (IAP)
  + Remove aphids (by hand)
  + Let virus incubate in growth chamber (18:6 [L:D] h, 22+-2C, and 50+-5% RH)for 21-28 days
  + Take leaf samples
  + Do PCR

PCR (from proposal): BYDV infection status will be assessed using quantitative PCR (qPCR) after a 21-day incubation period. To detect and quantify BYDV, 100 mg of leaf tissue will be removed from two fully expanded young leaves. Extractions will be performed using Qiagen RNeasy plant mini kit. cDNA synthesis will be carried out on a Superscript First Strand Synthesis System for RT-PCR. BYDV titers will be quantified with qPCR, using a CFX Connect Real Time System, and SsoAdvanced™ Universal SYBR® Green Supermix (2x) (Bio-Rad) with forward and reverse primers according to Balaji et al. (2003). The percentage of plants positive for the BYDV strain, i.e., transmission success, will be estimated. R. padi will be used as a control for both BYDV strains, anticipating high transmission efficiency for BYDV-PAV. Transmission success will be compared between virus strains and aphid species with generalized linear mixed models, assuming a binomial distribution and a logit link function.

Make sure seeds you’re using are untreated

S. E. Sadeghi et al showed that PAV there was no transmission, we used SGV strain too

Need to find different strains of BYDV, Who has published recently and can send us samples to infect?

Virus strain names based on what species of aphids are best at transmitting

Both strains common in the area? Literature, ask Hanu

Do transmission in 2 blocks, 15-20 reps for everything, can increase for 2nd block