Protocol for pilot transmission experiment

Variety Lambert winter wheat

14 plants

- 8 plants with BYDV (+) BCOA

- 4 plants with BYDV (-) BCOA

- 2 plants with no aphids (empty clip cages)

2 days before starting the experiment, tissue samples from colony plants were collected and tested with qPCR to verify infection status of BCOA colonies. Individual winter wheat (var. Lambert) plants were set on trays filled with water to create physical barrier to prevent unintentional aphid movement (escape). Clip cages with 10 adult BYDV (+) BCOA were placed on 8 treatment plants, while clip cages with 10 adult BYDV (-) BCAO were placed on 4 positive control plants. Clip cages with no aphids were placed on 2 negative control plants. OR Clip cages with 10 adult BCOA were placed on 8 reps of experimental and 4 reps of positive control plants. Treatment plants received BYDV (+) aphids, and positive control plants received BYDV (-) aphids. Empty clip cages with no aphids were placed on 2 reps of negative control plants. Each plant was supported by a bamboo skewer onto which the clip cages was tied. Aphids were transferred to each clip cage with a fine paintbrush, after which the clip cages were secured onto the most recently emerged full leaf.

Cages were left on plants for a 72 hour inoculation period, after which the clip cages were removed and surviving aphids were removed by hand. Plants were allowed to grow for 14 days and were monitored closely for any aphids left behind. After 14 days, infection status of plant was checked using qPCR.

BYDV (-) adult Mfc aphids were then added to 2 clip cages per plant (25 in each cage) on 5 treatment and 2 positive control plants, and BYDV (-) BCOA adults were added to 2 clip cages per plant (25 in each cage) on 3 treatment and 2 positive control plants. After a 5 day acquisition period, the clip cages were removed and aphids transferred to a new set of 14 day old winter wheat plants. 15 aphids from each plant were divided so 5 aphids were on each new plant. (15 (+) Mfc plants, 9 (+) BCOA plants, 4 (-) Mfc plants, 4 (-) BCOA plants). An additional 5 aphids per plant were collected and placed on damp filter paper to empty gut contents. Aphid tissue was then tested to confirm virus acquisition using qPCR. Aphids were left on the plants for a 5 day inoculation period. After this inoculation period, the clip cages were removed and surviving aphids were removed by hand. After 2 weeks, 100mg of leaf tissue was collected from a whole fresh leaf to test infection status of the plant using qPCR. After another week, 100mg of leaf tissue was collected to test infection status of the plant using qPCR.

Test individual aphids with empty guts to see if Mfc can actually acquire the virus

End plants are tested to see if Mfc can also transmit the virus.