Microbes Bionematicides Product Evaluation Report
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Trial Duration: 01/28/2021 – 03/04/2021
Crop: Tomato (cv. Rutgers)
Organism: *Meloidogyne hapla* (Northern Root-Knot Nematode)

Methods

Target organism colonies were maintained in the MSU Plant Science Greenhouses on Michigan State University's main campus. Original target individuals were collected from an infested parsnip field in September 2019, and colonies were reared in gallon-sized polystyrene pots on tomatoes (cv. Rutgers). Inoculum for this trial were collected from tomato colonies using the root extraction methods described in Hussey and Baker (1973).

A greenhouse trial was established in February 2021 to evaluate the potential efficacies of four product solutions against the target organism, *Meloidogyne hapla*. Steam sterilized soil was mixed with play sand at a 30:70 ratio and approximately 500cc of the soil mixture was placed into each of the 46 fresh polystyrene pots. Four seeds of a susceptible variety of tomato (cv. Rutgers) were seeded into pots. Four days later, plants were thinned to two seedlings per pot. Any pots containing plants that contained less than two seedlings or which showed signs of unthrifty growth were removed prior to inoculation. Remaining pots were each inoculated with 2000 *M. hapla* eggs (N=36). One week following, treatments were applied as a soil drench. Fifty ml of distilled water was applied to six positive control pots. Velum Prime was applied to six pots at a reduced rate equivalent to 6.5 oz/A and labelled as negative controls. Next, 50 ml of respective solutions (MN11.1, MN11.2, MN21.1, MN21.2) were applied to the respective remaining pots, each with six replicates. Pots were labelled correspondingly and arranged in a CRBD on the greenhouse bench and monitored weekly. Each week, plants were evenly fertilized with a 20-20-20 fertilizer solution and monitored for presence of phytotoxicity.

After trial conclusion, above ground plant heights and weights were measured, and roots were separated from soil. Soil and roots were collected into plastic bags and transported to the Applied Nematology laboratory for further processing. Soil was homogenized within the bags and immediately processed using the sugar centrifugal floatation method (Fig. 8). Roots were gently cleared of soil, rated on the Gall Indices Rating developed by Hussey and Janssen (2002): 0 indicating no galls and up to 5 indicating over 75% of the root system contains galls. Root systems were then weighed and recorded. One gram of roots was collected from each pot and individually extracted using the Hussey and Baker (1973) method (Fig 2). Pots that contained less than one gram of roots were marked to be scaled up accordingly. Soil was left in the processing room at room temperature (22 °C) to monitor hatching. Three days afterwards, soil was again processed. All processed samples were stored in refrigerator conditions until readings.

Soil (N=72) and root samples (N=36) were read using a counting dish and an inverted microscope a week after processing. Due to high sample concentrations, a one-ml subsample was read and scaled up to 10ml. Data was then entered into Microsoft Excel, and data analysis was run using RStudio version 1.3.1093, a one-way ANOVA followed by Tukey's HSD to determine significance.

Results+Discussion

Phytotoxicity

During trial duration, we observed no noticeable phytotoxic defects to plant coloration or growth for any of the treated pots.

Above-Ground Plant Height

Treatments M11v2, MN21v1, MN21v2, and Velum-applied plants were significantly higher than untreated positive control pots.

Root-mass

Treatments MN21v2 and Velum-applied plants were significantly heavier than untreated positive control plants ($P<0.05$, Tukey HSD; Figure 2).

Root Gall Index Rating for Nematode Infestation

Higher rating plants indicated more severe infestations and presence of galling on roots. Untreated control plants were significantly more galled than treatments M11v2, MN21v1, MN21v2, and Velum-applied plants ($P<0.05$, Tukey HSD; Figure 3).

Root-knot Nematode Concentrations
All treatments were significantly lower than concentrations in positive control plant roots. Both versions of MN21 were significantly lower than MN11v1 (*P<0.05*, Tukey HSD; Figure 4).
Root-knot hatching
Hatching between treatments was not significantly different (*P>0.05*, Tukey HSD; Figure 5) after soil was incubated for three days at room temperature (22 ˚C).

Overall, results for treatments MN21v1 and MN21v2 were significantly effective at reducing symptoms and signs of root-knot nematode infestations, similar to the application of Velum Prime. MN11v2 also shows potential efficacy, but it is notably lower than that of the nematicide Velum Prime. Root-knot nematode hatching was not significantly impacted by any of the treatments. Comparisons of above ground growth by treatment are visible in Figure 7. It is notable to mention that greenhouse nematicide trials involve a uniform, well controlled environment which limits confounding variables, so further evaluation is necessary in determining true product efficacy.

Citations:
Hussey RS, Barker KR. A comparison of methods of collecting inocula of *Meloidogyne spp*., including a new technique. *Plant Disease Reporter.* 1973; 57:1025–1028.
Hussey RS, Janssen GJW. Root-knot nematode: *Meloidogyne* species. In: Starr JL, Cook R, Bridge J, editors. *Plant Resistance to Parasitic Nematodes.* Wallingford, UK: CAB International; 2002. pp. 43–70.
Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep., 48:692.

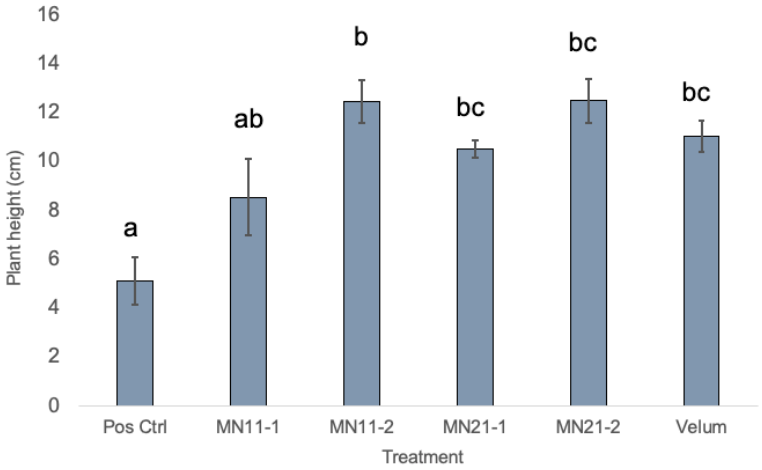

Figure 1. Average above ground heights of tomato plants (cv. Rutgers) across treatments. Standard error bars reflect standard error of the means. Treatments with unique letters indicate significance (*P<0.05*, Tukey HSD).

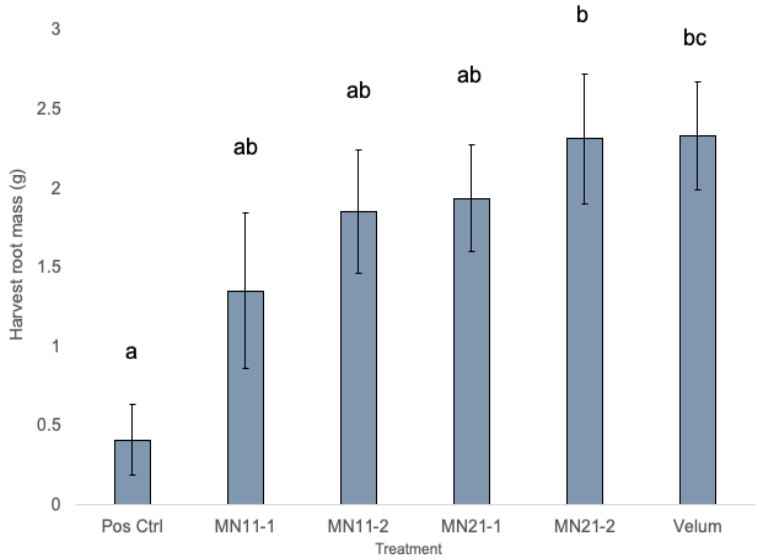

Figure 2. Average root mass of tomato plants (cv. Rutgers) across treatments. Standard error bars reflect standard error of the means. Treatments with unique letters indicate significance (*P<0.05*, Tukey HSD).

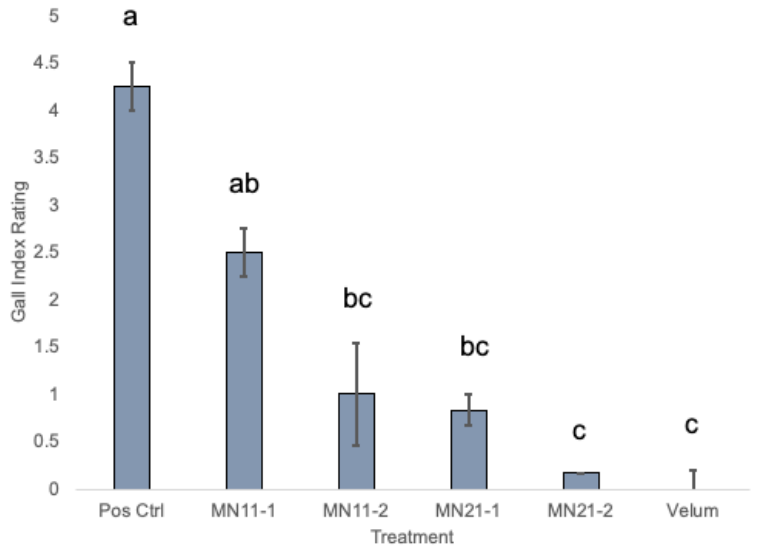

Figure 3. Average gall index rating of tomato plants (cv. Rutgers) across treatments. Standard error bars reflect standard error of the means. Treatments with unique letters indicate significance (*P<0.05*, Tukey HSD).

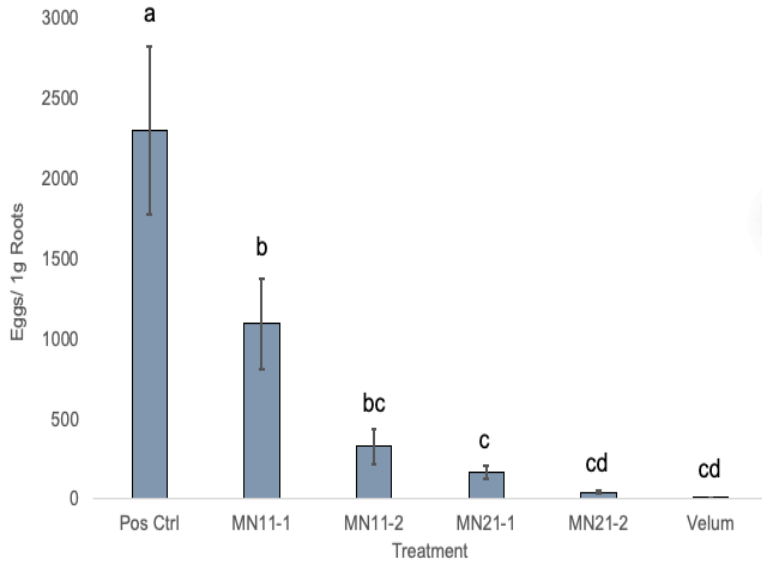

Figure 4. Average concentrations of root knot nematode eggs from 1g of tomato roots across treatments. Standard error bars reflect standard error of the means. Treatments with unique letters indicate significance (*P<0.05*, Tukey HSD).

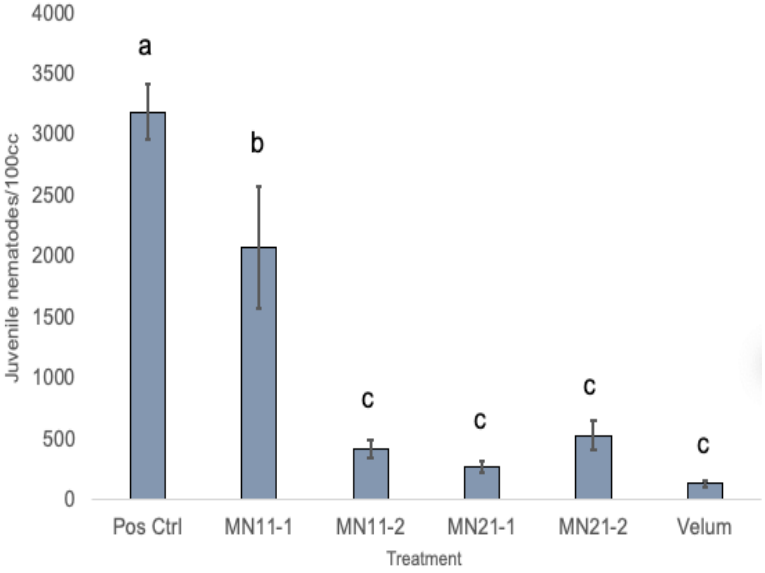

Figure 5. Average concentrations of juvenile root knot nematodes extracted from 100cc of soil treatments. Standard error bars reflect standard error of the means. Treatments with unique letters indicate significance (*P<0.05*, Tukey HSD).

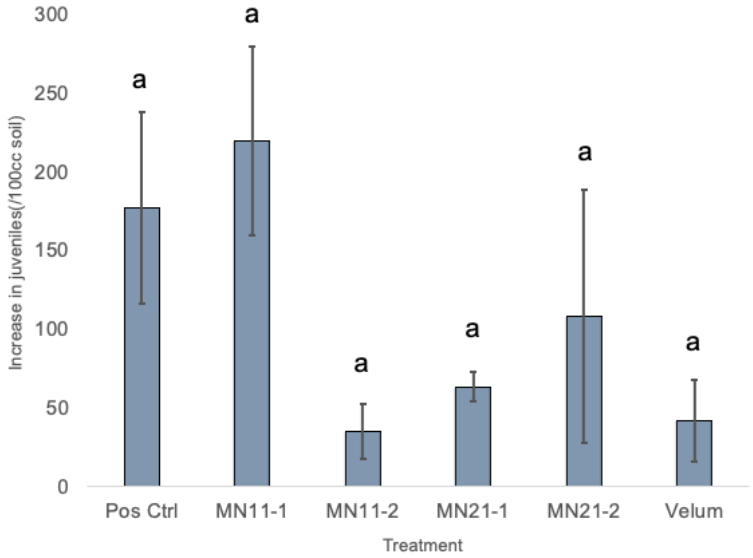

Figure 6. Average hatching by treatment in incubated soil. Standard error bars reflect standard error of the means. Treatments with unique letters indicate significance (*P<0.05*, Tukey HSD).

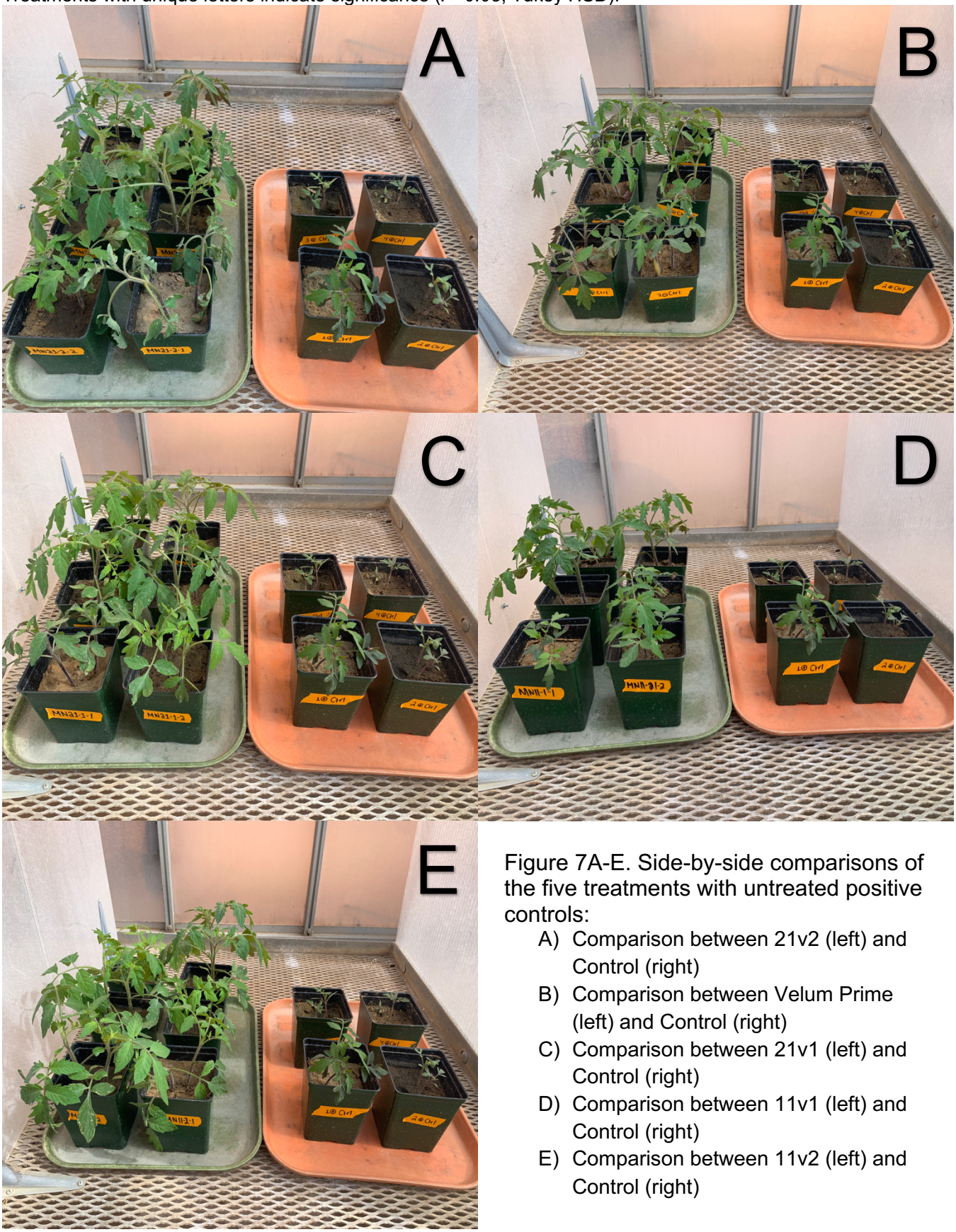

Figure 7A-E. Side-by-side comparisons of the five treatments with untreated positive controls:

A) Comparison between 21v2 (left) and Control (right)
B) Comparison between Velum Prime (left) and Control (right)
C) Comparison between 21v1 (left) and Control (right)
D) Comparison between 11v1 (left) and Control (right)
E) Comparison between 11v2 (left) and Control (right)

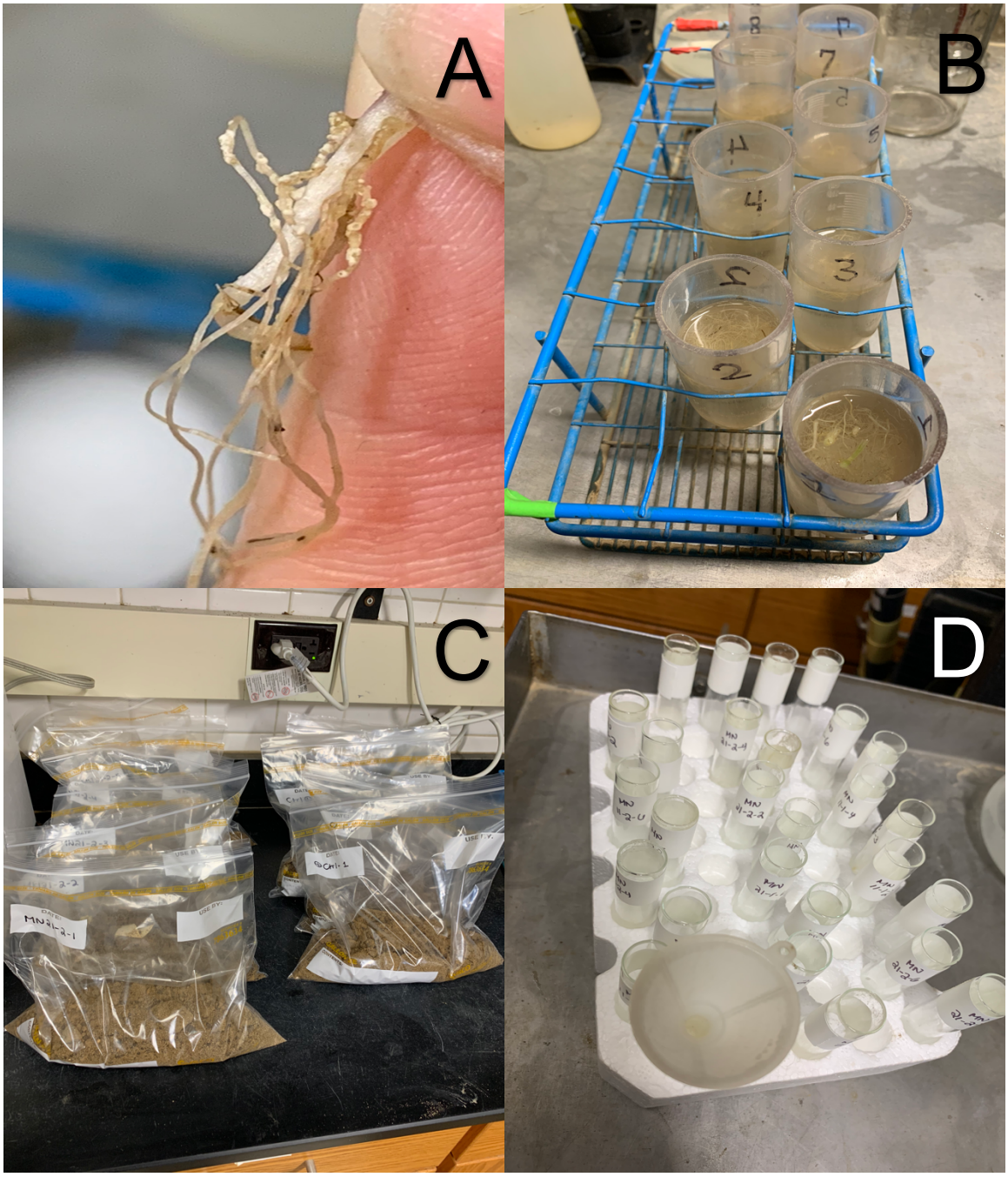

Figure 8A-D. Various processes involved in trial deconstruction, including measurement of root galling index and extraction(A,B) and soil processing (C,D).