Supplementary Materials and Methods

Seed disinfection treatment

1. Steam vacuum

During steam vacuum pasteurisation by Bejo Zaden B.V., the combination of vacuum conditions and saturated steam ensured the effective killing of bacteria and fungi under controlled temperature conditions. Although this procedure has been developed for the elimination of seed transmitted pathogens, it was adapted for application to the seeds of four different crops, namely beetroot, onion, spinach and pepper. This adjustment allowed reducing the seed microbiome while maintaining the germination potential of the different seed lots.

Under vacuum conditions, applying saturated steam on seeds causes the steam to condensate on the seed surface. Consequently, applying additional vacuum pulses makes the seed skin evenly permeable to the hot steam. While static vacuum is maintained, the seeds are then subjected to a heat treatment. Eventually, an additional series of vacuum pulses is applied (without addition of steam) thus leading to the evaporation of the steam and the cooling down of the seed batch under treatment.

2. Hot water

For coriander seeds, CN Seeds Ltd. used a hot water treatment to kill microorganisms present on the seeds. The treatment temperature and duration ranged between 30-60°C and 10-60 minutes depending on seed size. The hot water bath used for the treatment was cleaned between seed lots to limit cross-contamination. After treatment, the seed lots were dried at 10°C in low humidity for 24 h.

3. Sodium hypochlorite

DLF Seeds B.V. disinfected red fescue and perennial ryegrass seeds by spraying a 0.5% sodium hypochlorite solution on them using a small rotostat seed treater. Shortly afterwards, the seeds were placed in cheesecloth bags and rinsed with sterile water. They were then left to dry at 25 °C for 24 h.

Bioassay experiment set-up

1. Beetroot – *Pythium ultimum* (Bejo Zaden B.V.)

The inoculum was prepared by mixing 10 agar plugs containing *Pythium ultimum* (strain RC1007) mycelium with autoclaved soil substrate (550 g silversand, 175 g potting soil, 10 g of organic oatmeal flakes and 37.5 g water). The inoculum was incubated at room temperature in the dark for three weeks and mixed weekly.

Beetroot seeds were then sown in containers of infected potting soil containing 0.5% w/w *P. ultimum* inoculum. The bioassay included four replicates per treatment (treated,

untreated) per seed lot and each replicate comprised of 20 seeds sown in a row. Seed lots were randomised across containers but treatments were paired, and so, for every untreated seeds replicate, a replicate of treated seeds was also present in the same container. The containers were covered with lids and kept in controlled conditions (15 °C and 16 h light) for 14 days. Pre- and post- emergence damping off were recorded at the end of the experiment as number of non-emerged and number of infected seedlings respectively.

2. Onion – *Setophoma terrestris* (Nunhems Netherlands B.V. (BASF))

The inoculum was prepared in 1L flasks, using blended cultures of *Setophoma terrestris* strain PT-2 in Czapek Dox growth medium, grown 21 days at 22 °C. The pathogen originated from cultivated onion collected in the USA and tested positive for pathogenicity on onion. Cultures were homogenised and an aliquot of liquid mycelial suspension was then thoroughly mixed with sand (200 ml/L sand) and a sterile soil mix (5% OM/L sand).

Onion seeds were sown in closed bottom boxes of either infested substrate prepared as mentioned above or non-infested substrate. For the latter, non-infested growth medium was mixed with the sand and sterile soil mix. Seeds were covered with sand and were then watered and fertilised. The bioassay included two replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 50 seeds sown in a line. Treatments were paired, and so, for every replicate of untreated seeds, a replicate of treated seeds would also be present in the same box. A line of seeds from a resistant and a susceptible commercial onion variety was also sown in every container as an additional control of disease uniformity across boxes.

Plants were grown at 28 °C with 14 h of fluorescent light/10 h darkness. Phenotyping and symptom scoring took place 3 and 4 weeks post inoculation. Based on the percentage (%) of plants in decline due to pink root rot, leaf symptoms were scored using an ordinal scale from 1 (high resistance; below 5% decline) to 10 (susceptible; over 75% decline). Root symptoms were scored using an ordinal scale from 0 (high resistance; below 5% decline) to 5 (100% decline).

3. Onion – *Fusarium oxysporum* f. sp. *cepae* (Bejo Zaden B.V.)

The inoculum was prepared by adding 3 agar plugs of *Fusarium oxysporum* f. sp. *cepae* (strain P6432) mycelium 500 ml CDB-Y in a 1L Erlenmeyer flask and incubated on a shaker at 25 °C for 5 days to collect a spore suspension of 10^8 spores/ml.

Onion seeds were then sown in containers of infected potting soil containing 1.3×10^6 *F. oxysporum* spores per gram of soil. The bioassay included four replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 20 seeds sown in a row. Seed lots were randomised across containers but treatments were paired, and so, for every untreated seeds replicate, a treated seeds replicate was present in the same container. The containers were covered with lids and kept in controlled conditions (25 °C and 16 h light/8 h darkness) for 13 days. Pre- and post- emergence damping off were recorded at

the end of the experiment as number of non-emerged and number of infected seedlings respectively.

4. Spinach – *Pythium ultimum* (Germains Seed Technology)

Spinach seeds were sown in containers of field top soil that was collected in the area of Breda, the Netherlands from a field that was naturally infected with *Pythium ultimum*. Several *Pythium* isolates from this location have been identified as *Pythium ultimum* in previous internal research studies done by Germains Seed Technology (Germains Seed Technology personal communication). A 1 cm layer of potting soil was placed over the seeds to cover them after sowing. The bioassay included four replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 63 seeds. Each container only contained one such set of 63 seeds. As an additional control of disease uniformity, seeds coated with Thiram and Apron fungicides as well as seeds coated with a control filmcoat of no additives were also sown and evaluated.

The test was done in a growing chamber (50% humidity 20 °C day/15 °C night, 12 h light/12 h darkness) in which treatments were randomised. The number of germinated seedlings as well as the number of seedlings presenting post-emergence infection symptoms were scored 6 and 7 days after sowing.

5. Spinach – *Fusarium oxysporum* f. sp. *spinaciae* (under the authority of Pop Vriend Seeds B.V.)

Spinach seeds were sown in individual peat soil plugs. To test for post emergence sensitivity, 12 plugs with uniform seedlings (at 3 – 4 true leaf stage) were transferred to test containers and each plug was inoculated with *Fusarium oxysporum* f. sp. *spinaciae*. The inoculum was prepared in Czapek Dox broth on an agitator during one week. Two sets of bioassays were used: one set with a moderately virulent strain (Fus322) and one set with a highly virulent strain (Fus254). Plants from three spinach hybrids of known sensitivity to *F. oxysporum* were also included in each bioassay as a control to validate the trial. Per *F. oxysporum* strain, the bioassay included two replicates per treatment (treated, untreated) per seed lot. Each replicate comprised of 12 plants.

Treatments were paired, and so, for every replicate of untreated plants, a replicate of treated plants would also be present in the same container. The test was done in a climate room (25 °C day/20 °C night, 16 h light/8 h darkness). Post-emergence infection symptoms (cotyledon/leaf yellowing, plant collapse due to root rot) were scored using an ordinal scale from 1 to 10, with 1 being 'very resistant' and 10 being 'very susceptible' 14 days post inoculation.

6. Coriander – *Pythium* sp. (CN Seeds Ltd.)

The inoculum was prepared by growing *Pythium* sp. (CN Seeds Ltd. isolate; originating from infected coriander plant tissue) on agar plates and collecting zoospores using water

containing Tween20. The zoospore suspension was mixed with the autoclaved potting soil (~50,000 spores of *Pythium* per gram of soil) and left overnight.

Coriander seeds were sown in containers of the inoculated potting soil the following day. The bioassay included four replicates per treatment (treated, untreated) per seed lot. Per replicate, 50 coriander seeds were sown. Each one comprises of two viable seeds (the contents of the husk) adding up to 100 individual seeds.

Although treatments were not paired, seed lots were randomised across containers, with each container containing 8 random replicates. The containers were kept in controlled conditions in a climate chamber (20 °C day/18 °C night, 16 h light/8 h darkness; 72% relative humidity). The number of germinated seedlings as well as the number of seedlings presenting post-emergence infection symptoms were scored two and three weeks after sowing.

7. Pepper (seedlings) – *Phytophthora capsici* (Nunhems Netherlands B.V. (BASF))

The inoculum was prepared by growing *Phytophthora capsici* on V8 20% agar plates at 27 °C in the dark until the entire plate was covered (7-8 days). Zoospores were subsequently produced following an existing protocol (Ristaino, 1990). Under sterile conditions, each mycelium-covered plate was divided in 3-4 parts which were then moved in separate sterile empty petri dishes. There, the pieces of mycelium-covered agar were cut into small plugs (approximately 0.5-1 cm^2) and enough sterile distilled water was added to cover them. The water-filled plates were incubated at 27 °C under constant light for 24 h (induction of sporangia production), then placed at 4 °C for 45 minutes and then returned to the 27 °C incubator under constant light for 45 minutes (thermal shock). In this way, sporangia were opened and the released zoospores were collected by filtering the suspension through cheesecloth.

Pepper seeds were sown in containers in a mixture of peat and vermiculite (ratio 5:1 v/v) and incubated in a growth chamber (25 °C day/20 °C night, 8 h light 16 h darkness; 70% relative humidity). Inoculation with *P. capsici* took place 1 week after sowing. Inoculation was done using 5 ml of zoospore suspension at a concentration of 2×10^3 zoospores/ml. The bioassay included three replicates per treatment (treated/untreated) per seed lot and each replicate comprised of 8 plants. An extra set of 8 plants per treatment, per seed lot was left uninoculated as a control. Seeds from a resistant and a susceptible pepper line were also sown in every container as an additional control of disease uniformity per container.

Treatments were not paired. Disease was evaluated in three time points: 4, 7 and 14 days post-inoculation. At every disease evaluation time point each of the 8 seeds sown per replicate were classified as 'seedling', 'dead after germination' or 'missing' (non-emerged).

8. Pepper (plants) – *Phytophthora capsici* (Nunhems Netherlands B.V. (BASF))

Inoculum preparation followed the protocol described in bioassay (7) Pepper (seedlings) – *Phytophthora capsici*.

Pepper seeds were sown in containers in a mixture of peat and vermiculite (ratio 5:1 v/v) and incubated in a growth chamber (25 °C day/20 °C night, 8 h light 16 h darkness; 70% relative humidity). Inoculation with *P. capsici* took place 6 weeks after sowing. Inoculation was done using 5 ml of zoospore suspension at a concentration of 2×10^3 zoospores/ml. The bioassay included three replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 8 plants. An extra set of 8 plants per treatment, per seed lot was left uninoculated as a control. Seeds from a resistant and a susceptible pepper line were also sown in every container as an additional control of disease uniformity per container.

Treatments were not paired. Post-emergence infection symptoms were scored using an ordinal scale of three levels, namely 9 (dead/seriously compromised), 5 (necrotic ring in the crown) and 1 (plants without symptoms). Since infection was scored per plant, the median of a replicate could also take the values 3 and 7. Symptoms were recorded 1, 2 and 3 weeks post-inoculation. To validate bioassay quality, it was ensured that all susceptible variety replicates gave a score of 9.

9. Red fescue – *Laetisaria fuciformis* (DLF Seeds B.V.)

The inoculum was prepared by growing *Laetisaria fuciformis* (DLF Seeds B.V. isolate; originating from infected plant tissue from sport grass field in Denmark) on 50% PDA for 7 to 10 days and then collecting fungal plugs from the actively growing edge of the colony, near the borders of the petri dish.

Red fescue seeds were sown in test tubes containing sterile sports field soil mixture. Each tube was inoculated by adding a fungal plug 10 days after sowing. The bioassay included three replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 20 seeds.

All replicates were incubated in the same growth chamber in saturated relative humidity. The conditions differed for pre-inoculation (30 °C day/20 °C night, 8 h fluorescent light/16 h darkness; 10 days) and post-inoculation (20 °C day/15 °C night, 8 h fluorescent light/16 h darkness; additional 20 days). Post-emergence infection symptoms were scored based on the total infected area relatively to the total leaf area using an ordinal scale from 0 to 5, with 0 being 'healthy' and 5 being 'most diseased/dead'. Symptoms were recorded 20 days post-inoculation.

10. Perennial ryegrass – *Laetisaria fuciformis* (DLF Seeds B.V)

Perennial ryegrass seeds were used in this protocol, which is in all other aspects identical to bioassay (9) Red fescue – *Laetisaria fuciformis*.

11. Perennial ryegrass – *Puccinia* species (DLF Seeds B.V.)

A mix of different undetermined *Puccinia* strains was selected and tested for virulence on perennial ryegrass, then used for the study (*Puccinia* sp. DLF Seeds B.V. isolates; originating from infected plant tissue from sport grass field in the Netherlands). The pathogen strain mix was grown on perennial ryegrass leaves for 2 weeks. Spores were collected by washing the leaves with sterile water to prepare a 1×10^5 spores/ml spore suspension.

Perennial ryegrass seeds were sown in test tubes containing sterile sports field soil mixture. The bioassay included three replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 20 seeds. Each tube was inoculated 10 days after sowing by adding 1 ml of the spore suspension.

Incubation and data collection was identical to bioassays (9) Red fescue – *Laetisaria fuciformis* and (10) Perennial ryegrass – *Laetisaria fuciformis*.

<u>Germination tests</u>

1. Beetroot (Bejo Zaden B.V.)

Germination was tested as per ISTA (2021), at alternating temperatures T= 20 °C of 16 h and T= 30 °C for 8 h and saturated relative humidity. The test included four replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 50 seeds placed in a box between pleated paper. These boxes were placed in a climate cell with light. The percentage of germinated seeds was recorded 4 days (germination energy) and 7 days after sowing (germination capacity).

2. Onion (Bejo Zaden B.V.)

Germination was tested as per ISTA (2021), at temperature T= 18 °C and saturated relative humidity. The test included four replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 50 seeds placed in a box inside blotter paper that had been folded in two. These boxes were kept in the dark. The percentage of germinated seeds was recorded 6 days (germination energy) and 8 days after sowing (germination capacity).

3. Spinach (Pop Vriend Seeds B.V.)

Germination was tested as per ISTA (2021), at temperature T= 10 °C and saturated relative humidity. The test included four replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 50 seeds placed in a box between pleated paper. These boxes were placed in a climate cell with light. The percentage of seeds with radicle emergence was recorded 7 days after sowing (germination energy) and the percentage of normal germinated seedlings was recorded 21 days after sowing (germination capacity).

4. Coriander (CN Seeds Ltd.)

Germination was tested as per ISTA (2021), at temperature T= 20 °C and 72% relative humidity. The test included four replicates per treatment (treated, untreated) per seed lot. Per replicate, 50 coriander seeds were placed on a single piece of filter paper. Each seed comprises of two viable seeds (the contents of the husk) adding up to 100 individual seeds. The percentage of germinated seeds was recorded 14 days after sowing.

5. Pepper (Nunhems Netherlands B.V. (BASF))

Germination was tested as per ISTA (2021), at alternating temperatures (30 °C day/20 °C night, 8 h fluorescent light/16 h darkness) and saturated relative humidity. The test included two replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 100 seeds placed on a single rectangular piece of filter paper in a transparent tray. The percentage of germinated seeds was recorded 14 days after sowing.

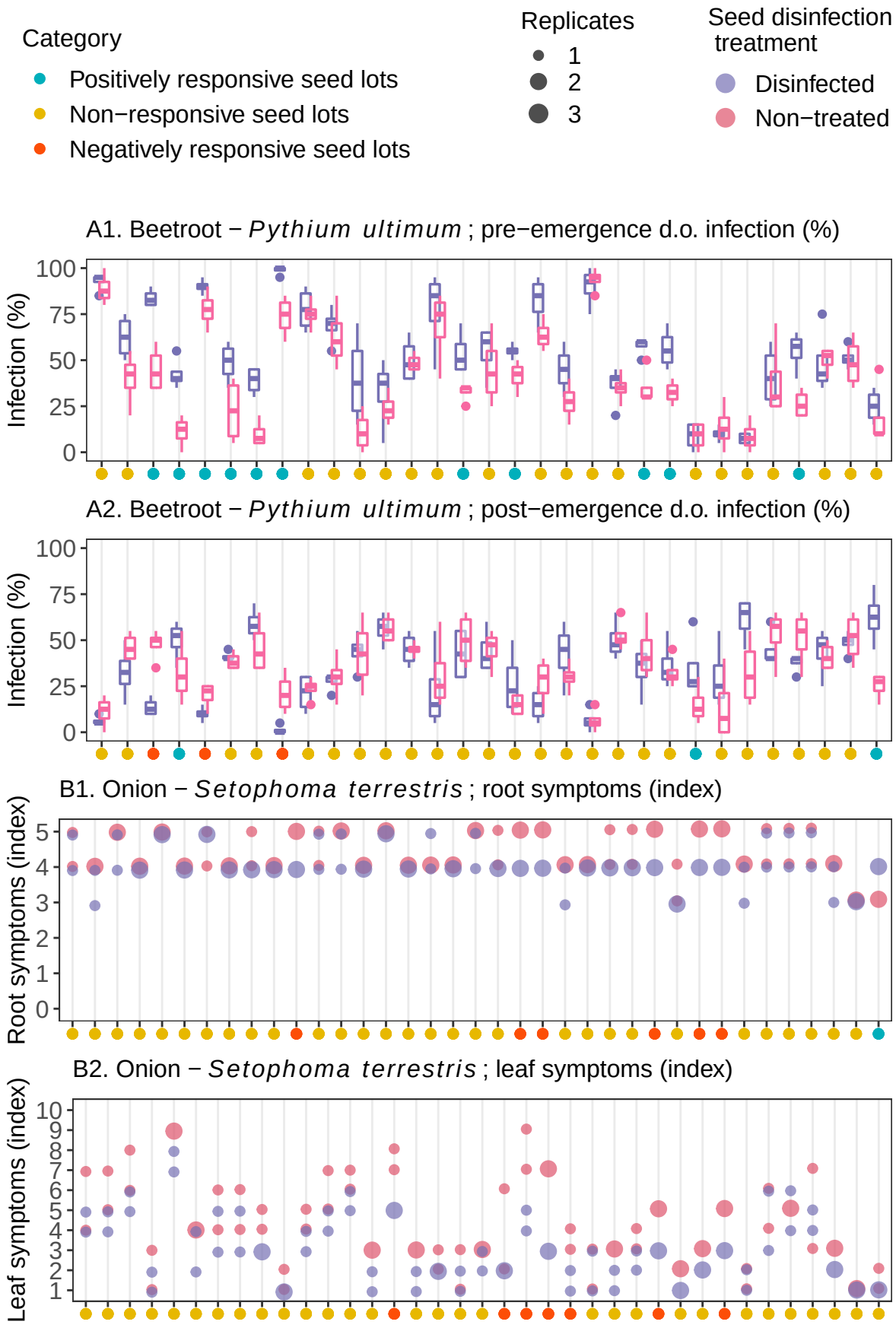
6. Red fescue (DLF Seeds B.V.)

Germination was tested as per ISTA (2021), using the method TP20-30 (Top of Paper, saturated relative humidity, 30 °C day/20 °C night, 8 h fluorescent light/16 h darkness). The test included three replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 100 seeds. The percentage of germinated seeds was recorded 14 days after sowing.

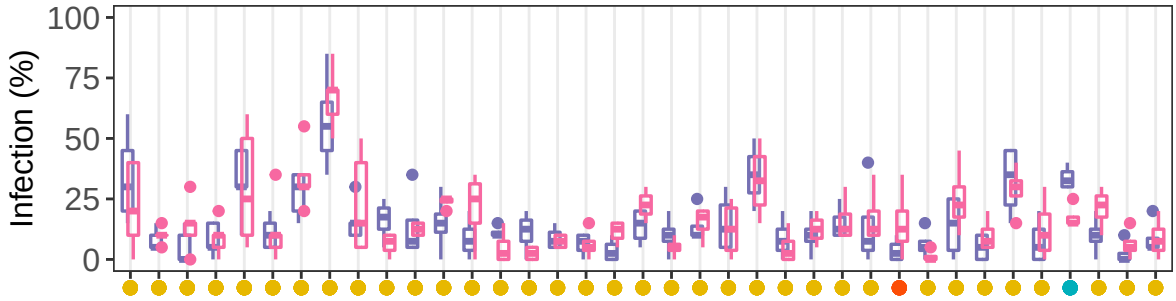
7. Perennial ryegrass (DLF Seeds B.V.)

Germination was tested as per ISTA (2021), using the method TP20-30 (Top of Paper, saturated relative humidity, 30 °C day/20 °C night, 8 h fluorescent light/16 h darkness). The test included three replicates per treatment (treated, untreated) per seed lot and each replicate comprised of 100 seeds. The percentage of germinated seeds was recorded 10 days after sowing.

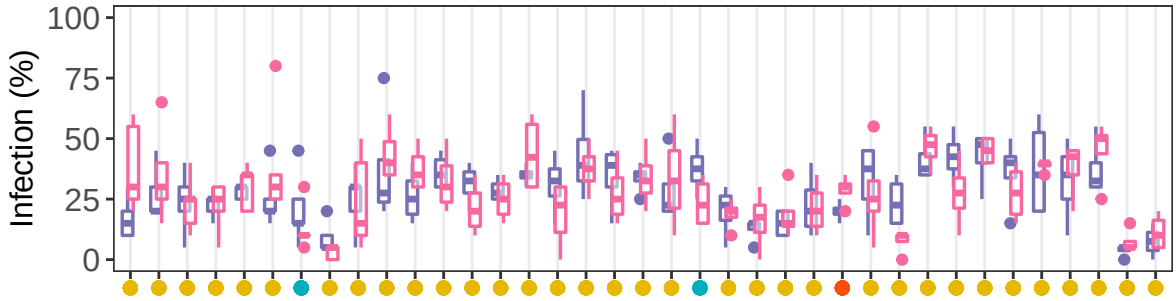
Supplementary Figures



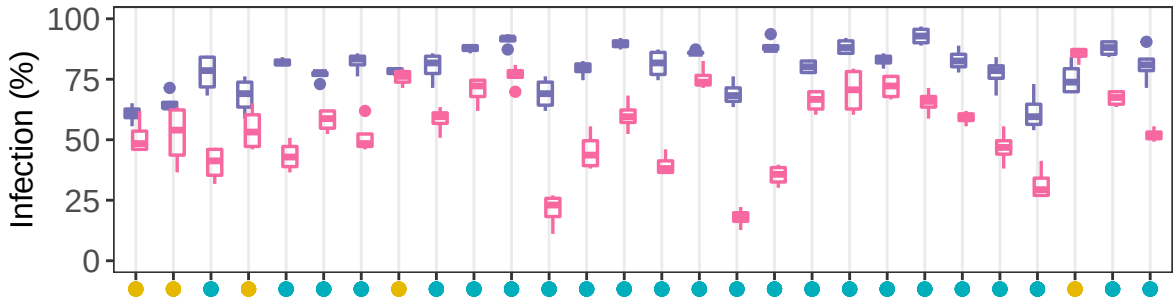
C1. Onion − *Fusarium oxysporum* ; pre−emergence d.o. infection (%)



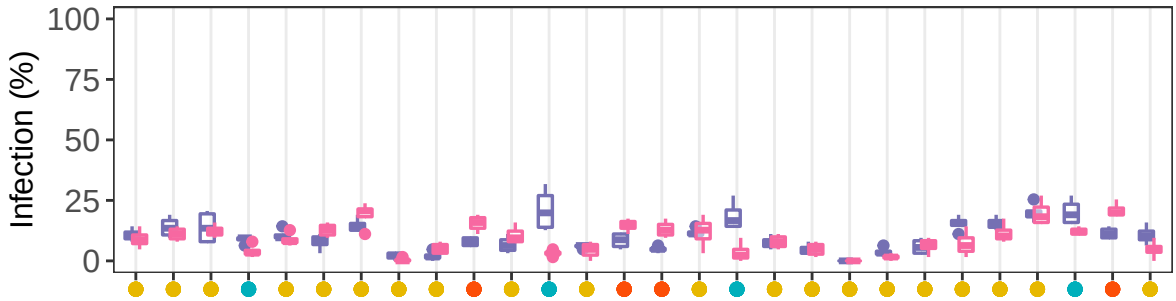
C2. Onion − *Fusarium oxysporum* ; post−emergence d.o. infection (%)



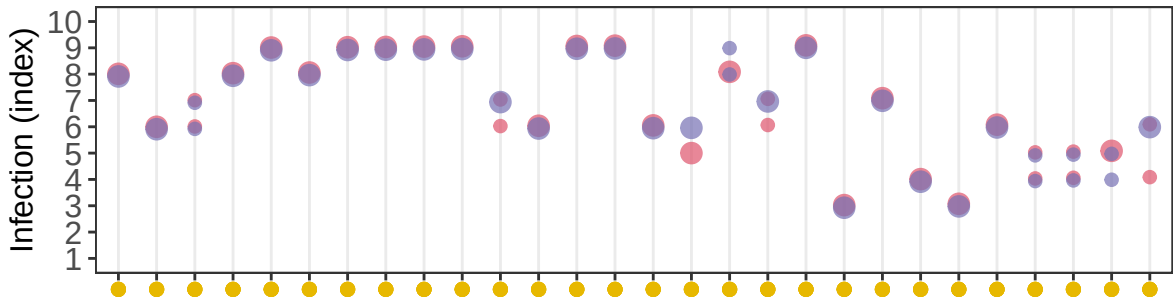
D1. Spinach − *Pythium ultimum* ; pre−emergence d.o. infection (%)



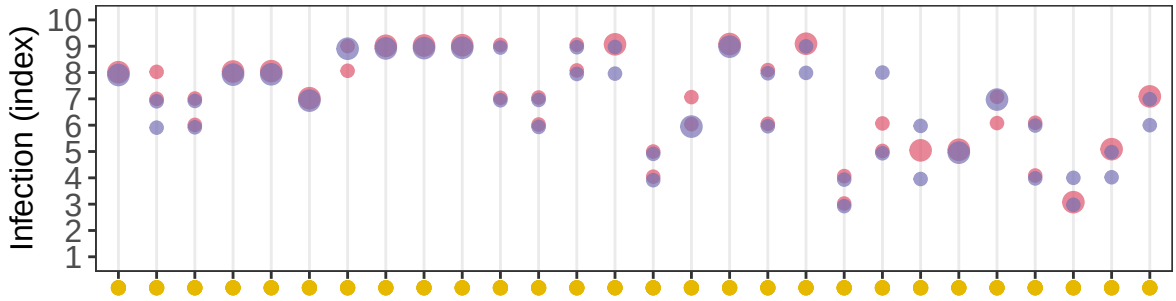
D2. Spinach − *Pythium ultimum* ; post−emergence d.o. infection (%)



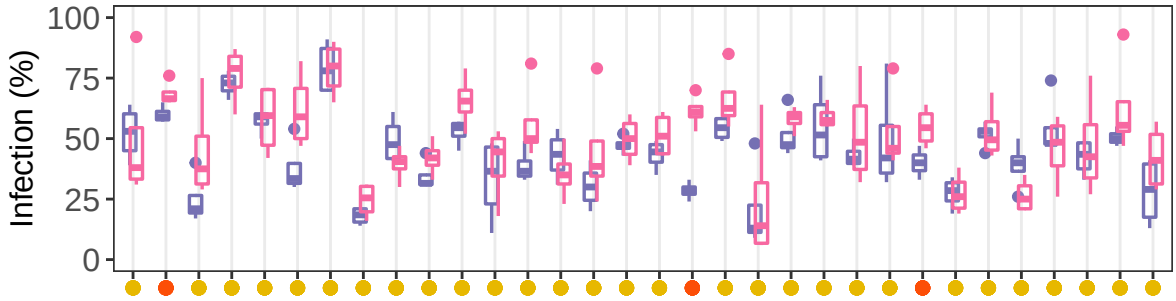
E1. Spinach − *Fusarium oxysporum* ; strain Fus322 infection (index)



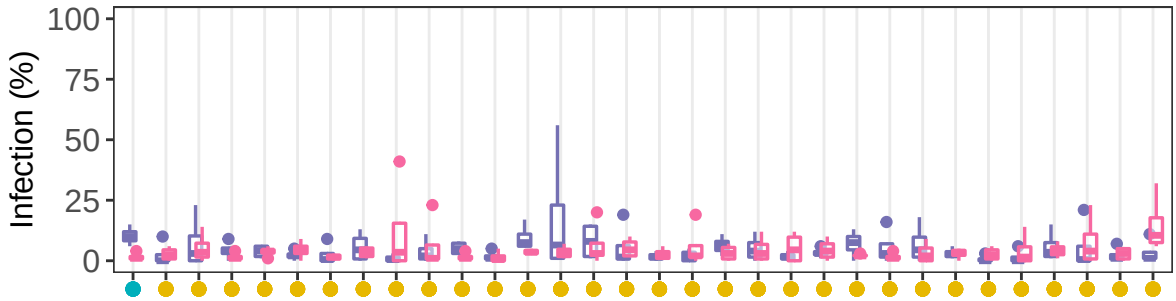
E2. Spinach − *Fusarium oxysporum* ; strain Fus254 infection (index)



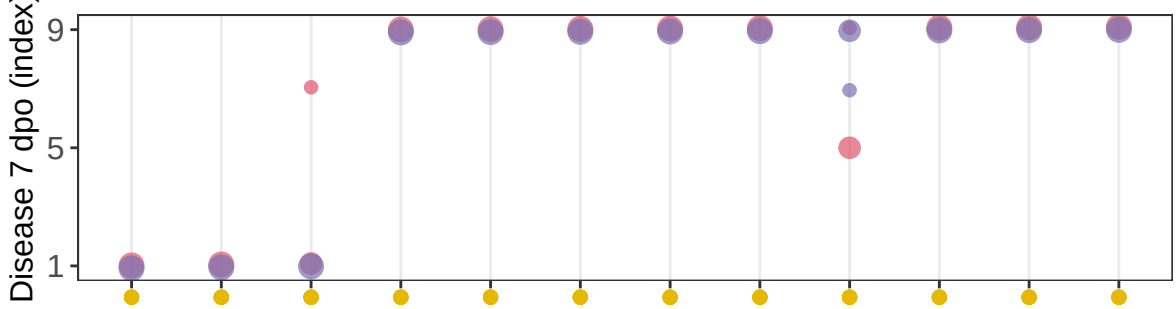
F1. Coriander − *Pythium* sp.; pre−emergence d.o. infection (%)



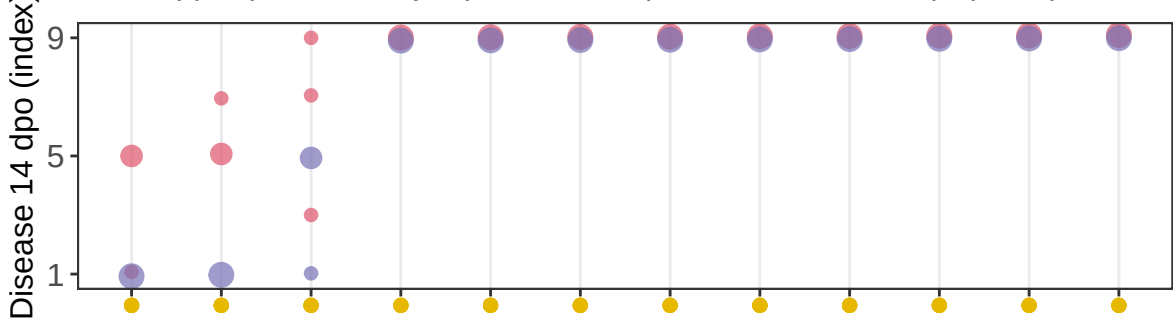
F2. Coriander − *Pythium* sp.; post−emergence d.o. infection (%)



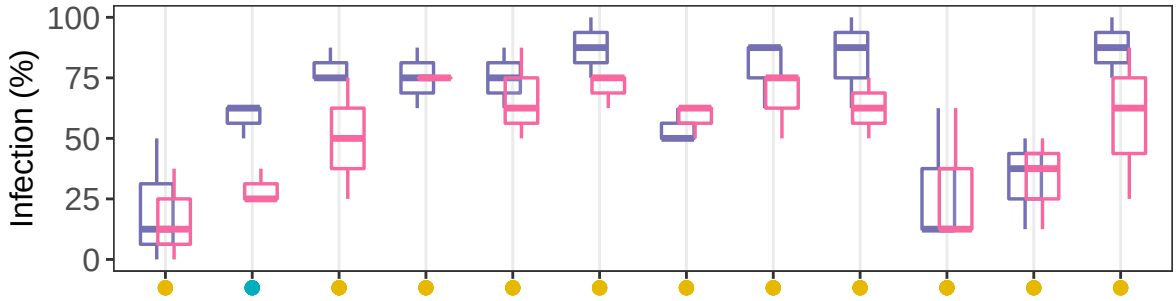
G1. Pepper plants − *Phytophthora capsici* ; disease 7 dpi (index)



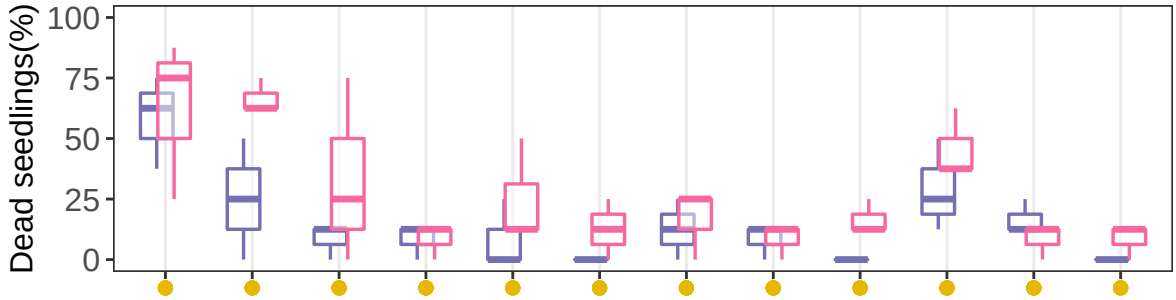
G2. Pepper plants − *Phytophthora capsici* ; disease 14 dpi (index)



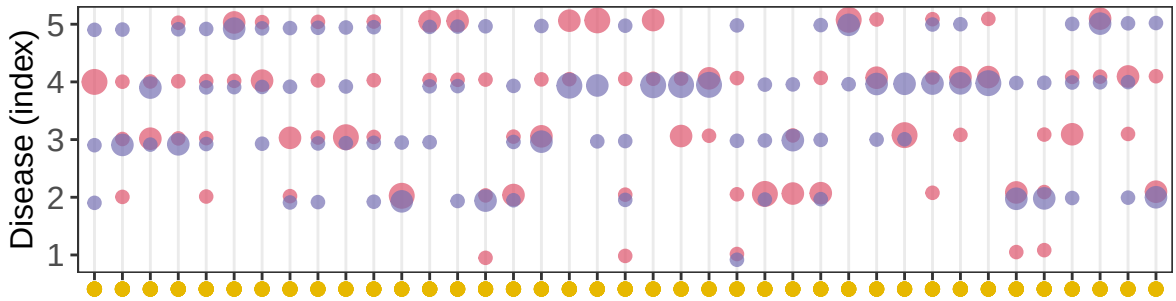
H1. Pepper seedlings − *Phytophthora capsici* ; pre−emergence d.o. infection (%)



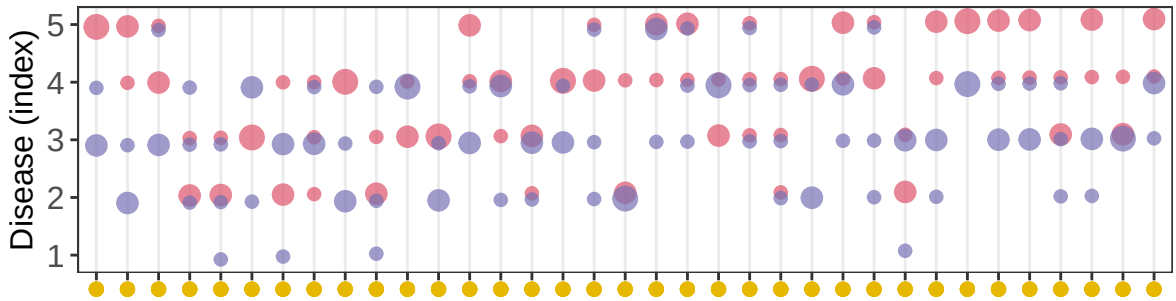
H2. Pepper seedlings − *Phytophthora capsici* ; dead seedlings (%)



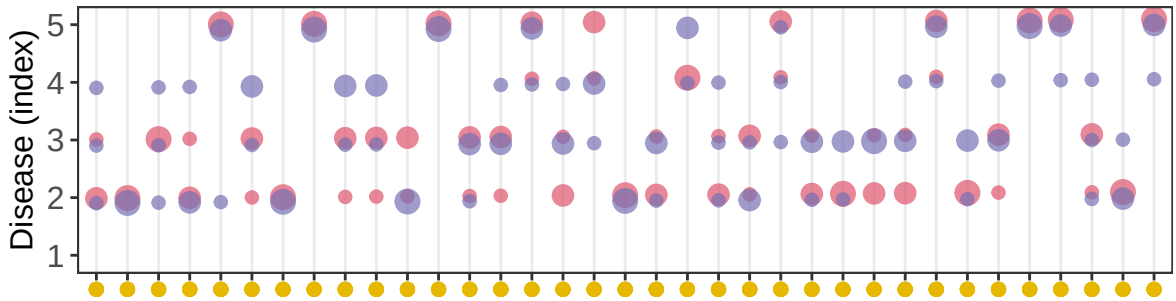
I. Red fescue − *Laetisaria fuciformis* ; Leaf symptoms (index)



J. Perennial ryegrass − *Laetisaria fuciformis* ; Leaf symptoms (index)



K. Perennial ryegrass − *Puccinia* sp.; Leaf symptoms (index)



Supplementary Figure 1: Detailed view of seed lot performance per bioassay as categorised for different disease variables. Each graph compares the performance of treated (purple) and untreated (pink) seeds for a given disease variable (y axis); the vertical lines serve for visual guidance since they group the box plots or data points per seed lot (x axis). The coloured points below the x axis annotate the seed lots per category (positively responsive, non-responsive and negatively responsive). Count data (generalised linear model, α = 0.05; false discovery rate correction α = 0.10) are presented with box plots. Each seed lot is represented by two box plots which partially overlap. Ordinal data (generalised linear model, α = 0.05; false discovery rate correction α = 0.10; exceptions: pepper *P. capsici* bioassay proportional odds logistic model, α = 0.05; onion – *S. terrestris* and spinach – *F. oxysporum* bioassays cut-off threshold of 2 in 10 or 1 in 5 scale index levels) are presented with overlapping point counts, where point size represents number of replicates with the same y value. Higher levels signify increased infection/presence of symptoms across all bioassays. To assist data visualisation of overlapping points, these are slightly displaced vertically (using ggplot2 position_dodgev).

References

ISTA (2021) I*nternational rules for seed testing*.

Ristaino JB (1990) Intraspecific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. *Phytopathology* **80**,1253–1259.