

## Research Paper

**Cite this article:** Diakaki M *et al* (2022). Beetroot and spinach seed microbiomes can suppress *Pythium ultimum* infection: results from a large-scale screening. *Seed Science Research* **32**, 274–282. <https://doi.org/10.1017/S0960258522000186>


Received: 13 March 2022  
Accepted: 29 July 2022  
First published online: 7 September 2022

**Key words:**

disease suppression; plant–pathogen bioassay; seed disinfection; seed microbiome

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# Beetroot and spinach seed microbiomes can suppress *Pythium ultimum* infection: results from a large-scale screening

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**Abstract**

Seed health is an indispensable prerequisite of food security. While the toolkit of plant protection products is currently limited, evidence suggests that the seed microbiome could protect seeds from pathogens. Thus, given their possible disease suppressive potential, we tested 11 different pathosystems to achieve the following proof-of-concept: seed microbiomes can be beneficial for seed health through conferring disease suppression. This study focused on beetroot, onion, spinach, pepper, coriander, red fescue and perennial ryegrass seeds, with each crop being challenged with one or two from a total of six pathogens, namely *Pythium ultimum* (or a *Pythium* sp.), *Setophoma terrestris*, *Fusarium oxysporum*, *Phytophthora capsici*, *Laetisaria fuciformis* and a mix of *Puccinia* sp. isolates. Each seed lot of each crop was tested with and without treatment with a disinfectant as a proxy for comparing intact seed microbiomes with seed microbiomes after partial elimination by disinfection. We found disease suppression in two pathosystems. Beetroot and spinach seed lots were able to suppress disease caused by *P. ultimum* when their microbiomes were intact but not after seed disinfection. We speculate that this relates to the microorganisms residing on and in the seed. Yet, seed microbiome disease suppression was not found in all pathosystems, highlighting the variation in seed morphology, plant cultivars, pathogens and seed disinfection treatments. A holistic understanding of the characteristics of seeds that harbour suppressive microbiomes as well as the pathogens that are sensitive to suppression could lead to more targeted and informed seed processing and treatment and, consequently, to the sustainable management of seedling diseases.

**Introduction**

Succeeding in feeding an expanding global population while simultaneously mitigating the consequences of climate change, requires securing the foundations of global food production, including the production of healthy seeds for food and feed (McGuire and Sperling, 2011; Husenov *et al.*, 2021). Mass production of seeds for agriculture is currently primarily the task of specialized companies which ensure the delivery of robust, healthy seeds to farmers. Among other steps, seed processing might include seed disinfection, which aims at killing seedborne pathogens without damaging the seed (Copeland and McDonald, 2001). Disinfection can be complemented by coating seeds with products that can protect them during germination. However, multiple plant protection products that can be applied on seeds have recently been banned from use in the EU while the authorization of alternative options progresses very slowly (Sundh and Eilenberg, 2021).

This limitation has sparked an interest in the role of seed epiphytes and endophytes in seed health. Together, these two types of seed-inhabiting microbes comprise the seed microbiome. Given the latter's functional potential and possible role in disease suppression (Links *et al.*, 2014; Kumar *et al.*, 2017; Verma and White, 2019; Solanki *et al.*, 2021), it can be hypothesized that, in the absence of chemical or other protection on the seed or in the soil, an intact microbiome may act as a barrier, protecting the seed from infection by plant pathogens in the field. Since disinfection treatments typically are non-specific, given that they are developed to eliminate bacterial and fungal pathogens from seed samples (Maude, 1996; Black *et al.*, 2006), they can serve as a tool for reducing the microbial load of seeds and, subsequently, allow investigating seed health in seeds bearing a reduced microbiome. In fact, as reviewed by Berg and Raaijmakers (2018), there is scientific evidence that disinfectant seed treatment applications can negatively affect the seed microbiome.

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Because of the variation in seed types, pathogens and seed disinfection treatments, exploring the disease suppressive potential of seed microbiomes will benefit from large-scale screening using different pathosystems (plant–pathogen combinations). Although the disease is expressed and evaluated on the plant that develops from a seed, rather than the seed itself, we chose to refer to ‘susceptible seeds’ rather than ‘susceptible seedlings developing from seeds’ for simplicity throughout this paper. In this systematic study, disease susceptibility was compared between disinfected and non-treated seeds of *Beta vulgaris* (beetroot), *Allium cepa* (onion), *Spinacia oleracea* (spinach), *Capsicum annuum* (pepper), *Coriandrum sativum* (coriander), *Festuca rubra* (red fescue) and *Lolium perenne* (perennial ryegrass). This was done in a total of 11 bioassays in which 20 to 40 seed lots per crop were challenged with a pathogen of interest for the seed industry. The seed disinfection methods were chosen based on practices used in the seed industry. This large dataset has been created for unravelling the potential role of seed microbiomes in seed health and was used to achieve the following proof-of-concept: seed microbiomes can be beneficial for seed health through conferring disease suppression.

## Material and methods

### Crops and seed lots

For beetroot, onion, spinach, coriander, red fescue and perennial ryegrass, 40 seed lots were used per crop, while for pepper, there were 20 seed lots, adding up to 260 seed lots in total. These were selected to represent a diversity of cultivars and harvest locations spanning a diverse range of seed lots and thus, presumably, also a diverse range of seed microbiomes. Seed lots were provided by Bejo Zaden B.V., Nunhems Netherlands B.V. (BASF), Pop Vriend Seeds B.V., CN Seeds Ltd., DLF B.V. and Sakata Vegetables Europe S.A.S.

### Bioassays

In order to study the performance of seeds with a reduced microbiome, a seed disinfection treatment was applied to each crop

(Table 1; details in Supplementary Materials and Methods). For every seed lot, disinfected and non-treated seeds were compared for their susceptibility to a given pathogen. Crop–pathogen combinations tested as well as bioassay design and number of replicates were selected on the basis of the availability of pre-validated protocols (Table 1; details in Supplementary Materials and Methods). A maximum of two disease variables per bioassay were incorporated in the data analysis. These are considered the most relevant and are summarized below in Table 2.

Although the experimental design of the 11 bioassays differed, they all aimed at detecting a difference in disease susceptibility between disinfected and non-treated seeds of the same lot. There were three possible outcomes for every seed lot per bioassay, namely higher, lower or unaltered disease susceptibility due to seed disinfection. Each of these outcomes allowed defining a category to describe the performance of a seed lot when challenged with a given pathogen after seed disinfection (Table 3).

### Seed germination tests

Seed germination was tested for all 260 seed lots for both disinfected and non-treated samples of seeds. The tests followed the protocols established by the International Seed Testing Association (ISTA, 2020) and were done on filter paper (see Supplementary Materials and Methods). They served as a control for possible physiological side-effects of the disinfection treatments on the seeds. For each seed lot, the germination of disinfected and non-treated seeds was compared. For beetroot, onion and spinach, data for both germination capacity and energy were used, while for coriander, pepper, red fescue and perennial ryegrass only germination capacity data were generated for analysis. Germination capacity is defined as the percentage of seeds able to germinate under optimal conditions, excluding abnormally formed seedlings. Germination energy refers to the percentage of fast-germinating seeds. Since the number of days after seed sowing when germination capacity and energy are scored differs per crop, these specifications are available in the Supplementary Materials and Methods. Seed germination following the ISTA protocols was examined to ensure that a difference in seed

**Table 1.** Crops, seed disinfection treatments and pathogens used to assess the potential effects of the seed microbiome on disease suppression<sup>a</sup>

Bioassay	Crop	Disinfection treatment	Pathogen
1	<i>Beta vulgaris</i> (Beetroot)	Steam vacuum	<i>Pythium ultimum</i>
2	<i>Allium cepa</i> (Onion)	Steam vacuum	<i>Setophoma terrestris</i>
3	<i>Allium cepa</i> (Onion)	Steam vacuum	<i>Fusarium oxysporum</i> f. sp. <i>cepae</i>
4	<i>Spinacia oleracea</i> (Spinach)	Steam vacuum	<i>Pythium ultimum</i>
5	<i>Spinacia oleracea</i> (Spinach)	Steam vacuum	<i>Fusarium oxysporum</i> f. sp. <i>spinaciae</i>
6	<i>Coriandrum sativum</i> (Coriander)	Hot water	<i>Pythium</i> sp.
7	<i>Capsicum annuum</i> (Pepper); seedlings	Steam vacuum	<i>Phytophthora capsici</i>
8	<i>Capsicum annuum</i> (Pepper); plants	Steam vacuum	<i>Phytophthora capsici</i>
9	<i>Festuca rubra</i> (Red fescue)	Sodium hypochlorite	<i>Laetisaria fuciformis</i>
10	<i>Lolium perenne</i> (Perennial ryegrass)	Sodium hypochlorite	<i>Laetisaria fuciformis</i>
11	<i>Lolium perenne</i> (Perennial ryegrass)	Sodium hypochlorite	<i>Puccinia</i> sp.

<sup>a</sup>See Supplementary Materials and Methods for details.

**Table 2.** Selected disease variables and data types in the different bioassays used to assess the potential effects of the seed microbiome on disease suppression

Bioassay	Disease variable 1	Disease variable 2	Data type
1 Beetroot – <i>Pythium ultimum</i>	Pre-emergence damping-off (number of non-emerged seedlings)	Post-emergence damping-off (number of infected emerged seedlings)	Count data
2 Onion – <i>Setophoma terrestris</i>	Root symptoms (root disease symptom severity)	Leaf symptoms (leaf disease symptom severity)	Ordinal data
3 Onion – <i>Fusarium oxysporum</i> f. sp. <i>cepae</i>	Pre-emergence damping-off (number of non-emerged seedlings 13 days post-inoculation)	Post-emergence damping-off (number of infected emerged seedlings 13 days post-inoculation)	Count data
4 Spinach – <i>Pythium ultimum</i>	Pre-emergence damping-off (number of non-emerged seedlings)	Post-emergence damping-off (number of infected emerged seedlings)	Count data
5 Spinach – <i>Fusarium oxysporum</i> f. sp. <i>spinaciae</i>	Strain Fus322 infection (disease symptom severity)	Strain Fus254 infection (disease symptom severity)	Ordinal data
6 Coriander – <i>Pythium</i> sp.	Pre-emergence damping-off (number of non-emerged seedlings)	Post-emergence damping-off (number of infected emerged seedlings)	Count data
7 Pepper seedlings – <i>Phytophthora capsici</i>	Pre-emergence damping-off (number of non-emerged seedlings)	Dead plants (number of emerged dead seedlings)	Count data
8 Pepper plants – <i>Phytophthora capsici</i>	Disease (disease symptom severity 7 days post-inoculation)	Disease (disease symptom severity 14 days post-inoculation)	Ordinal data
9 Red fescue – <i>Laetisaria fuciformis</i>	Leaf symptoms (disease symptom severity)	–	Ordinal data
10 Perennial ryegrass – <i>Laetisaria fuciformis</i>	Leaf symptoms (disease symptom severity)	–	Ordinal data
11 Perennial ryegrass – <i>Puccinia</i> sp.	Leaf symptoms (disease symptom severity)	–	Ordinal data

emergence in a bioassay could be accredited to pre-emergence damping-off infection rather than a difference in germination caused by a physiological side-effect of the treatment. Hence, seed lots showing a significant difference ( $\alpha = 0.05$ ) in germination between disinfected and non-treated seeds were excluded from the bioassay data analyses.

### Statistical analysis

All statistical analyses were performed with R version 4.0.2 (R Core Team, 2020). Given the heterogeneity in data structure, the datasets were analysed using different models. All datasets and scripts used for the analyses can be found in the 4TU database (doi 10.4121/20004842) which can be accessed at <https://data.4tu.nl/portal/>.

Count data such as number of germinated, emerged or diseased seedlings were analysed using generalized linear models with the glm function of the multcomp package (Hothorn et al., 2008). The dispersion of count data was examined using the testDispersion function of the DHARMA (Hartig, 2019) package. In order to account for data over- or under-dispersion, a quasibinomial distribution was used in the generalized linear models.

Ordinal data for the pepper (plants) – *P. capsici* bioassay were analysed with the polr function of the MASS package (Venables and Ripley, 2002) fitting a proportional odds logistic regression model to the data. The ordinal data of bioassays focusing on grass species (red fescue – *L. fuciformis*, perennial ryegrass – *L. fuciformis* and perennial ryegrass – *Puccinia* sp.) could not be modelled in the same manner: disease symptom data were recorded on an ordinal scale of six levels, with many levels having

**Table 3.** Definition of seed lot categories based on susceptibility of disinfected seeds compared to non-treated seeds in a bioassay to assess the potential effects of seed microbiomes on disease suppression

Category	Bioassay outcome
Positively responsive seed lots	Non-treated seeds are significantly less susceptible to infection than disinfected seeds. An intact seed microbiome is beneficial.
Negatively responsive seed lots	Non-treated seeds are significantly more susceptible to infection than disinfected seeds. An intact seed microbiome is disadvantageous.
Non-responsive seed lots	There is no significant difference in infection between non-treated and disinfected seeds. The seed microbiome does not influence pathogen infection rates or disease severity.

no data after all replicates were scored. The six levels were thus grouped into two: level A included replicates with scores of 0, 1 and 2 (healthy or with few symptoms) while level B included replicates with scores of 3, 4 and 5 (severe symptoms or dead plants). Consequently, the data were modelled using the glm function of the multcomp (Hothorn et al., 2008) package to fit a logistic regression in the model (binomial distribution; logit link). Data were visualized with the ggplot2 package (Wickham, 2009).

The Benjamini–Hochberg procedure was used to control for false discovery rate (FDR) at level  $\alpha = 0.10$ , given that the analysis comprised multiple comparisons. FDR was controlled in the analysis of the bioassay datasets to ensure a correction for type I

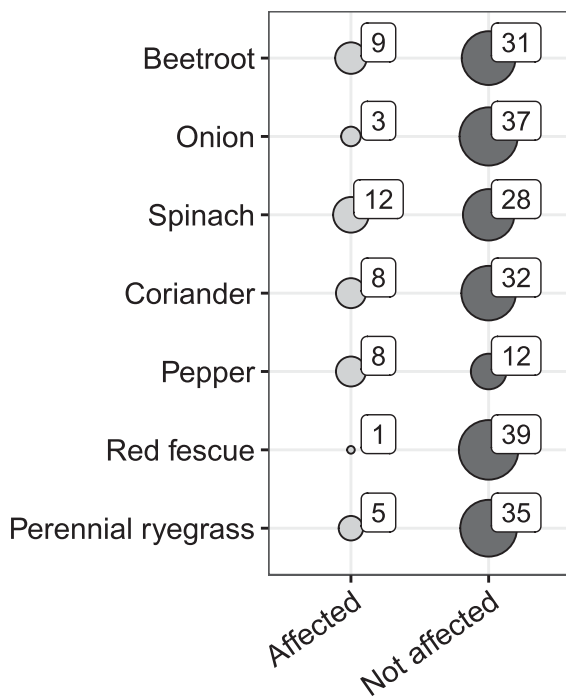
errors (falsely considering a result significant). When analysing germination data, it was important to minimise type II errors (falsely considering a result non-significant) and so the results were not corrected for FDR.

Three datasets were not analysed statistically because less than three replicates per seed lot per treatment were available. These included the onion – *S. terrestris* bioassay, the spinach – *F. oxysporum* f. sp. *spinaciae* bioassay and the pepper germination data. For the first two bioassays, disease severity of disinfected and non-treated seeds would be considered the same (not ‘significantly’ different) when their medians differed by <2 out of 10 index levels (spinach – *F. oxysporum* f. sp. *spinaciae* disease variables and onion – *S. terrestris* leaf score) or less than 1 out of 5 index levels (onion – *S. terrestris* root score). For the pepper germination data, an empirically austere cut-off threshold of 3% was established for deciding which seed lots were accepted for data analysis.

## Results

### Seed germination tests

Seed lots that showed a significant difference in germination rate between disinfected and non-treated replicates of seed samples (generalized linear model,  $\alpha = 0.05$ ; exception: 3% cut-off threshold for pepper, Fig. 1) were excluded from further analysis. Consequently, data are shown for 214 of the 260 seed lots that were examined. Specifically, these included 31 beetroot, 37 onion, 28 spinach, 32 coriander, 12 pepper, 39 red fescue and 35 perennial ryegrass seed lots (Fig. 1). Forty-six seed lots were excluded from the study due to either a positive or negative disinfectant treatment effect on seed germination.



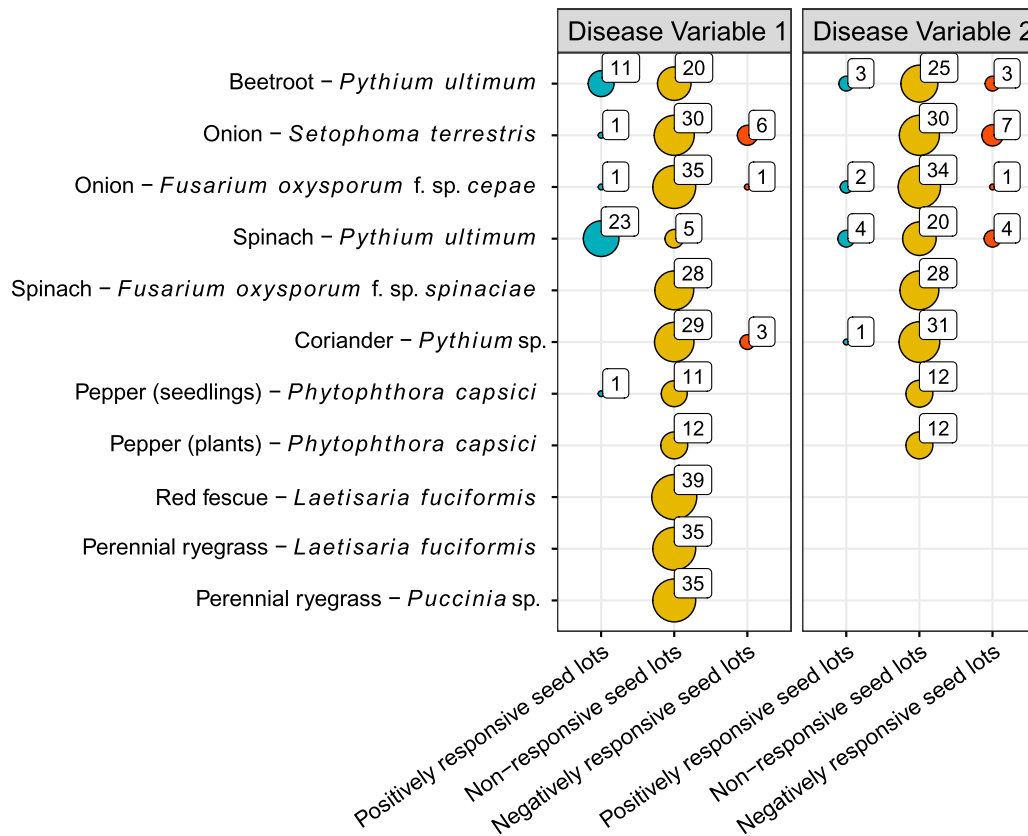
**Fig. 1.** Total number of seed lots per crop for which seed germination was not affected by the seed disinfection treatment (generalized linear model,  $\alpha = 0.05$ ; with one exception: pepper germination cut-off threshold of 3% difference). The size of each circle is proportional to the number of seed lots.

### Bioassays

Focusing on the 214 seed lots for which seed germination was not affected by the respective disinfection treatment (Fig. 1), bioassay results are shown for a total of 326 seed lot – pathogen combinations. The effect of seed treatment on each lot was determined by either a generalized linear model ( $\alpha = 0.05$ ) or a proportional odds logistic model ( $\alpha = 0.05$ ), with the latter only used for the data of the pepper (plants) – *P. capsici* bioassay.

Overall, the number of seed lots in each category varied across bioassays (Figs 2 and 3; Supplementary Fig. S1). Figure 3 visualises the mean (count data) or median (ordinal data) level of disease for non-treated ( $x$ -axis) and disinfected ( $y$ -axis) replicates per seed lot per bioassay, thus facilitating comparisons of the two within and across pathosystems. Given the large amount of data reported in this work, a detailed representation of all data points per replicate can be found in Supplementary Fig. S1. Both figures include an annotation of seed lots per category. In the bioassays examining coriander, pepper, red fescue and perennial ryegrass, the disinfection treatments had no effect on susceptibility for all or almost all seed lots for both disease variable 1 and 2. For disease variable 1, only 3 out of 32 coriander seed lots and 1 out of 12 pepper seed lots showed significant results when challenged with a *Pythium* sp. and *P. capsici* (seedling bioassay), respectively (Fig. 2). For disease variable 2, only 1 out of 32 coriander seed lots showed significant results when challenged with a *Pythium* sp. This was also the case for onion and spinach seed lots when challenged with *F. oxysporum* f. sp. *cepae* and *F. oxysporum* f. sp. *spinaciae*, respectively. Two and 3 out of 37 onion seed lots showed significant results for disease variable 1 and 2, respectively, while none of the 28 spinach seed lots showed significant results. When onion was challenged with *S. terrestris*, however, 6 and 7 out of 37 seed lots were negatively responsive for disease variable 1 and 2, respectively (data not analysed statistically; see Supplementary Materials and Methods). The two bioassays with the most positively responsive seed lots were those of beetroot and spinach when challenged with *P. ultimum*. In these two bioassays, 11 out of 31 beetroots and 23 out of 28 spinach seed lots were categorized as positively responsive for disease variable 1 (pre-emergence damping-off infection %). For disease variable 2 (post-emergence damping-off infection %), there were equal numbers of positively and negatively responsive seed lots for both bioassays.

For bioassays in which both pre- and post-emergence damping-off infection by the oomycetes *P. ultimum*, a *Pythium* sp., and *P. capsici* were measured, both disinfected and non-treated seeds showed higher levels of pre-emergence damping-off infection in comparison to post-emergence damping-off. This was evident for the bioassays of pepper seedlings – *P. capsici*, beetroot – *P. ultimum*, spinach – *P. ultimum* and coriander – *Pythium* sp. (Fig. 3). For example, in the spinach – *P. ultimum* bioassay, pre-emergence damping-off infection of both disinfected and non-treated seeds was >50% for all seed lots (Fig. 3.D1), while for post-emergence damping-off, it was < 25% for all seed lots (Fig. 3.D2). Bioassays in which pathogen inoculation took place after seedling emergence could not test for pre-emergence damping-off infection. These bioassays were spinach – *F. oxysporum* f. sp. *spinaciae*, pepper plants – *P. capsici*, red fescue – *L. fuciformis*, perennial ryegrass – *L. fuciformis*, and perennial ryegrass – *Puccinia* sp. These five bioassays resulted in no seed lots being categorized as either positively or negatively responsive (Fig. 3).



**Fig. 2.** Total number of seed lots per bioassay considered positively responsive (blue), non-responsive (yellow) or negatively responsive (red) based on the outcome of each bioassay per seed lot (generalized linear model,  $\alpha = 0.05$  and false discovery rate correction  $\alpha = 0.10$  for all bioassays; exceptions: proportional odds logistic model,  $\alpha = 0.05$  for pepper plants – *P. capsici* bioassay; onion – *S. terrestris* and spinach – *F. oxysporum* f. sp. *spinaciae* bioassays cut-off threshold of 2 in 10 or 1 in 5 scale index levels). Specifications for each disease variable can be found in Table 2. The size of each circle is proportional to the number of seed lots.

## Discussion

### Seed disinfection may deprive seeds of natural disease suppressive potential

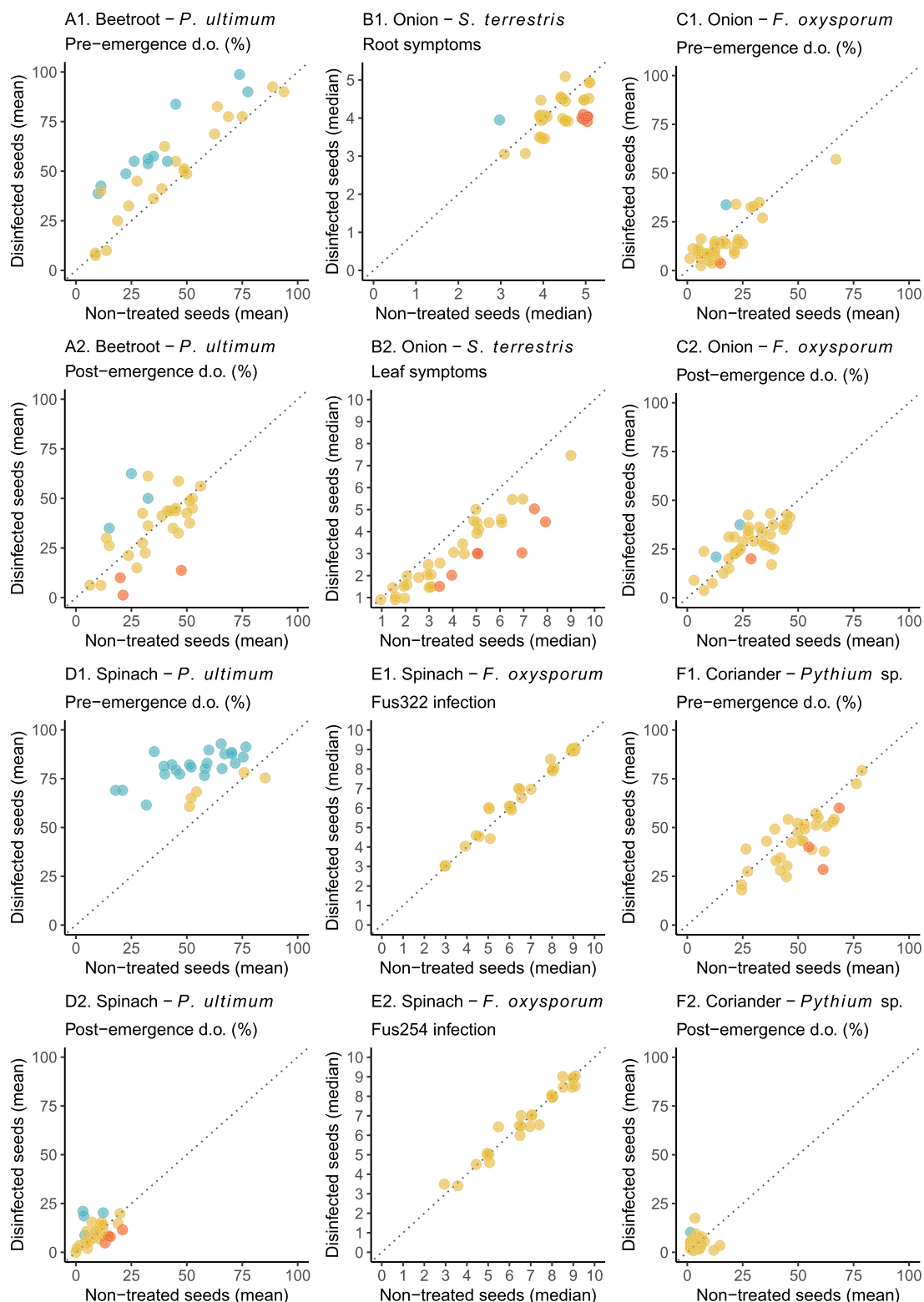
The role of the seed microbiome in seed health and its possible relevance to disease suppression have recently gained the attention of the scientific community (Rezki et al., 2016; Rybakova et al., 2017; Jack and Nelson, 2018; Nelson, 2018; Matsumoto et al., 2021). Certain microorganisms are known for their ability to confer disease suppressiveness to plants, as part of soil microbiomes, for instance. Suppressives soils have an innate ability to regulate specific soilborne pathogens as a result of various soil properties, including microbial biomass and activity (Schlatter et al., 2017; Jayaraman, 2021).

To assess the role of seed microbiomes in disease suppression, a total of 260 seed lots of different production locations and cultivars were examined across 11 bioassays in this study. Out of the total of 326 seed lot–pathogen combinations analysed, seed lots were categorized as positively responsive for 6 of 11 bioassays, predominantly as a result of the beetroot – *P. ultimum* and spinach – *P. ultimum* bioassays. For these two cases, the partial elimination of the seed microbiome by seed disinfection led to an increase in disease susceptibility, implying that the microbiomes of these seed lots may have included pathogen-suppressive microorganisms. Interestingly, a recent study reported that rice plants of the same cultivar could be differentiated for their resistance to the seedborne pathogen *Burkholderia plantarii* based on the presence of the bacterial seed endophyte *Sphingomonas melonis* (Matsumoto et al., 2021).

The results of our study confirm the proof-of-concept that seed microbiomes can confer disease suppression. Two of the 11 bioassays demonstrated a beneficial effect of the seed microbiome on seed health. The lack of significant findings for the other nine bioassays suggests that this type of suppressiveness is pathosystem-specific rather than a general phenomenon. However, it is also possible that suppressiveness was not detected due to characteristics of the various pathogens used in the bioassays. For example, red fescue and perennial ryegrass were challenged with airborne pathogens. Out of 31 beetroot and 28 spinach seed lots representing different seed production locations and different cultivars, 11 and 23, respectively, were categorized as positively responsive, while others showed non-responsive results when challenged with *P. ultimum*, suggesting that seed microbiomes vary in disease suppressive potential. Indeed, seed microbiome composition is known to be affected by plant genotype as well as environmental conditions at the location of cultivation (Klaedtke et al., 2016; Rochefort et al., 2021), and seed microbiomes differ at the plant species level (Torres-Cortés et al., 2018) as well as plant cultivar level (Rybakova et al., 2017; Wolfgang et al., 2020).

### Beetroot and spinach seeds can harbour microorganisms that suppress *P. ultimum*

Given the evidence for soil suppressiveness against members of the genus *Pythium* (Van Os and Van Ginkel, 2001; Scheuerell



**Fig. 3.** Seed lot performance per bioassay per disease variable (generalized linear model,  $\alpha=0.05$  and false discovery rate correction  $\alpha=0.10$  for all bioassays; exceptions: proportional odds logistic model,  $\alpha=0.05$  for pepper plants – *P. capsici* bioassay; onion – *S. terrestris* and spinach – *F. oxysporum* f. sp. *spinaciae* bioassays cut-off threshold of 2 in 10 or 1 in 5 scale index levels). The performance of non-treated and disinfected seeds is plotted on the x and y axis, respectively. Each data point represents the mean (count data) or median (ordinal data) value of an individual seed lot. Data points are colour-coded according to category. Higher levels signify increased infection/presence of symptoms in all graphs. Days post-inoculation is abbreviated as 'dpo' and damping-off is abbreviated as d.o.

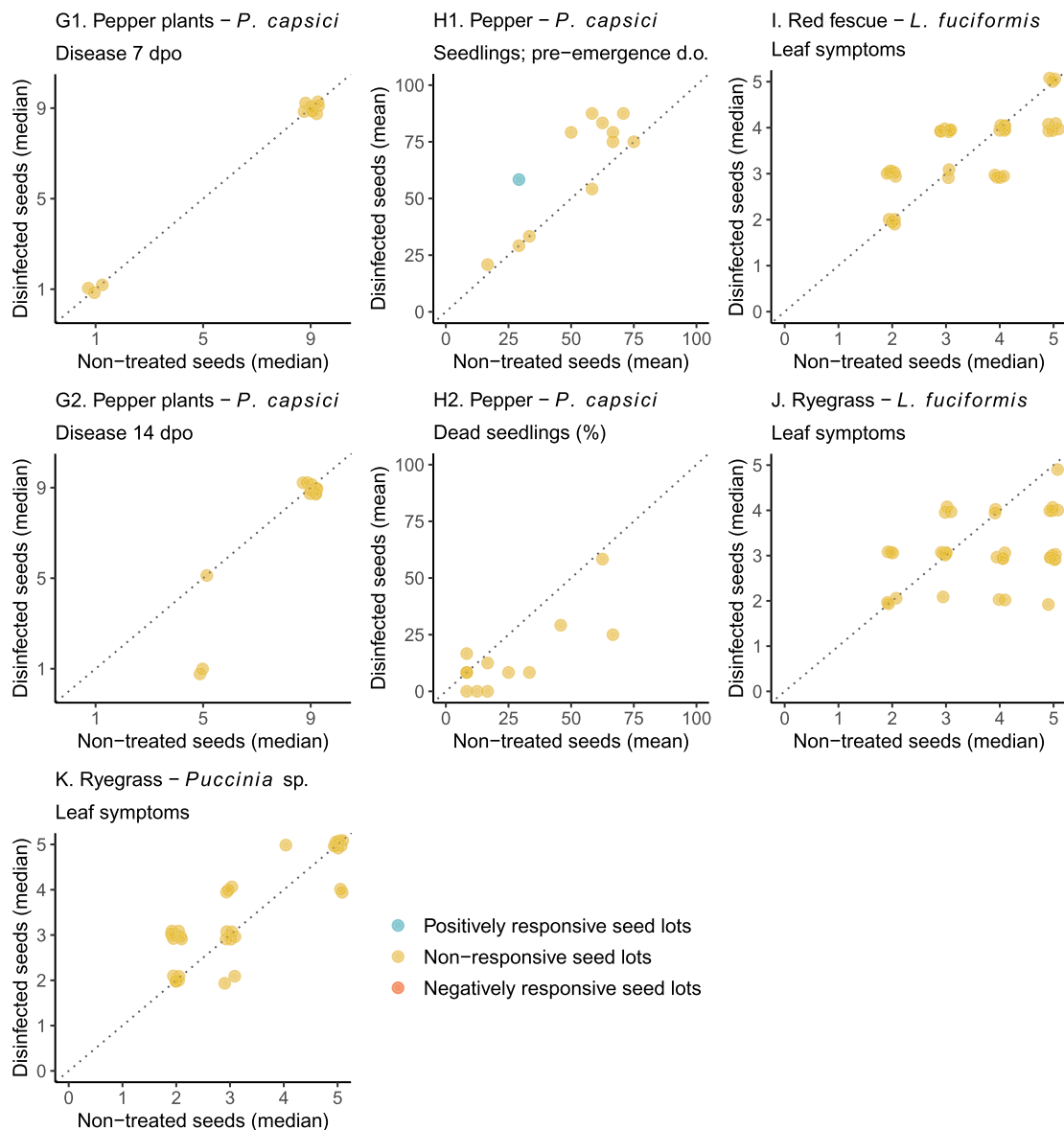


Fig. 3. Continued.

et al., 2005; Gravel et al., 2015), it was expected that this oomycete would be sensitive to seed microbiome suppressiveness as well. Both beetroot and spinach have been the subject of studies showing that different organic amendments in the soil led to *P. ultimum* suppressiveness (Cummings et al., 2009; He et al., 2012).

Beetroot and spinach were the two crops for which the highest level of infection was recorded for disinfected seeds, especially pre-emergence damping-off, with more than 75% infection recorded for 8 of 31 beetroot and 21 of 28 spinach seed lots (Fig. 3.A1, D1). This is in line with recent findings of pre-emergence damping-off being more discriminative than post-emergence damping-off when comparing spinach seed lots for tolerance to *P. ultimum* (Magnée et al., 2021). Additionally, the 3 of 31 beetroot and 4 of 28 spinach seed microbiomes that were categorized as negatively responsive to post-emergence damping-off infection by *P. ultimum*, were categorized as positively responsive to pre-emergence damping-off. Nonetheless, when looking at pre- and post-emergence damping-off

cumulatively, the overall response was positive for these three beetroot and four spinach seed lots (Supplementary Fig. S1).

The increased susceptibility of disinfected seeds compared to non-treated seeds of the same lot could also have been the outcome of micro-niches on the seed surface becoming available to *P. ultimum* after the elimination of microorganisms. This would be in line with a comparably greater microbial biomass in soils suppressive to *Pythium* in comparison to non-suppressive soils (Bongiorno et al., 2019). Regardless of the ecological mechanisms in place, it can be concluded that seed disinfection can deprive seeds of natural suppressive potential against specific pathogens, in this case, *P. ultimum*, and that the proof-of-concept of this study has been validated.

Lastly, suppressiveness towards *Pythium* sp. was not similar in all pathosystems, with no suppressiveness found for the coriander seed lots tested. This could be due to factors such as the different disinfection treatments used, pathogen inoculation techniques and pathogenic strains used for the various crop-pathogen

combinations evaluated (see Supplementary Materials and Methods). In the case of coriander seed lots, the hot water treatment could have facilitated the developing seedlings evading the pathogen by improving seed vigour and causing disinfected seeds to germinate faster (Copeland and McDonald, 2001; Bölek et al., 2013). Additionally, *Pythium* zoospores were used for the coriander - *Pythium* sp. inoculations. Since zoospores are attracted by seed exudates during germination (Nelson, 1991), the latter could have influenced the results of this bioassay. Although the hot water treatment used did not affect the germination capacity for the 32 coriander seed lots examined, we cannot exclude the possibility that the treatment might have increased the leakage of seed exudates. It is also possible that suppressiveness towards *Pythium* is observed for beetroot and spinach seed lots but not for coriander seed lots due to differences in the crop seed microbiome composition or seed morphology. Beetroot and spinach are both members of Amaranthaceae and produce seeds that are, in fact, utricles, a type of indehiscent fruit. In utricles, a papery pericarp structure loosely surrounds the true seed (Copeland and McDonald, 2001; Magnée et al., 2020). Coriander, on the other hand, belongs to Apiaceae and is different morphologically, with the dry fruit comprising two seeds, each enclosed in a separate mericarp inside the sclerotified pericarp (Diederichsen, 1996; Arora et al., 2021).

### Seed microbiome suppressiveness is not persistent across pathosystems

Apart from the multiple positively responsive beetroot and spinach seed lots in the respective *P. ultimum* bioassays in this study, there were only a few other seed lots with significant differences between disinfected and non-treated seeds in the other nine bioassays. All bioassays in which pathogen inoculation took place after seedling emergence led to seed lots being categorized exclusively as non-responsive. This was the case for the spinach - *F. oxysporum* f. sp. *spinaciae* bioassay as well as all bioassays on pepper, red fescue and perennial ryegrass seed lots, with the exception of a single seed lot in the pepper seedling - *P. capsici* bioassay. These results suggest that the role of the seed microbiome on disease suppression may be more pronounced at the earliest stages of germination, and confirms that pre-emergence damping-off infection is an appropriate metric for evaluating seed microbiome disease suppressive potential. Lastly, the presence of negatively responsive seed lots could imply that certain seed lots do not benefit from an intact microbiome, possibly due to the presence of harmful microorganisms (plant pathogens) on the seeds (Maude, 1996).

### An invitation for scientific groups to examine their seed systems of interest for disease suppression

The results of this study highlight the value of examining a large range of seed lots and pathosystems to gain insights into the role of the seed microbiome in disease suppressiveness. The outcomes of all bioassays performed are reported, including data sets with non-significant findings as well as data that could not be analysed statistically, to make the whole set of results available publicly. Elucidating the multitude of seed types and pathosystems that different research groups are working with would require a collective

effort. This study serves as an invitation for those with an interest in seed microbiome suppressiveness to examine and report findings using the systems of their expertise. Given the conclusions that can be drawn from this study, we speculate that disease suppressiveness could be present in seed lots of other plant species, especially these with utricles such as members of Amaranthaceae. We also speculate that bioassays testing for pre-emergence damping-off infection will be more likely to detect differences between disinfected and non-treated seeds. Recording the data as counts rather than based on ordinal scale will also increase the depth of the information available and, consequently, the statistical power of subsequent analyses.

### Conclusions

In this study, a diverse set of pathosystems was explored for seed microbiome disease suppressive potential. While our findings suggest that the seed microbiome can harbour beneficial microorganisms able to suppress seedling-stage disease, this phenomenon was not persistent across pathosystems. Instead, it was specifically detected for some seed lots of two crops that produce seed as utricles: beetroot and spinach. The pathogen against which suppressiveness was detected was *P. ultimum*, an oomycete for which suppressiveness by soil or plant microbiomes has already been the subject of numerous studies. As part of unravelling the role of the seed microbiome in disease suppression, we will now use culture-dependent and molecular techniques to validate the differences in seed microbial abundance and taxonomic composition between disinfected and non-treated seeds. Advancing our knowledge on this topic could offer useful insights to assist the seed industry at safeguarding the microbiomes of seed lots that could benefit from their presence, thus contributing not only to seed health in a sustainable manner but also to food security.

**Supplementary material.** To view supplementary material for this article, please visit : <https://doi.org/10.1017/S0960258522000186>.

**Acknowledgements.** The project 'Changing the system of seed health; An initiative of industry and research towards a paradigm shift' (LWV19097) receives financial support from the Top Sector Horticulture and Propagation Materials. Within the Top Sector, the business community, knowledge institutions and the government work together on innovations in the field of sustainable production of healthy and safe food and the development of a healthy, green living environment. We acknowledge the funding by the Dutch Ministry of Agriculture, Nature and Food Quality, Bejo Zaden B.V., CN Seeds Ltd., DLF B.V., e-nema Gesellschaft für Biotechnologie und biologischen Pflanzenschutz mbH, Germaines Seed Technology, Nunhems Netherlands B.V. (BASF), Pop Vriend Seeds B.V. and Sakata Vegetables Europe S.A.S. We also thank Dr Dennis te Beest for providing guidance for the statistical analysis of the data and Dr Anna Clocchiatti for her feedback on the above.

**Conflicts of interest.** Liesbeth van der Heijden is employed at Bejo Zaden B.V., Giovanni Lopez at DLF B.V., Anita van Nieuwenhoven at Pop Vriend Seeds B.V., Martje Notten at Nunhems Netherlands B.V. (BASF), Mirjam Storcken at CN Seeds Ltd. and Patrick Butterbach at Germaines Seed Technology.

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