Induction of soil disease suppressiveness against *Rhizoctonia solani* by *Brassica* green manure

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MSc Minor Thesis Report

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Abstract

Biofumigation is an alternative biological method to incorporate glucosinolate (GLS)-containing *Brassica* crops into the soil to control the soilborne disease. *Rhizoctonia solani* is a notorious soilborne pathogen and the common cause of root rot and damping-off in a wide range of agriculturally important crops. Hydrolysis products of GLSs have been reported as the active compound in biofumigation, but due to the complex soil environment, the suppressiveness of *Brassica* green manure to *R. solani* soil infection has not yet reached solid conclusions. In this thesis study, nine green manure treatments that differed in glucosinolate profile were tested for their potential to reduce soil disease transmission of *R. solani*, compared to the control treatment. These green manures included *Brassica rapa* morphotypes (leaf and tuber of fodder turnips and pak-choi), a *Brassica juncea* mustard accession, and *Hordeum vulgare* barley. Pathogen infection was observed by scoring disease symptoms in sugar beet (*Beta vulgaris* L.) seedlings over 26 days. The biofumigation effect of the green manure treatments in soil was analyzed by a reduced disease transmission. The result of this study showed disease reduction upon green manure incorporation and demonstrated that one fodder turnip genotype effectively suppressed disease development in sugar beet seedlings. Based on the previously reported GLS abundance in plant genotypes and tissue types, this study found gluconapin-high GLS composition possessing high potential in suppressing *R. solani* disease. To our knowledge, this study pioneered in correlating the effective GLS compositions of green manures with soil disease suppressiveness against *R. solani* in an ecological experimental setting. To this end, this thesis study indicated scientific values in confirming disease suppressiveness of gluconapin-high green manure, further investigating the effective hydrolysis products of GLS in soilborne disease control, and clarifying disease suppression mechanism in the soil-microbial scale.

Introduction

Rhizoctonia solani, a soilborne pathogen

Plant soilborne diseases are caused by pathogens that survive and act in the soil or residues on the soil surface. Plant root systems are debilitated by pathogens in the soil and the disease symptoms are often noticed only until extensive damage has been done to the crop. Soilborne pathogens include fungi, oomycetes, nematodes, viruses, and parasitic plants, with fungi and nematodes having the largest incidence and agricultural impact (Ampt *et al.*, 2019). The disease occurrence is rarely observed in nature and outbreak is mostly retained to farming systems and enhanced by agricultural practices, such as frequent monoculture cropping of susceptible crops, as well as irrigation, tillage, and fertilization (Park, 2003; Katan, 2017). Soilborne pathogens infect many agriculturally important crops, among them, are olive, tomato, potato, cucumber, carrots, lettuce, sugar beet, and brassicas (*Ruopoli*, 2015). In general, soilborne diseases reduce the yield of the infected crop by around 15-30% and can sometimes cause total crop loss (Oerke, 2006; Stirling *et al.*, 2016).

In particular, one of the economically most important soilborne pathogens is *Rhizoctonia solani*. It occurs worldwide and occupies 10% among main European soilborne pathogens (Ruopoli, 2015), causing some of the most widespread and serious plant diseases in more than 250 plant species (Sneh *et al.*, 1991). *R. solani* has a wide host range, including vegetables, flowers, shrubs, trees, grasses and field crops, which is related to *R. solani* existing of a species complex with different anastomosis groups (AG), that each have more specific host ranges (Postma & Schilder, 2015; Sharma *et al.*, 2005). These fungi cause serious losses on susceptible plants, as they attack the roots, lower stems, tubers, and corms, and are capable of infecting plants from early germination and seedling to mature stage (McCully & Thomas, 1977). The pathogen survives in adverse soil conditions as mycelium or sclerotia and is responsible for common diseases such as seedling damping-off, tuber scurf, root rot, sheath blight, bare patch and many other diseases (Lucas *et al.*, 1992; Stirling *et al.*, 2016). Besides extensive host range and persistence in soil, pathogenic *R. solani* is difficult to predict and control also due to their fast and complex adaptation to the environment. Although the detailed mechanisms remain unclear, *R. solani* disease incidence and severity rely on the interplay between soil abiotic and biotic factors, such as number of infective propagules, soil physicochemical properties, activity and composition of microbial communities, as well as ambient environmental factors such as temperature and moisture (Agtmaal, 2015; Campbell *et al.*, 1994).

Biofumigation and glucosinolate

Various methods are available and practiced for soil disease control, and traditionally they include seed treatment, fungicide application before planting, solarization, soil fumigants, etc. Since recent in Europe, non-chemical methods in agriculture production and bio-based production systems have become the fundamental topic of a new bio-economy and regulations concerning soil health are becoming increasingly strict. To highlight, soil fumigation using gas methyl bromide, one of the main substances that deplete the ozone layer, has since 2015 been banned completely from both use and production in the Netherlands (UN Environment, n.d.; The Annual Prophyta, 2017). With the renewed interest to maintain plant productivity with sustainable approaches, the alternative approach, biofumigation, has been gathering vast interest.

Conventionally soil fumigation is to treat the soil with volatile gas chemicals. The gas diffuses through open pore space throughout the soil as a pesticide (Martin, 2003). On the other hand, biofumigation is to apply

natural plant substances with a high biological activity into the soil for agricultural disease control (Kirkegaard & Matthiessen, 2004; Lazzeri *et al.*, 2013). The original idea of biofumigation specifically derives from disease suppression ability by glucosinolate-containing properties, which are found the most abundant secondary metabolites in the plant genus *Brassica* (family *Brassicaceae*) (Kirkegaard & Sarwar, 1998). Glucosinolates (GLSs) are a natural class of around 132 nitrogen- and sulfur-containing plant secondary metabolites (Agerbirk & Olsen, 2012). Despite a vast side-chain length and structural diversity (aliphatic, indole or aromatic), all GLSs share a chemical structure consisting of a β-D-glucopyranose residue linked via a sulfur atom to a (Z)-N-hydroxiaminosulfate ester, with a variable side chain R group derived from one of eight amino acids (Halkier, 2016). Upon tissue disruption, GLSs are hydrolyzed by endogenous myrosinase (thioglucoside glucohydrolases), yielding glucose and an unstable intermediate. Spontaneous rearrangement of the intermediate leads to the formation of a variety of products, including oxazolidinethiones, nitriles, indoles, thiocyanates and various forms of isothiocyanates (ITCs) (**Figure 1**) (Mérillon & Ramawat, 2017). These products have a wide range of biological activity, which include both positive and negative nutritional attributes and the mediation of plant-herbivore interactions (Hayes *et al.*, 2008; Tripathi & Mishra, 2007). Among the hydrolysis products, ITCs ("mustard oils"), giving the *Brassica* plants a pungent flavor or odor, are considered the most toxic and have a broad biocidal effect. They are commonly regarded as the key players in biofumigation (Dufour *et al.*, 2015).

In general, glucosinolate types, derivative compound profile, and toxicity level are highly variable. Around 16 GLSs were found common in *Brassica* while another 30 GSLs are present in different species of the genus (**Table 1**) (Mithen, 2001; Brown & Morra, 1997; Kirkegaard & Sarwar, 1998; Wittstock *et al.*, 2016). Meanwhile, individual plants mostly contain a limited number of major glucosinolate forms (Lou *et al.*, 2008), and the exact activity of each individual GLS, however, remains unknown.

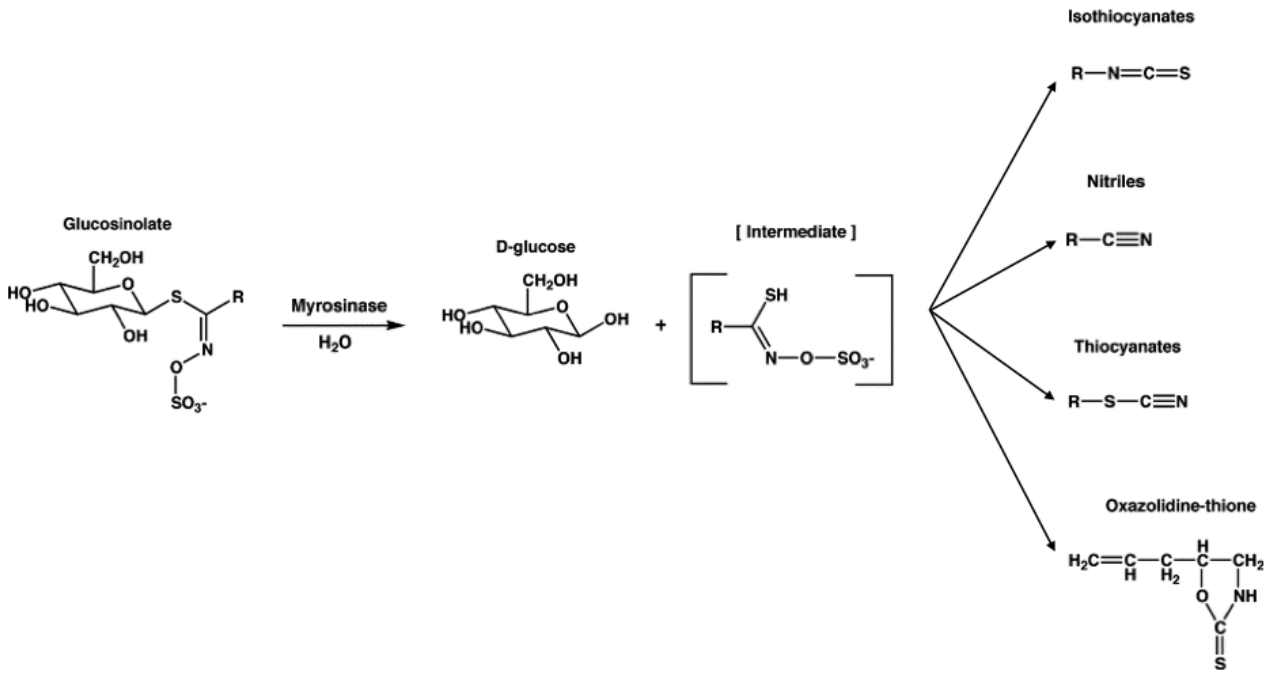

Figure 1. Generalized scheme of GLS myrosinase hydrolysis breakdown and example of derivatives (figure adapted from Vaughn & Berhow, 2005).

Table 1. 16 common GLSs in *Brassica* (Velasco *et al.*, 2016).

GLS name	Abbreviation	Structural name	Structural group
4-hydroxyglucobrassicin	4HBRA	4-hydroxy-3-indolylmethyl	Indole
4-methoxyglucobrassicin	4MBRA	4-methoxy-3-indolylmethyl	Indole
glucoalyssin	GAL	5-methylsulfinylpentyl	Aliphatic
glucoberteroin	BER	5-methylthiopentyl glucosinolate	Aliphatic
glucobrassicanapin	CAN	4-pentenyl	Aliphatic
glucobrassicin	BRA	3-indolylmethyl	Indole
glucoerucin	ERU	4-methylthiobutyl	Aliphatic
glucoiberin	GIB	3-methylsulfinylpropyl	Aliphatic
glucoiberverin	GIV	3-methylthiopropyl	Aliphatic
gluconapin	NAP	3-butenyl	Aliphatic
gluconapoleiferin	NAPOL	2-hydroxy-4-pentenyl	Aliphatic
gluconasturtiin	NAS	2-phenylethyl	Aromatic
glucoraphanin	GRA	4-methylsulfinylbutyl	Aliphatic
neoglucobrassicin	NBRA	1-methoxy- 3-indolylmethyl	Indole
progoitrin	PRO	2-hydroxy-3-butenyl	Aliphatic
sinigrin	SIN	2-propenyl	Aliphatic

By virtue of these glucosinolate compounds, *Brassica* crops have been used as biofumigant, green manure, cover crop or rotation crop (Kirkegaard & Sarwar, 1998). To be consistent, this study uses the terms “biofumigant” to refer to the GLS-containing organic compound and “green manure” for the crop that is applied into the soil.

Despite its wide application, however, owing to the complexity of environmental and ecological interactions, there is a lack of clarity of the true cause of the beneficial effect of biofumigation crop as a biological method for controlling soil-borne diseases. The mechanisms by which *Brassica* green manure can reduce soil disease and ultimately influence crop yield and quality are usually conferred by other benefits. These include the effects of non-GLS related disease suppression (for instance saponin avenacin, dimethyl-disulfide, dimethyl-sulfide and metha-nethiol), organic matter addition, soil erosion control, soil structure alteration, and nutrient regulation. *Brassica* green manure also shows potential to shape microbial communities by increasing disease-suppressive bacteria and disease protective fungi. Above that, the effectiveness can still be veiled by management techniques such as temperature, soil water contents, and optimal degradation period, incorporation timing and dosage (Kirkegaard & Matthiessen, 2004; Postma *et al.*, 2008; Vukicevich *et al.*, 2016). In order to confirm the effect of *Brassica* to *R. solani* disease suppression under controlled conditions, several greenhouse and field studies have been conducted (Manici *et al.*, 2000; Kasuya *et al.*, 2006; Larkin & Griffin, 2007; Yulianti *et al.*, 2006; Yulianti *et al.*, 2007; Motisi *et al.*, 2009; Cochran & Rothrock, 2015). Generally, a positive association was observed between *Brassica* green manure application with *R. solani* disease suppression, but the efficacies were varied by cases, encouraging further study.

Breeding of green manure *Brassica*

Since the last decades, the demand for green manure crops has increased among farmers. In the Netherlands, the market for green manure has tripled over the last few years, similar to trends in Germany

and the rest of Europe (The Annual Prophyta, 2017). This stimulated a strong interest in breeding for green manure crops with disease suppressive GLS composition.

Brassica vegetable crop breeding has so far focused on specific GLS levels to upgrade *Brassica* crop nutritional value, taste, and health beneficial effects for human. These include breeding for increasing glucoraphanin in broccoli, glucoerucin in rockets, sinigrin in mustards, cabbages and some Brussel sprouts, and gluconasturtiin in radishes and turnips (Padilla *et al.*, 2007; Hennig, 2013; Traka, 2016; Bonnema *et al.*, 2019), while reducing progoitrin and sinigrin in Brussels sprouts (Van Doorn *et al.*, 1998). Meanwhile, breeding efforts also aimed to reduce anti-nutritive GLSs. Progoitrin has anti-thyroid activity and promotes goiter disease. It also generates goitrogenic products in the seeds of oilseed *Brassica* used as animal feed, which damages the liver and kidney of animals (Mérillon & Ramawat, 2017; Tripathi & Mishra, 2007). Together, these knowledge obtained on GLSs content and level can be applied in breeding for interest in green manure *Brassica*.

Recent advances in plant genomics are rapidly leading to advances in understanding plant biosynthesis. In particular, transgenic modification of gene expression was stimulated by full sequencing knowledge of model plant *Arabidopsis thaliana*, a GLS-containing crucifer species. *Arabidopsis* and *Brassica* are two genera diverged from a common ancestor approximately 14.5-24 million years ago (Bowers *et al.*, 2003; Koch *et al.*, 2003). Conservation of gene sequences and gene order among taxa during their evolution enables knowledge of genetic functions of *Arabidopsis* genes easily transferred to studying *Brassica*. Subsequently, many important quantitative trait loci (QTLs) involved in the biosynthetic pathway of GLSs in *B. rapa*, *B. oleracea*, *B. juncea* and *B. napus* have been indicated (Bonnema *et al.*, 2019; Lou *et al.*, 2008; Carpio *et al.*, 2014; Aarts *et al.*, 2013; Lee *et al.*, 2013; Brown *et al.*, 2015; Sotelo *et al.*, 2014; Velasco *et al.*, 2016; Bisht *et al.*, 2009; Wittkop *et al.*, 2009; Schnug & Haneklaus, 2016; Liu *et al.*, 2012), and desirable GLS contents have been approached by candidate gene detection and transgenic selection. The genetic sources of QTLs involved in GLSs derive from a high diversity between families, genera, species, subspecies, and accessions of *Brassica* plants. Even within a single plant, the GLS composition strongly differs between tissues and developmental stages (Bonnema *et al.*, 2019). GLS profiling and genetic sequencing offer accurate tools to dissect GLS biosynthesis and have great potential in breeding for optimal GLS composition and content for *Brassica* green manure (Kirkegaard & Sarwar, 1999; Velasco *et al.*, 2016; Mazzola & Freilich, 2016).

Scope of the thesis

This minor thesis aimed to study *Brassica* green manure disease suppressiveness against a soilborne pathogen. Green manure crops, including turnips, pak-choi, mustard, differed in glucosinolate profile and were tested for their potential to reduce soil disease transmission of the soilborne fungus *R. solani*. The green manure effect was analyzed by reduced disease development in each sample consisting ten sugar beet seedlings. Disease suppressive levels of the *Brassica* green manures was related to the reported GLS substances in the turnips to search for causal factors. The thesis addressed to link GLS content of the different green manure crops to their disease suppression effect, in order to facilitate the breeding of *Brassica* green manure crops (**Figure 2**). Key hypotheses examined were: (1) green manures suppressed *R. solani* disease development in sugar beet seedlings; and (2) GLSs were the key chemical players in suppressing disease, and treatments with high GLS content were stronger in disease suppression.

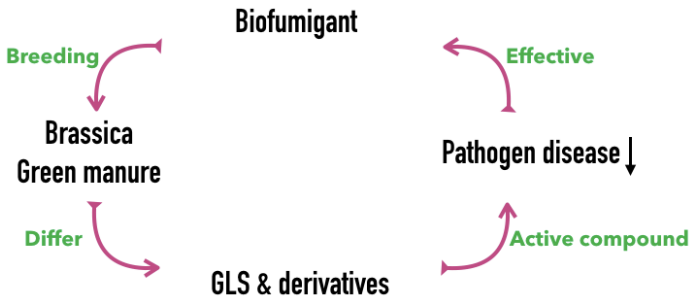

Figure 2. Schematic thesis overview.

Materials and methods

Materials

Soil — Sandy soil (organic matter: 4.8%, pH=6.60) was obtained from Droevendaal field site. The soil material was provided by Plant Ecology & Nature Conservation department.

Sugar beet — Sugar beet (*Beta vulgaris* L.) is a temperate biennial root crop cultivated for sugar production, forage and organic matter supply for soil. The seeds were produced at Sesvanderhave NV. They were coated in blue and did not contain fungicide against *R. solani*. The seeds were provided by the Biointeractions & Plant Health department.

***R. solani* inoculum —** Barley seeds infested with *R. solani* Kühn AG 2-2 IIIB was used as inoculum (Vagher *et al.*, 2010). AG2-2 IIIB is one type of the thiamine auxotrophic AG 2 subgroups and AG 2 is one of the 14 anastomosis groups (AG) in *R. solani* classification. AG 2-2IIIB severely hampers multiple crops and especially sugar beet cultivation, with an estimated affected area of 70,000 ha in Europe and causes ~15 M€ loss in the Netherlands (Postma & Schilder, 2015). The inoculum material was provided by the Biointeractions & Plant Health department.

Green manures — In total ten types of treatment were used in this study. These treatments included turnip, pak-choi, mustard, barley and control (**Table 2**). Two turnip (*B. rapa* ssp campestris, 2n=20) accessions with similar development timing, fodder turnip Ft 004 (CGN06678) from Denmark and Ft 086 (CGN07223) from Pakistan (provided by The Centre for Genetic Resources, the Netherlands) and their F_1 offsprings (crossings between mother Ft 004 and father Ft 086) were used as the main comparison group. Ft 004 and Ft 086 lines share highly synchronous growth while possessing contrasting GLS profiles, and within an individual plant, tissue parts also differ in GLS content, largely between the leaf and tuber (**Figure A15**). The relative abundance of some GLSs in F_1 offsprings was semi-dominant and resembles Ft 004 (Bonnema *et al.*, 2019). To compare the biocidal effect of GLS in *Brassica*, two accessions (pvBr 060403 & pvBr 051024) of pak-choi (*B. rapa ssp chinensis*) were mixed as low-GLS treatment; one cultivar SH3 of mustard (*B. juncea*) was used as high-GLS treatment. To observe the confounding factor of organic matter addition without GLS effect, KWS IRINA barley (*Hordeum vulgare*) was used as non-GLS treatment. Seeds were sown in the greenhouse and fresh greens were harvested (**Figure 3**). In addition, to examine the fundamental effect of plant treatment, one treatment without green manure addition was set as the control treatment. In this study, all green manure accessions were provided by the Plant Breeding department.

Table 2. Chart of green manure treatments in this experiment.

Treatment	Green manure plant	Scientific name	Accession	Tissue
1	Turnip	*Brassica rapa*	F_t 004	Leaf
2				Tuber
3			F_t 086	Leaf
4				Tuber
5			$F_1 = F_t$ 004*F_t 086 Cross 2 & Cross 4	Leaf
6				Tuber
7	Pak-choi (low-GS)	*Brassica rapa*	pvBr 060403 & pvBr 051024	Leaf

Treatment	Green manure plant	Scientific name	Accession	Tissue
8	Mustard (high-GS)	*Brassica juncea*	SM3	Leaf
9	Barley (non-Brassica)	*Hordeum vulgare*	KWS IRINA	Leaf
10	Control (no manure)	—	—	—

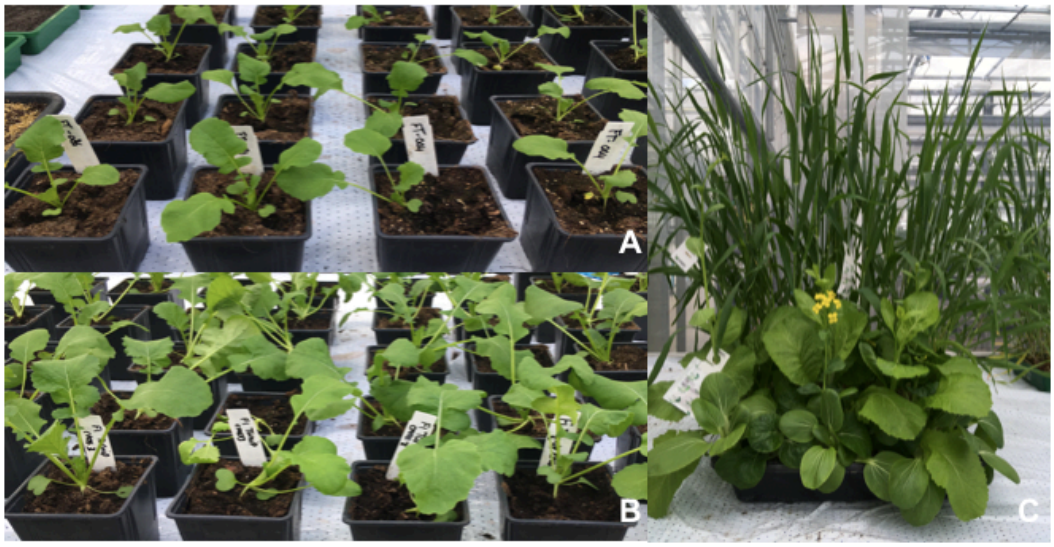

Figure 3. Green manure plants used in this study: A) Turnip Ft 086 & Ft 004 accessions; B) Turnip F_1 accessions; C) Pak-choi (bottom left), mustard (bottom right), and barley (top).

Experimental design

This thesis included three experiments. **Experiment 1** aimed to test sugar beet seed germination under normal soil condition, in order to assure good starting material (the seeds) and sowing method. Sowing depths (1 cm & 2 cm) and seed quality of sugar beet seeds were tested (**Table A1**). **Experiment 2** was a trial experiment to observe sugar beet seed germination and pathogen disease development under three degradation times and three dosages of a test green manure — pak-choi (leaf) (**Table A2**). The optimal conditions was used for **Experiment 3**. **Experiment 3** addressed the effect of green manure treatments, which differed among others in GLS composition, on soil suppressiveness against *R. solani* induced sugar beet diseases.

Green manures were sown, harvested, and incorporated in the soil in **Experiment 3** (**Table 3**). Seed sowing, cutting, and incubation of green manure plants in soil and subsequent sugar beet seed sowing were conducted in the greenhouse chamber 10.1 (Wageningen Unifarm). Pathogen inoculation and subsequent disease development observation were conducted in the growth cabinet 5 (Wageningen Unifarm). The experimental schedule was listed in **Table A4**.

Table 3. Experiment 3 disease suppression test of soils applied with green manure treatments against *Rhizoctonia solani* disease in the sugar beet seedlings (two blocks, three replicates per block; long day degradation time of green manures was changed to 14 days).

Time	Experiment	Place	Material
March 14	Sow green manure seeds		□ Pak-choi seeds (20) □ Mustard seeds (20) □ Barley seeds (80) □ Sowing boxes (2) □ Potting soil
April 11 & 12	• Harvest green manure • Mix into soil & incubate	Greenhouse chamber	□ Pak-choi fresh leaves (36 g) □ Mustard fresh leaves (36 g) □ Barley fresh leaves (36 g) □ Turnip fresh leaves (36 g per each treatment) □ Turnip tubers (36 g per each treatment) □ Droevendaal field soil (0.2 kg* 60 boxes = 12 kg) □ Mixer & scale
April 25	Sow sugar beet seeds in 14 days-soil		□ Soil box (10 treatments * 6 rep = 60boxes) □ Sugar beet seeds (10 seeds * 60 boxes = 600 seeds)
May 3	Inoculate pathogen into 14 days-soil	Growth cabinet	Rhizoctonia inoculum (5 barley seeds * 60 boxes = 300 barley seeds)
May 3 - 31	Observe seedling disease symptom	Growth cabinet	

In **Experiment 2**, the green manure dosages applied in previous studies were used as references to select for an appropriate range to be tested in this study. In laboratory tests, Kasuya *et al*. (2006) used 1% dried weight (DW), Zuluaga *et al*. (2015) used 1% DW; in greenhouse tests, Yulianti *et al.* (2006) tested 1%-10% FW and reported contrasting effect, and Pellerin *et al*. (2007) used 2.6% DW which represented around 1.7% in open-field; in open-field tests, Motisi *et al.* (2009) used 5% fresh weight (FW), Matthiessen & Kirkegaard (2006) used 3-4% FW, and Berbegal *et al.* (2008) used around 1.5% FW. Based on the reports, three dosages1%, 2%, and 3% were chosen and tested.

The biofumigation effect requires a proper release of GLSs from brassica tissues, and the release is maximized by disrupted tissue in wet and sealed soil (Stirling *et al.*, 2016). On the other hand, common field biofumigation practices recommend subsequent seed sowing after at least two weeks of green manure degradation (Van Os, 2016). The debate is to either maximize active GLSs in the soil or minimize the toxicity level and other negative factors for seed germination. Therefore, in **Experiment 2** three degradation times were tested, namely 0 day, 1 day and 20 days, to observe zero, short-term and long-term degradation effect on seed germination and disease development.

To test disease suppressiveness in green manure-incorporated soil, sugar beet seeds were planted in a line in a soil box with green manure treated soil. After sugar beet seed germination, which was approximately five days after seed sowing, *R. solani* AG 2-2 IIIB inoculum was placed at one edge of a rectangular soil box, around 2 cm away from the closest sugar beet seedling and same sowing depth as sugar beet seeds. Inoculum of *Rhizoctonia* consisted of five infected barley seeds, which were incorporated into the soil at the left end of each box (**Figure 4**). The pathogen generally transmitted through the soil with a speed of one cm per day. Above-ground symptoms were observed every day during the observation phase and the number of sick plants was noted. Disease development in each sample was calculated to compare treatment efficacy. Growth cabinet was featured by temperature 18-20 ℃, humidity 70% and light time 18 hours.

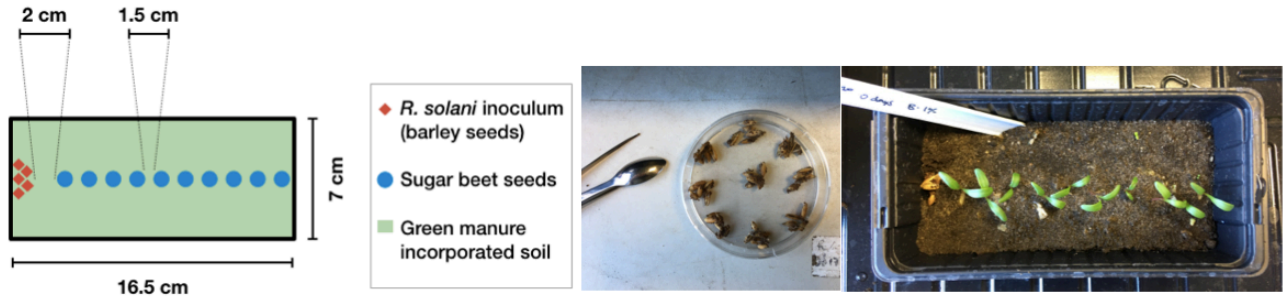

Figure 4. From left to right: I) Illustration of soil box design of each sample. Soil depth was 1.7 ± 0.2 cm; II) Inoculum, barley seeds infested with *R. solani*; III) inoculation into the soil.

Data Analysis

All data analyses were conducted in R (R Development Core Team, 2009). The building of the R codes was kindly instructed by Eline Ampt.

Data analysis — For each treatment, six samples were obtained from three replicates (soil boxes) and two blocks in a randomized complete block design. Germination rate and disease rate were calculated in each sample, and effects of the block, treatment, and germination rates were statistically tested by using analysis of variance (ANOVA).

AUDPC — The Area Under the Disease Progress Curve (AUDPC) was analyzed to quantify disease progress of *R. solani* in sugar beet seedlings in each sample. AUDPC is a well-established measure of disease in plant pathology research. It provides a measure in overall disease incidence where it describes the relationship of disease index and time in a linear framework (Schandry, 2017). In this study, AUDPC was calculated by integrating all the areas under the average disease rate between any given two neighboring time data points (dates) (packages: devtools & MESS). The AUDPC formula used was:

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

,

where y_i was the disease rate at given date point t_i. To compare green manure treatment effects, a linear mixed effects model was used to describe AUDPC (variate) by fixed effect (treatment and germination rate) and random effect (block). The block effect was not systematic in its contribution to the variate and the effect is not of our interest, thus the block effect was set as a random factor in the model (packages: nlme & car packages; figure visualization by ggplot2).

Post-hoc analysis — Based on the linear mixed model, multiple testing was conducted for pair-wise comparison of treatment effect. For 10 treatments, 45 pairs of comparison were calculated. In order to control the Type I error rate, Holm-Bonferroni correction was used to lower the significant threshold α to account for the number of tests conducted by:

$p = 1 - (1 - \alpha)^n$,

where α remained the same and the new (type I) error rate p was accounted for by the number of tests (n). The adjusted significance level is generally approximated as $p = \alpha/n$ to obtain an experiment-wise p-value of 0.05. Similarly, Holm-Bonferroni corrected was also used for the contrast tests between green manure treatments and the control treatment (package: emmeans).

Results

Experiment 1 — Test sugar beet seed germination

Germination of sugar beet seeds in the organic soil takes up 5-7 days-after-sowing (DAS) in the greenhouse (**Table A1**). Sowing depth did affect germination, as seeds sown 1 cm deep germinated 100% while sown 2 cm deep germinated 90% (**Table A3**). Therefore, 1 cm was chosen for the subsequent two experiments.

Experiment 2 — Test green manure degradation time and dosage

Germination

Sugar beet seeds that were sown in pak-choi leaf (test green manure) incorporated soil started germination around 7-8 DAS, with a range till 13-14 DAS (**Table A2**). Among the three degradation times (0 day, 1 day, 20 days), seeds sown in the soil after 20 days green manure degradation showed fast and uniform germination with the highest final germination rate, reaching 100% germination rate at 8 DAS (**Figure A1**).

Slower germination and lower final germination rate were observed in seeds sown in soils with green manure dosage of 2%, both with zero (0 day) (**Figure A2**) and short (1 day) degradation time (**Figure A3**). In general, seeds sown in soil with 3% pak-choi leaf green manure degradation showed more uniform germination rate.

Disease development

Due to time limitation, disease development was only able to be observed in sugar beet seedlings in the soil of 20 days green manure degradation time (there was not enough time to observe 0 day and 1 day degradation so no results were obtained).

As shown in **Figure A4**, disease development was observed 0-14 days-after-inoculation (DAI). In all dosages, disease symptoms of sugar beet seedlings initiated around 5-6 DAI and the pathogen progressed to infect sugar beet seedlings with a speed of ~1 cm/day (the distance between two seedlings was ~1.5 cm). There was no obvious difference between green manure dosages as to disease development, but still most observable in 3% treatment.

Combining the result of germination and disease development, this trial experiment showed that 20 days degradation time with a dosage of 3% green manure offers more uniform and solid germination and disease development. This combination was thus chosen for **Experiment 3**.

Experiment 3 — Test disease suppressiveness of green manure treatment

Germination

Seeds of all six genotypes (Ft 004, Ft 086, F_1, pak-choi, mustard, barley) were sown in Unifarm greenhouse. Due to limited tuber material of Ft 004, there were only two replicates of this material in block B instead of three, and thus making in total 59 samples (ten treatments * two blocks * three replicates). Germination of sugar beet seeds started from 5 DAS and lasted until 7-10 DAS (**Figure 5**). The average germination rate was 96.3% among all samples, and seeds sown in Ft 004 tuber treated soil had the lowest germination rate (**Figure 6**). In response to the final germination rate, no significant effect was discovered in the block, treatment or their interaction by two-way ANOVA test (**Table 4**).

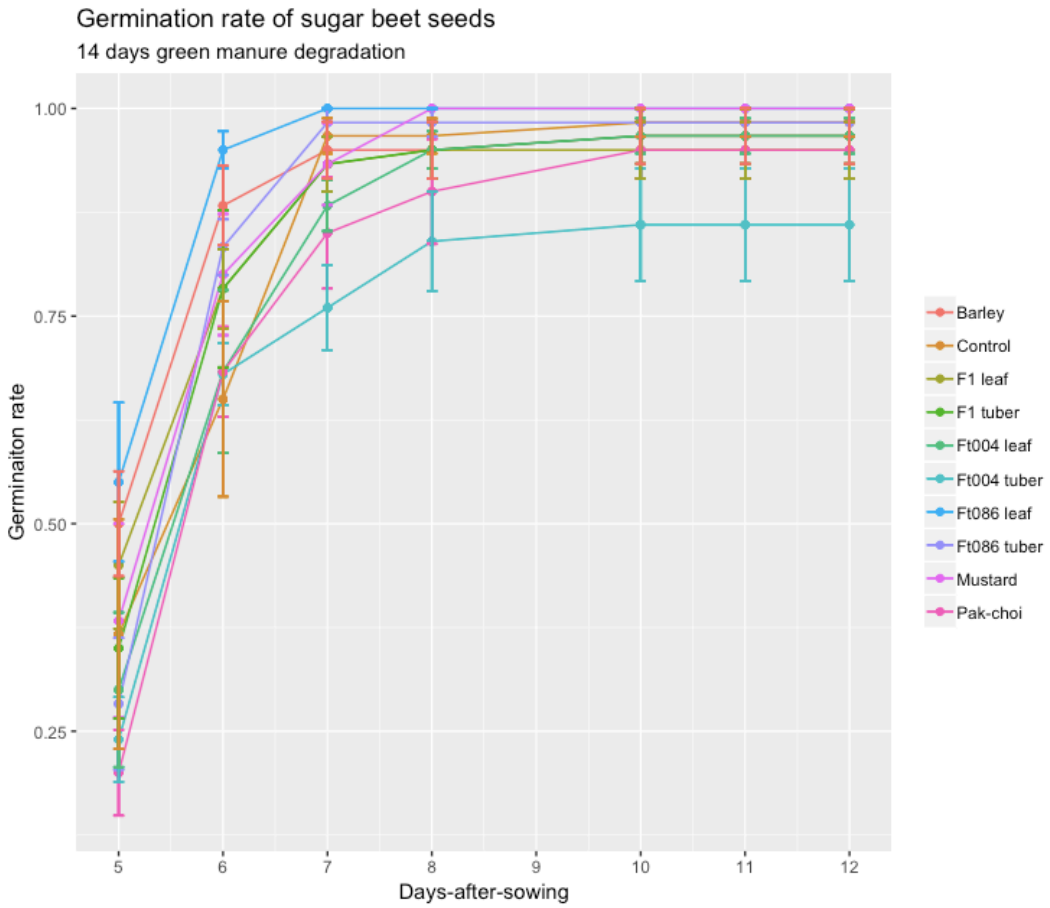

Figure 5. Germination rate of sugar beet seeds in ten treatments. Data are replicate means ± SE.

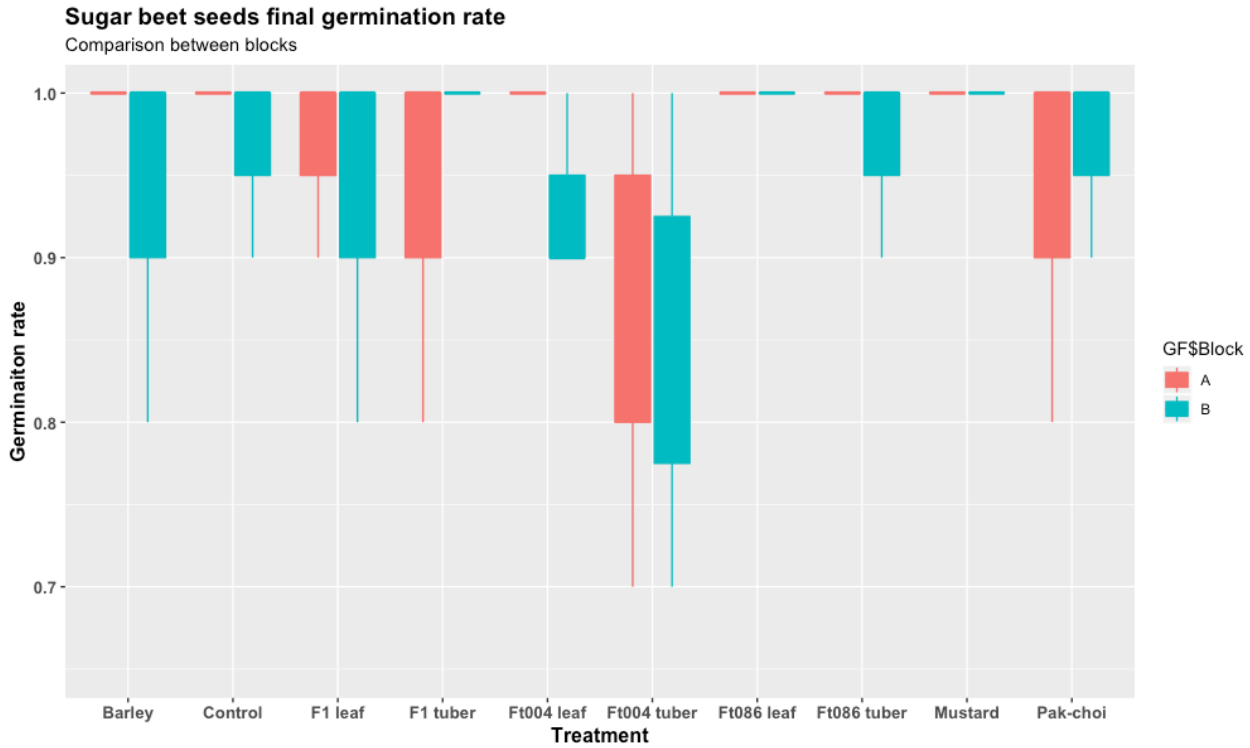

Figure 6. Box plot comparison of sugar beet seeds final germination rate between 2 blocks in 10 treatments. Data by average of 3 replicates.

Table 4. Summary of two-way ANOVA test of block, treatment and interaction effects in response to final germination rate.

	Df	Sum sq	Mean sq	F value	Pr (>F)	Significance
Block	1	0.00191	0.001909	0.321	0.574	
Treatment	9	0.07797	0.008663	1.458	0.198	
Block : Treatment	9	0.02371	0.002634	0.443	0.903	
Residuals	39	0.23167	0.005940			

Disease symptoms

Above-ground symptoms were used to decide whether a sugar beet seedling was infected by *R. solani*. In this study, three symptoms were decisive for disease (**Figure 7**): (1) damping-off was the most common *R. solani* symptom, where the entire seedling collapsed, with a dark, rotten root; (2) permanent wilting of leaves, where the leaves of infected plant were usually soft and dropping-off; (3) in a rarer case, a seedling stunted at an early stage and ceased subsequent growth. The seedling usually died shortly thereafter due to a weak health state.

Figure 7. Three symptoms caused by *R. solani* infection. From top to down damping-off, permanent wilting, and stunting at an early stage. The infected seedlings were marked in red. Photos were taken on May 7 (6 DAI).

Disease development

Due to the fact that germination rate was not 100% in all soil samples, the disease rates of each sample were thus described by the ratio of the number of observed diseased seedling to the number of final germination. Disease development was observed each day May 6-31 (DAI 5-30), in total of 26 days (**Figure 8**).

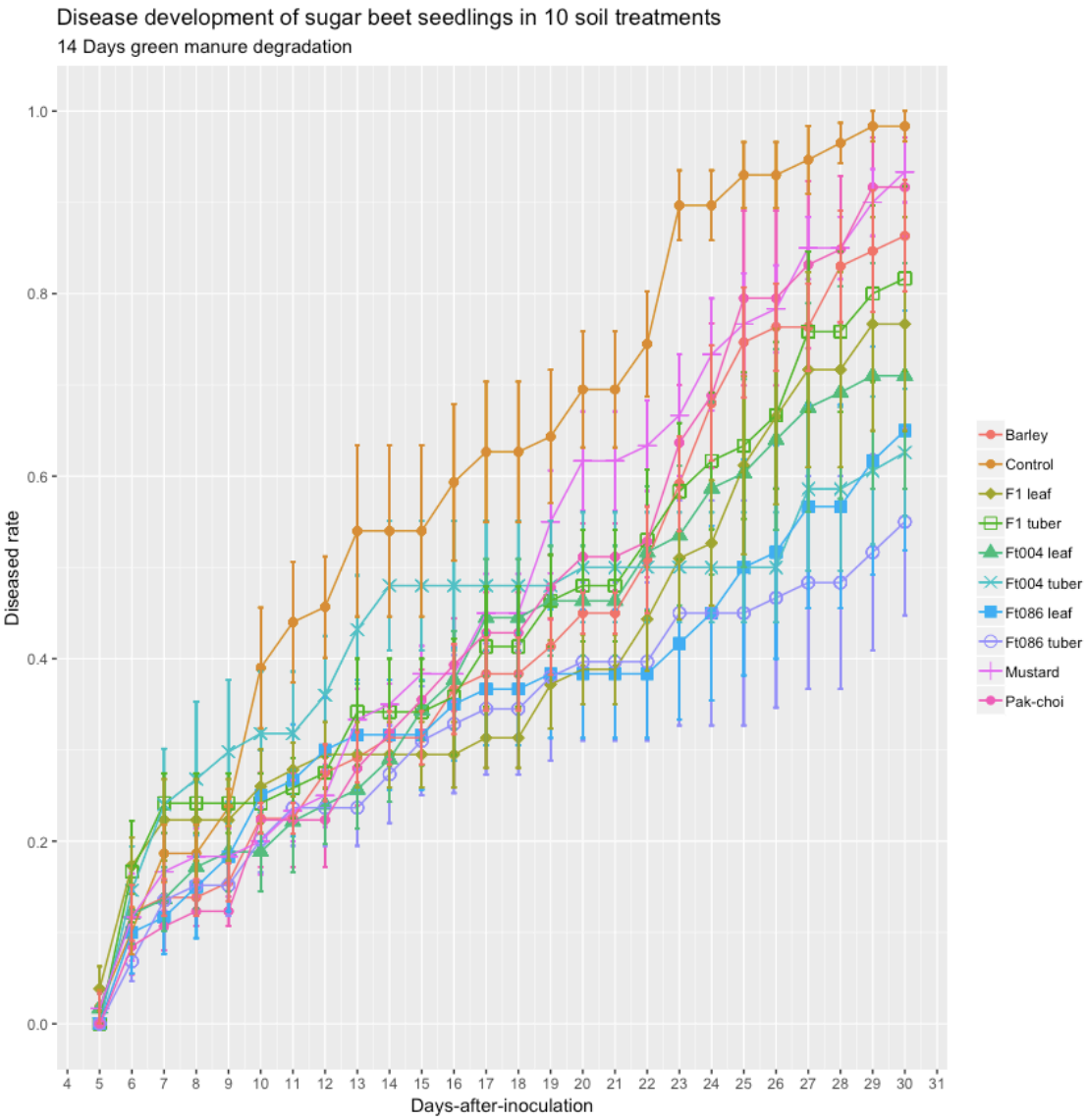

Figure 8. General disease development of sugar beet seedlings in 10 treatments. Data are replicate means ± SE.

In the linear mixed model, the germination rate was initially included as a fixed effect but it showed no effect in ANOVA test (**Table 5**). To keep the statistics simple and clean, the germination rate was removed from the linear mixed model (**Table 6**): Model= lme(AUDPC ~ Treatment, random = ~1|Block). Model assumptions of residue normality and homogeneity of variance were met (**Figure A5-A8**).

Table 5. Summary of two-way ANOVA type II test of treatment and germination rate effects in response to AUDPC. Significance levels were denoted as "" <1, "*" < 0.05, "**" < 0.01, "***" < 0.001.

	Chisq	Df	Pr(>Chisq)	Significance
Treatment	25.2318	9	0.002725	**
Germination rate	2.1555	1	0.142062	

Table 6. Summary of two-way ANOVA type II test of treatment effect in response to AUDPC. Significance levels were denoted as "" <1, "*" < 0.05, "**" < 0.01, "***" < 0.001.

	Chisq	Df	Pr(>Chisq)	Significance
Treatment	24.05	9	0.004222	**

A significant effect was detected in treatment in response to AUDPC (**Table 6**). For pair-wise comparison between green manure treatments with the control treatment, nine pairs of comparison were made and corrected by Holm-Bonferroni method. Reduced AUDPC was observed in all treatments compared to the control treatment (**Figure 9**) and significant difference occurred in F_1 leaf, Ft 004 leaf, and especially in Ft 086 tuber and leaf. No significant difference was detected between the nine non-control treatments. Other multiple comparison results are provided in **Table A5-A6**.

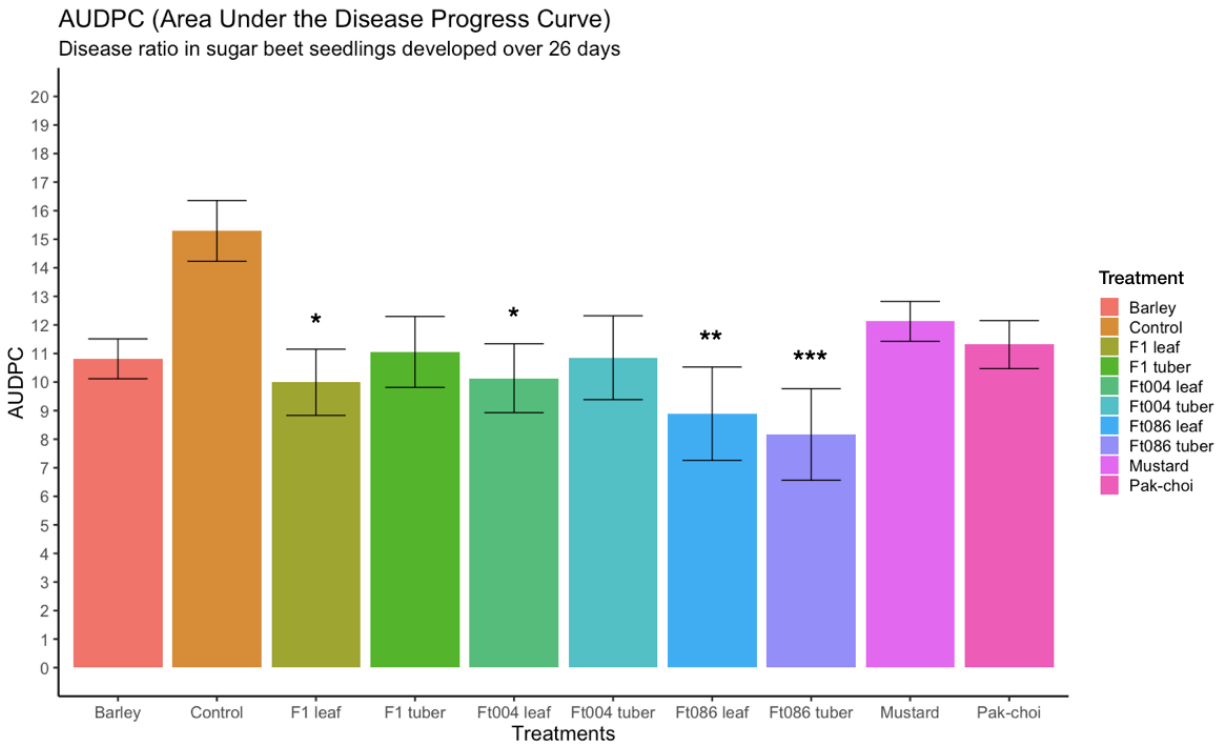

Figure 9. Bar plot of AUDPC among 10 treatments. Error bars are mean AUDPC in each treatment plus-minus standard error, calculated from 3 replicates in 2 blocks (59 samples in total). Significance levels ("" <1, "*" < 0.05, "**" < 0.01, "***" < 0.001) were denoted by pair-wise comparison to the control treatment.

Discussion

A fodder turnip Ft 086, both leaves and tubers, was able to significantly reduce disease transmission of the soilborne fungus *R. solani*. The candidate GLS for this effect was gluconapin.

Green manure dosage & degradation time

The appropriate green manure dosage depends on the types of the experiment (in vitro, greenhouse, open-field, etc), green manure tissue (fresh and dried leaf, root, seed pellets, etc), and pathogen (nematode, bacteria, fungi, etc.). The result of **Experiment 2** showed that with long (20 days) degradation time, the three green manure dosages induced similar result in germination and disease development, but with a short degradation time (0 & 1 day) of the green manure in the soil there was more variation in germination between samples. This was probably due to active oxygen-depriving hydrolysis and the release of GLS degraded compounds and other secondary metabolites when incorporating fresh-cut pak-choi into the soil, which has a short-term disruption to the soil environment (Neugart *et al.*, 2018; Vaughn *et al.*, 2006). *R. solani* damping-off suppression was observed in undecomposed/fresh and mature household composts (5-7 months degradation), which induced active microbial antagonism, while partially decomposed materials (one month degradation) were conducive (Tuitert *et al.*, 1998). In this study, 20 days degradation was suitable for fungal disease transmission and might thus have facilitated observation of effective disease suppressiveness of GLSs. Wider spans of degradation time should be involved in further experiments to conclude the relationship between *Brassica* green manure degradation to disease suppression effect.

R. solani disease infection in sugar beet seedlings

Damping-off was the most disease decisive symptom of *R. solani* infected sugar beet seedlings. However, damping-off could only be detected if the above-ground symptom was severe. Seedlings could also survive despite an infected root, mostly happened when plants were infected in later growth stages (**Figure A9**). Leaf wilting was observed as an early sign of pathogen infection, as water absorption in roots were permanently disturbed by pathogen infection. This symptom was either followed by damping-off directly or after several days. Less commonly occurred, early stunting usually happened to the first or the last seedling in a row. When it happened in the first position it was observed as infection. However, when it occurred in a seedling at the last position, an infection could not be judged until the death of the seedling. Other unhealthy symptoms were not decisive for infection, such as drought stress wilting (in which scenario the plant's vigor was able to recover by adequate water supply), necrosis lesion on the leaf surface (probably due to light/drought stress), and abnormal leaf growth (leaf shape etc).

The speed that *R. solani* transmitted through the soil was much reduced in treatments with green manure, and even in the control treatment, it was on average 0.6 cm/day. In addition, it was also observed that in some cases the pathogen passed over some seedlings, either temporarily or until the end of the experiment (**Figure A10**). This "jumping" phenomenon was first observed in two samples on May 12 (11 DAI). Through time the number of samples with jumping increased, and on May 21 (20 DAI) there were eight samples (out of 59) with healthy seedlings between diseased neighboring seedlings. The jumping cases were nonspecific to treatment or block and it reduced with increasing DAI. This could be caused by diffusive infection instead of one-directional. Fast-growing seedlings might have also gained physical resistance against pathogen infection by developing a stronger root system. Nonetheless, by the end of the experiment, most of the seedlings in all samples were infected, leaving few jumping cases.

GLS & green manure disease suppression

Reduced AUDPC was observed in all green manure treatments compared to the control treatment, indicating a positive disease suppression by organic amendment and secondary metabolites released from plant tissues, although a significant difference was only discovered in turnips. Green manure input in the soil reduces soil-borne disease possibly through increasing the diversity of antagonistic organisms, releasing compounds during decomposition that inhibit, deter or kill pathogen, enhancing plants nutritional status or defense mechanisms, and thereby improving its capacity to resist or tolerate attack by *R. solani* (Postma & Schilder, 2015; Stirling *et al.*, 2016).

Significantly reduced AUDPC was observed in F_1 leaf, Ft 004 leaf, and especially in Ft 086 leaf and tuber. A lower AUDPC indicated that disease suppressiveness of these genotypes/tissues was stronger and this may relate to their metabolite composition. One of the causal factors was the GLSs. In turnips, there was a high variation in genotype-based and tissue-specific GLS composition (Lee *et al.*, 2013; Bonnema *et al.*, 2019). Based on the difference in tuber GLS composition, 48 turnip accessions were grouped into four clusters, with Ft 086 grouped in Cluster 1 and Ft 004 in Cluster 3 (**Figure A11**) (Lee *et al.*, 2013). Comparing the two clusters, higher content of 4C-side chain (4C) aliphatic gluconapin, glucoerucin, and progoitrin and aromatic gluconasturtiin occurred in Cluster 1, and higher content of 5C-side chain (5C) aliphatic gluconapoleiferin, glucobrassicanapin, and glucoberteroin in Cluster 3 (**Figure A12**). In addition, there was a distinctive difference in relative GLS abundance between leaf and tuber tissues (**Figure A13,15**).

The most significant disease suppressiveness, by genotype Ft 086, could be attributed to its GLS profile with high abundance in 4C aliphatic gluconapin and progoitrin and aromatic gluconasturtiin (together described as “Group 1” in **Figure A14**), and disease reduction was especially distinctive in high gluconapin (NAP) content (**Figure A14-A15**). The highest gluconapin content in turnip Ft 086 was reported in 40-days tuber (Bonnema *et al.*, 2019), a harvest two weeks earlier than this study material. 40-Days tubers are thus expected to induce greater disease suppression and should be tested in the future study to confirm this result. So far to our knowledge, the biosynthesis of gluconapin in Arabidopsis plants requires the Gsl-alk locus that coverts precursor glucoraphanin to its alkenyl homolog gluconapin (Li & Quiros, 2003; Padilla *et al.*, 2007). In *Brassica campestris*, two key candidate genes BrAOP2 and MYB28 were proposed for gluconapin accumulation (Wang *et al.*, 2011). The hydrolysis products of gluconapin are isothiocyanates, nitriles, and epithionitriles (Klopsch et al., 2017) but no research has yet covered the mechanisms of gluconapin in soil disease suppression. Towards the breeding of green manure turnips, further investigation should focus on the efficacy of gluconapin in disease suppression and elucidation of the effective biocidal hydrolysis products of gluconapin against *R. solani*.

Likewise, significantly reduced AUDPC of genotype Ft 004 was characterized by a relatively high abundance of 5C aliphatic glucobrassicanapin, gluconapoleiferin, and the indole neoglucobrassicin and 4-hydroxyglucobrassicin (together described as “Group 2” in **Figure A14**). No leading GLS was found as a causal factor. In addition, the F_1 material in this study was a mixture of two F_1 hybrid genotypes (Hy2 & Hy4), both derived from a cross between mother (Ft 004) and father (Ft 086) accessions (Bonnema *et al.*, 2019). F_1 showed semi co-dominant inheritance of the 11 tested GLSs, which inherited more from the maternal line (Ft 004) than the paternal line (Ft 086) (**Figure A16**). When compared among the three accessions F_1, Ft 004, and Ft 086, the relative GLS composition was strongly and uniformly correlated with the reduction in

AUDPC. To our knowledge, this is the first study that confirmed a strong correlation of turnip GLS composition to the reduced soil disease progress of *R. solani*.

Despite the positive result obtained form this study, multiple previous studies have reported that biofumigant crops produced inconsistent levels of disease control and the effect was pathogen-dependent, based on the experimental results from multiple sites and years (Stirling *et al.*, 2016; Larkin & Lynch, 2018). Moreover, suppression of disease caused by *R. solani* is generally hard to predict, and the consistently leading antagonist has yet to be discovered (Bonanomi *et al.*, 2010). Therefore, the correlation found in this study should be further tested. To confirm the significant reduction of disease development by Ft 008, turnip tubers of the accessions grouped in Cluster 1 can be used (**Figure A11**). For example, Ft 086 is closely related to Vt 123, a vegetable turnip crop which originates from Japan, in tuber GLS. Ft 086 also shares a common tuber GLS composition with Vt 009, Ft 051, and Kt 04,05,06 share, while belonging to distinctive crop types and origins. It is expected that these turnip accessions would demonstrate high potential in suppressiveness against *R. solani* disease. Moreover, the effectiveness of the specific GLS composition to disease suppression can be verified in an F_2 progeny, selfed from the F_1 accession. Each F_2 offspring should have a unique and quantitative GLS segregation (Lou *et al.*, 2008). The new GLS compositions accumulated in F_2 turnip tuber and leaf tissues can be tested by High-performance Liquid Chromatography (HPLC) and their disease suppression against *R. solani* can be compared with Ft 086, Ft 004, and F_1, using this experimental set-up. Furthermore, the key myrosinase-dependent GLSs hydrolysis products that attribute to disease suppression should be analyzed and quantified in F_2 green manure.

Pak-choi and mustard were added as low and high GLS treatment and barley was added as non-GLS treatment. The result showed that no significant difference was observed in these three treatments compared to the control treatment. In the case of barley, the seeds used in this study were, unintentionally, coated with systemic fungicide and was thus expected to show high disease reduction. However, fungicide-containing green manure did not induce greater disease suppression compared to GLSs-containing green manures, indicating a positive effect of GLS in disease suppression. Contrary to our hypothesis, high-GLS treatment mustard did not show higher disease suppression. A strong conclusion was evaded by lacking GLS profile in pak-choi and mustard in this study material. The predominant GLSs in pak-choi are gluconapin and glucobrassicanapin (Lou *et al.*, 2008; Wiesner *et al*., 2013; Kim *et al.*, 2017), and *B. juncea* is characterized by aliphatic GLS, such as sinigrin (predominant in European cultivars), gluconapin (predominant in Indian cultivars), and glucobrassicanapin (Kirkegaard & Sarwar,1999; Sodhi *et al.*, 2002; Mérillon & Ramawat, 2017). Gluconapin was the key GLS candidate of disease suppression in turnips, but it did not contribute similarly in pak-choi and mustard, despite a potentially high abundance. One may suggest that *R. solani* disease suppression is strictly led by GLS compositions of either leading content in gluconapin or Ft 004 (F_1) leaf-like composition with glucobrassicanapin, gluconapoleiferin, and the indole neoglucobrassicin and 4-hydroxyglucobrassicin. In addition, the total GLS content is highly variable (Mérillon & Ramawat, 2017) and the effect of disease suppression can hardly be drawn without chemical analysis. Subsequent GLS profiling of the green manure materials would be very helpful to confirm effective GLS composition in disease suppression.

Soil microbiome interaction

This study demonstrated that certain GLS compositions in green manure effectively suppress soilborne disease caused by *R. solani*. The disease suppressiveness may not only come through the short-term direct effects of degradation products such as isothiocyanates (ITCs) (Lord *et al.*, 2011), which kill the pathogen

upon direct interaction, but also in longer-term alteration of the balance between pathogens and their antagonists. Recent studies incorporated next-generation sequencing and other 'omics' technologies and provided new insights into the microbial ecology of disease suppressive soils and the identification of microbial consortia and traits involved in disease control (Expósito *et al.*, 2017). Disease suppression is significantly correlated with increased soil microbial diversity and especially in populations of Proteobacteria, Firmicutes, and Actinobacteria (Mendes *et al.*, 2012), *Lysobacter* (Postma & Schilder, 2015), and Oxalobacteraceae, Burkholderiaceae, Sphingobacteriaceae and Sphingomonadaceae (Chapelle *et al.*, 2016), via the activation of antagonistic traits that restrict pathogen infection. Future study is warranted to quantify the green manure induced change in soil microbiome composition by using quantitative polymerase chain reaction (qPCR). In summary, in the interest of deciphering the code of GLS effectiveness in soil disease suppression, subsequent research should stress on the investigation of the hydrolysis products of the effective GLSs and the induced alteration in rhizobacterial community in longterm studies.

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Appendix

Table A1. Experiment 1 plan to test the appropriate sowing depth (1 cm & 2 cm) and germination quality of sugar beet seed material.

Time	Experiment	Place	Material
March 14	Sow sugar beet seeds	Greenhouse chamber	□ Sugar beet seeds (40) □ Sowing boxes (2) □ Droevendaal field soil □ Seed-sowing tools with 1 cm and 2 cm depth
March 18-20	Observe germination		

Table A2. Experiment 2 plan to test sugar beet germination and soil disease suppressiveness against *R. solani* under three dosages (1%, 2%, 3%) and three degradation times (0 day, 1 day, 20 days) of pak-choi green manure (three replicates).

Time	Experiment	Place	Material
March 13	• Harvest pak-choi • Mix pak-choi into the soil & incubate in plastic bags for 20 days	Greenhouse chamber	□ Pak-choi fresh leaves (30 g* 3 rep = 90 g) □ Droevendaal field soil (1.5 kg* 3 rep = 4.5 kg) □ Mixer & scale □ Small plastic bags (6* 3 rep = 18)
April 2	• Transfer 20 days soil mixture to soil box • Sow sugar beet seeds		□ Soil box (3 dosages* 3 rep = 9) □ Sugar beet seeds (10 seeds* 3 dosages* 3 rep = 90 seeds)
April 10	Inoculate with *R. Solani* infected barley seeds into 20 days-soil	Growth cabinet	Rhizoctonia inoculum (5* 3 box * 3 rep = 45)
April 10	• Harvest pak-choi • Mix pak-choi into the soil & incubate for 0 day and 1 day	Greenhouse chamber	□ Pak-choi fresh leaves (15 g* 3 rep* 2 times = 90 g) □ Droevendaal field soil (750 g* 3 rep* 2 times = 5 kg) □ Mixer & scale □ Soil boxes (3 boxes* 3 rep* 2 times= 18)
April 10	• Sow sugar beet seeds in 0 day-soil		□ Soil box (3 dosages* 3 rep = 9) □ Sugar beet seeds (10 seeds* 3 dosages * 3 rep = 90 seeds)
April 11	• Sow sugar beet seeds in 1 day-soil		□ Soil box (3 dosages* 3 rep = 9) □ Sugar beet seeds (10 seeds* 3 dosages * 3 rep = 90 seeds)
April 18	Inoculate with *R. Solani* infected barley seeds into 0 day-soil	Growth cabinet	Rhizoctonia inoculum (5 barley seeds * 3 box * 3 rep = 45)
April 19	Inoculate with *R. Solani* infected barley seeds into 1 day-soil		Rhizoctonia inoculum (5 barley seeds* 3 box * 3 rep = 45)
April 10 - 24	Observe seedling disease symptom		

Table A3. Experiment 1 results. Germination rates of two sowing depth observed 7 DAS.

March 21	Germinated	Germination rate
1 cm	20	100%
2 cm	18	90%

Table A4. Experiment calendar.

MON	TUE	WED	THU	FRI	SAT	SUN
March 11	12	13	14	15	16	17
		Mix pak-choi for 20D	Sow SB seeds			
			Sow Green manure seeds			
18	19	20	21	22	23	24
Observe germination				End observe germination		
25	26	27	28	29	30	31
April 1	2	3	4	5	6	7
	Sow SB seeds in 20D					
Cabinet —> 8	9	10	11	12	13	14
		Inoculate in 20D	Sow SB seeds in 1D	Observe disease in 20D		
		Mix pak-choi for 0D & 1D	Mix Green manure leaf for 14D	Mix Green manure tuber for 14D		
		Sow SB seeds in 0D				
15	16	17	18	19	20	21
			Inoculate in 0D	Inoculate in 1D		
				Observe disease in 0D & 1D		
22	23	24	25	26	27	28
	End observe disease in all	Decide days & dosage	Sow SB in 14D* 3%			
29	30	**May** 1	2	3	4	5
		Inoculate	Observe disease			
6	7	8	9	10	11	12
13	14	15	16	**—> Cabinet** 17	18	19
				Old plan		
20	21	22	23	24	25	26
27	28	29	30	31	**June** 1	2
				End observe disease		
3	4	5	6	7	8	9
	Clean-up & take root pictures					

*Green: Experiment 1； Blue: Experiment 2； Pink: Experiment 3

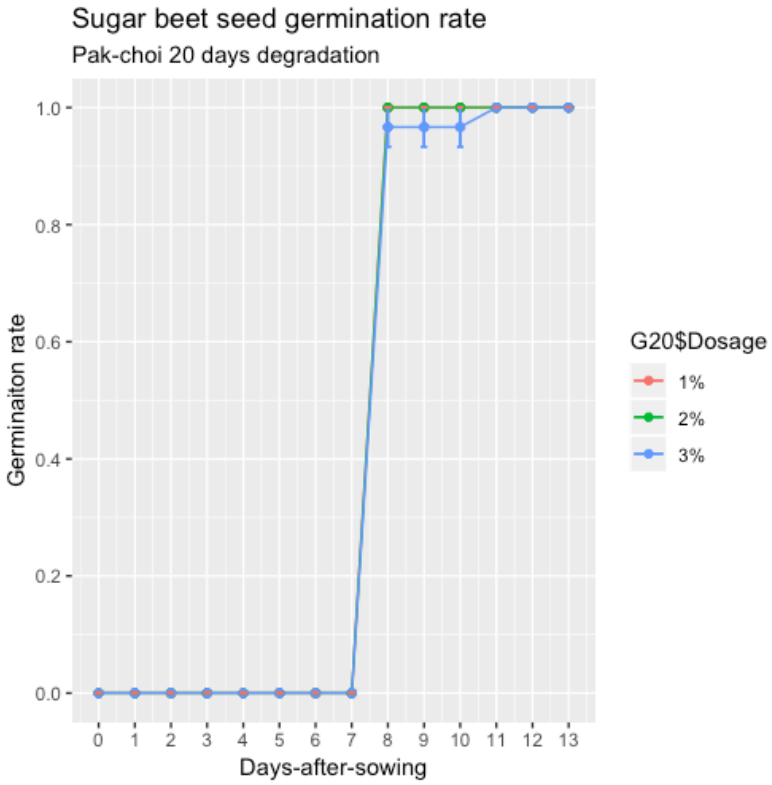

Figure A1. Experiment 2 result. Germination rates of sugar beet seeds in the soils of three dosages (1%, 2%, 3%) of 20 days degraded pak-choi treatment. Data are replicate means ± SE.

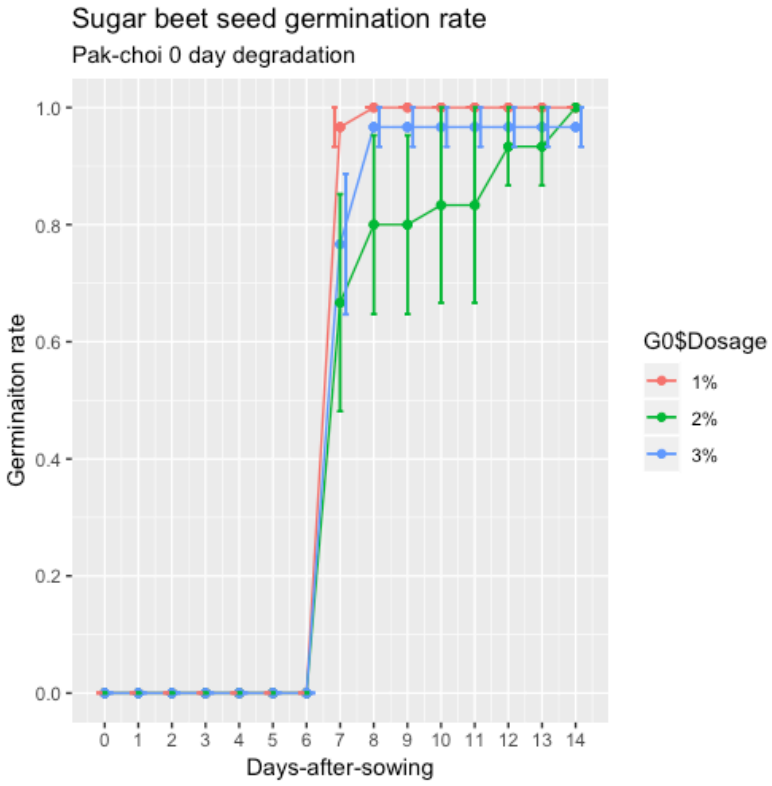

Figure A2. Experiment 2 result. Germination rates of sugar beet seeds in the soils of three dosages (1%, 2%, 3%) of 0 day degraded pak-choi treatment. Data are replicate means ± SE.

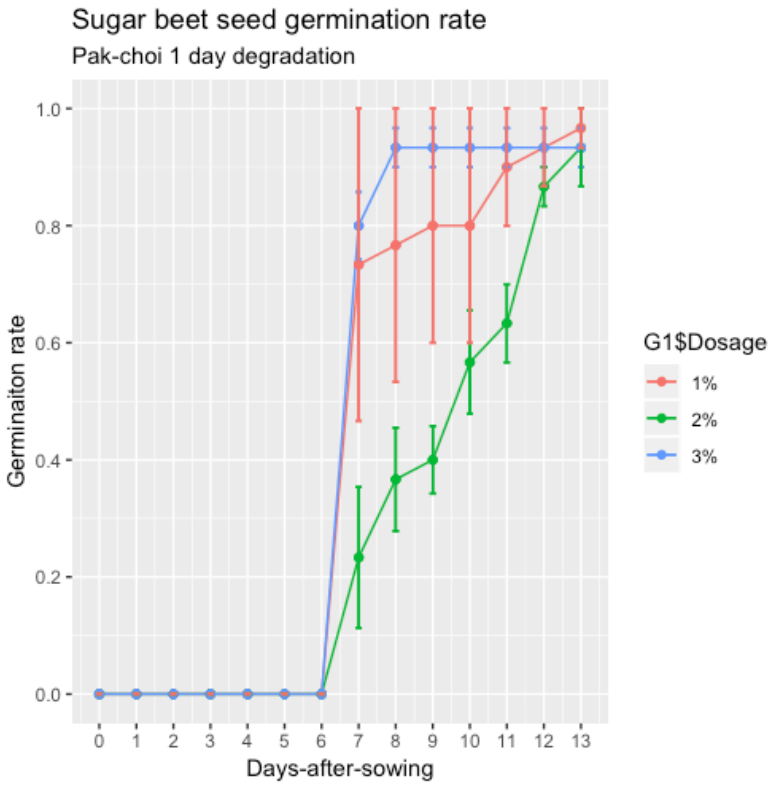

Figure A3. Experiment 2 result. Germination rates of sugar beet seeds in the soils of three dosages (1%, 2%, 3%) of 1 day degraded pak-choi treatment. Data are replicate means ± SE.

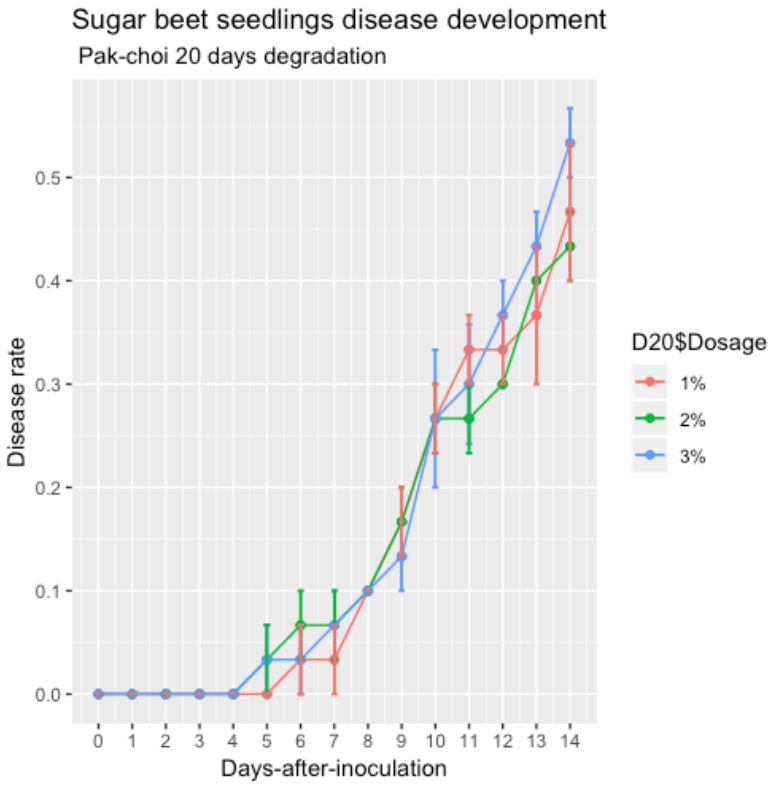

Figure A4. Experiment 2 result. Disease development of sugar beet seedlings in the soils of three dosages (1%, 2%, 3%) of 20 days degraded pak-choi treatment. Data are replicate means ± SE.

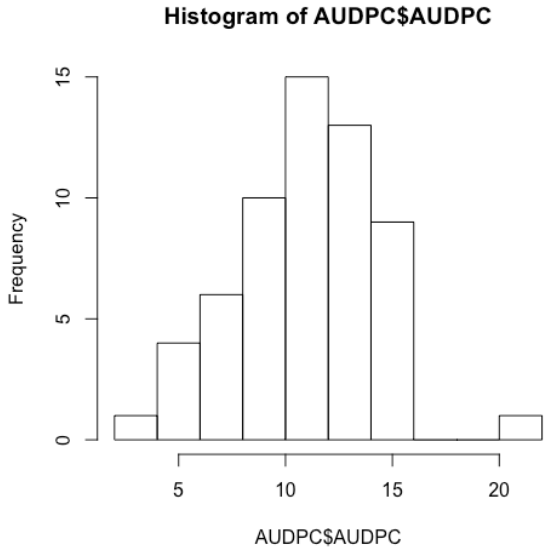

Figure A5. Histogram of AUDPC distribution of all 59 samples.

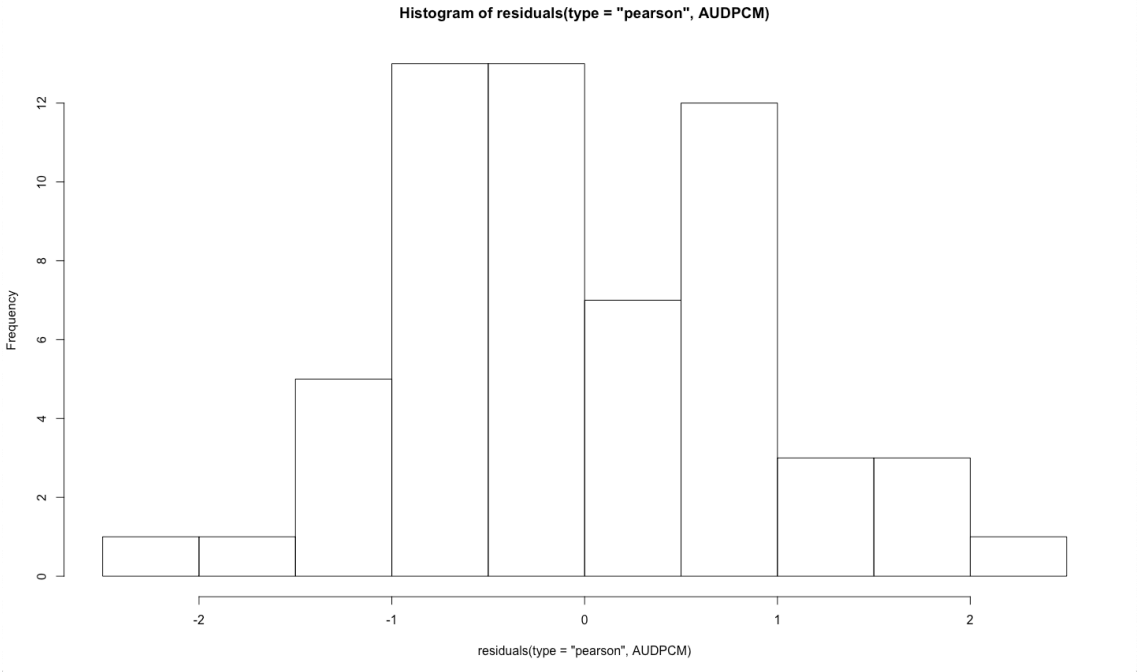

Figure A6. Histogram of residuals in the linear mixed model confirmed the assumption of normality.

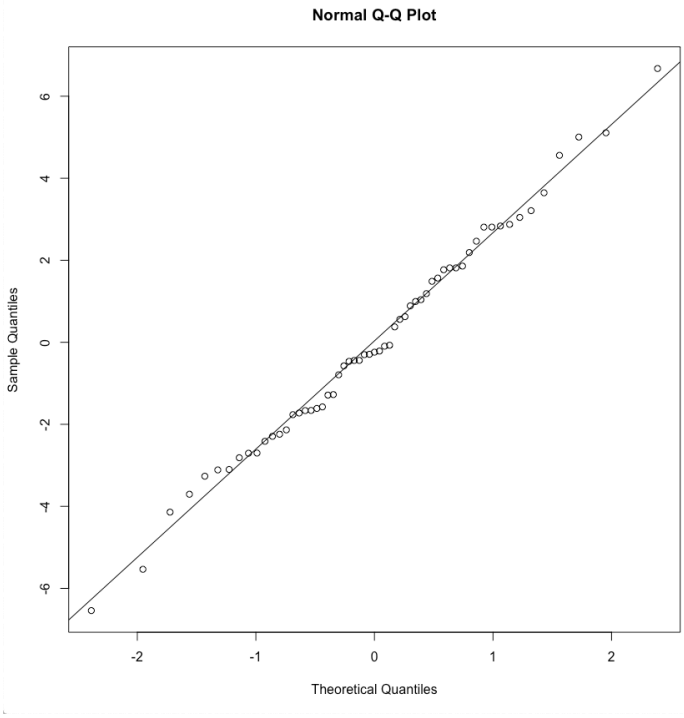

Figure A7. Quantile-quantile plot of residuals in the linear mixed model confirmed the assumption of normality.

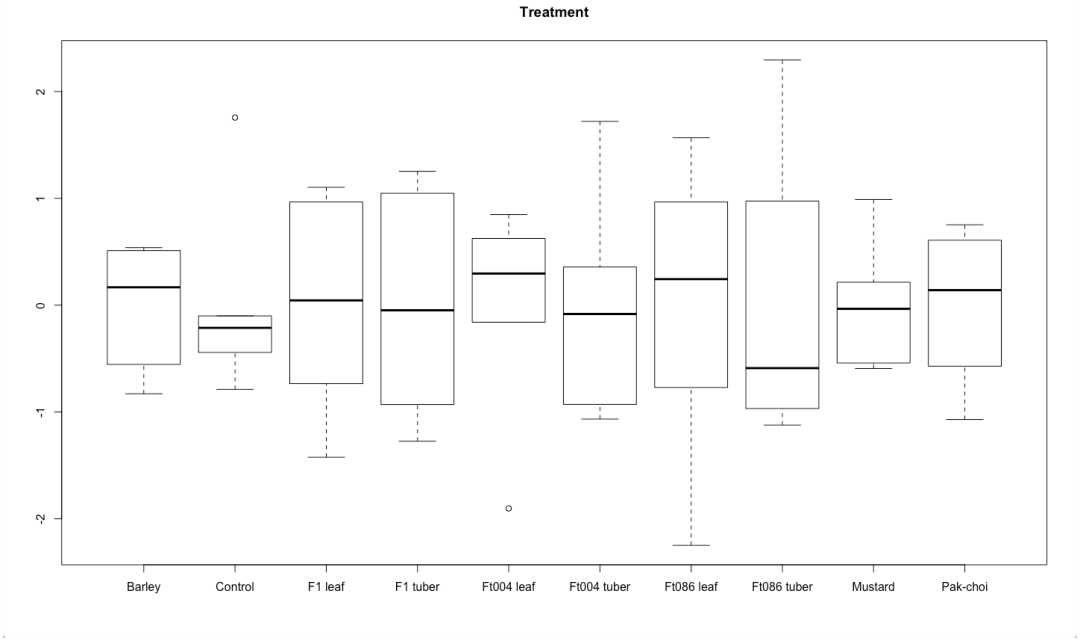

Figure A8. Bar plot of residuals in the linear mixed model confirmed the assumption of homogeneity.

Table A5. Pair-wide comparison of AUDPC by treatments with holm-bonferroni correction for multiple comparisons. Significance levels were denoted as "" <1, "*" < 0.05, "**" < 0.01, "***" < 0.01.

Contrast	Estimate	SE	df	t_ratio	p_value	Significance
Barley - Control	-4.4792	1.68	48	-2.667	0.4261	
Barley - F1 leaf	0.8225	1.68	48	0.490	1.0000	
Barley - F1 tuber	-0.2417	1.68	48	-0.144	1.0000	
Barley - Ft004 leaf	0.6783	1.68	48	0.404	1.0000	
Barley - Ft004 tuber	-0.0377	1.76	48	-0.021	1.0000	
Barley - Ft086 leaf	1.9217	1.68	48	1.144	1.0000	

Contrast	Estimate	SE	df	t_ratio	p_value	Significance
Barley - Ft086 tuber	2.6483	1.68	48	1.577	1.0000	
Barley - Mustard	-1.3117	1.68	48	-0.781	1.0000	
Barley - Pak-choi	-0.4983	1.68	48	-0.297	1.0000	
Control - F1 leaf	5.3017	1.68	48	3.157	0.1184	
Control - F1 tuber	4.2375	1.68	48	2.523	0.5995	
Control - Ft004 leaf	5.1575	1.68	48	3.071	0.1472	
Control - Ft004 tuber	4.4415	1.76	48	2.522	0.5995	
Control - Ft086 leaf	6.4008	1.68	48	3.812	0.0173	*
Control - Ft086 tuber	7.1275	1.68	48	4.244	0.0045	**
Control - Mustard	3.1675	1.68	48	1.886	1.0000	
Control - Pak-choi	3.9808	1.68	48	2.371	0.8293	
F1 leaf - F1 tuber	-1.0642	1.68	48	-0.634	1.0000	
F1 leaf - Ft004 leaf	-0.1442	1.68	48	-0.086	1.0000	
F1 leaf - Ft004 tube	-0.8602	1.76	48	-0.488	1.0000	
F1 leaf - Ft086 leaf	1.0992	1.68	48	0.655	1.0000	
F1 leaf - Ft086 tube	1.8258	1.68	48	1.087	1.0000	
F1 leaf - Mustard	-2.1342	1.68	48	-1.271	1.0000	
F1 leaf - Pak-choi	-1.3208	1.68	48	-0.787	1.0000	
F1 tuber - Ft004 leaf	0.92	1.68	48	0.548	1.0000	
F1 tuber - Ft004 tuber	0.204	1.76	48	0.116	1.0000	
F1 tuber - Ft086 leaf	2.1633	1.68	48	1.288	1.0000	
F1 tuber - Ft086 tuber	2.89	1.68	48	1.721	1.0000	
F1 tuber - Mustard	-1.0700	1.68	48	-0.637	1.0000	
F1 tuber - Pak-choi	-0.2567	1.68	48	-0.153	1.0000	
Ft004 leaf - Ft004 tuber	-0.716	1.76	48	-0.407	1.0000	
Ft004 leaf - Ft086 leaf	1.2433	1.68	48	0.740	1.0000	
Ft004 leaf - Ft086 tuber	1.97	1.68	48	1.173	1.0000	
Ft004 leaf - Mustard	-1.9900	1.68	48	-1.185	1.0000	
Ft004 leaf - Pak-choi	-1.1767	1.68	48	-0.701	1.0000	
Ft004 tuber - Ft086 leaf	1.9593	1.76	48	1.112	1.0000	
Ft004 tuber - Ft086 tuber	2.686	1.76	48	1.525	1.0000	
Ft004 tuber - Mustard	-1.274	1.76	48	-0.723	1.0000	
Ft004 tuber - Pak-choi	-0.4607	1.76	48	-0.262	1.0000	
Ft086 leaf - Ft086 tuber	0.7267	1.68	48	0.433	1.0000	
Ft086 leaf - Mustard	-3.2333	1.68	48	-1.925	1.0000	
Ft086 leaf - Pak-choi	-2.42	1.68	48	-1.441	1.0000	
Ft086 tuber - Mustard	-3.96	1.68	48	-2.358	0.8320	
Ft086 tuber - Pak-choi	-3.1467	1.68	48	-1.874	1.0000	
Mustard - Pak-choi	0.8133	1.68	48	0.484	1.0000	

Table A6. Pair-wide comparison of AUDPC with the control treatment. Significance levels were denoted as "" <1, "*" < 0.05, "**" < 0.01, "***" < 0.01.

Contrast	Estimate	SE	df	t_ratio	p_value	Significance
Barley - Control	-4.48	1.68	48	-2.667	0.0520	
F1 leaf - Control	-5.30	1.68	48	-3.157	0.0193	*
F1 tuber - Control	-4.24	1.68	48	-2.523	0.0599	
Ft004 leaf - Control	-5.16	1.68	48	-3.071	0.0210	*
Ft004 tuber - Control	-4.44	1.76	48	-2.522	0.0599	
Ft086 leaf - Control	-6.40	1.68	48	-3.812	0.0031	**
Ft086 tuber - Control	-7.13	1.68	48	-4.244	0.0009	***
Mustard - Control	-3.17	1.68	48	-1.886	0.0653	
Pak-choi - Control	-3.98	1.68	48	-2.371	0.0599	

Figure A9. Roots of survived seedlings in Ft 004 tuber treatment. Picture taken at June 4th.

Figure A10. An example of jumping infection on the seeding circled in red. Photo was taken on May 28 (27 DAI).

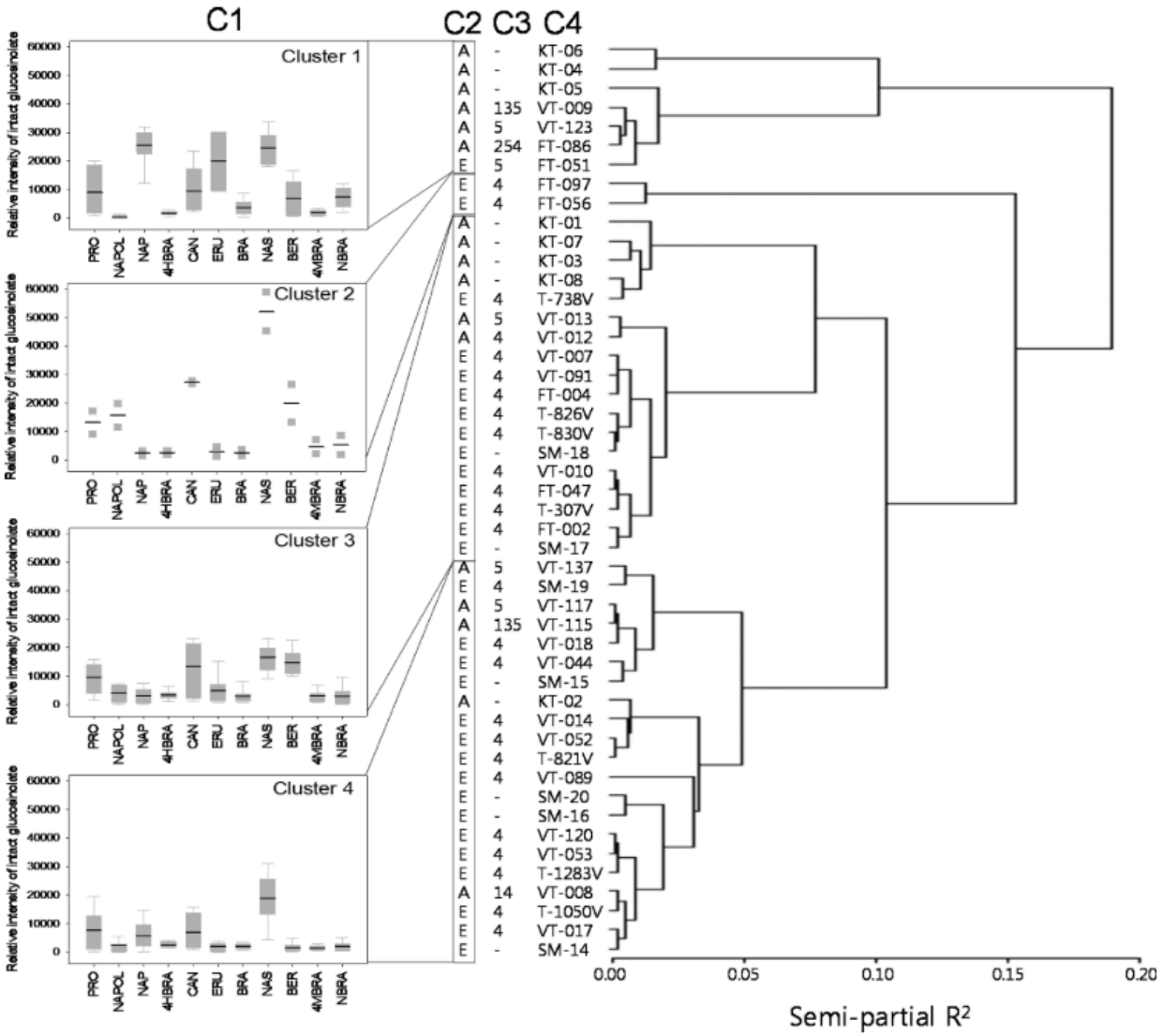

Figure A11. Clustering of turnip samples according to intact GLS composition (Lee *et al*., 2013).

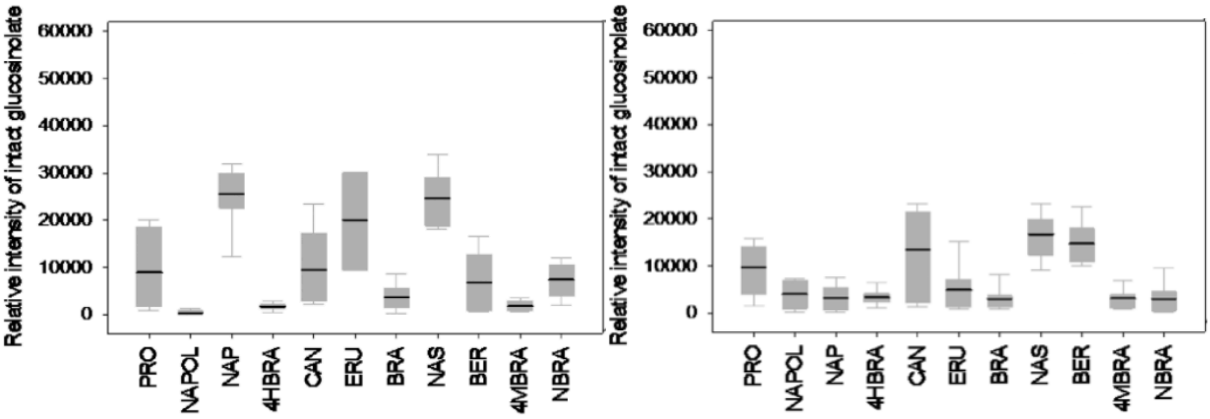

Figure A12. Relative composition and intensity of intact GLSs in Cluster 1 (left) and Cluster 3 (right) (Lee *et al.*, 2013).

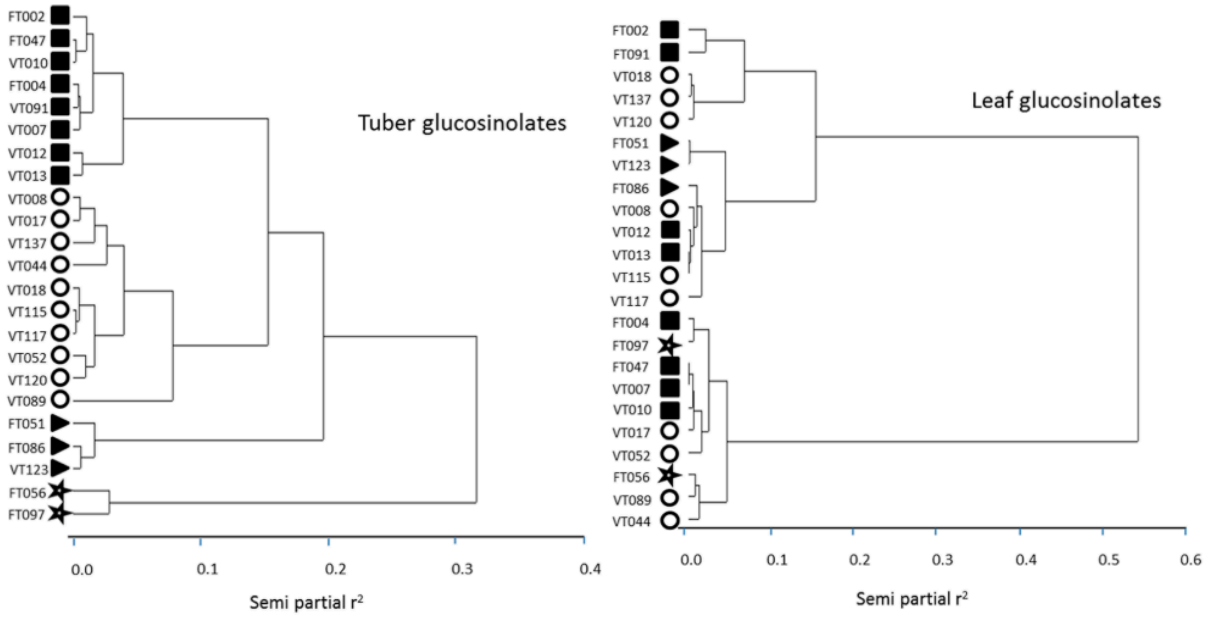

Figure A13. Clustering of turnip samples according to GLS composition in tuber and leaf tissues (Lee *et al.*, 2013).

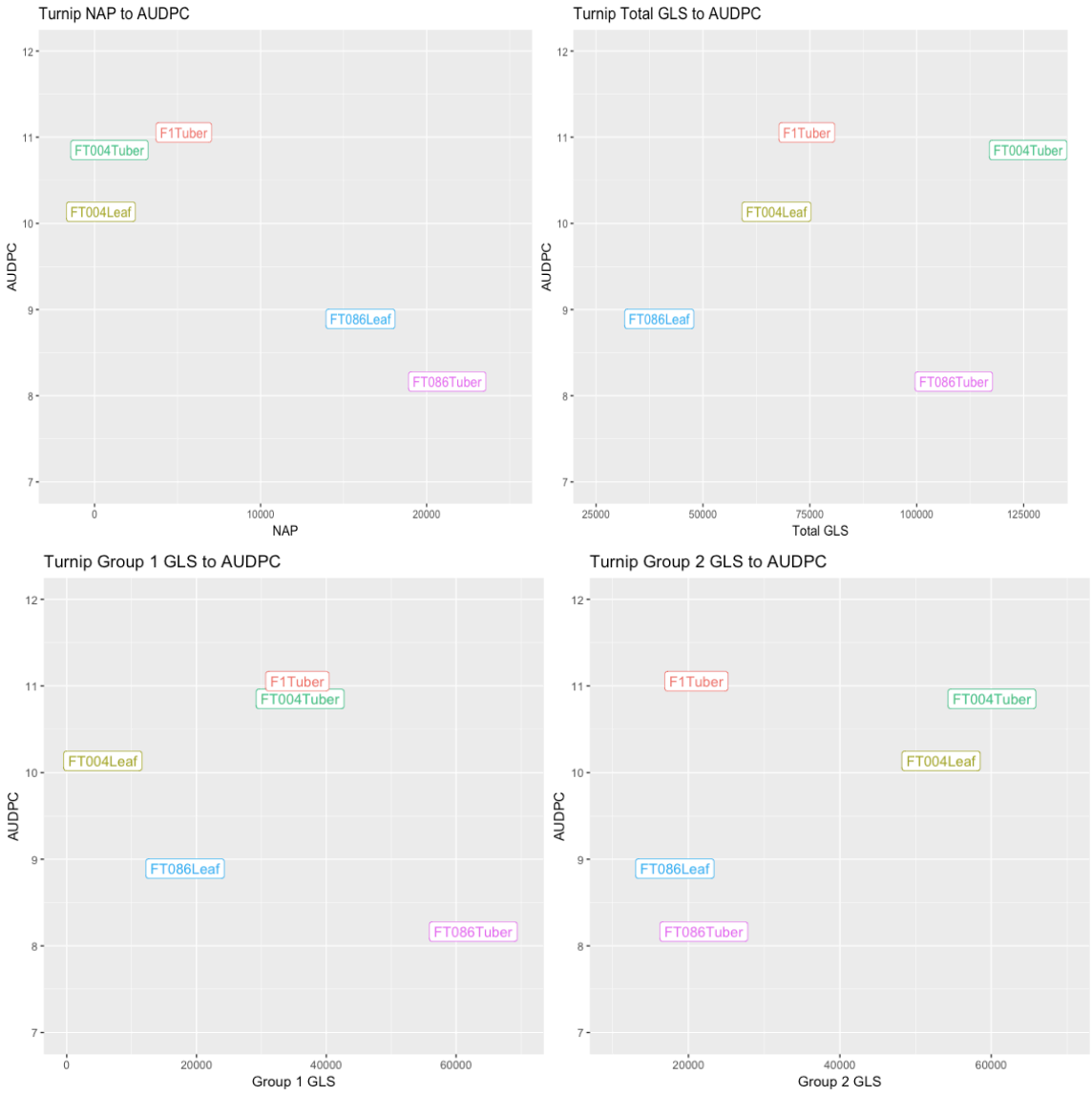

Figure A14. Visualization of correlation of the relative abundance of different GLS compositions to the final AUDPC response in turnips (based on data from Bonnema *et al.*, 2019).

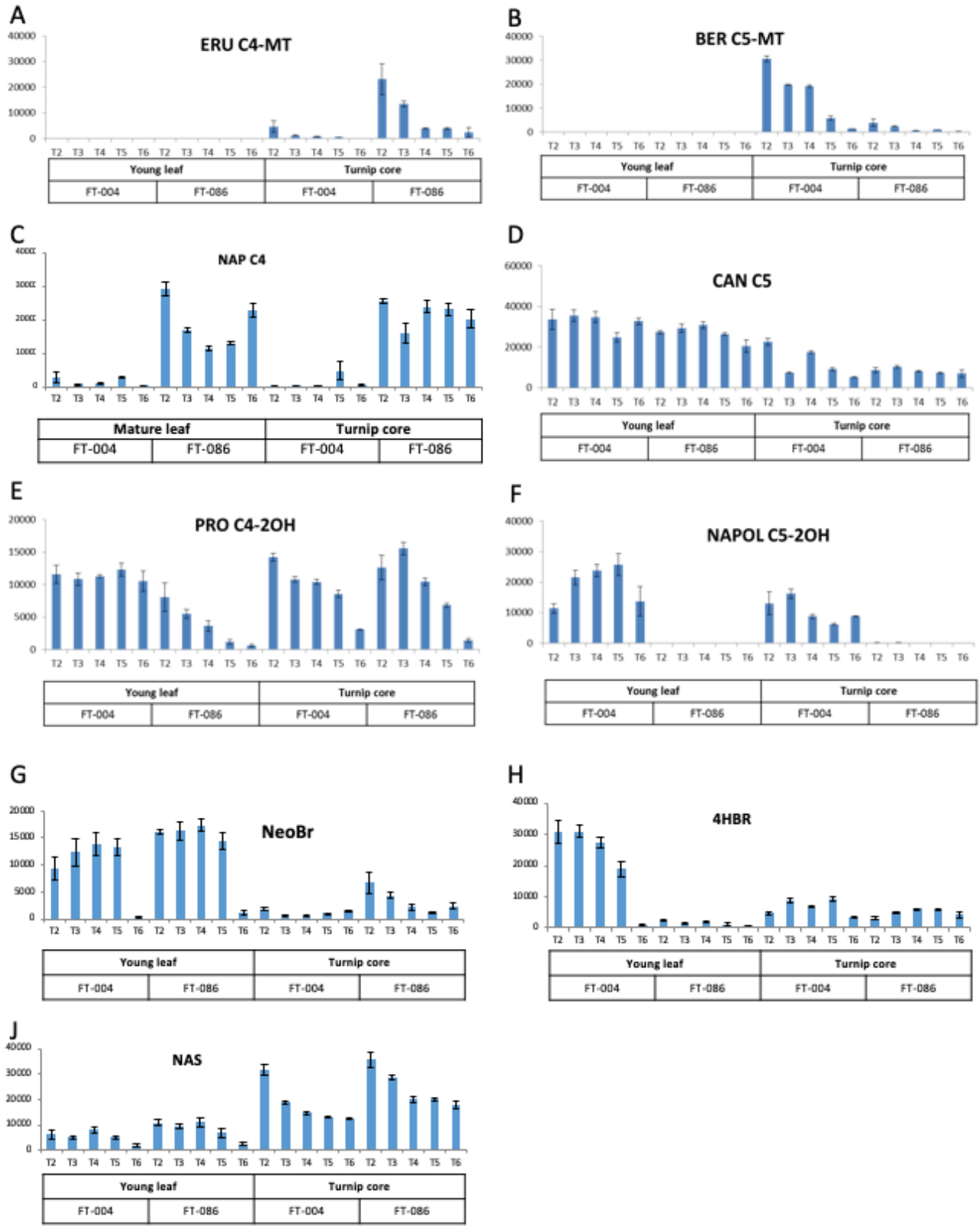

Figure A15. Relative quantity of individual GLSs in tissues of Ft-004 and Ft-086 turnip. The Y-axis shows the peak surface area measured in LC-MS for the indicated compound. Error bars indicate standard deviation (n=3) (data and figure retrieved from Bonnema *et a*l., 2019).

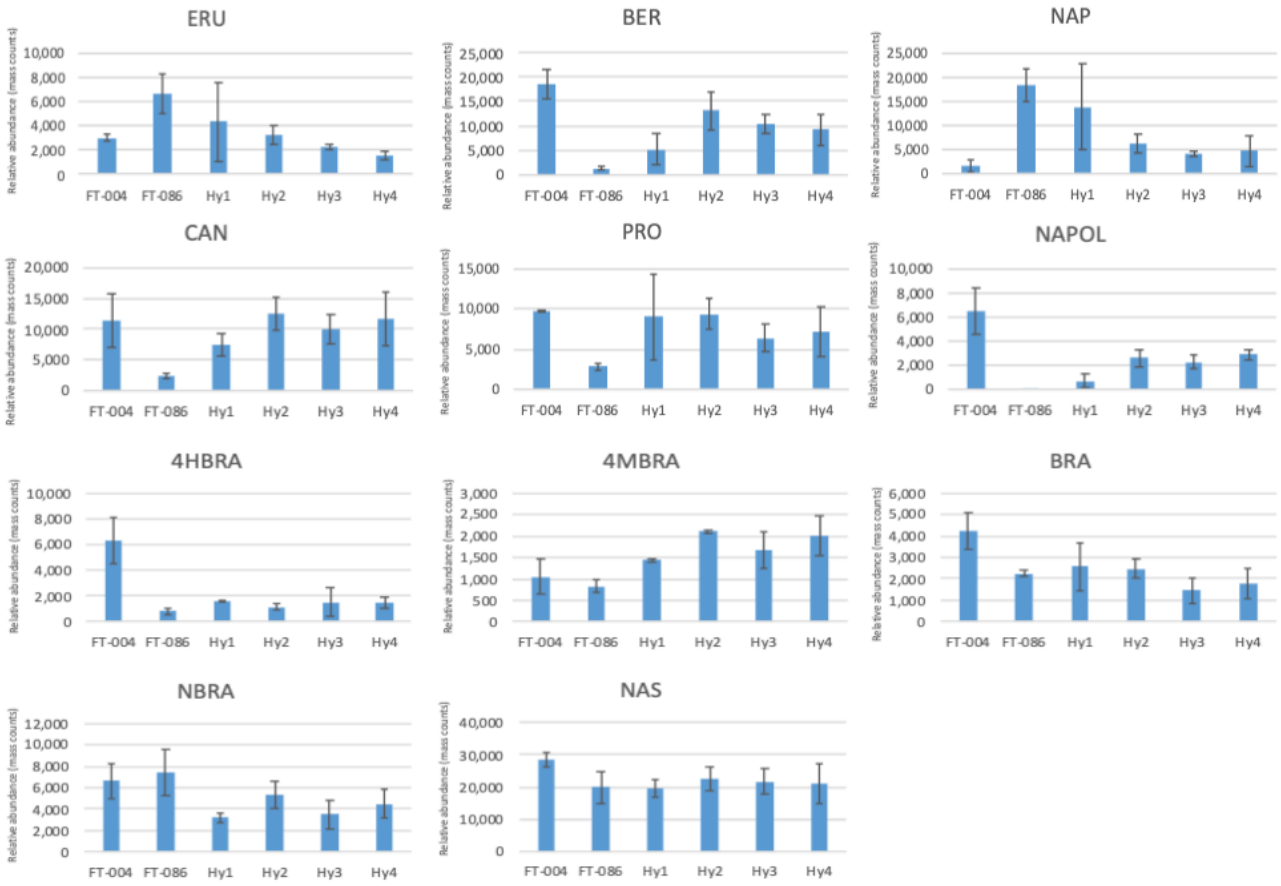

Figure A16. Relative abundance of 11 GLS in the tubers of two parental lines and four F_1 hybrids (figure retrieved from Bonnema *et al.*, 2019).