Quantification of differences in germination behaviour of pelleted and coated sugar beet seeds using X-ray Computed Tomography (X-ray CT)

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Abstract

Seed enhancement technologies i.e. priming, pelleting and coating have been extensively used throughout the last century to improve crop yield and to reduce losses associated with pest infestation. However, until recently, it has not been possible to non-destructively assess the effect of seed enhancement technologies belowground due to the opacity of soil. Using X-ray Computed Tomography (X-ray CT) we undertook a 4D visualisation of the germination process of four different sugar beet seed enhancement treatments (untreated / naked, coated, pelleted and pelleted + coated) in soil. The aim of this study was to improve the understanding of the germination process in the natural environment of the seed to inform future soil management and seed enhancement processes. Using X-ray CT we were able to quantify the germination and establishment process of different seed enhancement technologies in soil non-destructively for the first time. We observed a delay in seedling growth posed by the addition of a physical barrier, i.e. the seed coating. However, an enhanced radicle growth rate was observed in pelleted, as well as pelleted and coated seeds, after overcoming the physical barrier. The disadvantage posed by the addition of seed coating was overcome after four days of seedling growth. Further work should focus on refinements to the type and composition of the pelleting which we observed to have a retarded effect on seed germination.

Introduction

With an increasing global population (*ca.* 9.2 billion in 2050) and demand for food necessitates there is need to increase crop yield and efficiency across a wide range of plants (Lal, 2013) however, deterioration of suitable agricultural land for crop production is a significant problem (Monneveux *et al.,* 2013). Therefore, it is particularly important to identify crops that have a reduced need for nutrients and enhanced ability to overcome stress (Chapuis *et al.,* 2012) and to maximise yield. Crop seeds require certain soil properties (especially moisture and temperature) to initiate germination, a crucial stage which influences the success of establishment into full grown plants. The seeding material consists of a seed containing the perisperm and the embryo, as well as the surrounding fruitwall material. Both the seed and the fruitwall are referred to as the fruit. From this point the term 'seed' is not used in a botanically strict sense and includes both the fruitwall and the seed. Water absorption (imbibition) is the first and fundamental step in germination process (William *et al.,* 1995). During imbibition, the dry seed hydrates and swells which increases seed volume. Additionally, the fruit wall starts to soften which allows the radicle to penetrate the fruit wall and emerge into the soil (Lundgren, 2009). To enable the water absorption process from the soil, close seed-soil contact is important (Brown *et al.,* 1996). Germination occurs when embryo growth overcomes the constraints of the fruit wall surrounding the seed (Bewley *et al.*, 2013; Smykal *et al.,* 2014). Seed technology aims to sustain and improve the health and yield of crops as well as emergence and germination (Taylor *et al,* 1998; Ehsanfar and Modarres-Sanvay, 2005). Originally, seed enhancement technology i.e. seed pelleting was used to ensure successful precision sowing or to synchronise male and female inbred seeds (Vyn and Murua, 2001; Gorim, 2014). In addition to this, different enhancement technologies aim to improve performance to overcome growth restricting influences of the soil caused by temperature or moisture extremes. This may be through seed coatings that supply nutrients (Silcock and

Smith, 1982; Mašauskas *et al.,* 2008), hormones (Powell and Mathew, 1988), peroxides for oxygen provision or polymer coatings with hydrophilic abilities (Vyn and Murua, 2001; Gorim, 2014). Seed coating processes can be divided into two groups: seed pelleting and film coating. Pelleting consists of the addition of a relatively thick layer of material (often wood fibre) which is typically intended to increase the total volume and roundness of the seed to enhance the accuracy of planting. The added material may also contribute to increased water uptake and maintain sufficient moisture content during germination. Film coatings do not change the shape of the seed and are used to apply pesticides and fungicides (Hill, 1999).

Besides crop enhancement, our understanding of seed germination and seed-soil interaction is limited. Field trials provide information concerning how the plumule grows after germination (Chang, 2004). These field trials are limited to aboveground observations and hence do not provide insight into the germination process as the initial step of plant establishment. A Rhizotron enables a single slice 2D view of a root system facilitated by a glass window (Klepper and Kaspar, 1994). The resulting root structure however, is influenced by the boundaries posed by the glass windows which influences the growth behaviour and eventually the root architecture. Observation of seed germination is impeded as it is unlikely that radicle growth is observed through the glass window. If the seed would be placed next to the glass layer, the seed opening and therefore the germination process, would be influenced. X-ray CT as a non-invasive and non-destructive 3D imaging technique, has been increasingly used to visualise soil properties like porosity (Vogel, 2010) and *in situ* root systems (Tracy *et al.,* 2013; Mairhofer *et al.,* 2013). Magnetic Resonance Imaging (MRI) and X-ray CT have previously been used for 3D root quantifications and it has been reported that X-ray CT is better suited for smaller pot diameters (Metzner *et al.,* 2015). However, only few studies have been undertaken to examine seed germination in soil especially regarding the impact of seed

74 coatings. Most previous work on seed germination have been conducted on seeds grown
75 without soil (Gagliardi, 2011; Galhaut, 2014; Devarrewaere *et al.,* 2015). These studies have
76 shown that it is possible to visualise structural changes within the seed during germination.
77 Gregory *et al.* (2003) was the first to use X-ray CT to image wheat seedling growth in soil with
78 a resolution of 100 µm verifying the suitability of this technology to monitor seedling
79 establishment *in situ*. Soil generally has an opaque and heterogeneous structure which has
80 limited our ability to observe germination *in* situ (Brown *et al.,* 1996). Galhaut *et al*. (2014)
81 previously showed tissue detachment between radicle and cotyledons contributing to
82 hydration which was not previously observed in unprimed seeds using X-ray CT (however,
83 crucially not *in soil*).

84 Here we report the application of X-ray CT to quantify the impact of four seed enhancement
85 technologies (naked (N), film coated (FC), pelleted (P) and pelleted and film coated (P+FC)) on
86 the spatiotemporal germination of sugar beet seeds grown in soil. The aim of this study was
87 to assess differences in performance of different seed enhancement technologies to illustrate
88 the viability of X-ray CT for future germination studies in soil. Treatments were chosen based
89 on commercial interest (pelleted + coated) divided into their basic components (coating and
90 pelleting) compared to the untreated seed (naked). It was hypothesised that the enhanced
91 seeds with an applied pelleting would have a germination advantage over the naked seeds
92 whereas coated seeds might show a disadvantage due to the effect of the pesticide
93 components.

94 **Materials & Methods**

95 A Dunnington Heath series sandy loam soil (66.4% sand, 18.0% silt, 15.6% clay and 4.53%
96 organic matter) was collected from The University of Nottingham farm at Bunny,
97 Nottinghamshire (52.8586°, -1.1280°), air-dried and sieved to <1 mm. Sugar beet seeds were

supplied by Syngenta Seeds AB, Sweden. Four different treatment types have been chosen based on commercial availability using naked (uncoated) (N), film coated (FC), pelleted (P) as well as pelleted and film coated seeds (P+FC). The pelleted treatment is a Syngenta in-house development mainly consisting of woodmeal and clay. Seeds are coated with a standard fungicide and insecticide treatment. The precise coating and pelleting compositions are treated confidentially. All used seed treatments can be ordered at Syngenta Seeds AB, Sweden, by referring to this study.

It was imperative that different column packing methods had to be first pre-tested to ensure maximum comparability of field conditions as well as accurate reproducibility. Method A displayed in Figure 1 facilitates wetting via capillary action. An initial amount of dry soil was poured into the column and saturated with water from the bottom. The seed was placed centrally placed on the bottom layer and covered with dry soil. Capillary action saturated the top layer after a few minutes. In method B, the entire column was filled with dry soil and saturated with water. Then, a hole of the size of the seed was created, the seed placed into the hole and filled with dry soil. Method C was similar to method A but conducted only with dry soil. Therefore, the column was filled with dry soil, the seed placed centrally on this layer and covered with dry soil. The column was then saturated afterwards. To simulate a seed drill, in method D the column was prepared in a similar way than method B. After saturating the filled column, a hole was excavated and the seed dropped into it. The hole was then topped up with the same soil and compacted slightly. In method E, the soil stock was wetted to a set moisture content. This pre-wetted soil was used to fill the column to the sowing height. After placing the seed centrally on the bottom layer, the seed was covered with more pre-wetted soil. Further details are provided in the method development section of the results. The chosen method (method C) involved a single seed being placed centrally into a polypropylene

column with a height of 70 mm and 22 mm inner diameter which was previously filled with 22.8 g of soil to a height of 55 mm with four replications. After placement of the seed, another 6.8 g of soil was poured on top of the first layer to final height of 65 mm resulting in a bulk density of 1.2 g cm^{-3}. The column was saturated for 5 minutes using 30 ml water and drained for 30 minutes to achieve a gravimetric water content of 20%. The soil columns were incubated in a growth chamber with a day temperature of 20°C and a night temperature of 15°C applying 16 hours of daylight (dusk and dawn times were set to 1 hour). Moisture contents were monitored daily and maintained at 20% w/w. The size of the polypropylene columns were chosen to maximise the imaging resolution while not influencing the germination and establishment of the sugar beet seeds, a trade-off well known in CT. The radicle angle was very close to 90° in all samples and therefore the radicle was not touching the column wall and the radicle lengths did not at any point exceed the length of the column.

Columns were scanned daily using a Phoenix v|tome|x m 240 kV (GE Measurement & Control Solutions, Wunstorf, Germany). A potential energy of 130 kV with a current of 100 µA and a timing of 250 ms was applied collecting 2878 angular projection images in constant rotation mode (FAST SCAN), where image average and skip values were set to 1 and 0, respectively. Scans were performed at a spatial resolution of 20 µm with an acquisition time of 12 minutes each in a multi scan acquiring two sections. Acquisition images were reconstructed using the *phoenix datos|x 2rec* reconstruction tool (beam hardening was set to 8, region of interest and scan optimization has been automatically calculated) resulting in 16 bit data. The soil columns were scanned in the same order every day to reduce the impact of temporal influences and create a 24 hour difference between each scan. However, the germination was initiated at the same time for the whole sample set to raise the seedlings in the same day and night rhythm. Therefore there was difference of about 6 hours between the first and the last scan of the day,

however this had a negligible effect on the data interpretation as the shift in radicle length between replicate 1 and replicate 4 of each treatment was minimal and was mostly accounted for by natural variation which can be extrapolated to the 6 hour time difference.

Data processing was performed using *VGStudio Max® 2.2*. Seedlings were segmented using the 3D region growing tool and root lengths determined using the polyline tool as described by Tracy et al. (2012). The average thickness of the coating and pelleting was manually determined by using the distance tool on multiple sections of the seed in different 2D view orientations. The soil to air ratio for the different packing methods was determined by segmenting the seed as a solid object without inner air space and dilating the segmented area by 1 voxel (20 µm). The segmented seed was subtracted from the dilated seed so that a ring of 1 voxel thickness remained. A surface determination based on air space as background and several areas of soil as material was used to determine soil and air space volume which was used to calculate a percentage.

An additional destructive screening experiment was conducted to support the work with increased replication (20 per treatment). Half of the corresponding seedlings were excavated after two days of growth, the other half after four days. The excavated roots were washed and the root lengths determined using graph paper. All error calculations have been conducted using the standard error of the mean.

Results

Method development

Preliminary investigations were undertaken to assess the appropriate soil packing method (five in total) to create realistic field conditions (Figure 1). Figure 2A shows the capillary method was responsible for the formation of two distinct layers. The bottom layer consisted of a higher percentage of fine particles at the transition zone, whereas the top layer showed

a higher amount of coarser particles which resulted in a hydraulic disconnection causing the developing root and stem to push the top soil layer upwards. A ratio of 55.76% (±4.56) soil to 44.24% (±4.56) air was calculated within a distance of 1 voxel (20 µm) around the seed. Method B led to a higher seed-soil contact around the seed. Nevertheless, the filled region featured more pore space than the surrounding soil than would be considered ideal (Figure 2B). Method B showed the highest surrounding soil mass with a ratio of 68.86% (±2.09) soil to 31.14% (±2.09) air. Method C exhibited a high seed-soil contact throughout the whole region with the most uniform distribution of the fine soil particles with a ratio of 63.01% (±0.83) soil to 36.99% (±0.83) air (Figure 2C). Excavation of soil to insert the seed resulted in large air space pockets around the seed although the top layer was tapped downwards (Figure 2D). These air pockets resulted in a higher amount of air space around the seed (38.49% (±5.94) soil to 61.51% (±5.94) air). A similar appearance was observed in method E with a ratio of 32.86% (±12.31) soil to 67.14% (±12.31) air (Figure 2E). Method D and E had a significantly higher air space around the seed as well as a high variability within the replicates which was non-preferable. Method C was chosen for subsequent studies as it ensured greater reproducibility of packing and from field observations appeared to reflect a structural arrangement most similar to the field situation. However, one might expect a higher degree of heterogeneity in the field over large distances which would potentially increase variation in germination behaviour between seeds.

Comparison of seed enhancement technologies

X-ray CT scanning of a seed in air enables the distinction of different components (Figure 3). The scan of the bare seed ex situ enabled measurements of the thickness of the seed coating and pelleting. The seed coating had an average thickness of 0.03 mm evenly distributed over the seed surface with a volume of approximately 1.04 mm^3 (P+FC) and 1.09 mm^3 (FC). Applied

194 on the surface of a pelleted seed, the thickness varied between 0.03 mm and 0.06 mm with a
195 size of approximately 20.30 mm^3 (P) or 20.72 mm^3 (P+FC). The thickness of the pelleting varied
196 highly between 0.05 mm and 1.10 mm due to the shape of the seed. The applied pelleting had
197 a porosity of 18.03% for P and 15.82% for P+FC. Figure 4A shows an exemplar 2D image slice
198 of an X-ray CT scan of a 3 days old seedling *in soil*. The 20 µm resolution enabled a
199 differentiation of the fruit wall, the perisperm and the embryo for the first time *in soil*. The
200 shoot can be observed growing towards the soil surface and thereby pushing the soil particles
201 aside. Figure 4B shows a similar appearance for treatment P+FC. The formation of the apical
202 hook results in a region of higher compaction in comparison to the soil particles around the
203 seed. It appears that the number of small soil particles in immediate contact with the seed is
204 much higher in comparison to larger particles which can be precisely observed using X-ray CT.
205 The grey value intensity differences observed in the pelleting highlight the layers consisting of
206 materials with different X-ray attenuation coefficients where the lightest parts represent
207 mineral based components. On the outermost layer is a fine white line (high X-ray absorption)
208 which is due to the mineral content of the pesticide coating.

209 *Quantitative assessment of X-ray CT data*

210 Sugar beet radical growth characteristics for each seed treatment were measured daily for 4
211 days (Figure 5). Treatment N was shown to display a rapid growth response followed by 2 days
212 of steady growth (Figure 6A) whereas P+FC and P had an initial slower growth that increased
213 over the 4 days resulting in longer radicle length of P compared to N after 4 days of growth.
214 Specifically, the radicle lengths of P+FC were *ca.* 50% less in comparison to N on day 2 but
215 showed a similar length at day 4. FC displayed a delay in root growth of *ca.* one day and a
216 slower initial development compared to the P+FC. This was followed by a rapid growth

between day 3 and 4 but it did not subsequently achieve the same growth as the other treatments. The effect on the growth per day can be seen in Figure 6B.

Comparison of daily radicle growth (Figure 6B) showed a rapid growth at day 2 for N and P which decreased the following day. The P+FC seeds showed a continuous increase in radicle growth, whereas N and P decreased after the initial rapid growth. For FC almost no radicle growth was visible at day 2 but a rapid increase in growth occurred on the subsequent days.

A similar pattern was observed for the radicle volume between all treatments (Figure 6C). The volume determination suffers from an intrinsic potential error of up to 18-20% for a dilation by 1 voxel (addition of a one voxel layer) and up to 16-18% for an erosion of 1 voxel (subtraction of a one voxel layer) for the majority of the segmentations. N and P treatments showed a rapid increase in volume at day 2. The growth rate extenuated at day 2 but accelerated again at day 4. P+FC treatments showed an almost linear increase in radicle volume. A delay was observed for FC with a rapid acceleration on the last day. Figure 7 displays the ratio of radicle volume and radicle length to visualise the differences between the seed enhancement methods. There was a significant relationship interaction between sampling day and seed treatment ($p = 0.004$). A screening test was conducted to observe the variability within each treatment type (displayed in brackets is standard error): Day 2: N 8.4 mm (±2.4 mm), FC 0.2 mm (±0.2 mm), P 6.1 mm (±1.3 mm), P+FC 4.2 mm (±1.3 mm); Day 4: N 35.6 mm (3.5 mm), FC 18.7 mm (±5.2 mm), P 38.5 mm (±4.1 mm), P+FC 38.7 mm (±0.9 m). A comparison of the X-ray CT data and the screening data was conducted (Figure). The radicle lengths measured using the X-ray CT data were higher compared to the screening data in all treatments and both screening days.

Discussion

This study successfully highlighted subtle temporal differences in growth between different seed enhancement treatments using X-ray CT and hence verifying X-ray CT as a suitable tool for the quantification of the establishment process of plants i.e. our work was undertaken on sugar beet but is transferable to most seed types. Due to the nature of the *in situ* environment, the contrast of the collected images suffers in comparison to a scan of a seed outside of soil which is a limiting factor for observing the germination process in the seed, though we believe this is offset by the advantages of observing behaviour in soil. The results show for the first time in soil clear treatment differences in radicle growth characteristics over the first four days after sowing. Although the results generally showed low within treatment variability, an additional screening test of ten replicates was used to further understand the inherent variability of seedling establishment. Results indicated that the radicle length of all seed enhancement types have a high variability. It was noticed, however, that radicle lengths were higher when measured by X-ray CT compared to excavation measurement. It has previously been reported that radicle lengths can be underestimated using X-ray CT which contradicts these findings (Mooney *et al.,* 2012). However, in this study different plants were used for the X-ray CT and the destructive analysis so we attribute natural variation as the main reason for the difference. As the length differences were significant throughout all treatments, it may be possible that X-ray radiation had a small but beneficial effect in the small doses that are able to penetrate the soil (Shull and Mitchell, 1933). A further beneficial effect might be due to an inhibitory effect on pests that could be present in the soil or the seed itself (Ikram *et al.,* 2015) although this is less likely in the timescales of this study.

The use of FC seeds verified the assumption that the chemical coating, at least when applied directly to the seed, can inhibit early growth of the radicle. It has been reported that the

263 pelleting serves to increase the spatial distance to the coating besides its original purpose to
264 increase the ease of planting (Kaufmann, 1991; Hill, 1999; Taylor *et al.* 2001). The application
265 of pelleting resulted in a higher germination rate compared to other treatments based on root
266 length growth per day which might be due to the increased water uptake rate as a beneficial
267 side effect of the pelleting material consisting mainly of wood fibre and clay. The growth over
268 time showed a very rapid development of the radicle on day 2 for all treatments except for FC.
269 The fast radicle growth for the FC treatment started with a day delay which is likely due to the
270 proximity to the insecticides and fungicides. Standard pesticides used for sugar beet
271 protection include fungicides like Thiram or Tachigaren (active ingredient: Hymexazol) as well
272 as insecticides like thiamethoxan, imidacloprid or chlothianidin (KWS, 2017; Syngenta, 2016a;
273 Syngenta, 2016b). Redfearn and Osborne (1997) showed a quicker emergence using Thiram
274 compared to previous seed coating treatments with similar effectiveness. Hymexazol has been
275 reported to affect fungal RNA and DNA synthesis and should the transformed into glucosides
276 with fungi toxic effects as well as plant growth promoting effects rapidly after entering a plant
277 organism (Ypema, 2003). Since the 1990s, neonicotinoids (e.g. thiamethoxan) have been
278 widely used to reduce the risk of virus yellows and to control foliar and soil pests (Bayer, 2011;
279 KWS, 2017; Syngenta, 2016c). Though neonicotinoids, in particular, are controversial due to
280 the reported effects on bee populations (Rundlöf, 2015; BBRO, 2016). The addition of a
281 coating reduces the ability of the seed to open as quickly as a naked seed. The delay for FC
282 could therefore be due to the physical shell that was created during the coating process or the
283 resulting phytotoxicity posed by its proximity to the seed surface. Similar effects have been
284 reported for oil seed rape as imidacloprid and thiamethoxam supressed root system
285 development in the cotyledon stage (Huang *et al.*, 2015). Vyn and Murua (2001) found
286 uncoated seeds develop earlier than coated seeds, as found in this study. This reduction in

287 growth was observed only in FC and not in P+FC which may be due to the composition of the
288 pelleting material or to the swelling of the pellet during water uptake which might help to
289 overcome the physical shell of the coating by weakening the structure of the shell. It is likely
290 that the slower germination could be the influence of the coating material, which is not as
291 high for P+FC because the applied insecticides and fungicides are not directly in contact with
292 the fruit (the fruit includes both the seed and the fruitwall surrounding the seed) and therefore
293 the phytotoxicity impacting the seed is lower. Furthermore, a seed establishment delay was
294 observed in different coated turfgrass species using different irrigation techniques (Serena *et*
295 *al.,* 2012). Karnataka *et al.* (2008), however, described a higher germination rate, a higher
296 vigour index (vigour index = (root length + shoot length) x germination percentage) and an
297 increased field emergence in coated seeds after three months of growth. Therefore, FC
298 seedlings may overcome this delay later during their growth and give higher field emergence
299 compared to naked seeds based on the protection provided against negative effects like fungi
300 or insects. The growth rate comparison showed that P and P+FC radicle growth rates increased
301 after the initial rapid growth whereas the growth rate for N was constant. This may be due to
302 the enhanced water uptake during the first days of growth that would have increased water
303 storage inside the seedling in both P and P+FC, as well as an increased vigour caused by
304 increased water uptake (Gorim and Asch, 2015). A positive effect for different kind of
305 pelletings was previously shown during early establishment especially near moisture limiting
306 periods supporting this assumption (Scott, 1975).

307 Figure 7 showed that there are slight differences in volume per millimetre radicle length for P
308 and N in comparison to FC and P+FC but these were not significant. Also, the radicle volume
309 was almost the same at day 4 which suggests seed enhancement technologies like P+FC do
310 influence the radicle width shortly after germination in the same way as they influence radicle

311 length. The four growth stages (seed cracking; rapid radicle growth; formation of apical hook;
312 unfolding of apical hook) were visible in all four treatments although they were slightly
313 delayed for the FC. This observation confirms that physical seed enhancement using pelleting
314 or coating might not have altered the mechanism of emergence but rather the rate.

315 **Conclusions**

316 Seed enhancement technologies enable the alteration of the growth behaviour of the early
317 seedling. Although it appeared N has the highest growth rate over the first two days, the
318 addition of pelleting allowed a more rapid increase in root growth per day. The addition of a
319 coating is essential to ensure a consistent yield in the field due to the addition of protection
320 by applying insecticide and fungicide (FC). However, applying a coating reduced the seedling
321 emergence rate which is why a pelleting in addition to the coating is used to balance this
322 negative effect (P+FC). The positive effect of the pelleting as hypothesised was verified as was
323 the negative effect of the close proximity of the pesticide containing coating to the seed. This
324 study shows the benefits of X-ray imaging as a tool to compare different seed enhancement
325 technologies in soil supporting efforts of seed breeding companies to optimise their seed
326 enhancement compositions according to the seeds *in situ* performance.

327 The physical mitigation of a pesticide coating might be overcome by priming the seed prior to
328 pelleting which could be assessed in future studies. During priming, the germination process
329 is initiated and stopped before the seed starts to crack. This effect is hypothesised to
330 overcome the disadvantage of the coating delay. Future work is needed to evaluate the effects
331 of priming in comparison to the data presented here. Additionally, the effect of different
332 growth conditions (e.g. drought and compaction) could be monitored to gain a greater
333 understanding of the influence of the physical treatment as well as of the priming. Factors

such as these have important consequences in understanding seedling germination in a changing environment and may support sustainable agricultural practices.

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References

Bayer. (2011). Establishment pests. Expert Guide: Sugar Beet, 6–7.

BBRO. (2016). Opinion: The benefits of Neonicotinoid seed treatments in the sugar beet industry. British Sugar Beet Review, 84(2).

Bewley, J. D.; Bradford, K., Hilhorst, H., Nonogaki, H. (2013). Seeds.

Brown, A. D., Dexter, A. R., Chamen, W. C. T., & Spoor, G. (1996). Effect of soil macroporosity and aggregate size on seed-soil contact. Soil and Tillage Research, 38(3–4), 203–216. http://doi.org/10.1016/S0167-1987(96)01030-6

Chang, K. F., Hwang, S. F., Gossen, B. D., Turnbull, G. D., Howard, R. J., & Blade, S. F. (2004). Effects of soil temperature, seeding depth, and seeding date on rhizoctonia seedling blight and root rot of chickpea. Canadian Journal of Plant Science, 84(3), 901–907. http://doi.org/10.4141/P03-024

Chapuis, R., Delluc, C., Debeuf, R., Tardieu, F., & Welcker, C. (2012). Resiliences to water deficit in a phenotyping platform and in the field : How related are they in maize ? European Journal of Agronomy, 42, 59–67. http://doi.org/10.1016/j.eja.2011.12.006

Devarrewaere, W., Foqué, D., Heimbach, U., Cantre, D., Nicolai, B., Nuyttens, D., & Verboven, P. (2015). Quantitative 3D Shape Description of Dust Particles from Treated Seeds by Means of X-ray Micro-CT. Environmental Science & Technology, 49(12), 7310–7318. http://doi.org/10.1021/acs.est.5b02250

Ehsanfar, S., & Modarres-Sanavy, S. (2005). Crop protection by seed coating. Commun. Agric. Appl. Biol. Sci., 70(3), 225–229.

Gagliardi, B., & Marcos-Filho, J. (2011). Relationship between germination and bell pepper seed structure assessed by the X-ray test. Scientia Agricola, 68(4), 411–416. http://doi.org/10.1590/S0103-90162011000400004

Galhaut, L., de Lespinay, A., Walker, D. J., Bernal, M. P., Correal, E., & Lutts, S. (2014). Seed Priming of Trifolium repens L. Improved Germination and Early Seedling Growth on Heavy Metal-Contaminated Soil. Water, Air, & Soil Pollution, 225(4), 1905. http://doi.org/10.1007/s11270-014-1905-1

Gorim, L. (2014). Effects of seed coating on germination and early seedling growth in cereals. Dissertation, 1–132.

Gregory, P. J., Hutchison, D. J., Read, D. B., Jenneson, P. M., Gilboy, W. B., & Morton, E. J. (2003). Non-invasive imaging of roots with high resolution X-ray micro-tomography. Plant and Soil, 255(1), 351–359. http://doi.org/10.1023/A:1026179919689

Hill, H. J. (1999). Recent Developments In Seed Technology. Journal of New Seeds. http://doi.org/10.1300/J153v01n01_09

Huang, L., Zhao, C. I., Huang, F., Bai, Fang, H., Run-e, B. A. I., & Feng-ming, Y. A. N. (2015). Effects of imidacloprid and thiamethoxam as seed treatments on the early seedling characteristics and aphid-resistance of oilseed rape. Journal of Integrative Agriculture, 14(12), 2581–2589. http://doi.org/10.1016/S2095-3119(15)61140-6

Karnataka (2008). Effect of Seed Coating with Polymer Fungicide and Insecticide on Seed Quality in Cotton During Storage, 21(2), 137–139.

Kaufman, G. (n.d.). Tool for Stand Stimulus to Seed, 98–102.

Klepper, B., Kaspar T. C. (1994). Rhizotrons: Their development and use in agricultural research. Agron. J. 86, 745-753

KWS. (2017). Structure of pelleted KWS Sugar Beet Seed, accessed 12/06/2017, 11.00 a.m. https://www.kws-uk.com/aw/Seed-Production/Seed-Processing2121/Copy-of-Opbygning-af-et-f-rdigt-roefr-/~cfbn/

Lal, R. (2013). Ecohydrology & Hydrobiology Food security in a changing climate §. Integrative Medicine Research, 13(1), 8–21. http://doi.org/10.1016/j.ecohyd.2013.03.006

Lipiec, J., & Hatano, R. (2003). Quantification of compaction effects on soil physical properties and crop growth. Geoderma, 116(1–2), 107–136. http://doi.org/10.1016/S0016-7061(03)00097-1

Lundgren, J. (2009). Relationships of Natural Enemies and Non-prey Foods.

Mairhofer, S., Zappala, S., Tracy, S., Sturrock, C., Bennett, M. J., Mooney, S. J., & Pridmore, T. P. (2013). Recovering complete plant root system architectures from soil via X-ray μ -Computed Tomography, 1–7.

Masauskas, V., Masauskiene, A., Repsiene, R., Skuodiene, R., Braziene, Z., & Peltonen, J. (2008). Phosphorus seed coating as starter fertilization for spring malting barley. Acta Agriculturae Scandinavica, 582, 124–131.

Metzner, R., Eggert, A., van Dusschoten, D., Pflugfelder, D., Gerth, S., Schurr, U., … Jahnke, S. (2015). Direct comparison of MRI and X-ray CT technologies for 3D imaging of root systems in soil: potential and challenges for root trait quantification. Plant Methods, 11, 17. http://doi.org/10.1186/s13007-015-0060-z

Monneveux, P., Ramírez, D. A., & Pino, M. (2013). Plant Science Drought tolerance in potato (S . tuberosum L .) Can we learn from drought tolerance research in cereals ? Plant Science, 205–206, 76–86. http://doi.org/10.1016/j.plantsci.2013.01.011

Mooney, S. J., Pridmore, T. P., Helliwell, J., & Bennett, M. J. (2012). Developing X-ray Computed Tomography to non-invasively image 3-D root systems architecture in soil. Plant and Soil, 352(1–2), 1–22. http://doi.org/10.1007/s11104-011-1039-9

Redfearn, M., & Osborne, D. J. (1997). Effects of advancement on nucleic acids in sugarbeet (Beta vulgaris) seeds. Seed Science Research, 7, 261–267.

Rundlöf, M., Andersson, G. K. S., Bommarco, R., Fries, I., Hederström, V., Herbertsson, L., … Smith, H. G. (2015). Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature*, *521*, 77–80. http://doi.org/10.1038/nature14420

Scott, D. (1975). Effects of seed coating on establishment. New Zealand Journal of Agricultural Research, 18(1), 59–67. http://doi.org/10.1080/00288233.1975.10430388

Shull, A. C., & Mitchwell, J. . (1933). Stimulative Effects of X-rays on Plant Growth. Plant Physiology, 287–296.

406 Silcock, R., & Smith, F. (1982). Seed coating and localized application of phosphate for improving seedling growth
407 of grasses on acid, sandy red earth. Australian Journal of Agricultural Research, 33, 785–802.
408 Smýkal, P., Vernoud, V., Blair, M. W., Soukup, A., & Thompson, R. D. (2014). The role of the testa during
409 development and in establishment of dormancy of the legume seed. Frontiers in Plant Science, 5(July), 351.
410 http://doi.org/10.3389/fpls.2014.00351
411 Syngenta. (2016a). Force ST Seed treatment, last updated 23/03/2016. Accessed on 12/06/2017, 11.00 a.m.
412 https://www.syngenta.co.uk/product/crop-protection/seed-treatment/force-st
413 Syngenta. (2016b). Armure Seed treatment, last updated 01/06/2016. Accessed on 12/06/2017, 11.00 a.m.
414 https://www.syngenta.co.uk/product/crop-protection/fungicide/armure
415 Syngenta. (2016c). Cruiser SB Seed treatment, last updated 23/03/2016. Accessed on 12/06/2017, 11.00 a.m.
416 https://www.syngenta.co.uk/product/crop-protection/seed-treatment/cruiser-sb
417 Taylor, A. (1999). Seed enhancement. Journal of New Seeds1, 1(1), 103.
418 Tracy, S. R., Black, C. R., Roberts, J. A., & Mooney, S. J. (2013). Exploring the interacting effect of soil texture and
419 bulk density on root system development in tomato (Solanum lycopersicum L .). Environmental and
420 Experimental Botany, 91, 38–47. http://doi.org/10.1016/j.envexpbot.2013.03.003
421 Vogel, H.-J., Weller, U., & Schlüter, S. (2010). Quantification of soil structure based on Minkowski functions.
422 Computers & Geosciences, 36(10), 1236–1245. http://doi.org/10.1016/j.cageo.2010.03.007
423 Vyn, T. J., & Murua, M. (2001). Polymer Seed Coatings : Sufficient Risk Reduction for Early Plant Corn ? 56th
424 Annual Corn and Sorghum Research Conference., 1–11.
425 William, R. W., Lawnb, R. J., C, B., & Bythd, D. E. (1995). Studies on Water Damage in Mungbean . I . Effect of
426 Weathering on Seed Quality and Viability, 887–899.
427 Ypema, H. (2003). Fungicides, Hymexazol. In Encyclopedia of Agrochemicals. http://doi.org/
428 10.1002/047126363X.agr110
429

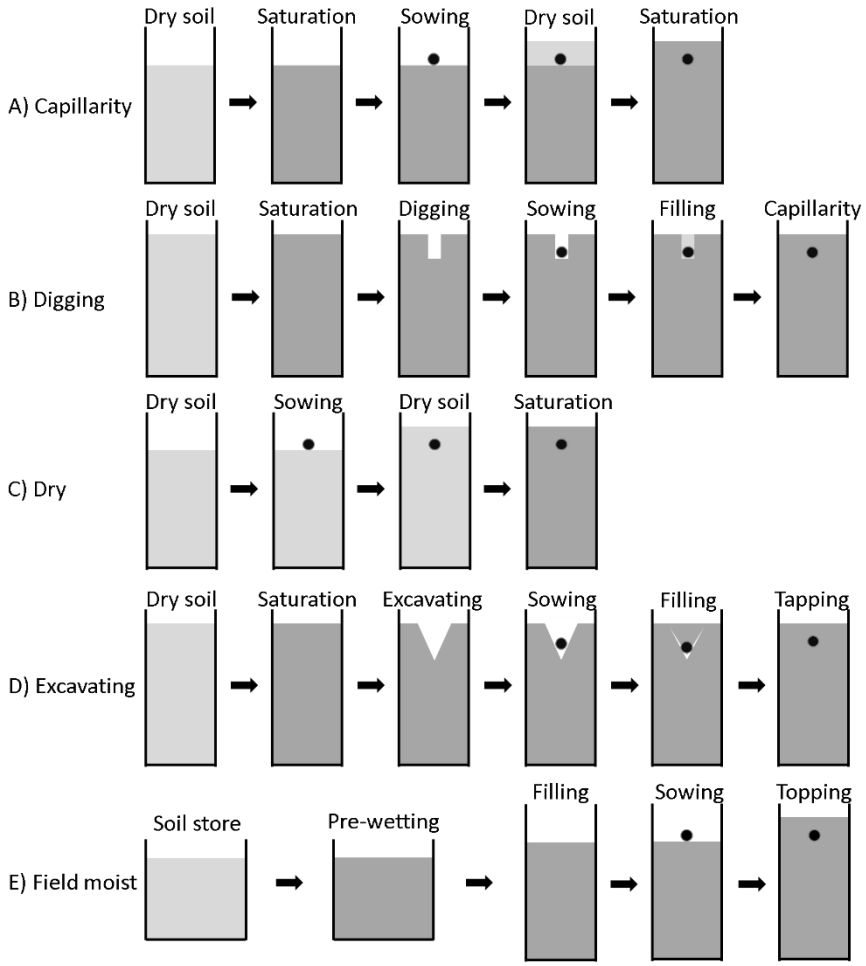
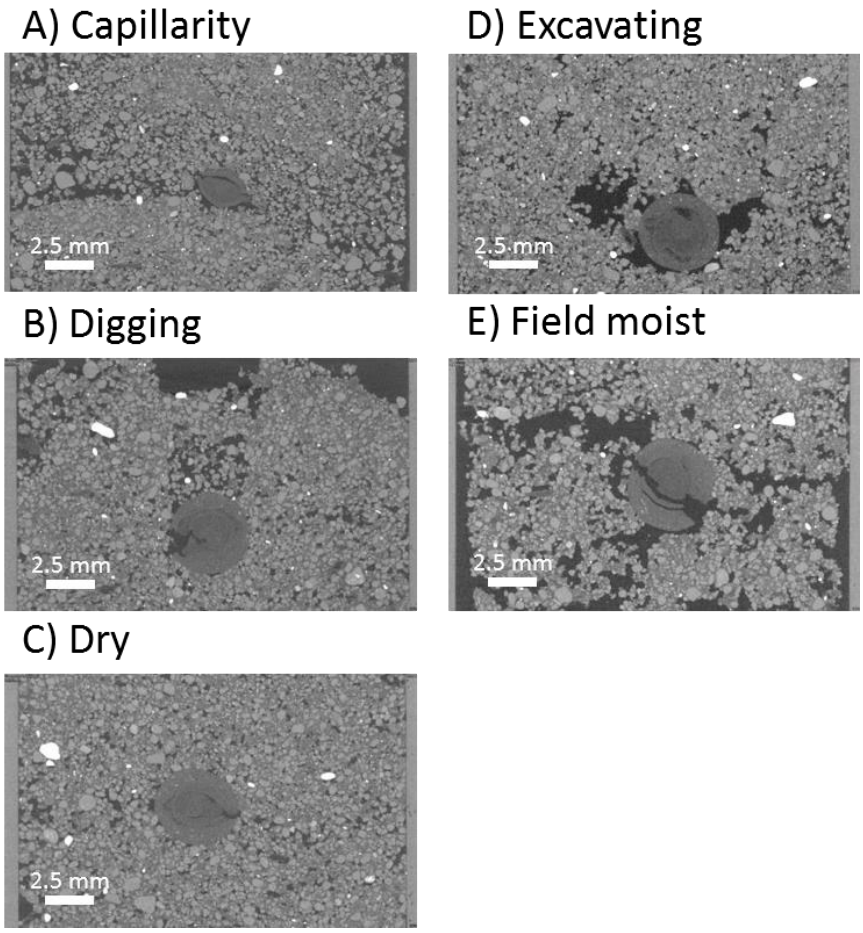


Figure 1: Packing methods used for the determination of a realistic field condition. Each method was set up using 3 replicates of a pelleted and coated seed. A) The column is filled with dry soil, saturated and the seed placed centrally on the layer. A second layer is poured onto the seed and the capillarity force saturates the second layer. B) The column is filled completely with dry soil and saturated. A hole in the size of the seed is dug and the seed placed into the hole. The hole is filled with dry soil and capillarity force saturates the filling. C) A first layer is packed dry, the seed sown on top the layer and a second layer applied dry as well. The whole column is saturated as the last step. D) The column is filled with dry soil and saturated. A larger portion is excavated and the seed placed into the hole. The hole is filled again with the previously excavated soil and compressed by tapping. E) The soil storage is pre-wetted and the column packed with this soil in two layers placing the seed in between the layers.



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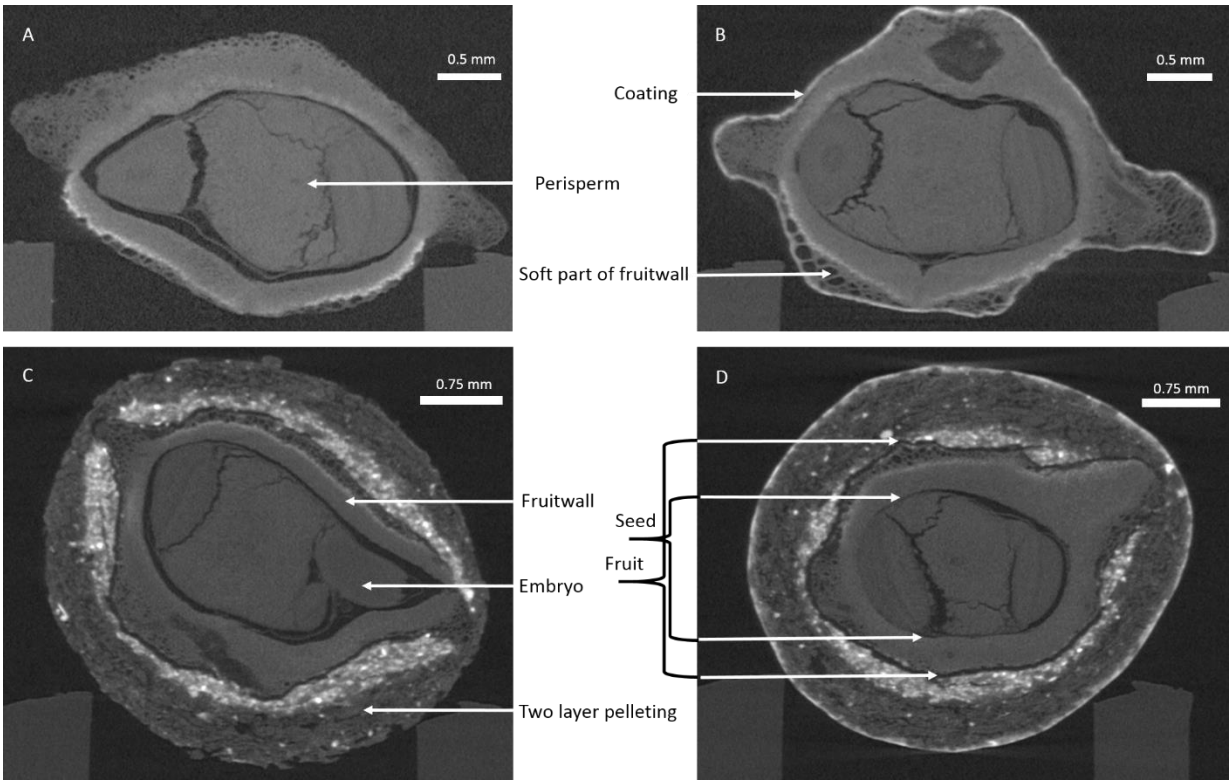
441 Figure 2: Results of the packing studies. The labelling of different methods refers to the previously explained methods of
442 Figure 1. The images have been taken from the top 2 cm of the column using the front orientation and the same scale. A)
443 The capillarity method resulted in the formation of a transition zone which forms a hydraulic disconnection between both
444 layers. B) The digging method results in a loose soil portion above the seed which is poorly connected to the surrounding soil.
445 C) The dry method results in uniform distribution of soil particles around the seed. D) The excavation method results in the
446 appearance of air pockets around the seed. E) The field moist method results in highly disconnected soil portions in the
447 column.

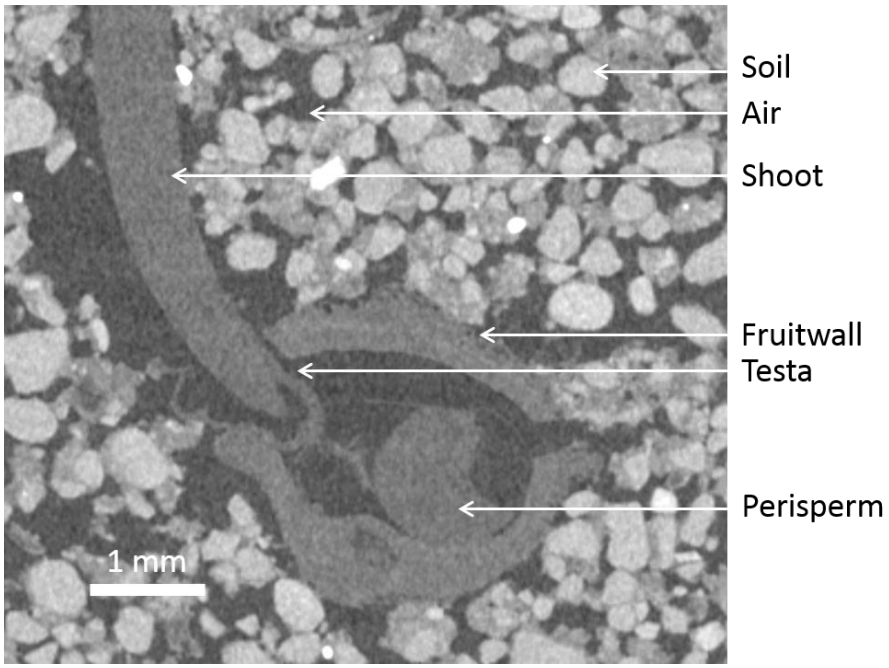


448

449 Figure 3: 2D images of non-germinated sugar beet seeds. A) Naked seed. B) Coated seed. C) Pelleted seed. D) Pelleted and

450 coated seed. This scan was taken with a resolution of 5 µm.

A



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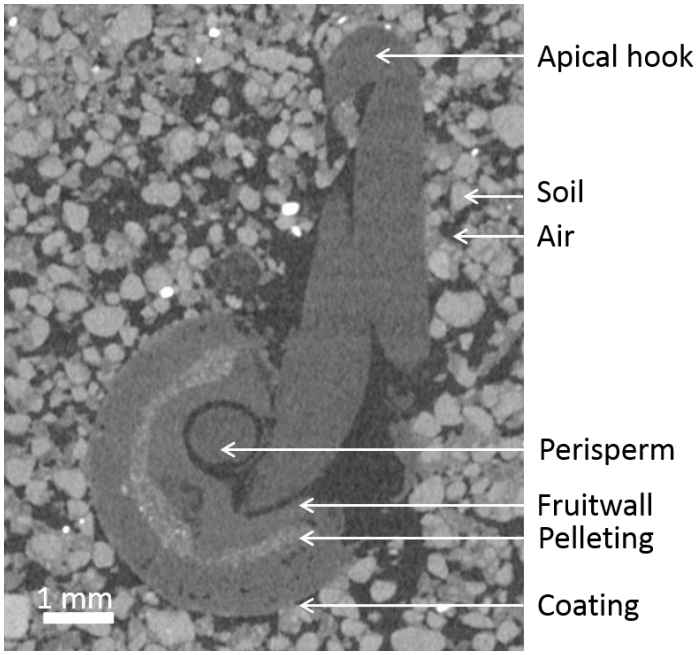
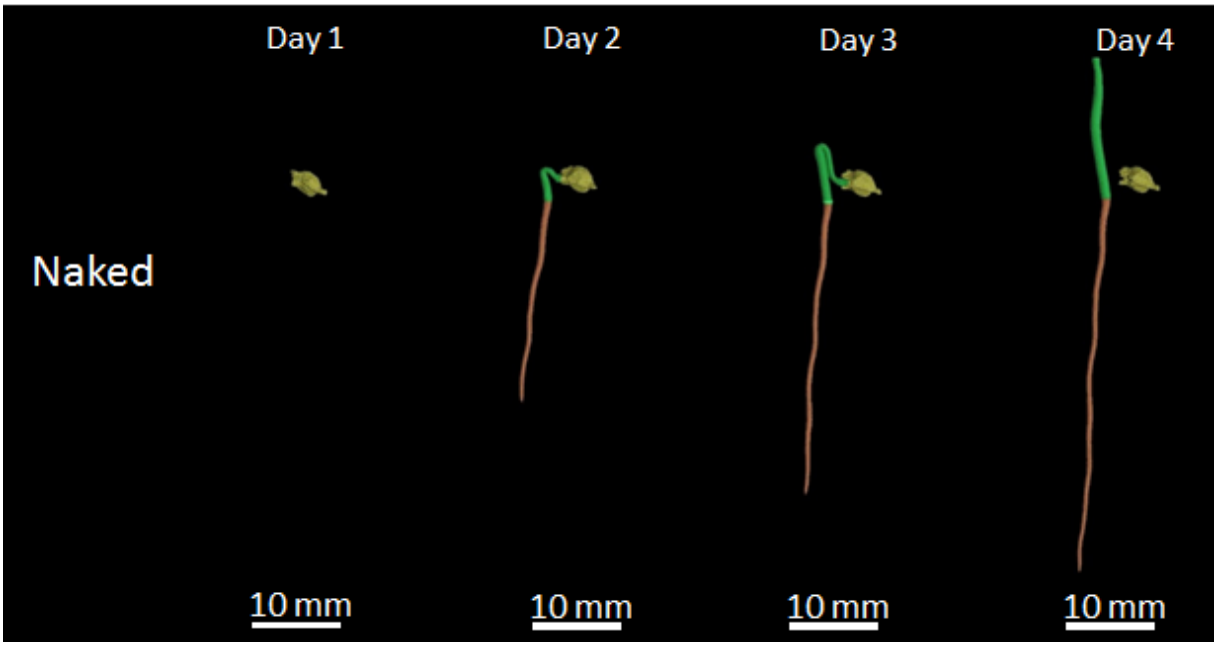


451 Figure 4: Exemplary 2D slices of day 3 scans. A) A 2D image of a naked seed on day 3. This scan was taken with a resolution

452 of 20 µm. B) A 2D image of a pelleted and coated seed on day 3. This scan was taken with a resolution of 20 µm.



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454 Figure 5: Exemplar temporal representation of 3D reconstructions of a naked sugar beet seed. The scans were taken at a

455 resolution of 20 µm.

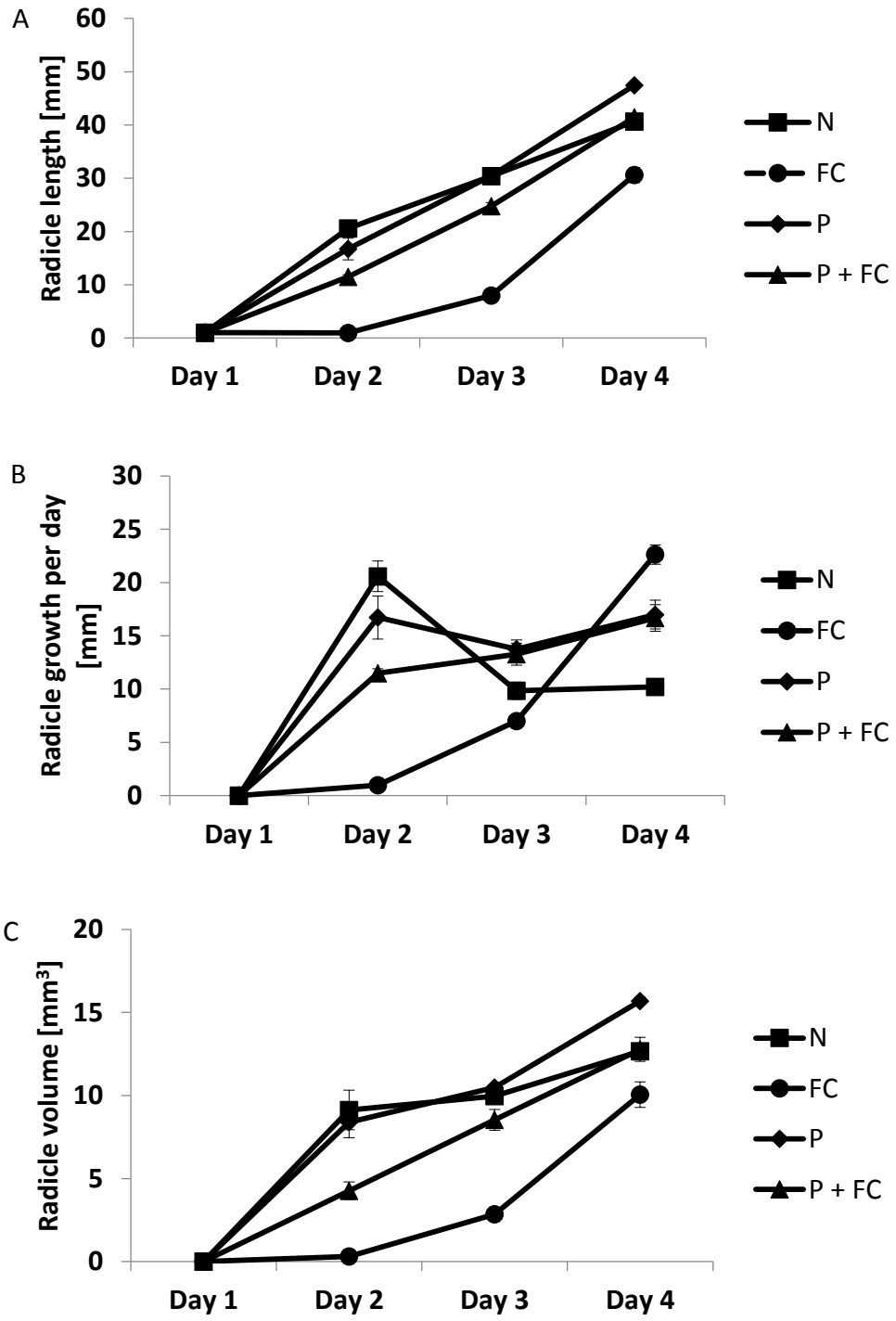
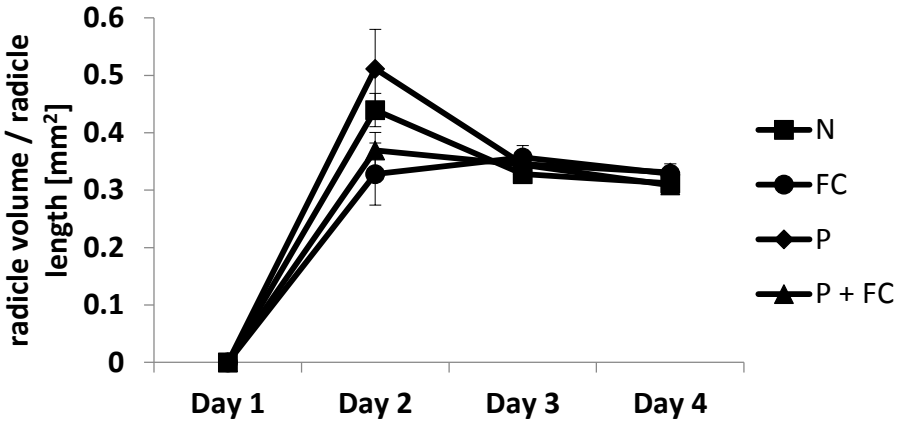


Figure 6: Analysis of radicle growth based on a growth period of four days. A) Radicle length comparison over 4 days. B) Radicle growth per day. Radicle growth per day is calculated as the subtraction of two consecutive days. C) Radicle volume change over time. Radicle volume is calculated automatically using VGStudio Max 2.2. N = 3, error bars are calculated using standard error of the mean.



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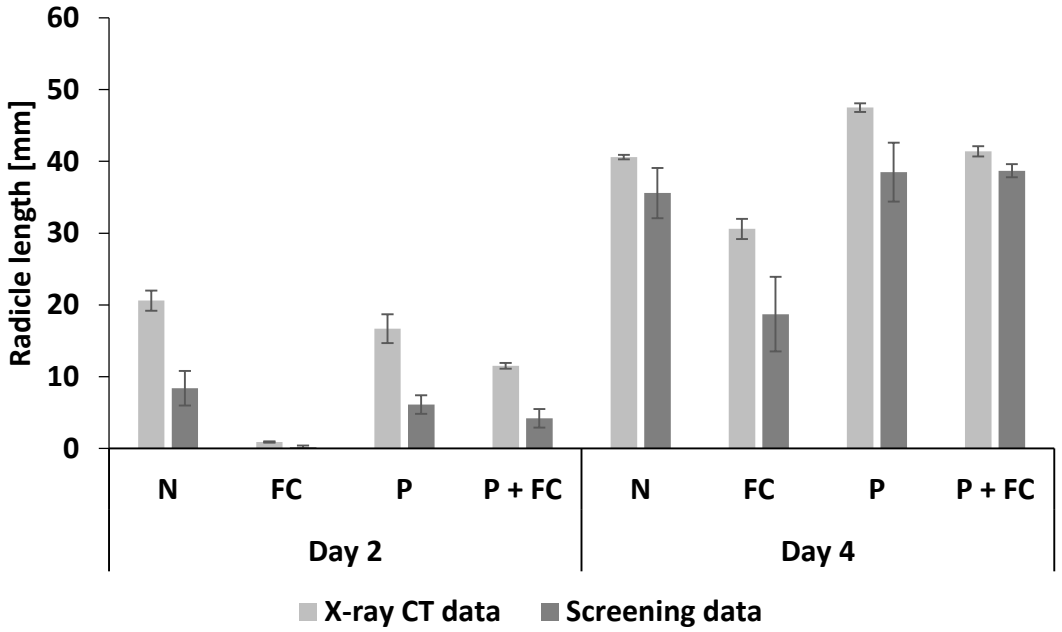
457 Figure 7: Ratio of radicle volume and radicle length. N= 3, error bars are calculated using standard error of the mean.



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459 Figure 8: Comparison of X-ray CT data and screening data after two and after four days of growth. The screening data was
460 gathered by excavating the seedling so that the seedlings for the day 2 and day 4 measurements for the screening data are
461 not based on the same plant like for the X-ray CT data. X-ray CT data N = 4; Screening data N = 10. Error bars are calculated
462 as standard error of the mean.