Seedling emergence through soil surface seals under laboratory conditions: effect of mechanical impedance and seal moisture

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Running title: Seedling emergence through soil seals

Abstract

It is well known that soil sealing strongly affects seedling emergence. The effect of soil sealing on the emergence of flax and turnip seedlings was studied in the laboratory. Seeds were sown in pots, watered, then covered with loamy soil and water was added. Three different doses of water were tested. Soil sealing was obtained with a paste of soil mixed with distilled water, added to each pot as a thick homogeneous, continuous and isotropic layer. The initial water content of the seal was measured. When seedling emergence was observed (or at the end of the experiment in the case of event failure), seal strength was measured in situ by a firmness pressure tester (used as a penetrometer). Relationships between water loss and initial moisture of the seal versus mechanical impedance were obtained. Differences in emergence success between species depended on the initial soil water content as well as on the initial seal moisture. A model of seedling emergence success of the two species, flax and turnip, as a function of the initial seal moisture content was obtained using a binary logistic regression model.

Key words: *Linum usitatissimum*, *Brassica rapa*, Loamy soil, Soil seal, Water content

Introduction

Soil sealing (also known as soil crusting) is a worldwide problem, occurring under a wide range of soil types and climatic conditions (Awadhwal & Thierstein 1985). Although physical and biological soil seals are an almost negligible portion of the soil, they have a number of crucial roles, especially where water is scarce (Maestre et al. 2011). Soil seals form the boundary between soil and atmosphere, and therefore control gas, water and nutrient exchange into and through soils (Belnap et al. 2005). Physical seals are formed by densely packed mineral particles resulting from either the disruption of soil aggregates and reorganisation of the disaggregated particles into "structural seals", or the formation of "depositional seals" (Valentin & Bresson 1992; Cerdan et al. 2001; Fox et al. 2004). In particular, soil sealing is a consequence of disruption of soil aggregates by water. Several mechanisms have been suggested as responsible for disruption of soil aggregates: the presence of electrolytes in the soil solution (associated with sodic soils); mechanical action (for example by raindrop impact, Agassi et al. 1981); or a combination of both mechanisms. In all cases, the wetting process (fast or slow wetting) contributes decisively to disruption of soil aggregates. In the Mediterranean region, the formation of physical seals on the soil surface is common (Singer & Le Bissonnais 1998), due to climate conditions, low soil organic matter content and poor structure and aggregate stability (Singer 1991).

Soil sealing strongly affects seedling emergence (Aubertot et al. 2002), and hence crop-stand establishment (Awadhwal & Thierstein 1985). Seedling emergence is affected by soil sealing through two mechanisms: physical impedance and changes in water evaporation rate which determines the moisture content in the seed bed (Rapp et al. 2000). The strength of a seal is affected by its moisture content and thickness, rate of drying, rainfall intensity and duration, soil texture, type of clay and bulk density (Awadhwal & Thierstein 1985). Those authors explained that a harder and less permeable soil seal develops under the following conditions: (1) high initial bulk density of the topsoil; (2) the soil does not contain organic matter and its clay content is high (3) the soil aggregates at the surface are smaller prior to wetting; (4) the upper layer water content is high and maintained for longer (slower drying). When the seal is sufficiently wet, the seedling deforms the material, and emergence takes place through penetration (Arndt 1965; Souty & Rode 1993). When the seal is dry, but not yet locally cracked by shrinkage, it may bend and break, if the exerted force is sufficient (Goyal et al. 1982; Souty & Rode 1993, 1994).

Seedling emergence depends not only on the soil seal strength, but also on the thrust that the seedlings can exert against the seal. It is generally assumed that the probability of seedling establishment depends greatly on seed size, e.g., the amount of reserves accumulated for early seedling development (Haig & Westoby 1988), and so seals could be especially critical for small-grain plants (Nuttall 1982; Gallardo-Carrera et al. 2006). Various methods of measuring the thrust strength of seedlings are used in laboratory trials, including the measurement of longitudinal deformation of a steel plate in direct contact with seedlings (Bouzaziz et al. 1990; Tamet et al. 1995), and measurement of the force that seedlings exert with sophisticate sensors (Gallardo-Carrera et al. 2007), indicating thrusts ranging from zero to around 0.5 N, depending on the species and the soil moisture.

This work intended to describe the emergence of seedlings of flax and turnip, two small seed crops, as affected by the impedance of laboratory-generated seals, and the initial soil and seal water contents. The mechanical characteristics of the soil seals depending on the degree of water loss were also studied and modelled, in order to predict the final emergence rate.

Material and Methods

A set of experiments were carried out using commercial seeds of flax (*Linum usitatissimum* L., *Linaceae*) and turnip (*Brassica rapa* L., *Brassicaceae*) whose ability to germinate under laboratory conditions had been previously established. We chose flax and turnip because they are crop species with relatively small seeds without sufficient reserves to overcome deep seeding or soil sealing (Forbes & Watson 1992). The weights of 1000 seeds were 5.86 g for flax and 2.10 g for turnip.

Only one type of soil was used throughout the experiments, a dried and sieved (< 2 mm) sandy clay loam (22% clay, 26% silt and 52% sand) obtained from the Ap horizon of a calcareous soil (Molina et al. 2016). In the field cracks are present in topsoil; however during the experiments crack formation was not observed in the experimental units, probably because of the small size in diameter. Each experimental unit was a plastic pot with a capacity of 145 mL (5.5 cm diameter and 6.1 cm height) that was filled according to the following procedure. First, a layer of sand 1.2 cm thick was placed at the bottom, and approximately half of the intended volume of water was added (I1). Then, the dried and sieved soil was added until the plastic pot was filled to 2 or 3 mm below the top, and one seed of flax or turnip which had been previously imbibed in distilled water (24 h) was placed on the surface, and the second half dose of water was added (I2). Finally, a thin layer (2 to 3 mm) of saturated soil paste was applied to completely fill the plastic pot.

This last layer became a seal when dry. Three total water amounts (I1+I2) were applied, 20, 40, and 50 g (equivalent to 8.6 mm, 17.1 mm, and 21.4 mm of water, respectively), to obtain three initial levels of soil moisture (IMSoil w/w). The saturated soil paste was prepared to obtain homogeneous and isotropic seals, with initial water contents (IMSeal - initial moisture of seal) of 0.213 to 0.337 (w/w), considering the water added to the dry soil to prepare the paste. The IMSoil moisture level of 8.6 mm is the minimum water volume needed to form a seal, while the moisture level of 21.4 mm was not only enough to form a water surface lamina but, at the same time, to ensure the wetting of all the soil contained in the pots by infiltration. These minimum and maximum water volumes would allow the generation of water infiltration to 1 cm and 5 cm in depth, respectively. These experimental conditions are very reproducible, although they may differ from some field conditions. 140 pots were thus prepared and subsequently monitored. 50 pots were used for each one of the IMSoil moisture levels 8.6 mm and 17.1 mm, including 5 different IMSeal moistures for flax and for turnip (with 5 replicates each one). However, the highest level of IMSoil moisture (21.4 mm) was represented with 40 pots, because only 4 different IMSeal moistures could be taken into consideration for each species.

Twice a day the seal was monitored to determine whether emergence had occurred. When a seedling emergence was observed, the following data were recorded: the final weight of each pot (g); the mechanical impedance of the seal (MPa); and the moisture of the seal (w/w), determined by drying one sample at 105 ºC to constant weight. The same measurements were made in pots where no seedlings emerged until the end of the experiment (this was when 24 h passed without recording further seedling germinations).

The mechanical impedance (MI) of the seals was measured using a firmness pressure tester (fruit sclerometer) with a cylindrical head of diameter 11 mm (95 mm^2 area). A continuous pressure was applied to the seal until it was broken. Water loss (WL mm) was determined by the difference between the weight of the pots at the beginning of the experiment and at seedling emergence, or at failure. The precision of weighing was 0.001 g. The thickness of the seals developed in one set of samples pots was measured with a slide gauge. The mean seal thickness formed (n = 50) was 3.5 mm (SE 0.4 mm, range 2.9–4.2 mm).

The data were first analysed statistically using a set of exploratory techniques to investigate linear, or nonlinear, relationships between measured parameters linked with soil moisture, seal moisture, water loss, mechanical impedance of the seal, seal water balance and water remaining in the soil sample, taking into account various groups of data characterized by the two species, the seedling emergence, and the initial moisture contents of soil and seals.

General linear models (GLM) and variance tests (ANOVA) were used to evaluate the influence of the main factors – species (flax / turnip), emergence of seedlings (yes / no), IMSoil (8.6, 17.1, 21.4 mm) and IMSeal -, on the measured mechanical impedance, as well as their interactions. The separation of means was evaluated using the Games-Howell method. Descriptive statistics were also calculated to characterize the mechanical impedance for the trials carried out according to the emergence or non-emergence of the seedlings.

Binary logistic regressions of the emergence of a seedling, a binary response variable, were performed with the initial seal moisture as a continuous predictor and for the various combinations of species and initial soil moistures studied. The models were fitted using an iterative reweighted least squares algorithm to obtain maximum likelihood estimates of the parameters, and the logit link function was used. To assess the performance of each fitted logistic regression model, various tests to determine the goodness-of-fit and some measures of association were used: (i) Deviance test and

Pearson test to determine whether the predicted probabilities deviated from the observed probabilities, (ii) Hosmer-Lemeshow test to compare the observed and expected frequencies of emergence, to assess how well the model fits the data, and (iii) Somers' D, c-statistic, Goodman and Kruskal's gamma, Kendall's tau which use the measurements of concordant pairs, discordant pairs and the total number of pairs to compare the predicted responses with actual responses.

Data were analysed using Minitab® Statistical Software (Minitab Inc. 2012). The probability level of significance was set at 0.05.

Results and discussion

In general, first seedling emergences were observed 3 days since the pots were prepared. The monitoring period lasted at least a week (7-10 days). After that, we considered the trial finished. The overall success rates of seedling emergence were 50% for flax and 51.43% for turnip (with a standard error (SE) of 5.97 % for both species), and the highest and lowest emergence rates were obtained with flax at IMSoil of 21.4 mm and 8.6 mm respectively (Table 1).

Fig. 1 shows the positive linear relationship between water loss and mechanical impedance. The p-value (< 0.001) for the linear regression model shows that the model estimated is significant, and that the mechanical impedance (MPa) can be predicted by water loss (mm), $MI = -0.3037 + 0.0614 \cdot WL$ ($r = 0.873$), displaying that the flow of humidity through the pot, which then evaporates or evapotranspiration results in the hardening of the seal. During the drying of the soil, surface tension compresses the particles together, forming a thick and strong layer (Sumner, 1992).

A GLM of the variable MI with the three main factors (species, emergence and IMSoil) and the covariable IMSeal, and all possible interactions, was established. Species was not a significant factor (p-value = 0.247) while the interaction of the other two factors (emergence and IMSoil) with the covariate (IMSeal) showed a significant effect on mechanical impedance (p-value < 0.001). The relationship between mechanical impedance of the seal and its initial moisture (IMSeal) is presented in Fig. 2, distinguishing between the emergence success or failure, and the three levels of IMSoil. Nevertheless, only in the case of IMSoil = 21.4 mm and non-emerged seedlings, did the fitted linear model between IMSeal and MI have a slope significantly different from zero (p-value < 0.001); the linear regression models of mechanical impedance versus IMSeal were not significant (the predictor IMSeal did not explain MI) for the other groups of data. Therefore, no relationship was detected between these two variables in the pots where seedling emergence was observed. Fig. 2 shows that emergence took place when the mechanical impedance was approximately below 0.4 MPa, whatever the initial seal moisture content. These values of seal mechanical impedance are in the lower band of field observations (Gallardo-Carrera et al. 2007). The seal impedance of pots where emergence did not occur that had been watered with 8.6 mm or 17.1 mm followed the same pattern, that is, the final mechanical impedance of the seal was not related with its initial moisture, and it varied approximately across the same range as in pots with seedling emergence (very few cases above 0.4 MPa). No significant differences were detected between the mean mechanical impedances corresponding to the three levels of IMSoil in pots with seedling emergence, and that corresponding to the lowest level of IMSoil in pots without seedling emergence (Fig. 2), with mean values around 0.15 MPa. Thus, failure to emerge in these cases does not seem attributable only to the IMSeal, although it was related with the seal strength. Moreover, when the IMSoil was highest (21.4 mm) the mechanical impedance of the seals in pots where emergence did not occur was clearly inversely related with their initial soil moisture, giving the widest range of seal mechanical impedances of the entire experiment (Fig. 2). It is notable that at similar values of seal mechanical impedance, some seedlings of both species emerged but others did not. These intra-species variations may be affected by the depth of the seed inside the pot, its size, and its speed of germination. Survival chances of seedlings depend, to a great extent, on the speed of germination after effective-rains, since fast germination ensures emergence before seal formation by the drying soil as well as the early establishment of a root system to tap water from deeper soil layers (Kigel 1995); moreover, larger seeds contain more reserves than the smaller seeds (Haig & Westoby 1988). It is thus possible that the large, quickly germinating seeds were able to break the seal, while the smaller, slower seeds were not.

The seedlings of both species develop with the cotyledons above ground (epigeous germination). The seedlings of flax have hypocotyls 1.5–4 mm long, epicotyls up to 1.5 mm long, elliptical-oblong cotyledons, 6.5–14 mm long and leafy. Those of turnip develop a taproot and lateral roots, have hypocotyls 5 cm long, epicotyls 2–4 mm long, cotyledons with petioles 2 cm long, blade cordate, 1–1.5 cm long, cuneate at base, and notched at apex (Corner 1976). Both species produce lipid-containing seeds (Al-Ani et al. 1985; Crawford 1992), and were considered to have low strength in terms of seal penetration (Forbes & Watson 1992; Gallardo-Carrera et al. 2007). In addition to their differences in morphology and seed size, flax seeds are compressed, 6–10 mm × 2–3 mm, while turnip seeds are globose, 1–1.5 mm in diameter (Corner 1976). Flax seeds are myxospermous, that is their coat produces mucilage when wet, while turnip seeds do not (Bengoechea & Gomez-Campo 1975; Crawford 1992). As mentioned above, the overall relative frequencies of emergence of the two species were very similar, but there were important differences in their emergence success depending on the initial water content as well as the initial seal moisture. Flax emerged more successfully when the initial soil moisture was greater, while turnip emerged more successfully when initial soil moisture was lowest (Table 1).

Binary logistic regression was used to determine the probability of emergence of a seedling in various conditions, and clarify the observed responses. These one-predictor models were fitted to the data to test the relationship between the likelihood that a seed emerges (π) and the initial moisture of the seal (IMSeal), according to $\pi = \exp(\alpha+\beta\cdot\mathrm{IMSeal}) / (1+ \exp(\alpha+\beta\cdot\mathrm{IMSeal}))$, where α and β are the regression coefficients. The results are shown in Table 1, and the regression coefficients were only significantly different from 0 for turnip seeds at the two higher IMSoil, although the directions of these relationships were opposite. For the remaining cases, the regressions showed that IMSeal was not a significant predictor of seedling emergence across the range of values studied; it is not possible to be certain that the IMSeal had an effect on the response. In the case of flax with intermediate IMSoil, IMSeal could be considered a significant predictor if the level of significance was set at 0.10 (but not at 0.05). Fig. 3 depicts these differences between the various cases, with the graphical representations of the estimated probability of seedling emergence depending on the initial seal moisture, considering the two species and the three levels of initial soil moisture separately. The results of the assessment of the model goodness-of-fit for all these regressions were also presented in Table 1; all the cases (except one of the statistical tests) were not significant, suggesting that the models were well fitted to the data. The best values for the measurements of association expressing the degree to which predicted probabilities agree with actual outcomes were obtained for turnip at the two higher levels of IMSoil, and the worst values also occurred for turnip, for the lowest level of IMSoil. For flax, the values obtained for these concordance statistics can be considered correct and acceptable.

The water content of the seal, in addition to that of the soil, was decisive in determining flax emergence at the intermediate level of IMSoil, where the probability of emergence increased when IMSeal increased; however, it seemed to play no role at the other two levels of IMSoil. Despite this, the percentage of flax emergence increased with IMSoil (Table 1). The results do not inform about the role of the mucilage layer, which differs between species (Crawford 1992; Kigel 1995), but it seems that, concerning flax, the seed imbibition and/or the seedling elongation were favoured when the water content increased.

On the other hand, turnip was more successful at emerging at IMSoil of 8.4 mm (Table 1), independently of IMSeal; at the intermediate IMSoil the probability of emergence increased when IMSeal increased, while at 21.4 mm of IMSoil the reverse was observed (Fig. 3). After imbibition there is a gradual acceleration of metabolism, and the resulting demand for oxygen exceeds the rate of replacement; seed endurance during this period of natural anaerobiosis is strictly limited, and when it is prolonged by excessive burial, flooding or compaction, emergence is greatly reduced (Crawford 1992). Al-Ani et al. (1985) and Crawford (1992) demonstrated that flax and turnip, both lipid-containing seeds, suffer at least a partial oxygen deficit during the initial stage of germination, but the percentage of post-anoxic survival was up to 15% greater in flax than in turnip, after 24h of anoxia (80% *versus* 65%). Thus, in this case, the water content of the seal seems to play a crucial role in successful seedling emergence by increasing its mechanical impedance over time, and by acting as an oxygen barrier. It could be very important in small seeds with a lipid-rich endosperm.

In those situations (combination of soil and seed) where the presence of a resistant seal limits the development of seedlings, the flow of water through the seal contributes to its hardening. The appropriate reduction of this flow (mulch, shading, simultaneity of two crops, etc.) can contribute to reduce this limitation.

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FIGURES AND TABLES

Table 1. Logistic regression analysis of the emergence of 140 seeds of the two tested species (Flax and Turnip) placed in soils with three different levels of initial moisture (IMSoil). The logistic regression model has the following form: logit(Emergence) = $\ln(\pi/(1-\pi)) = \alpha + \beta\cdot$IMSeal, where the variable Emergence is the dichotomous outcome variable (Yes / No), π is the probability of the outcome (emergence of the seedling), and IMSeal (initial moisture of the seal) is the continuous predictor.

Figure captions

Figure 1. Relationship between water loss and mechanical impedance for a set of pots used in the experiments.

Figure 2. Relationship between initial moisture of the seal and mechanical impedance, taking into account the emergence of the seedlings (no / yes) for the various initial soil moisture contents.

Figure 3. Fitted curves obtained using a binary logistic regression model for Flax and Turnip at 3 levels of initial soil moisture content. Continuous lines represent the fit obtained for the probability of the seedling emergence as a function of the initial seal

moisture content. Dashed lines show the 95% confidence interval bands for the prediction.

Table 1

	Flax			**Turnip**		
	IMSoil (mm)			IMSoil (mm)		
	8.6	17.1	21.4	8.6	17.1	21.4
Emerged seedlings	6	14	15	17	11	8
Number of seeds	25	25	20	25	25	20
Regression coefficients						
α	-9.07	-6.76	1.71	0.84	-13.25	21.16
(*p-value*)	(*0.177*)	(*0.100*)	(*0.837*)	(*0.867*)	(*0.008*)	(*0.032*)
β	25.1	28.5	-2.3	-0.3	52.7	-83.4
(*p-value*)	(*0.230*)	(*0.090*)	(*0.942*)	(*0.987*)	(*0.010*)	(*0.029*)
Chi-Square of Goodness-of-fit tests						
Deviance	25.87	30.92	22.49	31.34	24.57	20.22
(*p-value*)	(*0.307*)	(*0.125*)	(*0.211*)	(*0.115*)	(*0.373*)	(*0.321*)
Pearson	23.31	23.95	20.01	25.00	25.94	20.73
(*p-value*)	(*0.443*)	(*0.407*)	(*0.332*)	(*0.350*)	(*0.304*)	(*0.293*)
Hosmer-Lemeshow	2.03	3.40	7.14	2.94	2.21	2.45
(*p-value*)	(*0.566*)	(*0.334*)	(*0.028*)	(*0.400*)	(*0.530*)	(*0.294*)
Measures of association						
Somers'D	0.26	0.39	0.20	0.04	0.71	0.63
Goodman-Kruskal Gamma	0.32	0.46	0.24	0.06	0.81	0.73
Kendall's Tau-a	0.10	0.20	0.08	0.02	0.37	0.32
Concordance statistic	63%	69.5%	60%	52%	85.5%	81.5%

Figure 1.

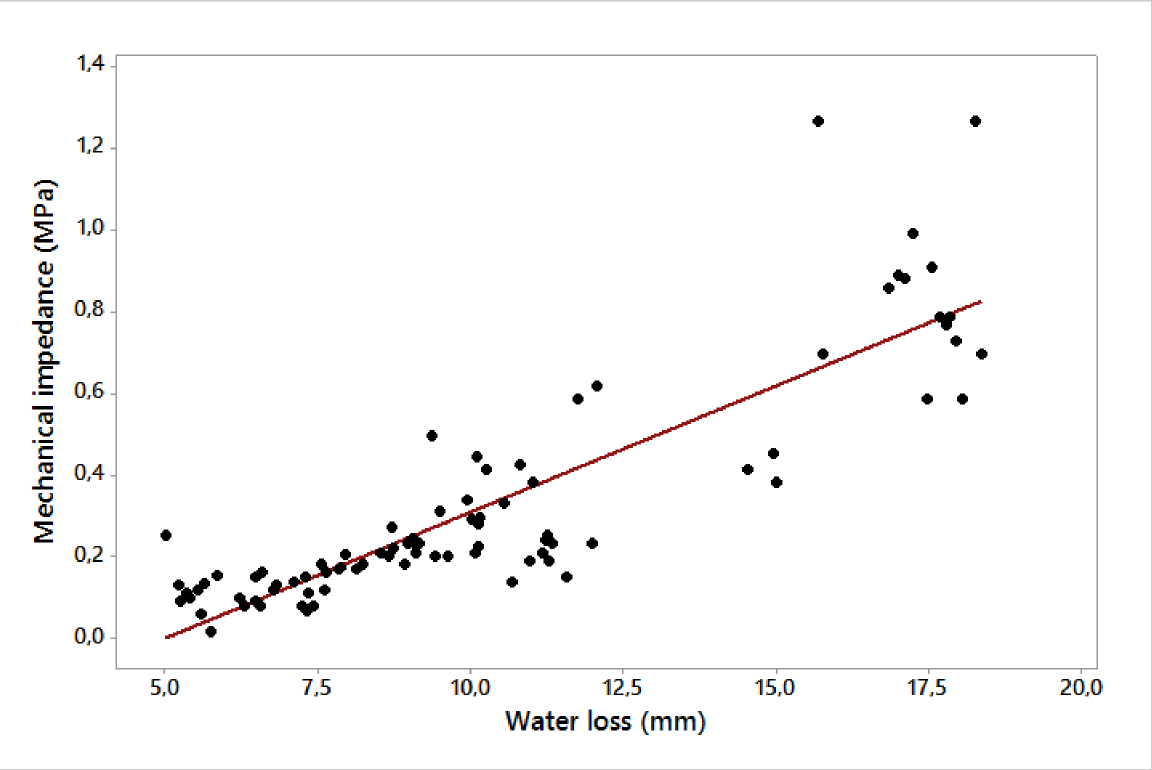


Figure 2.

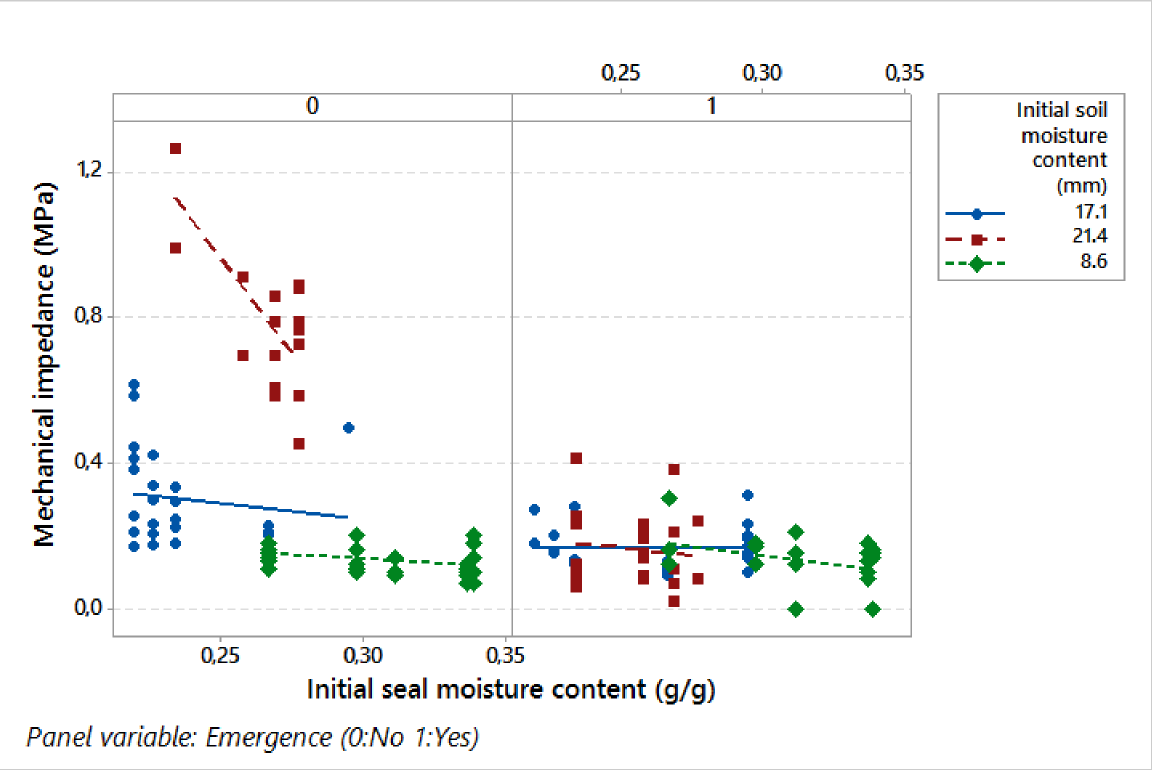
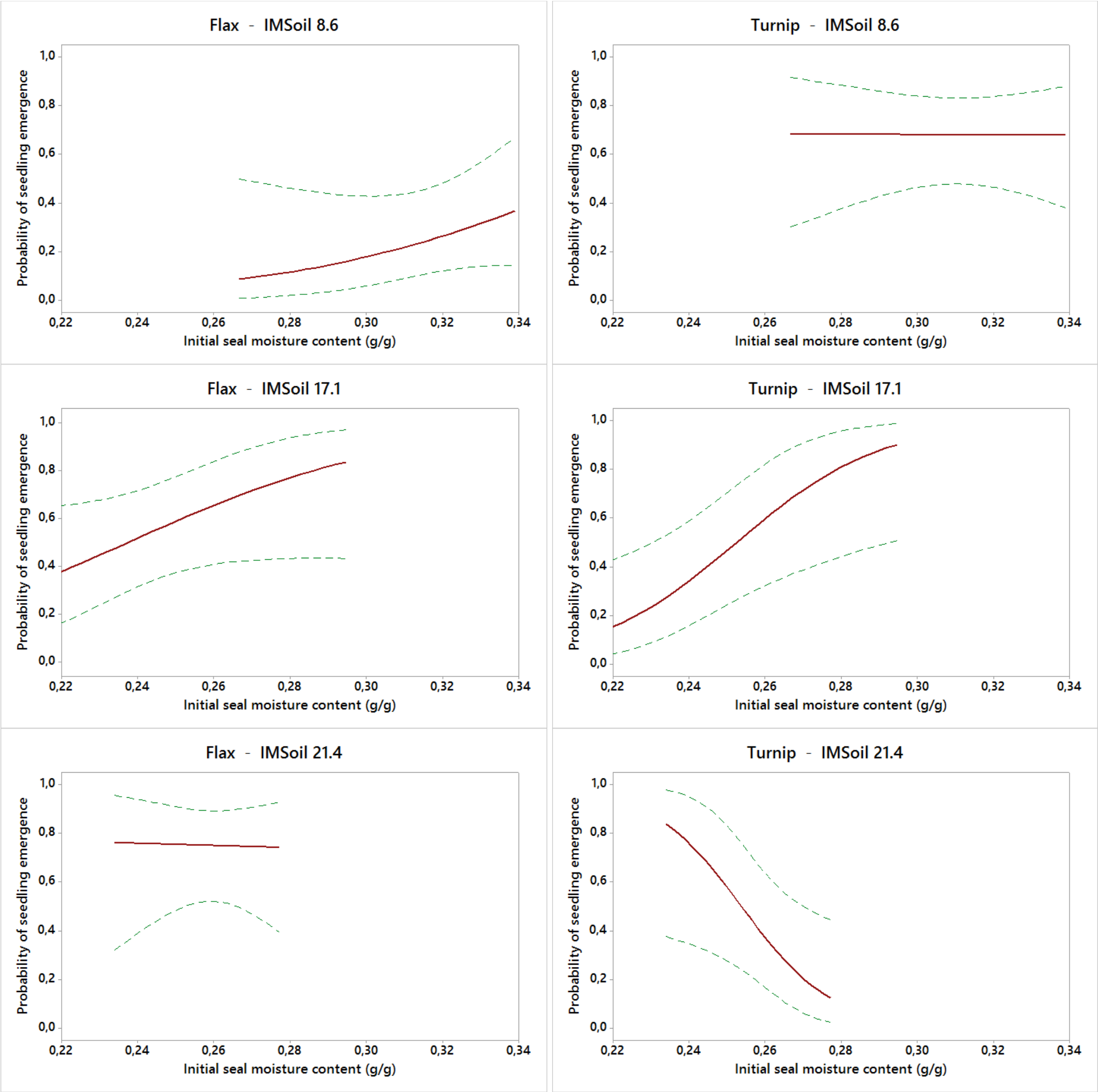


Figure 3.


Flax - IMSoil 8.6
Turnip - IMSoil 8.6
Flax - IMSoil 17.1
Turnip - IMSoil 17.1
Flax - IMSoil 21.4
Turnip - IMSoil 21.4
Probability of seedling emergence
1,0
0,8
0,6
0,4
0,2
0,0
0,22
0,24
0,26
0,28
0,30
0,32
0,34
Initial seal moisture content (g/g)