ABSTRACT
Planting depth effect on seedling emergence is an important concern for many crops grown around the world. Farmers in the low-precipitation (<300 mm annual) winter wheat (Triticum aestivum L.) (WW) production region of the Inland Pacific Northwest of the United States (PNW) plant seed as deep as 20 cm below the surface of summer-fallowed soils with deep-furrow drills to reach adequate seed-zone moisture. Seedlings need to emerge through 12 to 15 cm of soil cover, most often under marginal seed-zone water potentials. Successful stand establishment is the most critical factor affecting WW grain yield potential in the region. We developed a laboratory method to accurately assess WW emergence from deep planting depths in pots. To test the methodology, we first conducted a 4-yr field experiment to measure emergence of four WW cultivars having either standard-height or semi-dwarf growth habit. Depth of soil cover over the seed was 14 cm and seed-zone water potential over the 4 yr ranged from very dry (–0.69 MPa) to wet (–0.40 MPa). Next, a factorial laboratory pot experiment was conducted using the same WW cultivars and soil seed-zone water potentials similar to those during the 4 yr in the field. Statistical comparison between field and laboratory emergence data showed a strong correlation ($r = 0.71$, $p < 0.01$) for median time to emerge. We describe the step-by-step procedure for conducting a laboratory pot experiment to measure WW emergence from deep planting depths under a wide range of water potentials.

Core Ideas
- Stand establishment is the biggest factor affecting winter wheat yield in the low-precipitation region.
- Winter wheat seed is planted as deep as 20 cm below the soil surface to reach adequate soil moisture.
- Determination of a cultivar’s emergence ability in the field is limited to a short time window once a year.
- We developed a laboratory method to accurately measure emergence in pots from deep planting depths.
- Laboratory and field results were strongly correlated under a wide range of soil water potentials.

Laboratory Method to Evaluate Wheat Seedling Emergence from Deep Planting Depths
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D E E P P L A N T I N G of wheat after long fallow periods is common in many rainfed Mediterranean cropping regions of the world including the PNW (Douglas et al., 1992), several countries surrounding the Mediterranean Sea (Mahdi et al., 1998), southeastern and southwestern Australia (Rebetzke et al., 2007), and central Chile (Brunel et al., 2013). Farmers in the low-precipitation region of the PNW use deep-furrow drills to place seed 10 to 20 cm beneath the soil surface with as much as 15 cm of soil covering the seed.

A 2-yr winter wheat–summer fallow (WW–SF) rotation is practiced on >90% of the 1.56 million rainfed cropland hectares in the low-precipitation region of central Washington and north-central Oregon. Winter wheat is harvested in July through early August and the field then left fallow for 13 to 14 mo until WW is again planted in late August or early September into water stored in the soil during the fallow period. The climate is Mediterranean-like with cool, wet winters and hot, dry summers. Considerable surface soil drying occurs during July through September (Jury and Miller, 1974); thus the need for deep placement of WW seed to reach adequate seed-zone water (Donaldson, 1996).

If satisfactory WW stands cannot be achieved from deep planting in late August–early September, farmers will plant WW at a shallow depth of 2 to 3 cm into dry soil around mid-October and wait for the onset of fall rains. Such late planting reduces WW grain yields by 35 to 40% compared to early planted WW in eastern central Oregon (Higginbotham et al., 2011; Higginbotham et al., 2013), but this delay is not nearly as detrimental to grain yield potential in north-central Oregon (Bolton, 1983; Machado et al., 2015) where temperatures are warmer in the fall, winter, and spring.

Winter wheat cultivars grown in the PNW can germinate at water potentials as low as –1.25 MPa (Wuest and Lutcher, 2012; Singh et al., 2013), but a minimum water potential of –0.55 to –0.65 MPa is generally required for WW seedling emergence through 12 to 15 cm of soil cover (Lindstrom et al., 1976; Schillinger et al., 1998). Due to thick soil cover over the seed, it is not the coleoptile that emerges from the soil but rather the first leaf after pushing through the tip of the coleoptile. The first leaf is thin, has weak structural support and, since most often emerging under low soil moisture conditions, lacks much emergence force or lifting capacity (Arndt, 1965).

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Abbreviations: DAP, days after planting; PNW, Inland Pacific Northwest of the United States; SF, summer fallow; WSU, Washington State University; WW, winter wheat.
There is considerable variability in the capacity of PNW WW cultivars to emerge from depth. Winter wheat emergence is strongly correlated with coleoptile length (Schilling et al., 1998; Rebetzke et al., 2005, 2007, 2016). The coleoptile provides a protective sheath to the subcrown internode as it elongates to set the crown near to the soil surface. In addition to coleoptile length, work by Mohan et al. (2013) suggests that other, yet undefined, genetic traits are also important for WW seedling emergence.

Field experiments to evaluate emergence of WW cultivars and advanced numbered lines from deep planting have been conducted in east-central Washington since the early 1970s (Lindstrom et al., 1976; Schilling et al., 1998; Giri and Schilling, 2003; Mohan et al., 2013; several others). However, these field experiments can only be conducted during the very short “early planting” time window and, in some years, rain showers of 3 mm or greater cause surface soil crusting (Awadhwal and Thierstein, 1985; Feng et al., 2013) through which few if any elongating WW seedlings can penetrate.

There are a multiple reports in the literature on the correlation between wheat seedling emergence in the field with factors such as speed of wheat germination, soil water potential, planting depth, and seed priming (Lindstrom et al., 1976; Jame and Cutforth, 2004; Wang et al., 2009; Al-Mulla et al., 2014). We found nothing in the literature, however, describing laboratory procedures that mimic abiotic stresses found under field conditions.

The objective of this study was to develop a reproducible laboratory method for deep planting WW in pots under a wide range of water potentials that emulates emergence under field environments. This paper describes: (i) methods that were developed; (ii) steps for conducting a laboratory pot emergence study; and; (iii) the statistical correlation of seedling emergence in field vs. laboratory experiments.

**MATERIALS AND METHODS**

**Overview**

Field experiments were conducted for 4 yr at the Washington State University (WSU) Dryland Research Station near Lind, WA, to measure seedling emergence of four commonly grown WW cultivars from deep planting. Seed of these four cultivars was used in a laboratory study to validate a laboratory pot method that had been developed.

The four WW cultivars were Buchanan (Donaldson, 1993), Moro (Rohde, 1966), Eltan (Peterson et al., 1991), and Xerpha (Jones et al., 2010). Buchanan is a standard height (i.e., no dwarfing genes) hard-red cultivar widely grown in the driest portion of the region that receives <225 mm annual precipitation. Moro is a standard height soft-white cultivar released 51-yr ago, but is still planted by some farmers today in dry years due to excellent emergence ability, despite its modest grain yield potential, weak straw that causes lodging, and poor disease resistance. Eltan and Xerpha are semi-dwarf soft-white cultivars that contain the Rht1 dwarfing gene (Mohan et al., 2013) to reduce plant height. Eltan and Xerpha have higher grain yield potential than Buchanan and Moro, but appear to have reduced emergence capability from deep planting compared to standard-height lines Moro and Buchanan. Singh et al. (2013) quantified seed germination of these four WW cultivars in a laboratory study under seven water potentials and reported that Moro consistently exceeded the other cultivars for speed and extent of germination at water potentials as low as –1.00 to –1.25 MPa.

**Soils**

The soil used in all field and laboratory studies was a Shano silt loam (coarse-silty, mixed, superactive, mesic Xeric Haplopts) with a textural distribution of 390, 510, and 100 g/kg fine sand, silt, and clay, respectively. The Shano silt loam soil and its close relative, the Ritzville silt loam soil (coarse-silty, mixed, superactive, mesic Calcic Haploxerolls), are widely distributed throughout the WW–SF region of east-central Washington (Feng et al., 2013). These soils are permeable, well-drained and have uniform texture to the underlying basalt bedrock.

**Field Experiments**

Field experiments were planted in late August from 2007 to 2010 on land that had been fallowed for 13 mo (i.e., since the last harvest of WW). Slope was <2%. Average annual precipitation at the site is 244 mm.

Soil volumetric water content in the seed zone of summer fallow was determined gravimetrically at time of planting. These measurements were obtained in 2-cm increments, avoiding wheel tracks, to a depth of 26 cm using an incremental soil sampler. Mean water content for each increment was determined from four soil cores using gravimetric procedures described by Topp and Ferre (2002).

Seed for the 4-yr field experiment was newly harvested (i.e., harvested the same year) from a rainfed field nursery at the WSU Dryland Research Station. Seed was stored in paper bags under ambient conditions until use (about 40 d from harvest to planting). Germination tests conducted with wet blotter paper in petri dishes every year showed germination rate of all four cultivars was consistently ≥96%.

For each cultivar, 100 uniform-sized seeds with no physical damage were planted per replicate using a four-opener deep-furrow drill in 3-m-long rows with 38-cm spacing between rows. The experimental design was randomized complete block with four replicates per cultivar. To account for any differences among drill openers, each cultivar was planted once with each of the four openers. Seeds were planted at an average depth of 16 cm below the pre-plant soil surface into moist soil and an average of 14 cm of soil covered the seed. Soil water potential at depth of seed placement ranged from –0.69 to –0.40 MPa (10.3–12.7% volumetric water content). Wheat emergence was determined by counting individual seedlings at 24-h intervals beginning 7 d after planting (DAP). Seedling emergence counts were obtained on 7, 8, 9, 10, 11, 12, 13, 14, and 21 DAP.

**Laboratory Procedure**

Seed used in laboratory experiment was obtained from the harvest of the 2010 field experiment. Every seed used in the laboratory study was inspected to ensure no physical or other damage.

Laboratory studies began in 1999 to determine the best method to simulate seedling emergence under field conditions. Results from some of these earlier laboratory pot experiments were reported by Giri and Schilling (2003) and Schilling (2011). Over the years, improvements in the methodology were continuously made. Tapered plastic greenhouse/nursery pots 17.8 cm tall, 15.2 cm diam. at the top, and 12.7 cm diam. at the base were used.
for the laboratory experiment (Fig. 1). The procedure involves a layering approach to pot preparation. Figure 1 shows a cross-section diagram of a pot divided into four layers. The following steps describe the procedure:

**Collection and Preparation of Soil from the Field**

In a summer-fallow field, collect the quantity of soil needed from the surface 15 cm with a tractor-mounted scoop shovel. Screen soil through a 6-mm mesh to remove clods and straw, and store in the laboratory at a constant 21°C temperature for 30 d before the onset of experiments. Mass soil water content after air drying ranged from 1.5 to 2.0% (–2.00 MPa).

**Calculation of the Quantity of Water Needed to Reach Target Water Potentials**

Determine the baseline water content of the collected “dry” field soil. This is obtained by weighing several soil samples and drying them in an oven at 105°C for 24 h to achieve zero water content, then reweighing to calculate the percent mass water content prior to drying. Figure 2 shows the relation of mass water content vs. volumetric water content (at bulk density of 1.35) to water potential for Shano silt loam soil at Lind, WA. Based on the baseline mass soil water content, calculate the quantity of water to be added to reach target water potentials for the reserve layer and the two bottom layers (Fig. 1). To obtain this value, the quantity of soil that will be used in each batch, as well as the target soil bulk density that the seeds will be placed into are needed (an Excel spreadsheet can be created to calculate the quantity of water needed). For the Shano silt loam soil in this study, the following is an example to demonstrate calculations needed to reach a target soil water content of 12% by volume (–0.50 MPa) for a soil batch size of 20.0 kg, where the baseline soil water content is 1.50% by mass and the final desired compacted soil bulk density is 1.35:

**Input**

A. Baseline soil water (% by mass) = 1.50  
B. Target soil water (% by volume) = 12.0  
C. Weight of soil batch at baseline water (kg) = 20.00  
D. Compacted soil bulk density = 1.35

**Results**

E. Actual amount of dry soil in batch (kg) = C/(1 + (A/100)) = 19.70  
F. Total water needed (kg) = G – E = 1.75  
G. Total soil batch weight (kg) = [(B/D)/100) × E] + E = 21.45

Additional water to be added to the soil batch (kg) = G – C = 1.45

**Adding Water to the Soil for the Reserve Layer**

Using a small rotating cement mixer, add the needed quantity of dry field soil for the number of pots to be used for each water potential. Add water as needed to reach target water potential using a backpack sprayer (the mist spray helps aid in the consistent application of the water throughout the batch of soil). After water has been added, continue operating the mixer for 2 min until water is thoroughly distributed in the soil. Be careful not to mix longer than necessary, as this could cause unwanted drying of the soil. Turn the mixer off and pour the moist soil into a container. Pass the soil through a 6-mm mesh screen as it is removed from the mixer (Fig. 3a) to break up any large aggregates formed in the mixing process. Take three random samples from the moistened soil, weigh,
and dry in an oven at 105°C. Reweigh these samples after drying to determine the exact mass water content of the moistened soil. Prepare the pots immediately after the mixing and screening process.

**Preparing the Reserve Layer in Pots**

To obtain a depth of approximately 2.5 cm and a soil bulk density of 1.35, place 525 g of moistened soil in each pot (Fig. 3b) and uniformly compress under 1380 kPa pressure with a hydraulic press (Fig. 3c). This creates the bottom reserve layer (Fig. 1) and, unlike field conditions, the water potential needs to be higher than the overlying seed layers due to drying of this layer that occurs over several days in pots under laboratory conditions (confirmed during “trial and error” process). The reserve layer in our experiment had a water potential of ~0.25 MPa (15% by volume).

**Preparing the Seed Layers in Pots**

To reach the target water potential(s) for the compacted seed layer (Fig. 2), add the required quantity of water with the same procedure used for preparing the reserve layer as outlined above. As was required for the reserve layer, take three random samples from the moistened soil, weigh, oven dry, and weigh again to ultimately determine if target water content was achieved. After the mixing and screening process, add 525 g of soil on top of the reserve layer. Compact with the hydraulic press with 1380 kPa pressure (Fig. 3c) to a depth of approximately 2.5 cm.

**Adding Dry Field Soil**

Using the dry field soil, carefully fill each pot to the brim without compacting and level evenly with a straight edge (Fig. 3e). The depth of this dry field soil layer is approximately 10 cm (Fig. 1).

**Placing Pots in Appropriate Blocking**

Arrangement on Lab Bench

After completing the pot preparation as described in the eight-step process outlined above, place pots on a laboratory bench to remain untouched and at constant 21°C air temperature. In this laboratory setting, arrange replicates of treatment combinations in a completely randomized block design (Hinkelmann and Kempthorne, 2008). This blocking arrangement is important as we have measured some differences in emergence among replicates, likely due to slight air flow differences over the laboratory bench.

**Counting Emergence of Seedlings**

Seedling emergence generally does not occur until at least seven DAP, even at the highest seed-zone water potentials. We measure seedling emergence at exact 12- or 24-h time intervals. A seedling is scored as “emerged” as soon as the tip of the first leaf is visible (Fig. 3f). Depending on water potential, the majority of seedlings emerge by 14 DAP but, at the lowest water potentials, this can extend to 21 DAP.

**Laboratory Experiment to Compare with Emergence in the Field**

In the laboratory experiment, four wheat cultivars were planted in pots at seed-zone water potentials very close to those measured in the 2007 to 2010 field experiments. These four laboratory water potentials were ~0.75, ~0.64, ~0.45, and ~0.36 MPa and spanned the range from what is considered extremely dry to very wet conditions. The laboratory experiment was comprised of 64 pots (four cultivars × four water potentials × four replicates). Emergence of these four cultivars at the four water potentials was measured at 24-h intervals from 7 to 14 DAP.
**Statistical Analysis**

Analysis of variance was performed on two responses in both laboratory and field experiments: (i) median time to emerge, and (ii) final stand. Median time to emerge was statistically determined using SAS PROC NLIN. The nonlinear regression program fit a Gompertz sigmoidal model (Ohishi et al., 2009) to the emergence curve for both field and laboratory experiments. The model was used to interpolate to the 50th percentile of the emergence distribution. The ANOVA tests were done at the 5% level of significance. Additionally, correlation analyses using Pearson’s product-moment coefficient ($r$) (Puth et al., 2014) was conducted on both responses across the four cultivars and four water potentials.

**RESULTS AND DISCUSSION**

**Speed of Emergence and Final Stands**

There is a widely accepted general rule among PNW dryland WW farmers and scientists that a minimum of 15% of seeds planted need to emerge to be considered an acceptable stand (i.e., no less than an average six seedlings per meter of row at 40-cm row spacing). Comparison among cultivars for median time to emerge in both the field and laboratory showed a general trend of Buchanan > Moro > Eltan > Xerpha (Fig. 4). Statistical differences in median time to emerge in the field only occurred at the highest (~0.40 MPa) water potential. Significant differences among cultivars in median time to emerge were detected at all water potentials in the laboratory experiment (Fig. 4).

Final stands in the field and in the laboratory are shown in Fig. 5. Buchanan had exceptional final stand in the field at the lowest water potential. The ability of Buchanan to emerge well under abiotic stress conditions has been verbally reported by many regional farmers and documented by Schillinger (2011). Only Buchanan and Moro had final field stands deemed acceptable (i.e., >15% of seedlings emerged) at all four water potentials (Fig. 5). Eltan had acceptable field stands at the two highest water potentials. Xerpha field stands were only acceptable at the highest water potential (Fig. 5). Emergence at 24-h intervals from 7 to 14 DAP for the four cultivars and four water potentials is shown for both the field and the laboratory in Fig. 6 and 7, respectively.

Likely reasons for the lower stands in the field compared to the laboratory are: (i) greater depth of soil cover (14 vs. 12 cm); (ii) presence of soil clods of varying size in the field that can prevent seedlings from elongating around them, causing seedlings to “kink” and die; and, (iii) rain occurring after planting but before emergence that can cause soil crusting.

The only rain during the field emergence period in any of the 4 yr was 3.8 mm that occurred 3 DAP in 2009. This was the year of the highest water potential (i.e., ~0.40 MPa). Any rain event >3 mm can substantially reduce WW emergence (Donaldson, 1996; Schillinger, 2011) by creating a surface soil structural crust (Awadhwal and Thierstein, 1985). Subsequent soil surface drying, especially with high air temperature under full sunlight enhances the formation of these fragile soil crusts (Baumhardt et al., 2004; Singer and Warrington, 1992) which elongating pre-emerged WW seedlings have trouble penetrating. However, the 3.8 mm of rain 3 DAP appeared to have minimal effect on emergence as all four cultivars continued to emerge through 10 DAP (Fig. 6). Maximum air temperature did not exceed 26°C and skies were...
partially overcast during this time period; this, combined with high water potential, being a likely reason for the emergence without apparent difficulty.

**Correlation of Seedling Emergence in Field vs. Laboratory**

The correlation ($r$) for median time to emerge across all four cultivars and water potentials between field and laboratory experiments was strong ($r = 0.71, p < 0.01$) (Fig. 8). Although seeds were covered with 2 cm additional soil in the field than in the laboratory, median times to emerge were similar (Fig. 4). We feel this is likely due to soil temperature at depth of seed placement. Although not measured in this field experiment, soil temperature at depth of seed placement in late August–early September at Lind, WA generally range from 22 to 25°C with little diurnal variation. These seed-zone soil temperatures are considered optimal for speed of WW emergence from deep depths (Lindstrom et al., 1976; Donaldson, 1996). In contrast, soil temperature at depth of seed placement in the laboratory experiment was 21°C.

The correlation for final stands between field and laboratory experiments was weaker ($r = 0.41, p < 0.12$) than for median time to emerge. Final stands in the field were considerably less than those in the laboratory across cultivars and water potentials (Fig. 5), likely due to the differences in depth of soil covering seed, clods, and possibly rain as discussed above. A natural log transformation of the $y$ axis “field emergence” improved the final stand correlation to $r = 0.54, p < 0.03$.

**CONCLUSIONS**

We developed a laboratory method to accurately and realistically determine WW emergence from deep planting depth in pots over a wide range of water potentials. Important factors such as soil bulk density below and above seed placement, as well as the loose, dry surface soil typical of field conditions in the dryland region of the PNW were accurately created.

By using methods and procedures outlined in this paper, WW emergence from laboratory pot experiments correlate to emergences measured in the field at the same water potentials. Statistical comparison between field and laboratory emergence data showed a strong correlation for median time to emerge and a lesser correlation for final stands. This laboratory method provides for several basic and applied research needs such emergence assessment of advanced WW breeding lines as well as a wide gamut of experiments related to seedling physiology.

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