Fate of Avena sterilis ssp. ludoviciana seeds under different burial depths and wheat residue loads

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Abstract
Ludo wild oats (*Avena sterilis* ssp. *ludoviciana*) is a problematic winter weed across both conventional and conservation agriculture systems where soil disturbance and crop residue retention differ. To better understand its persistence, we compared the fate of primary and secondary seeds placed at 0, 2, 8, or 15 cm burial depths, and under 0, 2, or 3 t/ha wheat (*Triticum aestivum* L.) residue loads over a 16-month period. The experiment revealed both burial depth and wheat residue load affected the fate of both types of seed. Seedlings of primary and secondary seeds were able to emerge from 15 cm burial depth (17 %), however maximum emergence occurred from the 2 cm depth (71 %) and under 3 t/ha wheat residue (80 %). After 16 months, it was observed that 17 % of the primary and 24 % of the secondary seeds remained viable, but in a quiescent or dormant state. Burying seeds to a depth of >15 cm will be required to help prevent *A. sterilis* ssp. *ludoviciana* seedling emergence and possibly to reduce seed viability in the soil. When strategic tillage is applied, it will reduce the surface residue load and thereby reduce seedling emergence.

Keywords
Conservation agriculture, reduced tillage, strategic tillage, wild oat emergence, wild oat viability

Introduction
Wheat, the main winter crop of Australia's northern grains region (NGR; comprising parts of Queensland and New South Wales), is predominantly cultivated with reduced tillage and residue retention approaches (i.e., using conservation agriculture principles). The wild oat species (*Avena sterili*s ssp. *ludoviciana* (Durieu) Nyman, and *Avena fatua* L.) continue to be two of the most difficult-to-control winter weeds in the NGR, following the adoption of conservation agriculture (CA) which has a reliance on herbicides for weed control (Dang et al. 2015). Two important survival mechanisms of these two wild oat species are their variable seed dormancy and long-term seedbank viability that allows them to set persistent seed banks. Due to the variation in seed dormancy, multiple cohorts of wild oats seedlings can emerge within a season from the soil seedbank and infest crops at different stages of their growth.

In the CA system, there is reduced soil disturbance and shed wild oat seeds are concentrated in a shallow soil seedbank. These seed experience large fluctuations in soil temperature and moisture conditions which accelerates viability decline (ref). However, residue retention in CA influences the soil surface environment (in terms of reducing temperature fluctuations and increasing moisture) that could affect seed viability (Bullied et al. 2003). Wild oats seedling emergence also depends on the nature of the dormancy mechanisms that are developed during their maturation on the mother plant. In addition, seed dormancy of wild oats can vary between the primary and secondary seeds of a spikelet (a spikelet contains a larger primary and a smaller secondary seed, seed with a filled caryopsis is considered as a filled seed) (Quail and Carter 1968). These environmental and genetic influences cause variability in dormancy status which ultimately lead to staggered wild oats germination.

To address the persistence issue of ludo wild oat (*A. sterili*s ssp. *ludoviciana*, here after referred to as *A. sterilis*) in the NGR, there has been interest in the occasional application of a strategic tillage (ST) event within the long-term CA system. Strategic tillage involves tilling a CA field every 5 to 10 years with, the aim, of burying weed seeds to a depth from where they cannot emerge (Walsh et al. 2019). However, information is lacking on what would be an effective burial depth to prevent *A. sterilis* seedling emergence, considering seeds of *A. sterilis* are relatively bigger than most other weed seeds (seed can have a 1,000-seed weight of 35 g) and contain sufficient food reserves to support seedling emergence from great depths. So, before applying a deep burial ST operation the survival strategies of *A. sterilis* seeds buried at great depths needs to be ascertained to determine the interval required between ST operations. This experiment, therefore aimed to reveal the fate of primary and secondary seeds of *A. sterilis* when they are retained on the soil surface or buried at different soil depths, and under different wheat residue loads. This information will help in the determination of methods to better control this weed within the NGR.

Materials and Methods

Seed collection and viability test of A. sterilis seed lot

Spikelets of *A. sterilis* were collected from Westmar, Queensland (27°47'15"S 149°42'27"E) during November 2018. After collection, filled primary and secondary seeds were separated from the spikelets and stored at 15±2 °C, 15±5 % relative humidity (RH) until used 26 weeks later. Before starting the experiment, the viability of both seed lots was determined by taking three replicate samples of 20 dehulled seeds and incubating them at a 15/5±2 °C (day/night) thermoperiod under a matching 12/12-hour (day/night) photoperiod for 42 days. After this time, the non-germinated seeds were pierced mid-way along their dorsal surface and imbibed with 10 µM of gibberellic acid (treatments that are known to overcome dormancy in this species). Following a further 20 days of incubation the remaining seeds that had not germinated were recorded as being dead. However, all seeds used in this test were found to be viable, but *ca*. 30 % of the primary seeds and *ca*. 50 % of the secondary seeds had primary dormancy. It should be remembered that the storage conditions and the duration of the seeds remaining in the seed store can accelerated after-ripening process of the seeds. After-ripening is known to reduce the intensity of the dormancy system(s) present in wild oats seeds (Adkins et al. 1986).

Experimental design, treatments, and data collection

The experiment was conducted at Gatton (27°33'14"S 152°20'21"E) under natural environmental conditions, for 16 months from May 2019 to September 2020, in a randomized block design and three replications using four soil depths (0, 2, 8 and 15 cm) and three wheat residue loads (0, 2 and 3 t/ha). The experimental units were individual black plastic tubs (50 × 40 cm: diameter/height), containing 50 kg of a black Vertosol soil (sieved through *ca*. 2 mm using a barrel grinder) obtained from the Research Farm at the Gatton Campus (27°34'01"S 152°20'16"E). The soil had an initial soil moisture of *ca*. 7 %. In a treatment (one burial depth × one wheat residue load), 60 viable primary seeds were randomly placed on one side of the tub, while 60 viable secondary seeds were randomly placed on to the other side of the tub. The two halves of a tub were separated by a vertically placed polyvinyl chloride divider. The wheat straw (leaf and stem sections cut into *ca*. 5 cm; cv. LRPB Lancer) used to cover the soil surface was collected from the Research Farm (27°32'43"S 152°20'08"E) 3 months after crop harvest. Before being used to the experiment, the straw was kept dry in a room (30/15 ± 2 °C day/night temperature, 60 ± 5 % RH) for 3 months (now 6 months old). Three unsown tubs were also set up and randomly placed into the experiment, to determine presence of any local wild oat seeds in the collected field soil (however, no seeds were found in these soil samples). The fate of the buried seeds was determined over time by counting seedling emergence at regular intervals, and especially following rainfall events. After counting, the emerged seedlings were removed from the pots. The remaining seeds were retrieved manually from the tubs at the end of the experiment to determine their dormancy and viability status. At the end of the experiment, the buried seeds were classified into three types; those that germinated but failed to emerge (fatal germination), those that had decayed (dead) and those that remained filled. These filled seeds were carefully removed from the soil and incubated under the germination condition described above. Seeds that germinated within 42 days were considered viable but had been imposed into a quiescent state (lacking a requirement for germination). The remaining seeds were then dehulled and their viability tested with the dormancy breaking treatment (as described above). Seeds that germinated following this treatment were considered viable but dormant. In each treatment, all seeds that had been buried at the start of the experiment were accounted for at its end. The total dead seed was the sum of seeds that had been found to have decayed at exhumation and dead during the germination test.

Statistical analysis

Fate of primary and secondary seeds of *A. sterilis* under different burial depth and wheat residue loads were analyzed individually using ANOVA performed in Minitab v. 8.1. Means were separated using Fisher's protected LSD test at $P < 0.05$. The graphs were prepared using SigmaPlot v. 13.0.

Results and Discussion

There were no two-way interactions between burial depth and wheat residue load observed for the different fates of the primary seeds except for quiescent seed numbers ($F_{6, 24} = 2.77$, $P = 0.035$; Figure 1). In total, 44 % of all primary seeds sown were able to emerge from different depths within the soil. The best seedling emergence from the primary seeds was observed at the 2 cm burial depth (71 %), followed by the soil surface (57 %). The lowest emergence (17 %) was found at the 15 cm burial depth, where many of the

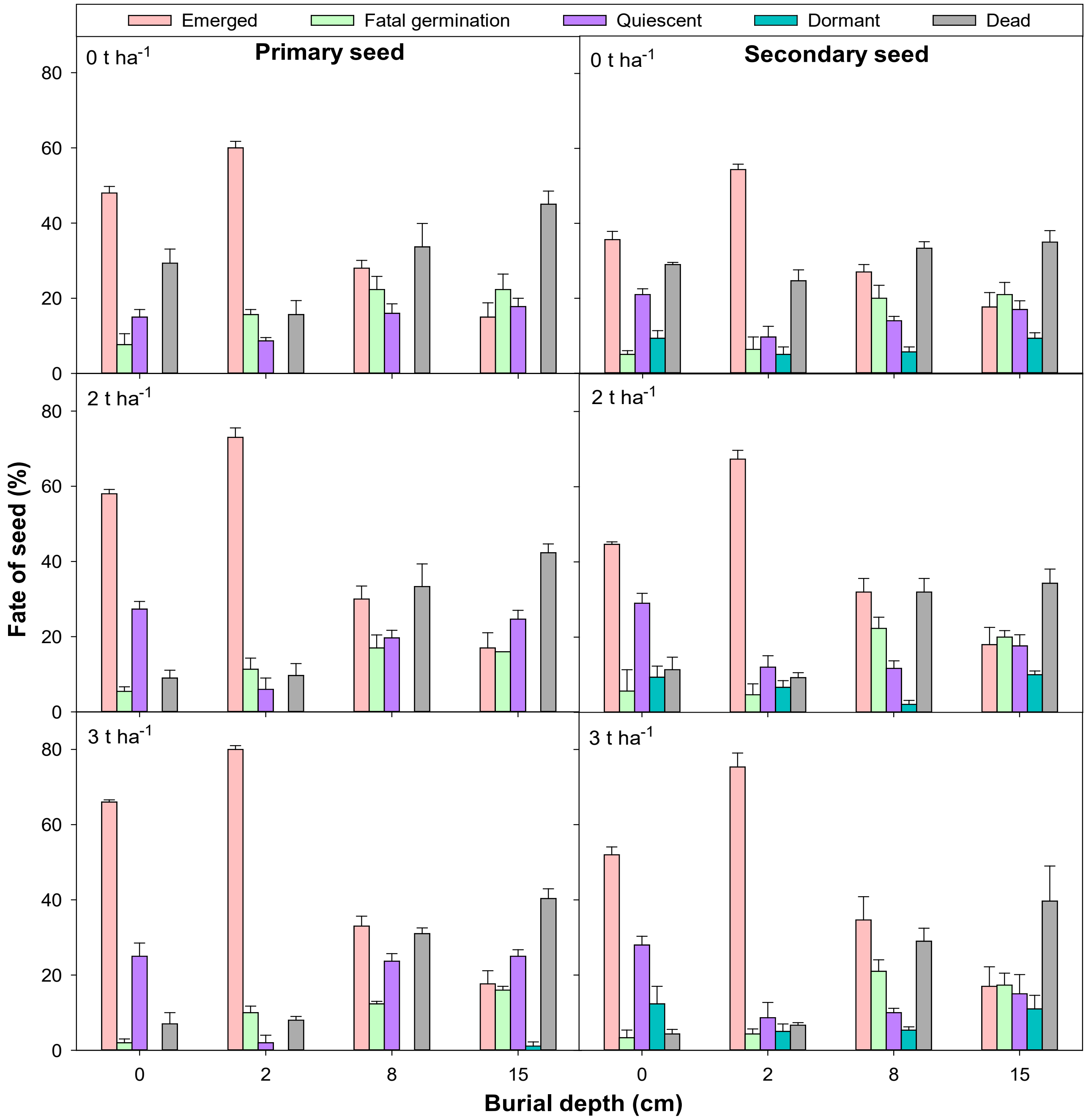

Figure 1. Fate of the primary and secondary seeds of *A. sterilis* during a 16-month trial with seed placed at different burial depths and using three wheat residue loads. Error bars represent standard errors of the mean of three replicate tubs of 60 sown both primary and secondary seeds.

primary seeds had died (42 %) or had attempted emergence and died (18 %). However, *ca.* 23 % of the primary seeds at this soil depth were also found to be alive but in a quiescent state. Therefore, these viable seeds are due to emerge at the next available opportunity when all requirements for germination are provided (that is during the cooler months of May and June). Gallandt et al. (2004) has reported that germination of the other species of wild oat (*A. fatua*) was higher in undisturbed soil as compared to conventional tillage (chisel ploughed up to 20 cm depth). Thus, it can be assumed that primary seeds, located at 0 and 2 cm soil depths should be able to emerge at a time when the next season's crop is to be planted. However, those seeds buried at a greater depth, could either emerge, or die or remain quiescent until a time they are brought closer to the soil surface.

The greater the residue load, the greater the number of seedlings that emerged from the primary seeds, especially from the 0 and 2 cm soil depths (Figure 1). Wheat straw residue loads presumably created an environment on the soil surface that encouraged more primary seeds to emerge from these two soil depths (*ca*. 66 % at 2 t/ha and *ca*. 73 % at 3 t/ha). In contrast, *ca*. 54 % of the primary seeds were able to produce seedlings from these two soil depths when the soil was not covered with any residue. However, the condition of no residue resulted in a greater primary seed death from these two top layers (35 %; 23 % dead with 12 % fatal germination), compared to those from the 2 t/ha (18 %; 10 % dead with 8 % fatal germination) and 3 t/ha (14 %; 8 % dead with 6 % fatal germination) treatments. When the burial depth was increased to 8 and 15 cm, the effect of the wheat residue load on different fates of the primary seeds was negligible.

Non-significant two-way interactions between burial depth and wheat residue load were observed for the fate of secondary seeds, except for the proportion of dead seeds ($F_{6, 24}$ = 3.49, P = 0.013; Figure 1). Emergence and viability of secondary seeds were also greatly impacted by the different burial depths and wheat residue loads, like that observed for primary seeds. However, the emergence of seedlings from secondary seeds was found to be *ca*. 5 to 15 % less than for primary seeds (Figure 1). Surprisingly, *ca*. 10 % of the secondary seeds from 0 and 15 cm soil depths, and *ca*. 5 % of those from 2 and 8 cm soil depths were found to be dormant after 16 months of burial, irrespective of wheat residue load (Figure 1). The level of dormancy present in the secondary seeds may be attributed to the retention of their primary dormancy or to the induction of a secondary dormancy mechanism (Fenner 1985).

Thus, maximum emergence of *A. sterilis* seedlings occurred from the 2 cm soil depth. A further significant contribution came from the 0 cm soil depth with little from those seeds buried at 8 or 15 cm soil depth. Crop (wheat) residue also had a significant role in *A. sterilis* seedling emergence especially when the seeds were retained on the soil surface or buried at a 2 cm depth. Crop residue has been reported to decrease the soil surface temperature and increase soil moisture levels, creating a modified environment that aids in weed seed germination and seedling emergence in the top soil layers (Bullied et al. 2003). No primary seeds were found to retain their primary dormancy after 16 months of the experiment, whereas a few (8%) secondary seeds still remained dormant. The stronger dormancy behaviour of the secondary seeds, as compared to the primary seeds, may therefore be responsible for lengthening the seedbank life of *A. sterilis.*

Conclusion

Seeds of *A. sterilis* were shown to germinate and produce seedlings that could emerge from a range of soil depths (0 to 15 cm), and this was true for both primary and secondary seeds. Of the remaining seeds a good proportion remained viable for at least 16 months. In addition, the retainment of litter on the soil surface helped to retain seed viability, with the larger amounts of litter creating a microclimate that was better suited to viability retainment and subsequent germination. If a ST activity is applied, then it needs to bury seeds below 15 cm soil depth. In addition, no further ST operation should be applied until all deeply buried seeds have died and this may take at least 2 years. Further studies should now be conducted with freshly harvested seeds, with seeds produced under different environmental conditions which are known to reduce dormancy, and with seeds from a range of biotypes to better understand the genetic component of the soil survival mechanism under CA in the NGR.

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