Impact of late season heat stress on the phenology and reproductive biology of *Avena sterilis* ssp. *ludoviciana*

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Abstract
Avena sterilis ssp. *ludoviciana* (wild oat) is considered to be the most difficult-to-control winter weed in the no-tillage-based conservation cropping systems of Australia's northern grains region (NGR). The increasing frequency of hot periods during the late winter/early spring season might be responsible for early shedding and less dormant seeds. This greater rain of less dormant seed may be better suited to the non-burial conditions of no-tillage and will be ready to reinfest during a subsequent autumn-sown crop. To examine this possible mechanism of persistence, an experiment was conducted using wild oat biotypes collected from northern and southern areas of the NGR. At the time of wild oat panicle initiation a portion of the plants were transferred from an ambient temperature greenhouse (23/14°C day/night) to a high temperature glasshouse (29/23°C). The process of moving plants was repeated on three further occasions, each ten days apart. Plants exposed early to heat stress matured 17 days earlier and produced less filled (28% less), smaller (34% smaller) spikelets with less dormant seeds (34% less) compared with control plants. The biotypes coming from the northern NGR maturated five to eight days earlier than the biotypes from the southern NGR. It is hypothesised that the increasing frequency of late season hot periods, coupled with no-tillage-based conservation cropping systems that do not bury seeds deep in the soil profile, is facilitating the persistence of species like *A. sterilis* ssp. *ludoviciana* in the NGR.

Key Words
No-tillage, climate variability, wild oat survival, fecundity, winter crop

Introduction
Wild oats (*Avena sterilis* ssp. *ludoviciana* (Durieu) Nyman and *Avena fatua* L. are the most important winter weeds, in terms of their abundance and difficult-to-control nature (Nugent *et al*. 1999), in Australia's northern grains region (NGR; comprising central Queensland, southern Queensland and northern New South Wales). The prevalence of wild oats in the NGR has grown following the adoption of no-tillage conservation agriculture (Dang *et al.* 2015). A variety of survival mechanisms such as early seed shedding and variable seed dormancy contribute to their ability to persist in cropping land. Wild oats infest ~0.6 M ha of cropping land and cost growers ~$4.5 M annually due to control costs and production losses (GRDC 2017). Within the NGR, *A. sterilis* ssp. *ludoviciana* tends to be more prevalent than *A. fatua* (Nugent *et al.* 1999).

The exposure of *A. fatua* plants to sustained high temperature heat stress during their vegetative growth and seed development phases is known to promote production of small, early matured seeds with a lower level of dormancy (Adkins *et al.* 1987). This in turn affects the dormancy behaviour and emergence of the progeny seed and seedlings. Thus, it is possible that heat stress prior to or during seed set could cause *A. sterilis* ssp. *ludoviciana* plants to produce seed that will be non-dormant, mature early and shed more seed onto the ground before the cereal crop is harvested. As no-tillage conservation agriculture is the usual practice for growing wheat (*Triticum aestivum* L.) in the NGR, early shattered, less dormant seeds will remain on or near the soil surface, and will be capable of germinating at the time of planting the next crop. However, seed production and extent of dormancy may differ among biotypes of *A. sterilis* ssp. *ludoviciana*, as has been reported for *A. fatua* (Adkins *et al.* 1987).

Although much research has been conducted on different biotypes of *A. fatua*, little has been done on the more abundant *A. sterili*s ssp. *ludoviciana*. In particular, there is a scarcity of research on *A. sterilis* ssp. *ludoviciana* reproductive biology in response to environmental variables such as heat or water stress. This knowledge is critical for understanding its persistence and invasiveness within NGR cropping systems. Hence, this study is concerned with the phenology and reproductive biology of *A. sterili*s ssp. *ludoviciana* biotypes in the NGR, grown under different periods of heat stress during seed development.

Materials and Methods

Wild oat biotypes
Seed of four biotypes of *A. sterilis* ssp. *ludoviciana* were collected from four locations in the NGR *viz*. Biloela (northern NGR; Biloela 1: -24.35471 °S, 150.49773 °E and Biloela 2: -24.35048 °S, 150.497 °E), Toobeah and Jandowae (southern NGR; Toobeah -28.36792 °S, 149.52197 °E and Jandowae -26.66727 °S, 151.0246 °E). These biotypes were chosen from multiple locations within the NGR (two from the north and two from the south) to provide representative coverage of the area over which *A. sterilis* ssp. *ludoviciana* populations are found in the NGR.

Experimental design
The experiment was conducted during July to November 2018 using a completely randomized design with six replications. Five germinated caryopses of each biotype were transplanted into a 20 cm diameter × 19 cm height pot containing 4.5 kg of a black Vertosol soil obtained from Gatton, QLD and moistened to field capacity. After 30 days, young plants were thinned to three per pot. The 120 pots (four biotypes × five heat stress treatments including control × six replications) were maintained at field capacity and kept in a greenhouse (clad with single skin plastic film, with roof and side wall ventilation) under ambient conditions (average ~23/14°C day/night, determined from a TGP-4520 tinytag plus 2 temperature logger). The heat stress treatments were applied at panicle emergence.

Treatments
Six pots per biotype (24 pots in total) were used as control treatments (i.e. no imposed heat stress) and remained in the greenhouse under ambient conditions throughout the experiment. To simulate heat stress at different time points following panicle emergence, on four occasions (see Table 1), the pots were moved into a temperature-controlled glasshouse set at 29/23°C (12/12-hour day/night photoperiod) where they remained until harvest. Pot positions in both houses were re-randomized every two days until harvest.

Table 1. Time when the biotypes were moved to temperature-controlled glasshouse.

Heat stress (HS) treatments	HS1	HS2	HS3	HS4
Occasions of plant movement	1st (at panicle initiation)	2nd (10 days after panicle initiation)	3rd (20 days after panicle initiation)	4th (30days after panicle initiation)
Biotypes	Days after emergence			
Biloela 1	58	68	78	88
Biloela 2	58	68	78	88
Toobeah	63	73	83	93
Jandowae	65	75	85	95

Data acquisition
The number of days taken to reach maturity was recorded for all plants. Total numbers of fertile tillers and spikelets (filled and unfilled spikelets were determined using a Faxitron model MX20 X-ray machine) were counted for all plants. Maturity was defined as the time when 50% of the seeds coming from one plant were physiologically matured. Physiological maturity was the time when the seed was ready to shed from the plant. Physiologically mature spikelets were collected by hand and stored in paper bags at 15°C with 15% relative humidity until required for dormancy test. The 1000 spikelet weight was determined by taking five lots of 50 spikelets from the bulked seed lots (combined across replicates of each heat stress × biotype treatment) and dried in an oven for 80°C for a period of 96 hours. Once dry, they were weighed and values multiplied by four to reach 1000. To check the dormancy status of the freshly harvested filled primary seeds, three replicates of 20 primary seeds coming from the bulked seed lots of each treatment were incubated at 8°C under 12/12 hour light/dark condition for 32 days in a thermogradient-bar.

Statistical analysis
A. sterilis ssp. *ludoviciana* responses to the heat stress treatments were analyzed using ANOVA performed on Minitab v. 8.1. Means were separated using Fisher's protected LSD test at $P<0.05$.

Results and Discussion
No two-way interactions between *A. sterilis* ssp. *ludoviciana* biotype and the timing of heat stress imposition were observed except in the number of filled spikelets produced per plant (Table 2). However, significant

main effects of both heat stress and biotype were observed for days to maturity, the number of filled spikelet and sterile spikelet production per plant, and 1000 spikelet weight. Interestingly, a main effect of the heat stress treatment was only observed for the number of fertile tillers produced per plant and for the dormancy behaviour of the freshly harvested filled primary seeds in the thermogradient-bar.

Northern biotypes matured five to eight days earlier than southern biotypes (Table 2). This might be because northern biotypes have become adapted to higher seasonal temperatures in the northern NGR. The southern biotype, Jandowae, took the longest time to mature (103 days) and the northern biotype, Biloela 1, took the shortest time to mature (94 days). Control plants procured significantly longer to mature (106 days) compared with plants exposed to heat stress commencing at panicle initiation. Plants exposed to heat stress commencing at panicle initiation were the fastest to mature and produced mature spikelets more rapidly (89 days) than plants exposed to heat stress commencing 10-30 days after panicle initiation (Table 2).

The longer plants were exposed to heat stress from panicle initiation stage, the lower the numbers of fertile tillers and filled spikelets produced, the spikelets were smaller, and plants completed their life cycle in a much shorter period of time (Table 2). Shorter maturation time might mean newly produced seeds are less filled with food reserves and that the typical dormancy mechanisms of this species would not have had time to develop; hence the seeds are smaller and less dormant. Adkins *et al.* (1987) reported that constant higher temperature (25°C) decreased the duration of seed development, the number of mature seeds produced per plant, their dry weight and dormancy in Canadian populations of *A. fatua*. In an earlier study, Peters (1982) found that high temperature (20°C as compare to 15°C) reduced viable seed production of European populations of *A. fatua* and those seeds were less dormant compared to controls.

The thermogradient-bar study showed that 96% of primary seeds of the control plants of all biotypes were dormant compared with 62% of those that matured 17 days earlier than the control plants, a significant difference of 34% less dormancy (Table 2). Thus, it is evident that changes in growing environment like high temperature are responsible for producing less dormant smaller seeds and these fresh seeds are set to germinate and re-infest the crop in the next season. This mechanism of early shedding and less dormant seeds may be contributing to wild oat persistence in the NGR under conservation agriculture. Medd (1990) reported the regular annual input of new seed rather than the carryover of *A. fatua* seed (in the seed bank) is the main reason for its persistence in conservation agriculture.

Conclusion

Heat stress during the reproductive phase is responsible for production of lower numbers of early maturing seeds with low dormancy, ready to germinate when sowing the next crop. Such seeds under conventional tillage would be buried, an action likely to result in high seed mortality. The absence of burying within no-tillage systems may facilitate the persistence and spread of *A. sterilis* ssp. *ludoviciana* within northern grains region. Further studies should be conducted under both heat and water stress with more biotypes to better understand it's invasiveness under conservation agriculture and to develop effective management strategies.

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Table 2. Effect of heat stress (29/23°C day/night) applied at various times following panicle emergence on days to maturity, fertile tillers, filled and sterile spikelets per plant, 1000 spikelet weight and percent dormancy of

freshly harvested primary seeds of four *Avena sterilis* ssp. *ludoviciana* biotypes from the NGR. Main effect means for biotypes and heat stress (HS) treatments (in bold text) sharing the same letter do not differ significantly.

Heat stress treatments	Biloela 1	Biloela 2	Toobeah	Jandowae	Mean (HS)
			Days to maturity		
HS1	84	86	91	95	**89 e**
HS2	91	92	96	99	**94 d**
HS3	96	98	101	105	**100 c**
HS4	99	101	105	108	**103 b**
Control	103	104	108	111	**106 a**
Mean (biotypes)	**94 d**	**96 c**	**100 b**	**103 a**	
			Fertile tillers plant^{-1}		
HS1	5	4	5	5	**5 d**
HS2	6	6	6	6	**6 c**
HS3	7	6	7	7	**7 b**
HS4	7	7	7	8	**7 b**
Control	8	8	8	9	**8 a**
Mean (biotypes)	**6**	**6**	**7**	**7**	
			Filled spikelets plant^{-1} (letters within biotypes indicate significant differences)		
HS1	144 d	121 d	156 d	134 d	**139 e**
HS2	147 d	143 c	182 c	157 c	**157 d**
HS3	155 c	162 b	193 bc	172 b	**170 c**
HS4	169 b	182 a	201 ab	182 ab	**183 b**
Control	184 a	184 a	214 a	191 a	**193 a**
Mean (biotypes)	**160 c**	**158 c**	**189 a**	**167 b**	
			Sterile spikelets plant^{-1}		
HS1	41	45	40	41	**42 a**
HS2	36	37	31	34	**34 b**
HS3	31	31	34	28	**31 c**
HS4	35	24	26	22	**27 d**
Control	26	19	25	18	**22 e**
Mean (biotypes)	**34 a**	**31ab**	**31 ab**	**29 b**	
			1000 spikelet weight (g)		
HS1	33.9	44.8	48.8	50.5	**44.5 e**
HS2	40.8	48.0	54.1	60.3	**50.8 d**
HS3	52.5	49.9	59.4	66.9	**57.2 c**
HS4	58.9	61.4	63.2	72.3	**63.9 b**
Control	61.7	64.2	69.2	73.8	**67.2 a**
Mean (biotypes)	**49.6 d**	**53.7 c**	**58.9 b**	**64.8 a**	
			Percent dormancy of freshly harvested filled primary seeds		
HS1	60	62	60	67	**62 d**
HS2	75	75	78	77	**76 c**
HS3	78	77	78	78	**78 c**
HS4	87	83	88	83	**85 b**
Control	93	97	93	100	**96 a**
Mean (biotypes)	**79**	**79**	**80**	**81**	

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