

# Early silique-shedding wild radish (*Raphanus raphanistrum* L.) phenotypes persist in a long-term harvest weed seed control managed field in Western Australia

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## Abstract

**BACKGROUND:** This study introduces a wild radish population collected from Yelbeni in the Western Australian grainbelt that evolved an early silique abscission (shedding) trait to persist despite long-term harvest weed seed control (HWSC) use. In 2017, field-collected seed (known herein as Yelbeni) was compared to surrounding ruderal and field-collected populations in a fully randomized common garden study.

**RESULTS:** The Yelbeni population exhibited a higher rate of silique abscission when compared to the ruderal populations collected from the site before wheat (*Triticum aestivum* L.) harvest (assessed at soft dough stage, Zadoks 83). A similar common garden study was conducted in the subsequent season (2018) using progeny reproduced on a single site without stress. The HWSC-selected progeny (Yelbeni P) shed 1048 ( $\pm 288$ ) siliques before wheat maturity at the soft dough stage (Zadoks 83) compared to 25 ( $\pm 7$ ) siliques from the pooled control populations. The Yelbeni P population only flowered 6 days earlier (FT<sub>50</sub> as determined by log-logistic analysis) than pooled control populations, which is unlikely to fully account for the increased rate of silique abscission. The Yelbeni P population also located its lowest siliques below the lowest height for harvest interception (10 cm), which is likely to increase HWSC evasion. The mechanism inducing early silique-shedding is yet to be determined; however, wild radish is known for its significant genetic variability and has demonstrated its capacity to adapt to environmental and management stresses.

**CONCLUSION:** This study demonstrates that the repeated use of HWSC can lead to the selection of HWSC-avoidance traits including early silique-shedding before harvest and/or locating siliques below the harvest cutting height for interception.

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**Keywords:** harvest weed seed control; integrated weed management; weed control evasion; sSeed-shedding; flowering time

## 1 INTRODUCTION

Wild radish (*Raphanus raphanistrum* L.) is the second most economically damaging weed of Australian grain production.<sup>1</sup> Over-reliance on herbicides has resulted in the widespread evolution of wild radish populations resistant to multiple herbicide modes-of-action (MoAs).<sup>2,3</sup> To control these resistant wild radish populations, growers have become increasingly reliant on nonherbicidal, integrated weed management (IWM) techniques that either reduce wild radish fecundity or intercept seed at harvest to minimize soil seed bank inputs, termed harvest weed seed control (HWSC).<sup>4,5</sup> Most weed seeds intercepted by the harvester are retained in the chaff fraction, which is then processed/crushed through impact mills attached to the harvester, to kill the seeds, or fed out the back of the harvester into a chaff-cart (chaff piled for sheep feed/burning) or concentrated into narrow rows, which are either left *in situ* or burnt.<sup>5</sup> HWSC is a highly effective IWM technique at controlling wild radish with 82–99% of the seed retained on the plant until grain harvest.<sup>6</sup>

Of that retained seed, almost all seed is unviable following HWSC treatment.<sup>7,8</sup>

Evolutionary theory suggests that the recurrent use of highly effective weed control techniques, such as HWSC, applies an intense selection for any survival enhancing trait.<sup>9</sup> Individuals which can survive chemical (herbicide) or cultural (agronomy) weed control techniques, persist as they have advantageous biochemical, life history or growth traits that allow individuals to survive and proliferate.<sup>10,11</sup> Aside from the widespread and well-documented selection of herbicide resistance,<sup>11</sup> adaptive life-history traits such as increased seed dormancy and change in

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flowering time have been identified in weed species in response to intensive weed management.<sup>12–14</sup>

Here we report on the behaviour of a Yelbeni wild radish population collected from the central grainbelt of Western Australia (described hereinafter as the Yelbeni population), which was found to persist despite >20 years of annual HWSC use. Further inspection found that individuals prematurely shed their seed before harvest. In this study, we compare the flowering time, seed-shedding and seed location traits of a putative HWSC-evading Yelbeni population against multiple populations collected from adjacent fields and ruderal locations to identify the causes of HWSC evasion in this population.

## 2 MATERIALS AND METHODS

### 2.1 Field survey and seed collection

A wild radish population suspected to be evading HWSC was collected from a field in Yelbeni in October 2016, before the scheduled grain harvest in December (Table 1). The Yelbeni site contained a patch of wild radish, at a high density, that had persisted despite >20 years of HWSC use (using a Tecfarm chaff cart, Tecfarm Engineering, Bruce Rock, Western Australia). This distinct patch of wild radish in the paddock was found to have shed a large proportion of their siliques when assessed at the wheat (*Triticum aestivum* cv. Mace, Australian Grains Technologies) soft dough stage-Zadoks scale 83. Siliques were counted and gathered from the soil surface (October 2016) over an area of  $\approx 6$  m<sup>2</sup> (known hereinafter as the Yelbeni population). The number of plants on the site were counted before the seed retained on the wild radish plants were collected separately to calculate the proportion of silique shed per plant. The site where the putative HWSC-evading Yelbeni population was collected was >100 m from the field edge and away from headlands, erosion contours and rock outcrops. The growth of the wheat crop at the site was consistent with that in the rest of the field, suggesting that confounding effects resulting from soil properties were unlikely to be important. Likewise, samples from two neighbouring fields that did not use HWSC were collected (known hereinafter as control population C1 and C2). The C1 and C2 populations were collected within 2 km of the Yelbeni sample site from sporadically distributed wild radish plants within the field. The cropping history of Yelbeni, C1 and C2 sites were similar except for contrasting exposure to HWSC (Yelbeni 20 years *versus* 0 years for C1 and C2 populations). As wild radish is an outcrossing and genetically variable species,<sup>15</sup> another control population was collected from a ruderal roadside location (control population C3)  $\approx 1$  km from the Yelbeni site. The Yelbeni and C1–C3 control collection sites

had a grey-brown loam soil type with  $\sim 40\%$  gravel, a soil pH (CaCl<sub>2</sub>) of 5.3 ( $\pm 0.2$ ) in the surface layer (0–10 cm), and a long-term average annual rainfall of 308 mm.<sup>16</sup> The Yelbeni, C1 and C2 locations had a continuous cropping history for the previous 10 years, consisting predominantly of wheat, lupin (*Lupinus augustifolius* L.) and canola (*Brassica napus* L.). The site was managed using conservation farming techniques,<sup>17</sup> with wild radish control reliant on herbicides and HWSC. The progeny of two additional ruderal wild radish populations (known herein as C4P and C5P; Table 1), were added later, in 2018, to increase the number of control populations for comparison.

### 2.2 Common garden study

Two common garden studies were conducted in 2017 and 2018, during the winter cereal growing season, at Shenton Park field station of the University of Western Australia ( $-31.950875$  S,  $115.793127$  E). The aim was to compare the morphological traits, seed production and silique-shedding characteristics of the collected wild radish populations, in the absence of crop competition or abiotic (moisture or nutritional) stress. In May 2017, the field-collected putative silique-shedding population (Yelbeni) and three control wild radish populations (C1–C3) ( $n = 20$  per population) were grown in the experiment without crop competition. Seeds with >5 mm of emerged radicle were planted at a soil depth of 5 mm. Using a completely randomized design, each plant was located at 2-m intervals in the centre of 1-m-wide rows lined with plastic to eliminate inter and intraspecific competition. Concurrently with the primary experiment, 10 randomized locations at the trial site were sown with Mace wheat (cv. Mace, Australian Grain Technologies). This served as a control for assessing the timing of wheat maturity. Before planting, each seedling site was pre-fertilized with 6 g NPK Blue Special™ (CSBP Ltd, Kwinana, WA, Australia) [N 12% (NH<sub>2</sub> 8%, NH<sub>4</sub> 1.9%, NO<sub>3</sub> 2.1%), P 5.2%, K 14.1%, S 6%, Mg 1.2%, Ca 4.3%, B 0.02%, Fe 0.08%] and 2 g Yates Flowtrace™ (Yates, NSW, Australia) (Fe 24%, Cu 0.75%, Mn 0.5%, Zn 0.2%, Mo 0.04%, B 0.033%), placed 10 cm deep to ensure adequate separation from the emerging plants and to ensure adequate growth without nutritional constraint. Every 6 weeks following emergence, an additional 4 g ammonium sulphate (N 21%, S 24%), was applied at the base of each plant to maintain growth. Adequate soil moisture was maintained with supplementary irrigation, with fungicides and insecticides applied routinely as required. Plants were inspected every 2 days for flowering initiation (as marked by the protrusion of the corolla from the calyx) with the data presented as the days from transplanting to start of flowering. Once the wheat growing at the site had

**Table 1.** Location of collection of intensively harvest weed seed control (HWSC) selected (Yelbeni) and control (C1–C5) wild radish populations,  $n$  = number of plants collected from the field site

Designation	Population	Location	Silique shed from plant at the collection site (%)	$n$	Coordinates
Yelbeni	Yelbeni	South Yelbeni, Western Australia	86%	28	$-31.31144444$ S; $117.6761389$ E
C1	In field control 1	South Yelbeni, Western Australia	3.4%	20	$-31.29511111$ S; $117.6759444$ E
C2	In field control 2	South Yelbeni, Western Australia	0.8%	20	$-31.37166667$ S; $117.7033333$ E
C3	Roadside control 1	South Yelbeni, Western Australia	0%	20	$-31.24461124$ S, $117.6558763$ E
C4	Roadside control 2	Mingenew, Western Australia	0%	20	$-29.16451390$ S, $115.4052753$ E
C5	Roadside control 3	Coorow, Western Australia	0%	10	$-29.87054047$ S, $116.1299604$ E

reached the soft dough stage (Zadoks scale 83), the aboveground height of the lowest silique on a branch of the wild radish plants were measured. Wild radish plants were then manually harvested, with shed siliques defined as siliques that had separated from the plant, without external intervention and were located at the base of each plant at the time of wheat soft dough stage (Z83), while retained siliques were still attached to the plant. Seed predation from pests was minimized by growing the trial under bird netting and intensive baiting for rodents. The total number of seed shed and retained, and the total seed production was assessed by extracting the seed from the siliques using a modified grist mill. Extracted seed was counted using a S-25 seed counter (Data Technologies, Tzora, Israel).

At flowering, five randomly selected plants from each population were covered with insect exclusion netting to prevent the ingress of foreign pollen and hand cross-pollinated using the bee stick method<sup>18</sup> to produce progeny (Table 2). In May 2018, the initial common garden experiment was replicated in the absence of interspecific competition. This trial replication utilized offspring (known hereafter as Yelbeni P) derived from seeds produced by Yelbeni plants cultivated under insect exclusion screens in the previous year. Additionally, seeds from C1 to C3 populations cultivated concurrently under insect exclusion screens in a controlled environment, with no insect, fertility or pathogen stress, were used (known hereafter C1P–C3P) (Table 2). To increase the number of control populations for comparison in this study, the progeny of the field-collected populations C4P and C5P (which were also grown at the same Shenton Park site in 2017) were added to this study (Table 1).

### 2.3 Statistical analysis

Data analysis was performed using R STUDIO v2021.09.1. Assessments of interpopulation diversity for each trait were made by comparing the range, mean and standard error (SE) of the mean. Normality was tested via a Shapiro–Wilk test. As flowering time was normally distributed, analysis of variance (ANOVA) was used to test for statistical differences amongst populations and experiments/years. The traits lowest silique, silique shed and total seed production were not normally distributed so a nonparametric Kruskal–Wallis test was performed to assess variability between populations and each year. There were no significant differences between the paddock and roadside control populations (C1–C3; Table 3), so these populations were pooled.

In order to compare the flowering time response of the Yelbeni population to the pooled control wild radish populations, non-linear regression analysis was performed using the *DRC* package in R STUDIO 3.0.0.<sup>19</sup> The observed population flowering time was fitted to a four-parameter logistic model (Eqn 1):

$$Y = c + \frac{(d-c)}{1 + e^{b(\log x - \log F)}} \quad (1)$$

where *Y* denotes cumulative flowering expressed as a percentage of the total population. *F* represents the *FT*<sub>50</sub>, the time to a flowering response (days after emergence) that occurs midway between the maximum limit (*d*), set to the total percentage of the population observed to flower, and the minimum asymptotic value of *Y* (*c*), which denotes the initial percentage of the population that has flowered, preset to 0. The parameter *b* is indicative of the relative slope at *F*, giving the rate at which the percentage of flowering increases over time. The selectivity indices (SI) function within

RStudio was used to statistically compare ( $P \leq 0.05$ ) the *FT*<sub>50</sub> parameters of the Yelbeni and pooled control populations. A Lack-of-Fit test was also applied to each curve to ascertain the appropriateness of the model (Eqn 1) in R 3.0.0.

## 3 RESULTS

### 3.1 Inter- and intrapopulation diversity of traits

HWSC is a highly effective method for managing wild radish seed-banks. This is because wild radish typically retains a significant portion of its seeds until the crop reaches maturity. These seeds are often held above the standard cutting height used during harvest, making them ideal targets for interception and control through HWSC techniques.<sup>20</sup> At the Yelbeni collection site the persistent wild radish plants shed a mean of 86% ( $n = 28$ ) of their siliques onto the soil before the surrounding wheat crop reached the soft dough stage (Z83) (Table 1). This seed collected from the soil surface were labelled the Yelbeni population. The expected silique retention at wheat crop maturity (hard dough Z87) is 99%.<sup>6</sup>

#### 3.1.1 Flowering time

In 2017, the field-collected Yelbeni population initiated flowering 22 days earlier than the pooled control populations (C1–C3) at 45 days postemergence (774 GDD) ( $P < 0.01$ ; Table 2). Likewise, Yelbeni P in 2018, (the progeny of the field-collected Yelbeni population), flowered 44 days postemergence (667 GDD), which was 19 days before the pooled control populations (C1P–C5P) (63 days and 1043 GDD) ( $P < 0.01$ ) (Table 2). However, at a population level the difference in median flowering time was less, with a predicted time for 50% of the population to flower (*FT*<sub>50</sub>) being 71 days postemergence (1217 GDD) for the Yelbeni P population compared with 77 days (1343 GDD) for the pooled control populations (C1P–C5P) (Table 2; Fig. 1).

#### 3.1.2 Silique height

For HWSC to be effective, the silique must be intercepted by the harvesting machinery. Siliques located >10 cm aboveground are considered likely to be intercepted by the harvest operation.<sup>5</sup> In this study the lowest silique was consistently closer to the ground in the Yelbeni population at 16.2 cm ( $\pm 3$ ) compared with the pooled control populations (C1–C3) at 33.1 cm ( $\pm 3.7$ ) ( $P < 0.01$ ). Whilst the Yelbeni population had a lowest silique height of 16.2 cm, above the nominal cutting height of 10 cm, it was found that some individuals in the population had siliques located as low as 5 cm off the soil surface, which was likely to limit HWSC interception. Using shed seed for the progeny, it was found that the Yelbeni P population located its lowest silique at 1 cm ( $\pm 0.6$  cm) compared with the pooled progeny control populations (C1P–C5P) at 14.9 cm ( $\pm 2.3$  cm) owing to the proliferation of a more pendulous form ( $P < 0.01$ ; Table 3).

#### 3.1.3 Seed-shedding before wheat maturity

In 2017, the quantity of seeds shed per plant was greater for the Yelbeni population with a range between 0 and 10 376 seeds and shedding 2349 or 369% more seeds per plant compared to the pooled controls (C1–C3) for which the number of seeds shed ranged between 0 and 4832 ( $P < 0.01$ ; Table 3). When repeated in 2018 using the progeny from shed seed grown in a common environment to remove environmental factors, it was found that Yelbeni P shed 1048 ( $\pm 288$ ) seeds per plant with a range between

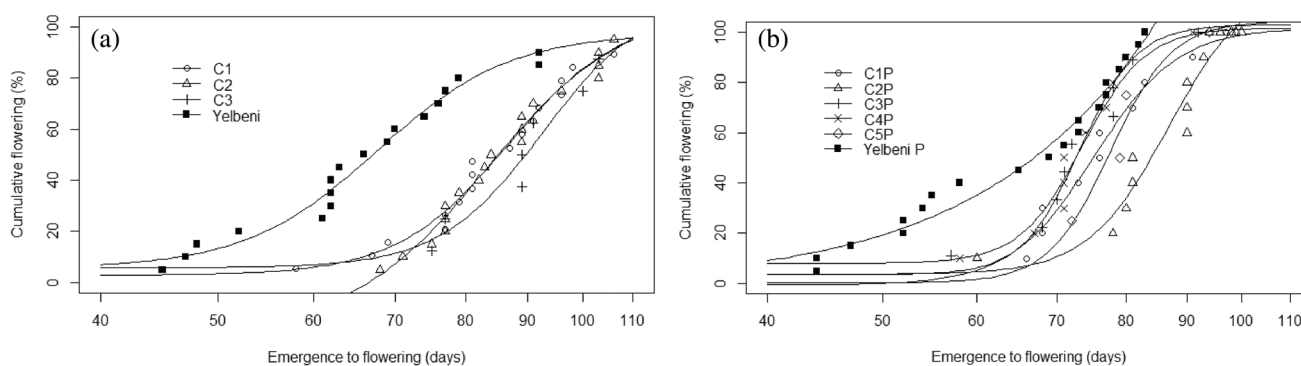
**Table 2.** Descriptive statistics and parameters estimates from the four parameter log-logistic model (Eqn 1) used to estimate the flowering time 50% (FT<sub>50</sub>). Standard errors for parameter estimates are in parenthesis for wild radish populations

Year	Population	Days from emergence to flowering			Parameter estimates from the four parameter log-logistic model (Eqn 1)				Predicted cumulative GDD (°C d) based on flowering time (FT <sub>50</sub> )	Population comparison P-value	
		Mean (SE)	Range (Min–Max)	Days to first flower	Cumulative GDD (°C day) for 1st flower	b (SE)	c (SE)	d (SE)			F Predicted flowering time (FT <sub>50</sub> ) (SE)
2017	C1-Control	87 (±3.5)	58–114	58	1027.0	–9.38 (1.44)	2.81 (4.72)	105.00 (6.15)	86.7 (1.4)	1545.9	0.83
	C2-Control	88 (±2.9)	68–114	68	1193.7	–7.75 (2.17)	3.84 (6.64)	108.09 (10.72)	83.2 (2.4)	1487.8	
	C3-Control	92 (±5)	75–114	75	1328.9	–10.26 (4.27)	4.74 (7.41)	109.47 (13.65)	92.5 (2.1)	1647.8	
	Pooled control results	88 (±2)	58–114	67	1183.2	–8.74 (1.13)	3.27 (3.62)	107.78 (5.67)	87.2 (1.2)	1566.4	
2018	Yeibeni	71 (±4.4)	45–114	45	774.9	–7.93 (1.21)	5.60 (4.21)	97.59 (3.63)	67.6 (1.1)	1506.8	0.81
	C1P-Control	78 (±3.4)	66–97	66	1148.0	–12.39 (2.57)	0.65 (6.19)	101.75 (7.97)	75.2 (1.5)	1306.9	
	C2P-Control	84 (±3.4)	60–94	60	975.3	–12.84 (2.74)	3.62 (4.87)	104.51 (8.09)	86.8 (2.1)	1428.5	
	C3P-Control	74 (±3.4)	57–92	57	917.3	–16.45 (3.90)	5.99 (7.08)	101.75 (8.50)	73.5 (1.4)	1183.8	
	C4P-Control	74 (±2.9)	58–91	58	1011.6	–17.93 (3.75)	3.44 (4.92)	103.08 (7.61)	73.1 (0.9)	1268.6	
Pooled control results	C5P-Control	81 (±5.3)	72–94	72	1164.1	–16.77 (5.13)	0.19 (6.61)	104.50 (9.04)	77.6 (1.3)	1361.2	<0.01
	Pooled control results	78 (±1.6)	57–97	63	1043.3	–8.38 (2.06)	–0.56 (5.58)	103.11 (7.64)	76.7 (0.4)	1343.3	
	Yeibeni P	66 (±3.1)	44–83	44	667.5	–3.16 (0.83)	–1.32 (11.21)	102.08 (4.36)	70.8 (1.89)	1217.3	

Abbreviations: GDD, Growing Day Degrees.

**Table 3.** Descriptive statistics for wild radish populations

Year	Population	Height of the lowest silique (cm)			Total number of seeds shed per plant			Total number of seeds produced per plant		
		Mean (SE)	Range (Min-Max)	<i>P</i> -value	Mean (SE)	Range (Min-Max)	<i>P</i> -value	Mean (SE)	Range (Min-Max)	<i>P</i> -value
2017	C1-Control	31.4 (±2.4)	14–54	>0.25	876 (±264)	8–4399	0.14	3037 (±500)	846–9439	0.27
	C2-Control	32.1 (±2.7)	15–53		1703 (±358)	0–6279		4093 (±442)	932–9135	
	C3-Control	39.8 (±6.3)	15–72		827 (±546)	0–4605		3472 (±798)	1414–7993	
	Pooled control results	33.1 (±3.7)	14–72	<0.01	1135 (±208)	0–4832	<0.01	3561 (±303)	846–9439	0.06
	Yelbeni	16.2 (±3)	5–42		3220 (±562.1)	0–10 376		5355 (±887)	160–16 513	
2018	C1P-Control	10.8 (±4.6)	0–40	>0.38	48 (±20.1)	0–169	0.18	4700 (±1131)	1016–10 631	0.22
	C2P-Control	21.2 (±3.8)	0–40		16 (±10)	0–88		3876 (±823)	1646–9469	
	C3P-Control	14 (±3.8)	0–30		3 (±2)	0–17		2327 (±445)	801–4471	
	C4P-Control	15 (±3.9)	0–28		34 (±18.9)	0–146		3701 (±470)	1695–5936	
	C5P-Control	11.3 (±7.6)	0–30		15 (±12.5)	0–46		2705 (±763)	971–3952	
	Pooled control results	14.9 (±2.3)	0–40	<0.01	25 (±7)	0–169	<0.01	3594 (±357)	801–10 631	<0.01
	Yelbeni P	1 (±0.6)	0–8		1048 (±288.3)	0–4087		8610 (±847)	3796–15 622	



**Figure 1.** The observed population response for flowering time for (A) wild radish population Yelbeni compared to the field-collected control populations C1, C2 and C3, and (B) wild radish progeny Yelbeni P compared to the progeny control populations C1P, C2P, C3P, C4P and C5P. Each symbol represents cumulative data points of 20 replicate plants. The plotted lines are predicted cumulative flowering date curves fitted to a four-parameter logistic model [Eqn (1)]. The parameter estimates can be found in Table 2.

0 and 4087 seeds, compared to 25 (±7) seeds per plant for the pooled control populations (C1P–C5P) with a range between 0 and 169 seeds ( $P < 0.01$ ). These results equate to the Yelbeni P population shedding seeds at a rate 4192% higher than the control populations (Table 3).

### 3.1.4 Total seed production

The total number of seeds produced per plant was greater for the Yelbeni population, with a mean of 5355 (±887) seeds, compared to the C1–C3 control with 3561 (±303) seeds ( $P < 0.01$ ). For the progeny, Yelbeni P produced 8610 (±847) seeds per plant compared to 3594 (±357) seeds per plant for the control populations (C1P–C5P) ( $P < 0.01$ ). The control populations did not significantly differ in fecundity from each other in both 2017 and 2018 ( $P = 0.37$ ).

The results show that the Yelbeni population was found to differ from the control populations in flowering time, silique height location, silique abscission rate and total seed production ( $P < 0.01$ ; Tables 2 and 3).

## 4 DISCUSSION

Harvest weed seed control was first adopted in Australia in the 1980s with the importation of chaff carts from Canada, which were originally used to collect the nutritious chaff (comprising partially filled grain, rachis and glume) fraction during harvest for use as stockfeed.<sup>21</sup> However, with the increase of multiple herbicide resistance in annual weed species in Australia,<sup>2</sup> growers quickly identified the potential of using chaff carts to intercept and manage weed seeds before they enter the soil weed seed bank,<sup>22,23</sup> thereby driving weed soil seed banks into decline.<sup>7</sup> However, as previously demonstrated with the use of herbicides,<sup>11</sup> the long-term use of effective weed control techniques such as HWSC, can inadvertently select for mechanisms that enable weeds to evade control measures.<sup>9,14</sup> Here a population of wild radish from Yelbeni in Western Australia was found to persist in cropping paddocks, despite an extended history (>20 years) of annual HWSC use. To persist, this Yelbeni population exhibited attributes including the development of siliques close to the ground, thereby below the nominal height for harvest



interception.<sup>6</sup> The Yelbeni population also had greater silique-shedding from the plant, largely before the crop (wheat) was mature for harvest. These traits were found to be heritable under conditions of no nutrient or moisture stress at the experimental site.

#### 4.1 Silique location and form

As per Walsh and Powles,<sup>6</sup> seed located below the minimum harvest cutting height is considered unlikely to be intercepted by the harvesting process. The Yelbeni P population was observed to locate its lowest silique at a mean height of 1 cm  $\pm$ 0.6 in the absence of crop competition as a consequence of a pendulous form which located siliques near the soil surface. This pendulous form was exhibited before flowering and was typical of the Yelbeni plants grown in the field, and therefore was not considered to be the result of silique weight on the branches. It is proposed that a more pendulous phenotype is more prevalent where HWSC has been routinely used.

Species shifts or the selection of prostrate forms that locate seed close to the ground to evade collection has been demonstrated in turf, which is subject to repeated mowing.<sup>24</sup> Whilst a less erect growth pattern may lead to HWSC evasion, reduced plant height is likely to have ecological costs within competitive field crops.<sup>25,26</sup> Within plant canopies, when crops are grown close together, they absorb a significant portion of the photosynthetically active radiation (PAR), leaving less available for competing plants such as weeds. This competition also alters the light spectrum within the canopy. This shift in light quality can lead to a decrease in overall plant biomass and reproductive potential. Additionally, it encourages plants to grow taller and more upright, potentially increasing the height of the siliques. Any change towards a more erect growth form makes the plants more prone to interception by HWSC.<sup>27–29</sup> Whilst this study was conducted in the absence of crop competition, it has been found that wild radish<sup>28,30</sup> and annual ryegrass (*Lolium rigidum* Gaudin) grown within competitive canopies have higher seed locations,<sup>31</sup> resulting in increased HWSC interception.

#### 4.2 Wild radish seed-shedding before crop harvest

Surveys of the Australian dryland cropping fields have found that HWSC-susceptible wild radish populations shed <1% of their total seed production before crop harvest, making wild radish an ideal candidate for HWSC.<sup>6</sup> However, this study demonstrated that the Yelbeni population shed 62% of its seed when grown without crop competition or abiotic stress. This trait occurred in the seed-shed progeny with 94% seed-shedding (Yelbeni P) when grown without stress. The significant increase in the percentage of seed-shedding in the progeny of the Yelbeni population, highlights the potential for purification and/or recombination of the trait when selected. Previous studies indicate that a plant mortality of 99% is required to effectively reduce a wild radish population.<sup>32,33</sup> Given the widespread occurrence of multiple herbicide resistance in wild radish populations,<sup>34</sup> it is expected that any trait reducing HWSC interception will be likely to result in the persistence of a wild radish population.

Recurrent selection studies on wild radish by Ashworth *et al.*<sup>14</sup> demonstrated the adaptive capacity of a small wild radish population to produce early-maturing phenotypes that had reduced height and biomass at maturity, leading to less competitive, more pendulous growth forms.<sup>14</sup> Flowering time is expected to be an important factor leading to silique-shedding, as earlier flowering allows for sufficient time for silique maturation before early

shedding.<sup>14,20,30</sup> The Yelbeni and Yelbeni progeny (Yelbeni P) had similar early maturity and pendulous growth forms as demonstrated by Ashworth *et al.*<sup>14</sup> The Yelbeni P population demonstrated a 19-day reduction in the start of flowering and a 6-day reduction in flowering at the population level (FT<sub>50</sub>) (Table 2; Fig. 1) compared to the pooled control populations. However, the flowering time changes in the Yelbeni population and Yelbeni P progeny are modest compared to flowering time changes reported by Ashworth *et al.*<sup>14</sup> The 6-day (FT<sub>50</sub>) reduction in flowering time (Yelbeni P) was within the range of flowering times expressed in the unselected populations (C1P–C5P) which do not have an early silique-shedding or pendulous form trait. As the control populations expressed flowering times across the range expressed in the Yelbeni/Yelbeni P populations, flowering time is unlikely to fully account for the change in silique-shedding or plant form as demonstrated previously by Ashworth *et al.*<sup>14</sup>

Continuous application of HWSC constitutes a significant selection pressure for HWSC-evading traits as >99% of the seed in wild radish populations can be intercepted by HWSC techniques.<sup>6</sup> The mechanism of seed-shedding by dicot species is determined by a complex array of plant signalling networks that includes hormones and gene regulators responsible for thickening and dissolving of cell layers within the silique abscission zone.<sup>35</sup> Previous studies on the model *Brassicaceae* sp. *Arabidopsis thaliana* L. found that seed-shedding is primarily genetically controlled, initiating the lignification and separation of the silique margin,<sup>36–38</sup> and changing protein deposition in the silique wall structure<sup>39,40</sup> leading to silique-shedding. The mechanism of silique-shedding for wild radish has yet to be identified and requires further study; however, seed shattering in plants is governed by a series of intricate physiological and genetic processes, which are further influenced by environmental conditions. While the underlying mechanisms of this process have been extensively studied and are relatively well-understood in cultivated crops, the knowledge regarding these mechanisms in most wild and weedy species remains limited.<sup>35</sup>

Environmental conditions such as low humidity and high temperatures may exacerbate the effect of early silique-shedding by increasing the tension between the individual layers of the abscission zone.<sup>41</sup> Environmental factors such as low humidity<sup>42,43</sup> and high winds<sup>43,44</sup> have been shown to increase seed-shedding in canola (*Brassica napus* L.). The Yelbeni site in the central grainbelt of Western Australia is a mediterranean environment characterized by mild winter growing conditions and hot/dry spring conditions during maturation. These hot/dry conditions may exacerbate any seed-shedding characteristic within the silique abscission zone.

#### 4.3 Harvest weed seed control management consequences

HWSC is currently adopted in Australia and North America to control seedbank inputs of annual weed species such as annual ryegrass (*Lolium rigidum*),<sup>31</sup> wild radish (*Raphanus raphanistrum*)<sup>6</sup> and weedy amaranths (*Amaranthus* spp.)<sup>45,46</sup> as they retain much of their seed on the plant until crop harvest. Many other weed species retain a high proportion of seed until harvest including tall waterhemp (*Amaranthus tuberculatus*) (94% retention),<sup>46</sup> giant ragweed (*Ambrosia trifida*) (>80% retention),<sup>47</sup> jointed goat-grass (*Aegilops cylindrica*) (76% retention),<sup>48</sup> downy brome (*Bromus tectorum*) (>75% retention),<sup>6</sup> field mustard (*Sinapis arvensis*) (>65% retention),<sup>49</sup> chickweed (*Stellaria media*) (>55% retention),<sup>45</sup> corn spurry (*Spergula arvensis*) (>45% retention)<sup>50</sup> and black-bindweed

(*Fallopia convolvulus*) (>40% retention),<sup>50</sup> making HWSC an important weed control tool globally. Species with high seed retention rates impose a greater selection intensity for HWSC-evading traits, therefore seed-shedding should be monitored. In addition, to maintain the effectiveness of HWSC techniques, attention needs to be given to combining the use of HWSC techniques with agronomic practices that will put HWSC-evading traits at an ecological disadvantage. Early emerging weed cohorts have a greater time to mature and shed silique before harvest, therefore the effective use of residual pre- and postemergent herbicides is important.<sup>20</sup> It also is imperative to maximize crop competitiveness, as this encourages erect growth of the weed and is likely to put less erect growth forms at an ecological disadvantage.<sup>30</sup> Designing crop rotations that allow different herbicide MoAs to be used and diversify crop maturity timing will be likely to be useful in reducing the predictability of harvest techniques, limiting the selection of seed-shedding traits. This diversity may also be further improved by adding divergent cultivar maturity timing, pre-harvest swathing or weed desiccation within short-term pasture phases.

#### 4.4 Limitations of the study

This study introduces the traits of a wild radish population that has persisted in a Western Australian field with long term HWSC. The wild radish populations were assessed for silique-shedding at a single point in time when wheat planted at the experimental site was at the soft dough stage (Z83), replicating the growth stage (Z83) at the field site in Yelbeni in 2016. However, the length of the wheat growing season can change according to location and cultivar choice, therefore the proportion of siliques shed per population may change. Other traits such as flowering time, inflorescence height and plant form may also change with location, seasonal conditions and management practices, and therefore additional studies are required. Importantly, Walsh and Powles<sup>6</sup> demonstrated that environmental factors such as temperature, rainfall, growing season length, and inter- and intraspecific competition, did not affect wild radish silique retention on HWSC-susceptible populations. This aforementioned study was assessed at wheat maturity (Zadoks 93) across nine geographically distant locations of the Australian grainbelt.

This study demonstrates that a population of wild radish that differs in life-history traits can persist within HWSC-managed fields; however, further consideration should be given to the origin of these adaptations. Whilst the recurrent selection of advantageous HWSC-evading traits from a genetically diverse population can occur, the observed differences in HWSC-evading traits within the Yelbeni population may also be the result of the ingress of offsite genetics from either gene flow or field contamination. Field contamination may also result in the founder effect where a new colony is started by a few members. An isolated (>100 m from field edge) small population of nongenetically representative individuals may purify advantageous HWSC-evading traits, leading to rapid genetic divergence. Weed populations can colonize fields through the use of contaminated machinery or poorly cleaned crop seed.<sup>51–53</sup>

This study was conducted in the absence of interspecific competition to demonstrate the lifecycle adaptations of the Yelbeni wild radish population. It is possible that the expression of these life-history traits may differ if studies were performed in a competitive environment.<sup>28,30,31</sup>

## 5 CONCLUSION

Here, we introduce a population of wild radish that has evolved HWSC-evading traits including silique positioning below the harvest cutting height and increased silique-shedding onto the soil before harvest. The combination of these phenotypic changes provided an effective survival strategy when under recurrent selection from HWSC. With HWSC research and adoption increasing globally to control multiple herbicide resistance-prone species,<sup>54</sup> it is imperative to protect HWSC through the implementation and integration of crop rotations and pastures, herbicide rotation, increased crop competition and the diversification of weed seed set reduction techniques.

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## CONFLICT OF INTEREST

No conflicts of interest have been declared.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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