

Recurrent selection on wild radish (*Raphanus raphanistrum* L.) flowering time leads to rapid adaption of reproductive phenology

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Summary Harvest weed seed control (HWSC) is an emerging tool that offers much needed diversity from the sole use of herbicides to control the growing number of multiple resistant weed species worldwide. However the efficiency of HWSC is contingent upon the effective collection of weed seeds at harvest. Earlier flowering biotypes have been considered likely to lead to HWSC evasion through early seed shedding prior to harvest. This study demonstrates that when flowering time selection is applied to an unselected population of wild radish (*Raphanus raphanistrum* L.), flowering time (FT) can halve from 60 days after emergence (DAE) (FD₅₀) in the commencing population (G0) to 29 DAE (FD₅₀) following five generations of early FT selection.

Keywords Recurrent selection, wild radish, flowering time, harvest weed seed control.

INTRODUCTION

With no new herbicide modes of action likely to be discovered in the near future (Duke 2012), the widespread evolution of herbicide resistance in over 430 species (Heap 2014), has necessitated the development of alternative, non-herbicidal weed control strategies that manipulates niches in the biology of species for control (Murphy 1998, Madafiglio *et al.* 2006, Walsh and Powles 2007). One novel, non-herbicidal technique for weed management is harvest weed seed control (HWSC), which aims to intercept, concentrate and treat weed seeds that are retained on plants during the commercial grain harvest (Walsh *et al.* 2013).

One weed species that is biologically suited for HWSC is wild radish (*Raphanus raphanistrum* L.). In Australia, wild radish is considered to be the most problematic dicot weed species (Alemseged *et al.* 2001, Borger *et al.* 2012) causing significant yield losses in commercial dryland and horticultural crops (Code and Donaldson 1996, Blackshaw *et al.* 2002), evolving resistance to multiple herbicide groups, such as inhibitors of acetolactate synthase (ALS) (Hashem *et al.* 2001), phytoene desaturase (PDS) (Walsh *et al.* 2004), photosynthetic electron transport (Hashem *et al.*

2001), synthetic auxin herbicides (Walsh *et al.* 2004) and inhibitors of EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) (Ashworth *et al.* 2014).

HWSC techniques apply an intense selection for evasion. As the effectiveness of the HWSC is contingent upon weed seeds being remaining on plants above the cutting height at harvest (Walsh *et al.* 2012), it has been suggested that HWSC control may select for or earlier seed shedding biotypes (Baker 1974) with earlier flowering considered likely to increase the risk of fruit abscission prior to harvest (Panetos and Baker 1967, Panetta *et al.* 1988).

As earlier flowering biotypes are considered more likely to shed seed prior to harvest, this study investigated the potential for a previously unselected wild radish population to adapt its FT in response to recurrent FT selection. Changes in the height and biomass of the population were also assessed.

MATERIALS AND METHODS

This selection study was conducted using the wild radish biotype WARR7 (referred herein as G0), collected prior to the development of harvest weed seed control practices (Walsh *et al.* 2004). Using this commencing population (G0), five successive generations of recurrent early FT selection was conducted in October 2011 (EF1), December 2011 (EF2), March 2012 (EF3), July 2012 (EF4) and December 2012 (EF5). During each selection, concurrently grown control populations were maintained in the absence of selection (CE1–CE5). Where possible, the previous selection was re-grown to check the phenotypic advancement of each selection and produce progeny to ascertain the heritability of each selected generation (EF1C–EF4C).

Flowering time (FT) selections were made from a commencing population of 1300 plants with successive selections made from populations of 250 plants. Wild radish seeds (G0) greater than 2.2 mm in diameter were pre-germinated on solidified water agar (0.6% w/v) in darkness for 2 days. Pre-germinated seeds were transplanted into pots containing standard potting mixture. Pots were maintained in an outdoor growth facility at the University of Western Australia

(Crawley; Perth, Western Australia) during their normal growing season (June–October). All pots were watered regularly and fertilised weekly with 2 g Scotts CalMag grower plus™ soluble fertiliser. Initial selections (13 plants; 1%) were selected, based upon the number of days from emergence to the opening of the first flower. Subsequent selections based upon 25 plants (10%). Selected plants were isolated to ensure cross pollination Newly opened flowers were crossed using the Beestick method as outlined by Williams (1980), ensuring a random pattern of cross pollination (panmixia). At maturity, the same number of siliques were harvested from each plant in the population and bulked. Mature siliques were then processed using a modified grist mill.

The rate of FT progression was evaluated by growing the commencing (G0), all selected (EF1–EF5), all control (CE1–CE5) and all selected progeny generations (EF1C–EF4C), at the same time within temperature regulated glasshouse conditions during a period of stable to gradually increasing day length (June onward). For the duration of the experiment, temperatures were maintained above the base temperature for wild radish growth (4.5°C) (Mekienian and Willemsen 1975, Reeves *et al.* 1981), to a nominal setting of 25°C day and 15°C at night.

Measurements The date of first flowering and the height of the first flower were recorded. Above ground biomass at the initiation of flowering was harvested and dried at 65°C for 7 days before weighing.

Statistical analysis To compare the FT response of recurrently selected wild radish populations, non linear regression analysis was performed using the drc package in R 3.0.0 (R Development Core Team 2011; <http://www.R-project.org>) (Streibig *et al.* 1993 and Price *et al.* 2012). The observed population FT was fitted to a four-parameter logistic model (1):

$$Y = c + (d - c / (1 + \exp \{b (\log x - \log e)\})) \quad (1)$$

where Y denotes cumulative flowering as a percentage of the total population, e is the FD_{50} , denoting the time or accumulated temperature producing flowering response is half-way between the upper limit, d (fixed to the total percentage of the population collected) and c which is the lower asymptotic value of Y (set to 0). The parameter b denotes the relative slope around e.

The relative importance of accumulated heat units (GDD) to flowering was compared between each selection as described by Marcellos and Single (1971) using equation:

$$GDD = \sum (T_{\max} + T_{\min}) / 2 - T_{\text{base}}$$

where T_{\max} is the daily maximum temperature, T_{\min} is the daily minimum temperature, T_{base} is the base temperature for wild radish (4.5°C) (Mekienian and Willemsen 1975, Reeves *et al.* 1981, Norsworthy *et al.* 2010). The accumulated photoperiod (hours) prior to flowering was reported as the accumulated photoperiod 7 d prior to flowering as described by Norsworthy *et al.* (2010).

RESULTS

Following five generations of early FT selection, FT was halved at the population level (FD_{50}) from 59 DAE (G0) to 29 DAE (EF5) (Table 1), reducing the thermal requirement prior to flowering (GDD), from 634°C d (G0) to 344°C d (EF5). These FT reductions were considered to be heritable with FT in all selected population progenies (EF2C–EF5C) not appreciably shifting from the parental lines (EF1–EF4) (Table 1). In the absence of selection, the concurrently grown control generations (CE1–CE5) negligibly changed FT compared to the commencing population (G0) (Table 1).

DISCUSSION

This study clearly demonstrates that an unselected wild radish population (G0) contains sufficient genetic diversity to rapidly adapt its FT in response to FT selection. Five generations of early FT selection, halved FT at the population level (FD_{50}). These rapid changes in FT concur with observed field and glasshouse studies in wild mustard (*Brassica rapa* L.) (Franke *et al.* 2006, Franks *et al.* 2007, Franks 2011).

The rate of fruit abscission was not determined in this study. However as a consequence of early FT selection, the number of individuals in the population carrying well matured pods at harvest would rapidly increase. This is expected to increase the probability of silique abscission prior to harvest, especially during periods of water deficit, high temperature or wind (Taghizadeh *et al.* 2010, 2012).

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Table 1. Parameter estimates (days to flowering) following early flowering time selection using the four parameter logistic model (1) used to estimate FD_{50} parameters. Standard errors for parameter estimates are in parentheses. Selection ratios were calculated based upon FD_{50} values for the unselected commencing population (G0) and respective selected or unselected control progeny.

		d	b	e FD_{50} (DAE)	e FD_{50} Selection ratio	Shift from G0 (days; FD_{50})	Flowering range (days)
Commencing	G0	100	-9.79 (0.2)	59 (0.2)	–		52
Selected	EF1	100	-9.65 (0.2)	51 (0.1)	0.8	-8	35
	EF2	100	-19.19 (0.5)	57 (0.1)	0.9	-2	31
	EF3	100	-11.70 (0.3)	45 (0.1)	0.7	-14	19
	EF4	100	-11.38 (0.4)	37 (0.1)	0.6	-22	19
	EF5	100	-14.38 (0.4)	29 (0.1)	0.5	-30	13
Unselected	CE1	100	-16.93 (0.6)	61 (0.1)	1.0	2	34
	CE2	100	-10.54 (0.3)	57 (0.1)	1.0	-2	32
	CE3	100	-11.10 (0.3)	59 (0.1)	1.0	0	37
	CE4	100	-9.96 (0.3)	58 (0.1)	1.0	-1	45
	CE5	100	-12.63 (0.4)	62 (0.1)	1.0	3	41
EF1 progeny	EF2C	100	-10.76 (0.3)	46 (0.1)	0.9	-5 [#]	27
EF2 progeny	EF3C	100	-26.07 (1.0)	60 (0.1)	1.1	3 [#]	32
EF3 progeny	EF4C	100	-11.82 (0.3)	48 (0.1)	1.0	3 [#]	20
EF4 progeny	EF5C	100	-12.09 (0.4)	38 (0.1)	1.0	1 [#]	23

[#]Progeny shifts compared parental population (EF2C to EF1, EF3C to EF2, EF4C to EF3 and EF5C to EF4).

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