Refinement of weed risk assessments for biofuels using *Camelina sativa* as a model species

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Summary

1. Biofuel production has the potential of reducing CO_2 emissions while decreasing global dependence on fossil fuels. However, concerns have been raised on the invasiveness of biofuel feedstocks. Estimating invasion potential remains a challenge because of inconsistencies and inherent limitations of using first-tier qualitative weed risk assessment (WRA) protocols singularly.

2. We evaluated the usefulness of second-tier quantitative WRA methods using a recently introduced oilseed crop, *Camelina sativa*, as a model species. First, we subjected *C. sativa* to the qualitative Australian WRA and found that it should not be allowed entry. We then used demographic models fit with field-estimated parameters as a second-tier approach to quantitatively evaluate its invasion potential. Data on disturbance (two herbicides, mechanical, none) and seeding season (autumn, spring) relative to *C. sativa* demography were obtained over 2 years in two rangeland ecosystems in Montana, USA. Population growth (λ) was forecast by developing population dynamics models using field data.

3. Emergence rates were greatest when *C. sativa* was spring-seeded; all survivors to maturity occurred only in mechanically disturbed plots. Population growth rate never exceeded 0·03, and the maximum time to extinction was 6 years. Perturbation analyses indicated that consistent propagule pressure and biologically improbable rates of seed survival are necessary to sustain *C. sativa* populations, indicating that the risk of invasion by this species in the studied ecosystems is low.

4. *Synthesis and applications.* Although more site-years of demographic data would strengthen our conclusions about the invasion potential of *C. sativa*, we contend that the methods developed provide a useful contribution to WRA. If applied to proposed plant biofuel species, our second-tier quantitative refinements will elucidate important population dynamics often overlooked by qualitative WRAs and, in turn, may reduce the frequency of invasions or rejection of potentially useful species.

Key-words: biofuels, *Camelina sativa*, demographic models, invasive species, plant invasions, population dynamics, quantitative weed risk assessment

Introduction

Biofuel production has the potential to reduce CO_2 emissions (Fargione *et al*. 2008) (but see Walker 2010) while decreasing global dependence on fossil fuels. Despite the motivation for supplementing fossil fuels with renewable fuels, biofuel feedstocks should not be cultivated without first determining their potential to escape and become invasive (Davis, Brainard & Gallandt 2008). Cultivation of these species has been questioned because several of their agronomic characteristics coincide with invasive plant attributes such as drought and low soil-fertility tolerance, short life cycle and rapid accumulation of biomass (Raghu *et al*. 2006; Barney & DiTomaso 2008). Additionally, biofuel feedstocks possess a high likelihood of becoming invasive because of sustained propagule pressure associated with large-scale cultivation (Mack 2000; Buddenhagen, Chimera & Clifford 2009; Davis 2009; Minton & Mack 2010). Unfortunately, identifying potential invaders and estimating the damage that may be incurred if a plant is introduced remains a challenge to invasion biologists.

Weed risk assessment (WRA) protocols are tools used for identifying potential non-native plant invaders to minimize the undesirable effects of invasions (Caley, Lonsdale & Pheloung

2006; Buddenhagen, Chimera & Clifford 2009). The goal of WRA is to create an objective, consistent and transparent process with which to determine a plant's invasive potential. WRAs are constructed so they can be performed relatively quickly, often with a limited amount of information, and by a multitude of assessors, each with a different level of expertise. By answering qualitative questions about species' biological traits, as well as characteristics of the native and proposed regions of introduction, an assessment outcome is obtained that can be used to influence decisions on species introductions.

Traditional risk assessment frameworks often rely on a tiered approach in which lower tiers are characterized by qualitative or quantitative assumptions that often are data-poor, rely on deterministic analyses and have high uncertainty. Higher tiers are characterized by numerous quantitative data, refined exposure assumptions and probabilistic analyses including statistical assessments of uncertainty and variability (NRC 1983). The risk assessment process is recursive so that when a risk characterization using lower-tier assumptions reveals concerns, higher-tier and more realistic assumptions are used (NRC 1983). In this tiered process, then, the risk assessment is refined.

Some qualitative WRA protocols are an effective first tier in the risk assessment process because they can be accomplished with reasonable accuracy and in a timely manner (Daehler & Carino 2000) while creating long-term net economic benefits (Keller, Lodge & Finnoff 2007). The Australian WRA (A-WRA) is widely accepted as the best system and has been adopted with little modification in other parts of the world, including New Zealand, Hawaii and the Pacific Islands, Africa, Canada and the Mediterranean (Daehler *et al.* 2004; Gassó, Basnou & Vilà 2010; McClay *et al.* 2010). On average, accuracy estimates of the A-WRA are 90% for identifying major invaders and 70% for correctly identifying noninvaders (Gordon *et al.* 2008).

There is consensus that the A-WRA is useful; however, limitations exist. Specifically, criticisms about assessor subjectivity and inconsistency, and challenges of defining 'invasive' and 'noninvasive', have been raised (Onderdonk *et al.* 2010). These confusions lead to mistakes, and ultimately, some invasive species will pass the assessment, naturalize and become invasive. However, these negative aspects of the A-WRA, and methods to minimize them, have been addressed and suggestions presented by Onderdonk *et al.* (2010) and Gordon *et al.* (2010). An additional concern with the A-WRA is that nearly a quarter of assessed species are placed in the ambiguous 'evaluate' category, although this is reduced to approximately 8% with the addition of a second screening developed by Daehler *et al.* (2004). To our knowledge, however, this has not been widely implemented. The recent discussions clarifying ambiguous aspects of the A-WRA and the second screening system developed by Daehler *et al.* (2004) are helpful and necessary to maximize A-WRA usefulness. Regardless of how many improvements and safeguards are implemented, however, a single-tiered approach will always be limited in its prediction accuracy (Cousens 2008; Davis *et al.* 2010). Therefore, multi-tiered assessments, particularly for high-value/high-risk biofuel feedstocks, may need to be used to maximize economic benefits and reduce chances of environmental degradation.

This study evaluated the usefulness of a quantitative second-tier WRA methodology using *Camelina sativa* (L.) Crantz (camelina, gold-of-pleasure, large-seeded false-flax), a proposed biofuel feedstock in the northern Great Plains (NGP), USA, as a model species. It possesses characteristics common to many plant invaders such as tolerance to adverse growing conditions (nutrient-poor soils, frost and drought), resistance to pests and disease, rapid life cycle and prolific production of small seeds (Putnam *et al.* 1993). Furthermore, *C. sativa* has a non-native weedy congener, *C. microcarpa* Andrz. ex. DC. (littlepod false-flax), and the possibility of hybridization between these species increases its invasive potential (Ellstrand & Hoffman 1990).

As a first-tier approach, we assessed the invasion potential of *C. sativa* using the A-WRA model and found that it should not be allowed entry (Davis 2010). Recommendations of 'reject' and 'evaluate' were obtained depending on how questions were answered (see Table S1 of Appendix S1 in Supporting Information). Also, because propagule pressure will increase substantially through cultivation, concerns about its invasiveness were raised. Therefore, we undertook a more thorough investigation not only to assess this species' potential to become invasive in the NGP but more importantly to investigate the practicality of a quantitative approach to WRA to be used as a starting point for more rigorous in-field assessments, as discussed by Cousens (2008) and Davis *et al.* (2010). Minton & Mack (2010) have recently contributed methods to assess the effects of cultivation and population size and density, on the persistence of founder populations. To our knowledge, their study is the first attempt at employing field-based empirical approaches to assess invasion potential. To complement Minton & Mack (2010), we suggest methods to capture the demography of biofuel feedstocks, as well as modelling exercises, to elucidate important drivers of population dynamics. To accomplish this, we parameterized a demographic model from site-specific data, thus resulting in probabilistic determinations of *C. sativa*'s invasion potential under likely scenarios. Specifically, we quantified *C. sativa* emergence, survival and fecundity rates in response to common disturbances and seeding seasons in Montana rangelands. Through modelling simulations, we gained estimates of the population growth rate (λ) and, thus, invasion potential.

Materials and methods

STUDY SPECIES

Camelina sativa is an annual herbaceous oilseed plant species that was introduced to the Americas as a crop contaminant (Putnam *et al.* 1993). It has gained popularity in the NGP owing to its potential as a biodiesel feedstock. Also, the oil of *C. sativa* seeds contains omega-3 fatty acids (α-linolenic acid), an important component of human health (Zubr 2003). *Camelina sativa* is a promising crop because it has been suggested to have a low incidence of pests (Eidhin, Burke & O'Beirne 2003), tolerance to drought and frost and the ability to grow in many soil types (Shukla, Dutta & Artz 2002). It can be seeded

earlier than most crops, has a rapid life cycle (85–100 d) and exhibits prolific small seed production (Shukla, Dutta & Artz 2002). Similar to other biofuel candidates, many of the traits that make this crop attractive agronomically are invasive plant attributes (Baker 1974).

SITE DESCRIPTION

This study was conducted in two rangeland ecosystems in Gallatin County, Montana. The first was located at the Montana State University Fort Ellis Research Center (45°40·25′N, 110°57·48′W), 1506 m elevation, and the second was at the Montana State University Red Bluff Research Ranch (45°35·45′N, 111°36·95′W), 1420 m elevation. The soil at Fort Ellis is classified as a fine, mixed, superactive, Frigid Typic Argiustoll and at Red Bluff, the soil is a mixed, Frigid Aridic Ustipsamment. These sites represent historically disturbed rangeland ecosystems that are likely to receive escaped propagules (i.e. along a highway, adjacent to a highway gravel pit and close to agricultural fields). Both sites are south-facing with minimal slope, but Red Bluff and Fort Ellis differ in climatic and soil characteristics, as well as in plant species richness (Table 1).

EXPERIMENTAL DESIGN, APPLICATION OF DISTURBANCES AND SEEDING

The experiment was conducted in the 2008 and 2009 growing seasons following a randomized split-plot arrangement. Ten replications at Fort Ellis and eight replications at Red Bluff were established in 2008, and nine and six replications were used in 2009 at Fort Ellis and Red Bluff, respectively. There were five mainplots in each replication, each measuring 3·5 m by 1 m and subplots were 1·5 m by 1 m. Buffers between main- and subplots of 1 m and 0·5 m, respectively, minimized seed movement between treatments.

Three of the five main plots were randomly assigned one disturbance type (one of two herbicides, mechanical or no disturbance), applied each autumn before seeding. Mechanical disturbance was applied on 29 October 2007 and 9 October 2008 by using a shovel to overturn the top 15 cm of soil. The commonly used rangeland herbicide treatments, metsulfuron + chlorsulfuron (Cimarron Plus) (DuPont, Wilmington, DE, USA) and imazipic + glyphosate (Journey) (BASF Corp., Research Triangle Park, NC, USA), were applied separately with a backpack sprayer at rates of 55·75 and 531·6 g a.i. ha⁻¹, respectively, on 19 November 2007 and 26 October 2008. Of the two main plots that received no disturbances, one received *C. sativa* seeds to assess demography under natural conditions, while the other plot received no seeds (control) to determine whether *C. sativa* was present in the environment prior to this experiment. *Camelina sativa* can be sown either in the autumn, as a dormant seeding, or in the spring (Putnam *et al.* 1993). Consequently, each subplot was assigned to either an autumn or spring seeding treatment.

Prior to seeding, *C. sativa* seed germination rate was evaluated in a Conviron CMP 3244 growth chamber (Conviron, Winnipeg, Canada) at 20 °C and 24-h photoperiod. Four subsamples of 25 seeds were placed on hydrated blotter paper in clear boxes. After 5 days, *C. sativa* percentage germination was 98% ± 1% (mean ± SD). *Camelina sativa* seeds were distributed onto the soil surface of each subplot at a rate of approximately 6666 m⁻² (10 000 seeds per subplot) and stepped on lightly to reduce movement by wind or water. Autumn seeding for the first trial occurred on 6 and 7 December 2007 at Red Bluff and Fort Ellis, respectively. The spring seeding for the first trial occurred on 15 and 24 March 2008 at Red Bluff and Ft. Ellis, respectively. The second trial autumn seeding took place on 2 November 2008 at both sites, and spring seeding occurred on 21 March 2009 at both sites.

DEMOGRAPHIC DATA COLLECTION

Camelina sativa emergence was estimated using five 100-cm² rings, constructed from 2·5-mm wire and randomly placed within each subplot. Beginning in March of each year, *C. sativa* plants present in each ring were counted weekly to estimate emergence, survival to maturity, seeds produced per plant and over-winter seed survival. Emergence rates were estimated by summing the numbers of seedlings in the rings, scaling to 1 m², and dividing by the number of seeds sown m⁻² (6666). A seedling was considered to have emerged if cotyledons were visible. At maturity (August), all surviving *C. sativa* plants were harvested, thus providing survival rate estimates from emergence to maturity. For each subplot, all *C. sativa* plants were placed in one paper bag and stored at room temperature until samples were processed. Total plant biomass and seed weight for each subplot were weighed to the nearest 0·001 g. Using seeds harvested from randomly selected individual plants, total seed production for each subplot was estimated by regressing seed number with seed biomass. The r^2 values for the seed number/seed biomass relationship at Fort Ellis in 2008 and 2009 were 0·91 ($P < 0·001$) and 0·93 ($P < 0·001$), respectively. Lack of seed production at Red Bluff in 2008 precluded quantifying this relationship, but in 2009, the r^2 value was 0·81 ($P < 0·001$).

DATA ANALYSIS

Few consistent patterns of emergence were observed across sites and years, based on visual inspection of interaction plots. Consequently, analyses were conducted separately for each site-year. To minimize problems associated with nonconstant variance and non-normality, emergence rates were sin⁻¹ (square root)-transformed and subjected to ANOVA using the mixed model (PROC MIXED) procedure of SAS version 9·2 for UNIX (SAS Institute 2008). Results of this analysis indicated significant seeding season × disturbance type interaction ($P < 0·05$), and further pairwise comparisons were made using the

Table 1. Annual precipitation, temperature, soil properties and plant species richness at Red Bluff and Fort Ellis, MT. Soil samples to a depth of 15 cm were taken on 29 September 2008 at Red Bluff and Fort Ellis with a hand-held soil probe. Five soil cores taken from within each undisturbed subplot were aggregated and analysed. Plant species richness is the total number of species observed at a site and was acquired using a 1⁻ᵐ² frame placed inside and directly outside each 15 randomly selected subplots ($n = 30$ at each site)

	Annual precipitation – 30 year avg. (cm)	Annual mean temperature – 30 year avg. (°C)	Soil organic matter (%)	Soil NO_3 (kg ha⁻¹)	Plant species richness (number)
Red Bluff	40·41	6·5	1·4	3·36	11
Fort Ellis	49·02	6·2	4·6	7·86	26

SLICE option in SAS version 9.2. Differences in biomass of harvested *C. sativa* plants were evaluated relative to seeding season. A Type III ANOVA model was fit to the data using *R* statistical software v. 2·8·1 (R Development Core Team 2009). The model included seeding season and site and was blocked by year. This analysis did not include disturbance as a variable because *C. sativa* plants that survived to maturity occurred only in the mechanical disturbance treatment.

A demographic model was constructed using Visual Basic 2007 to investigate *C. sativa* population dynamics. Because of lack of plant survival beyond emergence in all other treatments, only the mechanically disturbed subplots and associated demographic parameters were included in the model. *Camelina sativa* population dynamics in response to this disturbance exhibited stark differences between the two field seasons, particularly in plant survival. Therefore, the model was constructed with the option to simulate data using three approaches for each site: using 2008 data only, using 2009 data only, or using the 2 years combined. Variability was incorporated by randomly selecting sets of vital rates during simulations.

All simulations began at time $t = 0$ under the assumption that propagules escaped in spring just before the growing season, for an initial spring seedbank (SBs) density of 6666 m^{-2}. The state variables in the model were seedlings (Sdl), seed-producing adults (A), seeds produced (SP) and autumn seedbank (SBa) and are linked by the vital rates: emergence from SBs (S_{emrg}), Sdl survival to A (S_t), seed produced per A (S_{pp}) and seed survival from autumn to autumn (S_s) (Fig. 1). In the simulations, all seeds that were produced were returned to SBa, as we assumed that seed immigration was equal to emigration. Our field observations suggest that spring Sdl from SBa grow as winter annuals. Therefore, the proportion of autumn seedlings that survived the winter was represented by the vital rate, F_{emrg}.

In the model, the numbers of individuals at each life stage subsequent to initial seeding of SBs were determined by randomly selecting one set of vital rates from a subplot. Multiplication of vital rates and the number of individuals in each life stage provided the population size of the next life stage (Table 2). At the end of each life cycle, the population growth rate, λ, was calculated as follows:

$$\lambda = \frac{N_t}{N_{t-1}} \qquad \text{eqn 1}$$

where N_t is the number of seedlings at the present time step and N_{t-1} is the number of seedlings at the previous time step. At the end of each simulation, the geometric mean of λ was calculated.

Table 2. Life stages and equations used in the *Camelina sativa* population dynamics model

Life stage	Symbol	Equation
Spring seedbank density	SBs*	–
Seedling	Sdl	$(SBs*S_{emrg}) + (SBa*F_{emrg})$
Seed-producing adult	A	$Sdl*S_t$
Subplot total seed production	SP	$A*S_{pp}$
Autumn seedbank density	SBa	$SP + (SBa_{t-1} - Sdl_t)*S_s$

*Initial seedbank density of 6666 seeds m^{-2} at time $t = 0$ for all modelling scenarios. Also refers to the amount of seeds supplied to the system (propagule pressure) for all $t > 0$ in modelling scenario three (perturbation analysis).

For each field site, the model was run separately under different scenarios to explore factors governing the population dynamics of *C. sativa*. First, the 'default' scenario projected the population using observed values. Each set of vital rates had an equal probability of being selected. The simulations were run 1000 times, each time projecting the population until a quasi-extinction threshold of one *C. sativa* seed km^{-2} was reached. The quasi-extinction threshold is commonly used in situations where the population is exponentially declining, yet will never reach extinction (Caswell 2001). After the last of 1000 runs, the geometric mean λ and times to extinction for each run were averaged. The second modelling scenario evaluated a 'worst case' situation (most likely to become invasive) where *C. sativa* population dynamics were modelled using the highest value of each vital rate observed over the two field seasons. These data are representative of the presence of safe sites, where *C. sativa* populations have the greatest potential to increase. There was no variability under this scenario which, therefore, required only one run. Each simulation was run until the quasi-extinction threshold was reached, and the geometric mean λ and years to extinction were calculated. The third modelling scenario was a perturbation analysis of two demographic parameters important in plant invasions, seed survival (S_s) and propagule pressure (Jordan *et al.* 1995; Westerman *et al.* 2005). Based on hypothetical changes in S_s, from 0·0 to 1·0, as well as propagule pressure (number of seeds m^{-2} added every spring for all $t > 0$), from 0 to 10 000, we determined which combinations of these two parameters would result in a geometric mean λ > 1 over 21 years. Beginning with an initial seeding rate of 6666 seeds m^{-2},
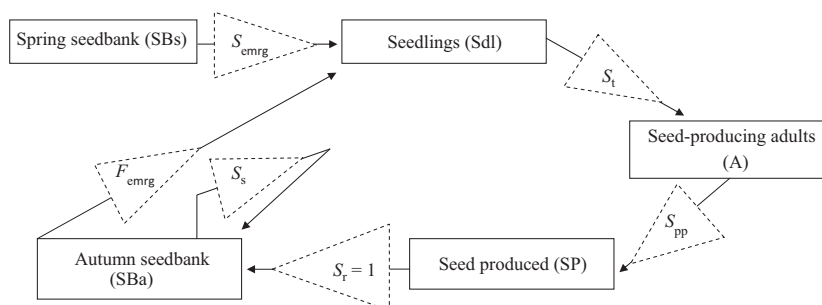

Fig. 1. Life cycle diagram illustrating the model structure used to assess the population dynamics of *Camelina sativa*. Solid boxes and dashed triangles represent state variables and vital rates, respectively. Vital rates are denoted: S_{emrg} = seedling emergence from spring seedbank, S_t = survival from emergence to maturity, S_{pp} = seeds produced per plant, S_r = seed rain (proportion of seed that is returned to the seedbank; always = 1), S_s = seed survival, F_{emrg} = seedling emergence in spring from autumn seedbank. Simulations always began under the assumption that propagules escaped in spring just prior to the growing season at time $t = 0$. With the exception of the perturbation analysis when seeds were added periodically, subsequent years ($t > 0$) did not include S_{emrg} from SBs, and the population transition rate to seedlings between years was regulated by F_{emrg} from SBa.

each parameter combination was simulated 500 times to achieve estimates of variability on λ.

Results

FIELD STUDY

Different patterns of *C. sativa* emergence were observed between the study sites. Irrespective of disturbance type or year, emergence of autumn-seeded *C. sativa* at the Red Bluff site was generally lower than when it was seeded in the spring (Fig. 2). Emergence rates of *C. sativa* in the mechanical disturbance were the lowest of all treatments at Red Bluff in 2008, averaging 0·014 ± 0·009 (mean ± SD) when seeded in the autumn and 0·021 ± 0·013 (mean ± SD) when seeded in the spring. In 2009, however, there was little difference in emergence among treatments regardless of seeding season.

At the Fort Ellis site, *C. sativa* emergence rates in 2008 and 2009 were lower when seeded in the autumn than when seeded in the spring (Fig. 3). Although not always significantly different compared to other disturbances, the lowest emergence rates were observed in the metsulfuron + chlorsulfuron treatment. In contrast to the patterns observed at Red Bluff, the mechanically disturbed subplots at Fort Ellis produced the highest emergence rates of all the treatments, especially when seeded in the spring. However, because of high variability in

2009, this emergence rate was no different from the glyphosate + imazipic or the undisturbed subplots.

The only *C. sativa* plants that survived to maturity were found in the mechanically disturbed subplots but displayed no consistent trends over sites and years (Table 3). For example, spring-seeded subplots in 2008 produced only three survivors at Red Bluff, but a total of 3325 mature plants were harvested at Fort Ellis. Although not as drastic in 2009, the opposite pattern occurred between sites, with more spring-seeded plants (93) surviving at Red Bluff and only 36 spring-seeded plants at Fort Ellis.

The biomass of mature *C. sativa* plants was greater in autumn-seeded subplots than in spring-seeded subplots at both sites ($P < 0.001$; Fig. 4), and *C. sativa* biomass and seed production were positively correlated ($r^2 = 0.82$, $P < 0.001$). With the exception of spring-seeded plots in 2009, total seed production was always lower at Red Bluff than at Fort Ellis. Plants from three spring-seeded subplots at Fort Ellis in 2008 produced seed in excess of the original 6666 m^{-2}. However, when averaged across subplots, seed production was never greater than the amount that was originally sown (Table 4).

POPULATION DYNAMICS MODEL

Under the 'default' scenario for Fort Ellis and Red Bluff, the geometric mean λ for 1000 simulations never exceeded 0·03
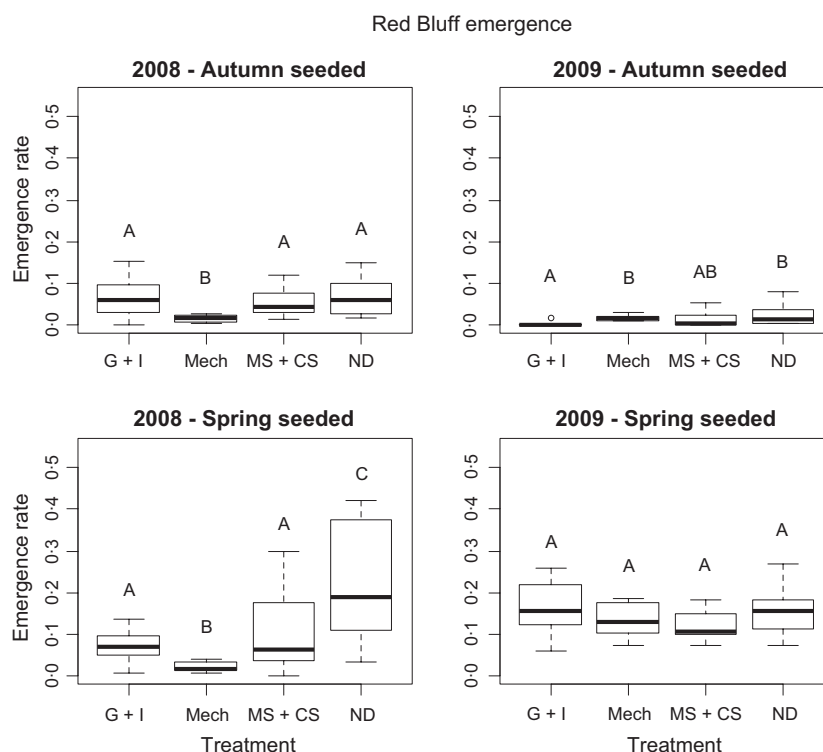
Red Bluff emergence

Fig. 2. *Camelina sativa* emergence values (untransformed) in response to different rangeland management practices conducted at Red Bluff, MT. 'G+I' = glyphosate + imazipic; 'Mech' = mechanical disturbance; 'MS + CS' = metsulfuron + chlorsulfuron; 'ND' = no disturbance. Letters within the plots represent significant differences among treatments at $P = 0.05$. The bold horizontal line in the boxes shows the median value, and the middle 50% of the data is within the box. The ends of the dashed lines (whiskers) show the minimum and maximum values unless there are outliers, in which case ○ signifies an outlier.
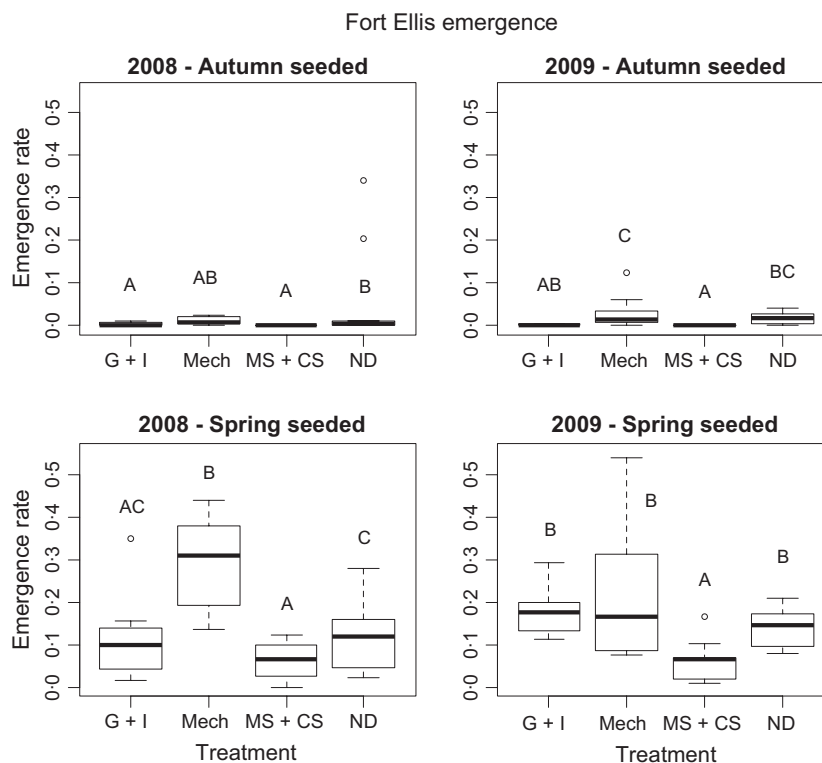
Fort Ellis emergence

Fig. 3. *Camelina sativa* emergence values (untransformed) in response to different rangeland management practices at Fort Ellis, MT. 'G + I' = glyphosate + imazipic; 'Mech' = mechanical disturbance; 'MS + CS' = metsulfuron + chlorsulfuron; 'ND' = no disturbance. Letters within the plots represent significant differences among treatments at $P = 0.05$. The bold horizontal line in the boxes shows the median value, and the middle 50% of the data is within the box. The ends of the dashed lines (whiskers) show the minimum and maximum values unless there are outliers, in which case 'O' signifies an outlier.

Table 3. *Camelina sativa* survival rates (mean ± SD) from emergence to maturity (S_t) and total number of plants harvested at the Red Bluff and Fort Ellis Research Centers, MT

Site	Year	Seeding season	S_t	Number of plants harvested
Red Bluff	2008	Autumn	0·0	0
		Spring	0·01 ± 0·01	3
	2009	Autumn	0·15 ± 0·07	93
		Spring	0·02 ± 0·02	88
Fort Ellis	2008	Autumn	0·16 ± 0·15	145
		Spring	0·11 ± 0·06	3325
	2009	Autumn	0·13 ± 0·09	347
		Spring	0·001 ± 0·004	32

and the maximum time to extinction was 6 years (Table 5). Data simulation under the 'worst case' scenario, where vital rates were assigned the highest observed values over the two field seasons, resulted in λ values of 0·35 and 0·03 and extinction times of 22 years and 7 years for Fort Ellis and Red Bluff, respectively. Results from the perturbation analysis revealed that extremely high rates of propagule pressure and seed survival (S_s) were necessary to obtain increasing population density (λ > 1·0) (Fig. 5). Average λ values using observed vital rates from Fort Ellis did not exceed 1 until $S_s = 0.6$ and

10 000 seeds were added for all $t > 0$ (1·01 ± 0·05 SD). Red Bluff required 6000 seeds m^{-2} to be added for all $t > 0$ and $S_s = 1.0$ to produce average λ values >1 (1·01 ± 0·007).

Discussion

The limitations of subjecting a non-native plant proposed for introduction to a single-tiered qualitative WRA protocol to determine invasion potential are well-established (Cousens 2008; Davis *et al.* 2010). Our research highlighted the practicality of refining WRAs to include quantitative approaches, by incorporating methods that are consistent with the tiered approach of traditional risk assessment (NRC 1983, 1996). Although this idea has been presented before (Davis *et al.* 2010), at the initiation of our experiment, no specific methods had yet been presented.

Population dynamics models have been used for many purposes in ecology (Caswell 2001). In a plant-specific context, models are used to assess the effects of management tactics in the conservation of endangered species (e.g. Menges 1990; Schemske *et al.* 1994; Griffith & Forseth 2005) and to characterize weed population dynamics in both natural and agricultural systems (e.g. Maxwell, Wilson & Radosevich 1988; Parker 2000; Davis *et al.* 2006). To our knowledge, no attempt has been made to use demographic modelling for the prediction of invasive biofuel feedstocks. This study shows that
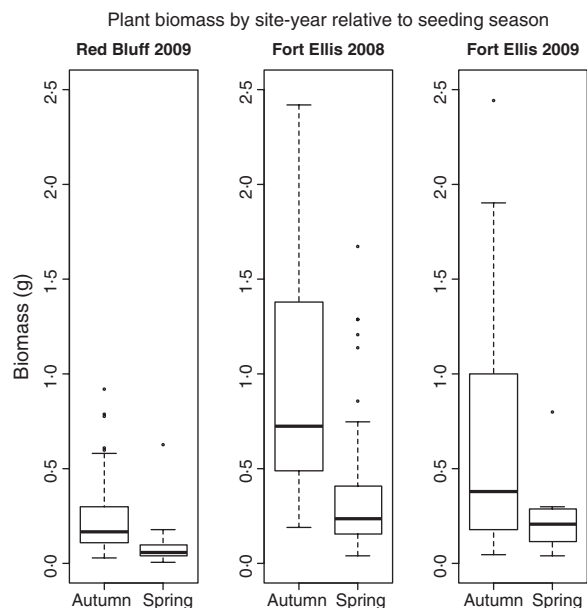
Plant biomass by site-year relative to seeding season

Red Bluff 2009   Fort Ellis 2008   Fort Ellis 2009

Fig. 4. Average *Camelina sativa* plant biomass (g plant^{-1}) at Red Bluff and Fort Ellis, MT. 2008 is omitted from Red Bluff because of lack of plant survival. Biomass of plants from spring-seeded plots was significantly less than biomass of plants from autumn-seeded plots ($P < 0.001$). The bold horizontal line in the boxes shows the median value, and the middle 50% of the data is within the box. The ends of the dashed lines (whiskers) show the minimum and maximum values unless there are outliers, in which case ○ signifies an outlier.

Table 4. Estimated *Camelina sativa* seed production (mean ± SD) at the Red Bluff and Fort Ellis research centers, MT relative to amount sown (6666 m^{-2})

Site	Year	Seeding season	Number of seed produced	% of amount originally sown
Red Bluff	2008	Autumn	0	0·0
		Spring	58 ± 116	0·8 ± 1·7
	2009	Autumn	395 ± 237	5·9 ± 3·5
		Spring	162 ± 169	2·4 ± 2·5
Fort Ellis	2008	Autumn	1595 ± 1953	23·9 ± 29·3
		Spring	5107 ± 3693	76·6 ± 55·4
	2009	Autumn	1087 ± 1810	16·3 ± 27·1
		Spring	38 ± 79	0·5 ± 1·2

Table 5. Average population growth rate (λ) values and maximum time to extinction results of the *Camelina sativa* population dynamics model after 1000 simulations under the 'default' scenario

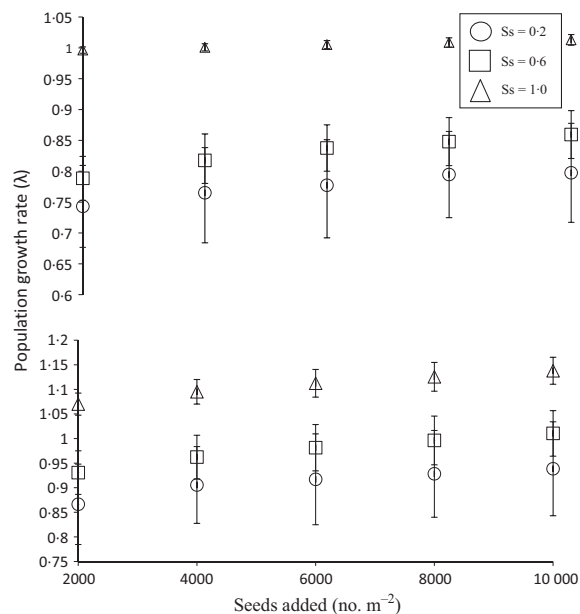| Site	Year	λ (mean ± SD)	Max. years to extinction
Red Bluff	2008	0·010 ± 0·025	4
	2009	0·002 ± 0·002	4
	08/09	0·005 ± 0·014	4
Fort Ellis	2008	0·017 ± 0·023	6
	2009	0·001 ± 0·002	4
	08/09	0·030 ± 0·064	4

Fig. 5. Sensitivity analysis results from the combined manipulations of seed survival (S_s) and propagule pressure on *Camelina sativa* population growth rate (λ) for data collected at the Red Bluff (top) and Fort Ellis (bottom) Research Centers, MT. Population growth rates were projected for 21 years for each of 500 simulations, and the geometric mean λ was calculated. Error bars represent ± 1 SD. The original amount of seed provided was 6666 m^{-2} at $t = 0$; the *x*-axis refers to the amount of seed added for all $t > 0$.

demographic models parameterized with field-based data offer valuable site-specific information otherwise overlooked by qualitative models. When used together in a multi-tiered process, qualitative and quantitative approaches may reduce the frequency of plant invasions.

In this study, perturbation analyses investigated the outcomes that could result from the combined manipulations of *C. sativa* seed survival and propagule pressure. Previous studies (e.g. Jordan *et al.* 1995) have provided evidence that seed survival is an important parameter in annual weed population dynamics and a significant factor in invasions (Timmins & Owen 2001; Radosevich, Holt & Ghersa 2007). In our simulations, however, manipulating seed survival alone, even increasing to biologically improbable rates, did not result in population growth. To accomplish λ > 1, it was necessary to add propagules each spring in combination with increasing seed survival rates beyond what we observed in our experiment, further suggesting low risk of *C. sativa* invasion into rangelands.

We received conflicting recommendations between our first- and second-tier assessments on whether to allow entry to *C. sativa*. In these cases, a competition experiment between the biofuel and native plants that grow adjacent to cultivated fields may be appropriate. Conveniently, these can be conducted simultaneously as demographic data are collected and can provide additional information to settle disputes between first- and second-tier assessments. We conducted a competition study between *C. sativa* and a widely cultivated nonweed, *Brassica napus* L. (canola), in the presence of the invasive weed *Bromus tectorum* L. (downy brome) under different soil

conditions. We found the competitive ability of *C. sativa* to be very low (Davis 2010), thus increasing our confidence in our modelling results.

Survival of *C. sativa* occurred in both years only in the mechanical disturbance, but with high temporal and spatial variability at both sites. Temporal variability in abiotic factors, namely temperature and precipitation, has been recognized as a driver of weed population dynamics (Freckleton & Stephens 2009) and could have affected the survival of *C. sativa*. However, the fact that the survival rates at Red Bluff and Fort Ellis displayed opposite trends between the two sites and the 2 years demonstrates the need for additional research on the factors driving *C. sativa* survival.

LIMITATIONS OF POPULATION DYNAMICS MODELS AS WRA TOOLS

Our model for *C. sativa* is based on highly variable data collected from only 2 years and two sites, and this lack of long-term data means this model is limited in its predictive power (Freckleton & Watkinson 1998). Minton & Mack (2010) reported similar results, as their study was conducted over 3 years, with unpredictable abiotic factors influencing their variable outcomes. These short time frames are major limitations of incorporating quantitative methods into the WRA process. However, in the absence of long-term data, perturbation analyses such as ours on seed survival and propagule pressure can offer insight into important demographic drivers. Also, substituting spatial diversity for temporal diversity can increase demographic knowledge without prolonging the assessment process unnecessarily (Wiens 1989). Another limitation of our approach is that both of our field sites were relatively similar in abiotic characteristics and were infested with non-native plants. Because of this, the inference space is limited to other semi-degraded ecosystems with similar biotic and abiotic conditions. Lastly, our data were collected immediately following a freshly created mechanical disturbance. Our population projections, therefore, assumed that the system is being disturbed annually. In reality, this frequency of disturbance is unlikely, as the ground that was disturbed would soon begin to be re-colonized by plants. Therefore, the already low population growth rates from our modelling exercises may overestimate the *C. sativa* growth rate that would be observed in reality.

Our model assumed no seed dispersal dynamics among *C. sativa* populations, yet this is a crucial driver of invasions and an important component of a comprehensive population dynamics model (Case 2000; Richardson *et al.* 2000). If we had found the invasion potential of *C. sativa* to be of concern, an effective additional step would be to refine this model by collecting dispersal data. For true pre-entry assessments, however, obtaining this information may not be an option because those field trials will need to be conducted under quarantine. In such cases, using data in the model from a closely related species may be an option.

Most biofuel feedstock candidates are longer lived than *C. sativa* and will grow in patches because they reproduce vegetatively; therefore, some important parameters that we used in our model for *C. sativa* will need to be substituted with others. For example, it might be more relevant to investigate propagule pressure parameters such as rhizome size and age classes and their effects on patch spread rate and within-patch shoot density. Subsequent demographic analyses should then focus on the relevant drivers of these substituted parameters (Maxwell, Wilson & Radosevich 1988).

Conclusions

In addition to assessing the usefulness of a quantitative second-tier WRA methodology, this study also provided answers to some previously uncertain biological and ecological characteristics of *C. sativa*. The amount of land devoted to its production in the NGP is projected to increase, yet its weedy characteristics were concerning. Clearly, it was necessary to evaluate this species' invasion potential by conducting a WRA using refinement procedures that included experimentally derived data on demography and population dynamics. Our results, although limited because of high variability and low number of site-years, provided preliminary evidence that escaped *C. sativa* populations are likely to be relegated to severely anthropogenically disturbed habitats. The current probability that this species will invade rangelands to cause ecological damage is low.

Most importantly, this risk assessment of *C. sativa* supports the idea that the current qualitative WRA paradigm can and should be improved with experimentally based approaches. This is especially relevant for biofuel crops because of the unique risks they pose. Qualitative model-based WRAs, although an effective first tier in the overall process, will always be limited in their capability to predict invaders when used singularly (Cousens 2008). However, if a system is implemented such that 'accepted' biofuel feedstocks or those that need to be 'evaluated' further after the first-tier process are subjected to an experimentally based quantitative second-tier WRA, it will greatly increase prediction accuracy (Davis *et al.* 2010). Researchers responsible for conducting WRA field trials must collaborate directly with plant breeders and other interested parties to facilitate effective communication among the different entities so that biofuel feedstocks are introduced responsibly. In turn, the probability of wrongly allowing entry to an invader or refusing entry to a noninvader will be greatly reduced.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Australian weed risk assessment for *Camelina sativa*.

Table S1. A-WRA questions and varied answers.

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