Australian Government
Department of Health
Office of the Gene Technology Regulator

The Biology of *Carthamus tinctorius* L. (safflower)

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Version 1.2: October 2019

This document provides an overview of baseline biological information relevant to risk assessment of genetically modified forms of the species that may be released into the Australian environment.

Version 1.2 is a minor revision to Version 1.1 to update information.

For information on the Australian Government Office of the Gene Technology Regulator visit our website.

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ABBREVIATIONS

ABARES	Australian Bureau of Agricultural and Resources Economics and Sciences
AOSCA	Association of Official Seed Certifying Agencies
APVMA	Australian Pesticides and Veterinary Medicines Authority
CMS	cytoplasmic male sterility
CSIRO	Commonwealth Scientific and Industrial Research Organisation
Cu	copper
F_1	first filial generation (of a hybrid cross)
FAO	Food and Agriculture Organisation (of the United Nations
FAOStat	FAO statistics division
Fe	iron
ft	feet
g	gram
GLA	gamma (γ) linoleic acid
GM	genetically modified
GMS	genetic make sterility
GRDC	Grains research and Development Corporation
h	hour
ha	hectare
HO	high oleic (acid) safflower
HSYA	hydroxysafflor yellow A
kg	kilogram
LA	linoleic acid
m	metre
mg	miligram
Mn	manganese
MUFA	monounsaturated fatty acid
NSW	New South Wales
NT	Northern Territory
ODS	Office of Dietary Supplements, National Institutes of Health, US Department of Health and Human Services
OECD	Organisation for Economic Co-operation and Development
PUBCRIS	Public Chemical Registration Information System (APVMA)
PUFA	polyunsaturated fatty acid
Qld	Queensland
RCT	randomised clinical trial
RNA	ribonucleic acid
RNAi	RNA interference
SA	South Australia
SHO	super high oleic (acid)
TAG	triacylglycerides
TGMS	thermo genetic male sterility
US	United States of America
USDA-APHIS	United States Department of Agriculture Animal and Plant Health Inspection Service
US-FDA	United States Food and Drug Administration
USSR	United Soviet Socialist Republic
Vic.	Victoria
Zn	zinc

PREAMBLE

This document describes the biology of *Carthamus tinctorius* L. (safflower), with particular reference to the Australian environment, cultivation and use. Information included relates to the taxonomy and origins of cultivated *C. tinctorius*, general descriptions of its morphology, reproductive biology, biochemistry, and biotic and abiotic interactions. This document also addresses the potential for gene transfer to occur to closely related species. The purpose of this document is to provide baseline information about the parent organism for use in risk assessments of genetically modified (GM) *C. tinctorius* that may be released into the Australian environment.

Version 1.2 of this document is revision to include updated information.

The common names for *C. tinctorius* vary with country, region, language and use, but it will be referred to as safflower or *C. tinctorius* in this document.

Safflower is a branching, spiny, thistle-like herbaceous annual plant. Originally, safflower was grown for its floral pigments for use as red (carthamin) and yellow (carthamidin) dyes and for medicinal purposes. Now, it is mainly cultivated in hot dry climates as an oilseed and to a lesser extent for meal, birdseed and seed for small animals such as mice and guinea pigs. Safflower is one of humanity's oldest crops, and yet it remains a minor crop compared to other oilseeds.

SECTION 1 TAXONOMY

Cultivated safflower (*Carthamus tinctorius* L.) is an annual oilseed crop that is a member of the family Asteraceae (Compositae), tribe Cardueae (thistles) and subtribe Centaureinae (Bérvillé et al., 2005). Asteraceae is recognised as the largest family of flowering plants and contains more than 1500 genera and 22,000 species ranging from annual herbs to woody shrubs. Safflower is known by many other names around the world kusum, kasunmba, kusumbo, kusubi, kabri, ma, sufir, Kar/karar, sendurgam, agnisikha, hebu, su, suban and many others. The Arabic usfur is thought to have been the root for the English name via a number of others – affore, asfiore, asfrole, astifore, asfiori, zaffrole or zaffrone, saffiore to finally, safflower – while in China it is known as hung-hua or 'red flower' (Chavan, 1961 and sources cited therein) and many others, as summarised by Smith (1996).

The taxonomy of *Carthamus* has changed substantially as data for this group has been obtained and interpreted (McPherson et al., 2004; Sehgal and Raina, 2011). There have been as few as four species in the genus (with related species in a separate genus) to as many as 25 species and subspecies divided in up to five sections. The sections were based on five chromosome groups identified by Ashri and Knowles (1960) n=10, 11, 12, 22 and 32. Safflower belongs to a *Carduncellus-Carthamus* complex and morphological and cytological characteristics have not been sufficient to delimit the species into discrete sections and genera. Depending on the taxonomist and the emphasis on particular morphological characteristics, species have been moved between the genera *Carthamus* and *Carduncellus* (McPherson et al., 2004). Determining species relationships is made more difficult by the low levels of genetic variation that occurs when clear morphological differences are present (Mayerhofer et al., 2011).

The classification scheme followed in this document is that of López-González (1990) (Table 1), which recognises 16 species within *Carthamus* and another closely related species, *Femeniasia balearica*. The species have been further divided into three sections based on chromosome numbers, the Section *Carthamus* (n=12), Section *Odonthagnathis* (n=10 or 11), Section *Atractylis* (n=22 or 32) and two species of uncertain placement.

Carthamus oxyacantha and *Carthamus persicus* were thought to be the parent species of *C. tinctorius* (Ashri and Knowles, 1960). More recent genetic analysis and geographic evidence indicate that

Carthamus palaestinus is the wild progenitor of safflower and originated in the Middle East, near Israel and is fully cross-compatible with safflower (Pearl et al., 2014).

Table 1 Taxonomic groups of *Carthamus* sensu[a]

Section	Species	Chromosome number	Recorded as present in Australia?
Carthamus L.	*C. tinctorius* L.	2n=2x=24, n=12	Yes
	C. oxyacanthus Bieb.	2n=2x=24, n=12	No
	C. palaestinus Eig	2n=2x=24, n=12	No
	C. persicus Willd. (basionym *C. flavescens* auct.)	2n=2x=24, n=12	No
	C. curdicus Hanelt.	2n=2x=24, n=12	No
Odonthagnathis (DC.) Henelt	*C. divaricatus* Beguinot & Vacc.	2n=2x=24, n=12	No
	C. leucocaulos Sm.	2n=2x=22, n=11	No
	C. glaucus Bieb.	2n=2x=20, n=10	Yes[b]
	C. tenuis (Boiww. & Bl.) Bornm.	2n=2x=20, n=10	Yes
	C. dentatus (Forssk.) Vahl	2n=2x=20, n=10	No
	C. boissierei Haláacsy	2n=2x=20, n=10	Yes
Atractylis Reichemb.	*C. lanatus* L.	2n=2x=20, n=10	No
	C. creticus L. (syn *C. baeticus* (Boiss & Reuter) Nyman)	2n=4x=44, n=22	Yes
	C. turkestanicus Popov	2n=6x=64, n=32	No
Uncertain placement	*C .nitidus* Boiss.	2n=6x=64, n=32	No
	Femeniasia balearica Susanna	2n=2x=24, n=12	No

[a] Based on the classification proposed by López-González (1990).

[b] Some uncertainty, see Section 8.2.

SECTION 2 ORIGIN AND CULTIVATION

2.1 Centre of diversity and domestication

Safflower is an ancient crop that is believed to have a single origin of domestication from approximately 4000 years ago in the Fertile Crescent (Pearl et al., 2014). This region ranges from southern Israel to Western Iraq (Chapman et al., 2010). Safflower has been grown for centuries in India, China and North Africa. Although safflower is considered a minor crop compared to other oilseed crops it is grown in over 20 countries, occupying over one million hectares of agricultural land and producing more than 850,000 tonnes of seed (FAOSTAT, 2019). The top four producers of safflower from 2015–2017 consistently included Russia, Kazakhstan, Mexico and the United States of America (US). Other significant producers of safflower include Turkey, India, Argentina and China (FAOSTAT, 2019).

Seven “centres of similarity” were identified by Knowles (1969), namely the Far-East, India, the Middle-East, Egypt, Sudan, Ethiopia and Europe. Ashri (1971) added more centres but these were not centres of diversity or origin, but of very similar safflower types. Considerable genetic diversity exists across different genotypes. When 60 representative genotypes from India and other countries were examined it was observed that plant height, seed yield, branching height and seed weight accounted for 80% of the diversity (Patel et al., 1989). Patel et al. (1989) identified 14 clusters of genetic diversity, but distribution into clusters was random showing that geographic isolation is not the only factor causing genetic diversity. Up to ten centres of similarity throughout the world were identified based on morphology. Nuclear microsatellite analysis of accessions suggests the presence of five genetic clusters, one in each of the following regions: Europe; Turkey-Iran-Iraq-Afghanistan; Israel-Jordan-Syria; Egypt-Ethiopia; and Far East-India-Pakistan (Chapman et al., 2010).

The different species of *Carthamus* are all believed to have one common ancestor, probably from Iraq and north-western Iran. With the exception of cultivated safflower, the species are all spiny weeds that grow in the wild. There appears to be three wild species that are closely related. *Carthamus flavescens* (= *C. persicus*) is usually found in wheat fields in Lebanon, Syria and Turkey. *C. oxyacantha* is a serious weed in the area from western Iraq to north-western India and northward into the southern parts of some former republics of the Union of Soviet Socialist Republics (USSR). *C. palaestinus* is found in the desert regions of Iraq, Israel, and Jordan. These species readily cross with *C. tinctorius* to produce fertile progeny. It is thought that early in its evolution, safflower spread to Egypt, Ethiopia, South Asia and the Far East, where distinct types have evolved (as reviewed by Smith, 1996).

Domestication of safflower has resulted in traits such as reduced shattering, smooth seeds, reduced duration of the early vegetative growth stage, restriction of branching to the upper part of the stem, and reduced seed dormancy (Bérvillé et al., 2005). Breeding programs have resulted in the release of cultivars with higher oil content and/or increased disease resistance in recent years (GRDC, 2010).

2.2 Commercial uses

Historically safflower was grown for the flowers or floral pigments that were used in making red (carthamin), orange and yellow (carthamidin) dyes for colouring fabrics until cheaper aniline dyes became available in the early 19th century (Li and Mündel, 1996).

Prior to the 1960s in the United States, the oil from seeds was used mostly as a base for paints and in Australia was introduced in the 1950's due to shortages in drying oils for the paint and resin industries (Smith, 1996). It is still used in paints and varnishes today because of its non-yellowing characteristic.

Worldwide the primary use for safflower is edible seed oil for use in cooking, salad dressings and margarine. The meal left over after extraction of oils from seeds can be used as a stockfeed for cattle and other livestock. The meal is unsuitable for monogastric animals such as swine and poultry, due to hulls not being removed resulting in a high fibre content (30–40%) (Li and Mündel, 1996).

In Australia the primary use is as an oilseed and birdseed (GRDC, 2017). White seed varieties in particular can be valuable in birdseed markets, however pricing can be volatile (GRDC, 2017). Whole safflower seeds are used in the birdseed industry, mainly for wild birds, especially for members of the parrot family and pigeons, with birdseed markets expanding through Canada, France, US, Japan and Egypt (Li and Mündel, 1996 and references cited therein). In Canada, most of the safflower produced is for the birdseed market (Mündel et al., 2004).

Cultivated varieties of safflower range in seed oil content from 20–45% of the whole seed (Li and Mündel, 1996). Standard oil content for oil types of safflower in Australia is 38 % with premium reduction or increase for levels below or above this level respectively (GRDC, 2017). There are two groups of safflower cultivars differing in seed oil composition, characterised by high linoleic acid (70–75% of total fatty acids) and high oleic acid (70–75%) (Singh and Nimbkar, 2006). Commercial safflower cultivars grown in Australia are either those high in the monounsaturated fatty acid (MUFA), oleic acid or those high in the polyunsaturated fatty acid (PUFA), linoleic acid. The safflower varieties that are high in oleic oil are used as heat stable cooking oil, cosmetics and infant food formulations (GRDC, 2017). The linoleic oil varieties contain nearly 75% linoleic acid which is used for edible oil products such as salad dressings and soft margarines (GRDC, 2010, 2017) and these varieties are also grown for seed (GRDC, 2017). Public awareness about the health benefits of certain fatty acids has already made safflower an important crop for the vegetable oil market (Li and Mündel, 1996).

2.2.1 Livestock feed

The use of safflower seed or seed meal as livestock feed is limited by several factors. Unless the seed or seed meal has been dehulled, the high fibre content present palatability and digestibility problems,

particularly for ruminants and poultry. Most commercial safflower meal includes hulls, and therefore has very high fibre content and a low protein content of 24%. However, decorticated meal, with most of the hulls removed, the fibre content is reduced while the protein content increases to 40% (Oelke et al., 1992). Compared to soybean meal, the quality of safflower protein is low due to its deficiency in lysine, methionine and isoleucine, the sulphur containing amino acids. Additionally, the protein fraction of the meal contains two anti-nutritional phenolic glucosides, the bitter-flavoured matairsinol-β-glucoside and the purgative 2-hydroxyarctiin-β-glucoside. However, these compounds can be removed by physical and enzymatic methods (Heuzé et al., 2015 and references cited therein).

A summary of information regarding the use of safflower seed and seed meal as livestock feed is presented below. Unless otherwise cited, the information below is from the Feedipedia website (Heuzé et al., 2015 and references cited therein).

RUMINANTS

Generally whole safflower seeds and hulled seed meal are less palatable than other common oilseeds. The incorporation of hulls can lead to a reduction in feed efficiency (because of low digestibility) unless the diet is supplemented with adequate energy and protein.

Palatability of the hulled seed meal is variable, sometimes presenting a problem for beef cattle but apparently not for dairy cattle or rams. In young sheep, supplementing poor quality diets with safflower meal resulted in increased weight gain and wool growth compared to a barley/urea supplement. Research has shown safflower meal to be a valuable ingredient for dairy cows, with no noticeable effect on flavour or odour of the milk produced. However, an oxidised flavour may develop in milk, if lactating dairy cows are fed more than 2–3 kg/day of high linoleic acid safflower (Mündel et al., 2004). Replacing cottonseed meal with safflower meal may increase milk fat content in Friesian cows and buffaloes.

Safflower could be used as forage, but there are many more productive crop/pasture options. Use of safflower as a forage could occur where seed may be of inferior quality, such as after an early frost or drought. Hay from safflower cut after flowering is likely best suited to sheep and goats, as they are not irritated by the spines. However, the hay produced from safflower is not recommended for consumption by cattle as they are more susceptible to mouth ulcerations caused by the spines (Mündel et al., 2004).

PIGS

Safflower meal is also not a suitable feed for pigs as a consequence of both the high fibre content and the low protein quality due to its deficiency in essential amino acids. It is not recommended to feed safflower meal to weanling pigs. Dehulled safflower meal is suitable for grower-finisher pigs, but only if supplemented with lysine. Up to 12% safflower meal can be included in the diet of growing pigs provided additional lysine is included. Pregnant sows may have up to 15% dehulled safflower meal in their diets, but this should be reduced to much lower levels when lactating.

POULTRY

Generally, due to the high fibre content, whole seed and unhulled seed meal are of low value. Partial or total dehulling can enable the use of safflower products in poultry but incurs the additional expense of the dehulling process.

Whole safflower seeds are used as feed for broiler chickens at levels of up to 20% with no effect on performance and carcass traits. In contrast, levels of up to 10% tended to lower performance of layers (not significantly) and can also increase the linoleic acid content in the yolk. Safflower seeds can be safely included in broiler and layer diets at a 10% level.

The use of dehulled safflower seed meal for poultry needs to include supplementation with some amino acids (lysine and methionine). For example, performance of broilers on a diet of 22% safflower meal (supplemented with lysine) was almost halved when the diet was without supplement. Layers would require supplemental lysine and methionine.

DOMESTIC ANIMALS - BIRDS AND SMALL ANIMALS

Safflower seeds are used as birdseed especially for members of the parrot family and pigeons. Safflower seed or seed meal can be included in the diet of rabbits, gerbils, hamsters and chinchillas (Mündel et al., 2004).

In Australia safflower seed is used for birdseed and small animal seed mixes, with bright white seed varieties preferred for their appearance (GRDC, 2017).

2.2.2 Medicinal uses

Safflower seeds, oils and flowers have a wide range of medicinal uses in many countries. Safflower has been used in China since the 2nd century B.C. almost exclusively for medicinal purposes (Li and Mündel, 1996). The flowers are used as tonics for a range of conditions such as dilation of arteries, reduction of hypertension and increased blood flow.

Seed decoctions are used as laxatives, for urinary tract infections and to reduce rheumatic pain (Mündel et al., 2004). Safflower teas, made from the foliage and flowers of the plant, have been developed in China and India and marketed globally as herbal health teas. Women in India and Afghanistan have used teas made from foliage to prevent abortion and infertility. The tea has also been used as a preventative measure against cardiac and cerebral vascular diseases (Emongor, 2010and references cited therein). It was expected that use of both seeds and flowers may increase profits for farmers and increase production areas in India (Singh and Nimbkar, 2006).

The oil is used in Iran to treat liver and heart ailments and in India to treat sores and rheumatism. It has also been used to treat cerebral thrombosis (Emongor, 2010) and has lowered blood pressure in over 90% of patients (Li and Mündel, 1996). Safflower decoctions have been used to successfully treat male sterility (Qin 1990, as cited by Li and Mündel, 1996). The oil of a GM safflower has been approved by the United States Food and Drug Administration (US-FDA) for use as a dietary supplement (Nykiforuk et al., 2012).

2.2.3 Industrial applications

Over the last decade, there has been increased demand for vegetable oils in food, feed and bio-based industrial materials. Vegetable oils consist of triacylglycerides (TAGs). The energy density of TAGs has made vegetable oils an attractive source of biodiesel, produced by transesterification of TAG fatty acids. Monounsaturated fatty acids such as oleic acid are highly heat stable and biodegradable and are well suited to use in the oleochemical industry (bio-based plastics, foams, and fluids) and could replace petroleum based sources in the manufacture of a number of industrial products such as lubricants, hydraulic fluids and biofuels (GRDC, 2010). Safflower varieties with a high linoleic content have potential to be included in industrial applications for use in paints (GRDC, 2017). Other minor industrial uses for safflower oil include cosmetics, soaps, and infant formula (GRDC, 2017). Recently a GM variety of safflower producing high levels of oleic acid has been commercialised. This variety was developed to produce oleic acid that can replace some petrochemicals in the manufacture of plastics, paints, resins and other industrial oils (GRDC, 2017).

The petals of safflower flowers contain carthamidine (yellow) and carthamin (red), which are extensively used across various industries as natural dyes. The red and yellow pigments are widely used in the food, beverage and pharmaceutical industries, as well as in the production of personal

care products and also used as dyes for fabrics used for a wide variety of applications (see references such as Chavan, 1961; Smith, 1996; Weiss, 2000; Zohary et al., 2012).

2.3 Cultivation in Australia

2.3.1 Commercial propagation

Safflower is an annual oilseed crop that is propagated by seed. It is either self- or insect-pollinated, with little to no pollination by wind. Outcrossing rates between adjacent plants can be quite high, approaching 100% in some varieties (see Section 9.1). Long distance outcrossing between safflower plants has been reported to occur at a rate of 0.01% at a distance of 100 m or not at all when plots were separated by 300 m (McPherson et al., 2009a). The Organisation for Economic Co-operation and Development (OECD) Seed Scheme for Varietal Certification, which applies in Australia and many other countries, requires that crops of certified safflower seed be grown with an exclusion distance of 200 m from other safflower crops, and that basic safflower seed (the source for certified seed) be grown with an exclusion distance of 400 m (OECD, 2013). The Association of Official Seed Certifying Agencies (AOSCA), which administers standards for certified seed production in the US, requires an isolation distance of 403 m (1,320 ft) for all classes of safflower seed (AOSCA, 2012).

2.3.2 Scale of cultivation

Safflower has been grown in Australia since the 1950s. It was introduced in response to shortages in drying oil in the paint and resin industries. Production expanded to 48,000 ha by 1968. Safflower was initially mainly grown in Queensland (Qld) until its decline in 1970s due to droughts and a severe disease outbreak of *Alternaria carthami*, a fungal pathogen causing leaf blight. Following the abolishment of quotas on the use of vegetable oils for margarine production in 1976, safflower production increased again peaking at 74,688 ha in 1979 when record prices were paid for safflower(GRDC, 2010). This represents less than 0.5% of total cropping area in Australia. Production did decline again, due to a combination of volatile market prices and competition from other oilseed crops such as cotton, canola and sunflower which developed in the 1960s and 1970s (Jochinke et al., 2008; Wachsmann et al., 2008).

In Australia, safflower production has shifted from northern New South Wales (NSW) and southern Qld to include the higher rainfall (> 450 mm) cereal growing regions of southern NSW, Victoria (Vic.) and South Australia (SA) (GRDC, 2010, 2017). This shift in production coincided with the introduction of two disease resistant cultivars released in the late 1980s (Jochinke et al., 2008). In 1987, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) released the varieties Sironaria (resistant to *A. carthami* and moderate resistance to *Phytophthora cryptogea*) and Sirothoria (resistant to *P. cryptoge*a and susceptible to *A. carthami*) (GRDC, 2010). The Australian industry was based primarily on Sironaria which has high linoleic acid content and is also suitable for birdseed markets. Additional cultivars were introduced in the 1990's such as S555 (high linoleic oil) and S517 (high oleic oil) (Jochinke et al., 2008). Other cultivars have been imported in recent years (GRDC, 2017) but production areas have been declining, with the Food and Agriculture Organization of the United Nations (FAO) calculating an area of approximately 5,000 ha per year between 2014 and 2017 (FAOSTAT, 2019). Safflower areas are no longer reported separately in Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) data, with last reported area in 2013 of 8,000 ha (ABARES, 2014).

2.3.3 Cultivation practices

In Australia, safflower is an annual plant with a long growing season. It is generally sown in June or early July in northern and central NSW and during July in southern NSW, Vic. and SA. Provided there is water available, sowing could occur as late as September and early October in parts of Vic. and SA.

However, yield is related to sowing time and is most reliable when the crop is sown in late June or early July (GRDC, 2010). Similar to other oilseed crops, the sowing date has been shown to affect seed oil content (Mirshekari et al., 2013). Safflower may be sown later than other winter crops, which allows it to be used for weed management or as an option when earlier planted winter crops have failed to establish (GRDC, 2010).

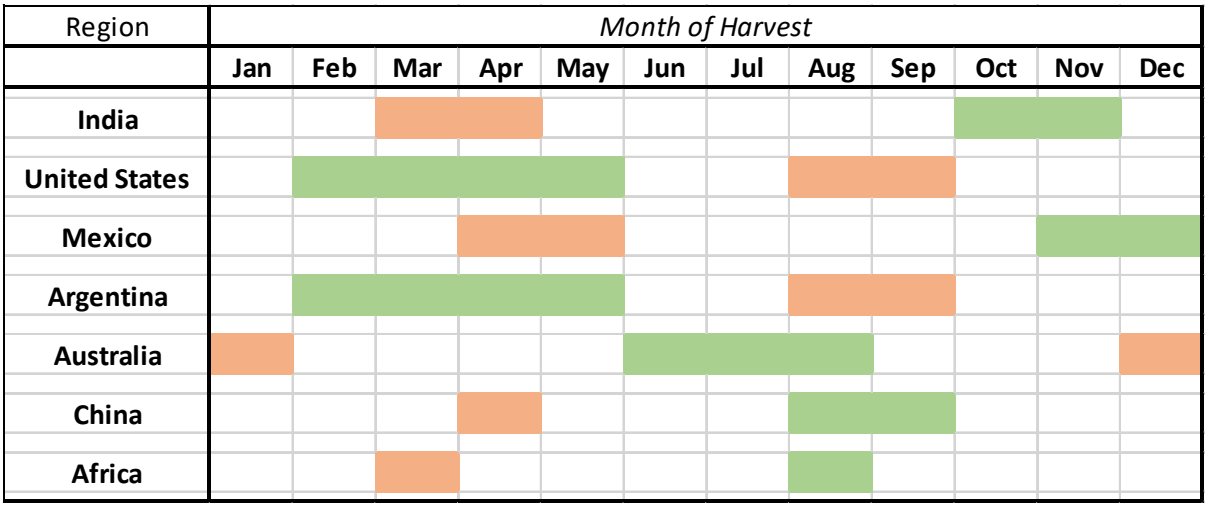

Figure 1 Sowing (green) and harvest (orange) dates of major global safflower growers (adapted from Gilbert, 2008).

Sowing rates depend on the region and moisture availability with rates ranging from of 12–15 kg/ha in northern NSW and 18–24 kg/ha in Vic. and SA, seeding rates would be lower in drier conditions (9 kg/ha in northern NSW) and as high as 25–31 kg/ha under irrigation (GRDC, 2010). Typical plant densities would be 20–25 plants m^{-2} in the northern and central NSW, 30–35 plant m^{-2} in southern NSW and 30–40 plants m^{-2} in Vic. and SA, with higher planting densities for irrigated crops and lower for dry conditions (GRDC, 2017). Safflower in the US is sown at a high seeding rate of 28–39 kg/ha, although the crop develops at a significantly higher density of approximately 65 plants m^{-2}, promoting better weed competition (Oelke et al., 1992).

Ideally sowing should be into moist soil, typically between 2 and 5 cm depth but this will vary with soil type and conditions. Delayed emergence and reduced early vigour can occur due to deeper sowing, leaving plants susceptible to pest, disease and competition from weeds (Mikkelsen et al., 2008). Safflower is normally planted with standard cereal sowing equipment in rows 18–36 cm apart. Narrower rows help suppress weeds, whilst wider spacing allows for better airflow for disease control (GRDC, 2010).

Seedlings emerge 1–3 weeks after sowing. Emergence takes longer under cooler temperatures, which also increases the risk of insect damage and disease. Plants spend 2–3 weeks in the rosette stage while growing leaves and are susceptible to frosts below -7 °C. The rosette stage is followed by stem elongation, branching and flowering stages. After flowering the time to maturity is about four weeks. The time from sowing to harvest is around 26–31 weeks, but varies with variety, location, sowing time and growing conditions. Timing of flowering is influenced more by day length than sowing date. In Australia, flowering of winter sown safflower generally coincides with wheat harvest (GRDC, 2010).

Safflower has a deep root system, which makes it ideal for rain-fed cropping systems (Singh and Nimbkar, 2006). Well-drained, deep, fertile, sandy loam soils provide maximum safflower yields (GRDC, 2010). In Australia, due to its deep tap root system, safflower is often used on problem soils to break up hard pans and to improve both water and air infiltration in the subsoil (GRDC, 2010).

Although safflower has high water requirements, it does not tolerate waterlogging well. Safflower has the ability to extract water from deeper layers of soil compared to many other crop plants due to its large tap root, which can elongate up to three metres (Li and Mündel, 1996; GRDC, 2010) and thus is considered quite drought tolerant. Irrigation can extend the growing season by two weeks, whereas maturity is reached earlier (hastened) by drought, salinity, increased temperatures or day length. Safflower is considered to have moderate to high tolerance to salinity, being similar to barley or cotton (GRDC, 2010). Safflower is moderately frost tolerant during the rosette stage, but is susceptible to frost damage from the stem elongation stage to maturity. It is also relatively resistant to hail or wind damage (Mündel et al., 2004).

Safflower has similar nitrogen requirements to cereals and international research has indicated that in terms of nitrogen use efficiency, it can be regarded as a low input crop, outperforming sunflower in seed yield on low nitrogen soils (GRDC, 2017). One tonne of safflower seed removes 25 kg nitrogen, 4.3 kg phosphorous and 4 kg sulphur from the soil. Most soils (with the possible exception of sandy soils) contain adequate levels of potassium and sulphur (GRDC, 2010). Although safflower can access nutrients from deeper than cereal crops, fertilisers can be applied to increase yields and oil levels, especially in irrigated or higher rainfall areas. Fertiliser application rates are dependent on expected yields based on available soil moisture (or irrigation), which also varies significantly between different cultivars. Application methods of nitrogen for safflower must consider safflower's ability to access nutrients from deeper in the soil profile (GRDC, 2017). For safflower grown in Pakistan, a study of different nitrogen application rates discovered that plant height, number of branches, number of capitula and total seed yield were all significantly increased with the application rate of nitrogen at 120 kg/ha (Siddiqui and Oad, 2006).

Safflower is a poor competitor with weeds, particularly during emergence through to the rosette stage of development, and weed management is essential when growing this crop. It is important to control the number of weeds as a means of reducing the potential negative impacts on yield. Cultivation can be used to control weeds when the safflower plants are seedlings, measuring 7–15 cm tall. There are some registered herbicides available for use in safflower cropping systems, which are typically used as either pre-planting or pre-emergence herbicides. These herbicides are used for the control of in-crop grass and broadleaf type weeds (see Section 7.1).

HARVEST

Safflower sown in winter is usually ready for harvest four to six weeks after wheat. Safflower is ready for harvest once all the leaves have turned brown and the latest flowering heads are no longer green (GRDC, 2017). At maturity the seeds should be white and easily threshed by hand (Oelke et al., 1992). For the major global safflower growers the harvest dates are variable, summarised in Figure 1 (above), which helps to ensure supply of safflower seed throughout the year. Harvest of safflower generally begins in late December in northern NSW and continues into March in the south east of SA. In Australia, it is recommended that seed moisture at the time of harvest should be less than 8% to avoid overheating and mould formation during processing and storage, thus most processors will not accept seed above this level (GRDC, 2010, 2017). It is also recommended that harvest occurs as soon as possible as rain can cause staining or early sprouting of the seed, both of which reduce value of the seed (Oelke et al., 1992; GRDC, 2010). In parts of Canada, seed is harvested at 12–15% moisture and then dried by aeration (Mündel et al., 2004).

Safflower is generally harvested without swathing. Safflower is suitable for harvest by direct heading since the capitula do not shatter easily. The same machinery used for cereals can be used for safflower but ground speeds are slower to reduce seed loss (Oelke et al., 1992; Thalji and Alqarallah, 2015). Periodic cleaning of equipment to remove bristles from radiators and hot engine components may be necessary to minimise the risk of fire (GRDC, 2010). In addition, harvesting in cooler or more humid

parts of the day is recommended both to reduce the risk of fire and to increase seed cleanliness (Jochinke et al., 2008). In Australia, seed loss during harvest (direct heading) is about 3 to 4% (GRDC, 2010).

2.4 Crop Improvement

Safflower produces some of the healthiest oils for human consumption and despite favourable agronomic traits, such as drought resistance and adaption to arid regions, it still remains a minor crop worldwide. In the past this has been due to its low oil content and yield relative to other oilseed crops, such as canola and cotton, and susceptibility to diseases and insect pests. Hence, the major breeding objectives have been to improve seed yield, seed oil content and disease resistance.

The primary end uses of safflower seed oil are for the edible and industrial oil markets and to a lesser extent the bird seed market (Knowles, 1989). Modern plant breeding has been used to develop cultivars with different fatty acid oil profiles, quantity and quality. This includes speciality oils thought to have beneficial health effects such as oils with high γ-linoleic acid (gamma linoleic acid, GLA) and increased tocopherol content (Velasco et al., 2005; Nykiforuk et al., 2012). Safflower oil also has potential in the biofuel industry (Patrascoiu et al., 2013) and as a platform for the production of pharmaceuticals in GM safflower seed (Mündel et al., 2004; Nykiforuk et al., 2012). See Section 2.4.2 for more detail.

2.4.1 Breeding

OIL CONTENT

The primary objective of safflower breeding programs over the years has been to increase oil content. Prior to 1942, seed of commercial cultivars had less than 28% oil per whole seed. Current varieties grown in Australia have up to 42% oil content (GRDC, 2010). Breeding programs in the United States have successfully developed cultivars with oil content of 45–55% (see review by Sehgal and Raina, 2011).

Selection from local varieties is the most common breeding method used for safflower cultivar development in India and several germplasm lines with desired traits have been developed (Singh and Nimbkar, 2006). This germplasm can then be used for breeding in other countries, through selection and/or hybridisation with local lines. In the 20th century, safflower cultivars were developed in the United States, Canada and Argentina using introduced germplasm from India, Russia and Turkey (Singh and Nimbkar, 2006).

Seed yield and oil content are the most complex traits in safflower and selection for them is hampered by large genetic-environment interactions (Golkar, 2014). Seed yield is positively correlated, but seed weight negatively correlated with oil content. The proportion of hull content is positively associated with seed weight but negatively associated with oil content. The thick pericarp keeps oil production low, so a reduction of the pericarp will increase oil content. Selection for high oil content can be performed using the thumbnail method, due to seeds with high oil content having thin hulls and are easily pressed using thumbnail pressure (Singh and Nimbkar, 2006)

HYBRID SAFFLOWER

Dominant and recessive genetic male sterility (GMS), cytoplasmic male sterility (CMS) and thermo sensitive genetic male sterility (TGMS) systems for producing hybrid safflower plants have been developed (Singh et al., 2008; Meena et al., 2012).

GMS safflower lines (both spiny and non-spiny flowered lines), which exhibit an increase of 20–25% in seed and oil yield are available in India. Similarly CMS and TGMS lines are commercially available in India (Meena et al., 2012). Average yield and oil content of CMS hybrid lines were greater than the open pollinated lines in trials run across sites in the US, Canada, Pakistan, Mexico and Spain (Li and

Mündel, 1996). In Australia, comparison of four US derived CMS lines with open pollinated lines was inconclusive with regards to yield (Wachsmann et al., 2003). Despite development of systems to produce hybrid safflower and testing of hybrids, globally speaking commercial production of hybrid safflower is considered largely elusive (Mündel, 2008).

MODIFIED FATTY ACID COMPOSITION

Safflower is an oilseed crop that is primarily grown for its high quality edible oil. Safflower seeds contain the fatty acids, palmitic acid, stearic acid, linoleic acid and oleic acid. Safflower lines have been developed with the following modified fatty acid compositions; increased palmitic acid, increased stearic acid, high to very high linoleic acid, high to very high oleic acid with reduced saturated fatty acids (palmitic and stearic acids) (Singh and Nimbkar, 2006; Hamdan et al., 2008).

Oleic acid and linoleic acid are the two major fatty acids in safflower seed oil accounting for 90% of fatty acids present. Cultivated safflower seed oil traditionally had a high linoleic acid content of about 70% but breeding since the 1940s has changed the ratio of oleic and linoleic acids to produce high linoleic (70–90%) and high oleic acid (HO - 75–85%) cultivars.

Breeding for modified fatty acid composition using a few genes has been successful. The allele *ol* has been bred into cultivars in the United States to produce two types of fatty acid composition modifications, including high oleic and high linoleic cultivars (Knowles, 1989). The *ol* allele was found to be associated with a defective microsomal oleate desaturase FAD2-1 (fatty acid desaturase) (Rapson et al., 2015). Vegetable oils high in oleic acid have increased nutritional value and industrial applications. The normal oleic acid amount in safflower is 10–15% with a natural mutant (*ol*) accumulating up to 70%. The *olol* allele has now been incorporated into safflower breeding programs worldwide, following the development of perfect molecular markers, and has resulted in the release of numerous high oleic acid safflower varieties including Saffola 317 (S-317) (Cao et al., 2013; Liu et al., 2013).

Safflower varieties introduced to Australia from the United States have included the HO variety Saffola 517 and the linoleic oil variety Saffola 555 (GRDC, 2010). HO cultivars have been developed by conventional breeding and by genetic modification (Section 2.4.2). Non-food applications or potential industrial uses of HO vegetable oils with high oxidative stability include uses in biodiesel, lubricants, and hydraulic oils, all products that require high oxidative stability (Vanhercke et al., 2013).

NON-SPINY VARIETIES

Safflower cultivars are generally spiny but in some countries, especially where hand picking of seeds is practiced, production is dominated by non-spiny cultivars, China and India for example. Non-spiny varieties introduced and developed in India in the past, such as CO-1 and JS-1, had poor yields. More recent non-spiny cultivars introduced in India, NARI-6 and NARI-NH-1 have comparable yields to spiny cultivars whilst also having increased tolerance to both foliar and wilt diseases (Singh and Nimbkar, 2006). These non-spiny cultivars can provide dual incomes to farmers as the florets can be collected easily after maturity, and then sold separately to the food and textile industries for natural dyes.

DISEASE RESISTANCE

Disease incidence is relatively low in safflower due to safflower being a rain-fed crop, although is often grown under irrigation which can increase the prevalence of disease (Nimbkar, 2008; Mirshekari et al., 2013). Under favourable conditions outbreaks can devastate safflower crops as seen with the *A. carthami* outbreak in India in 1997 (Singh and Nimbkar, 2006). In Australia, the fact that safflower is a minor crop is an important contributor to reduced disease incidence. Low production levels, the long time between successive plantings of safflower in the crop rotation, and the distance between safflower fields would all contribute to low levels of inoculum.

To make safflower more competitive as an oilseed crop, cultivars with increased disease resistance to foliar diseases were developed. The most devastating diseases worldwide are leaf blight (*A. carthami*) and wilt (*Fusarium oxysporum*), which are both caused by fungal pathogens and can cause production losses of up to 50% (Sehgal and Raina, 2011). Breeding safflower for disease resistance is the simplest method for controlling disease in the crop. Resistance to *A. carthami* and *F. oxysporum* are known to be due to single dominant genes. Germplasm line VFR-was developed with resistance to multiple diseases including wilt and root rot, which are caused by the fungal pathogens *Verticillium dahliae*, *F. oxysporum* and *Rhizoctonia solani*, respectively (Cook et al., 2002; Singh and Nimbkar, 2006; Singh et al., 2008).

The first commercial oilseed safflower variety grown in Australia in the 1950s was Gila, from Arizona. Gila was the main cultivar grown in most countries for three decades (1960–1990s). Although new cultivars have increased seed yields, their susceptibility to diseases and low seed oil content resulted in its decline in production (Mündel, 2008). In the 1970s and 1980s, Gila suffered severe losses due to leaf blight caused by *A. carthami*. This led to the development of disease resistant varieties by CSIRO, namely Sironaria and Sirothora in 1987. Sironaria is resistant to *A. carthami* and moderately resistant to *P. cryptogea* while Sirothora is susceptible to *A. carthami* and resistant to *P. cryptogea* (GRDC, 2010). Sironaria has lower oil content than newer varieties grown worldwide. Little research on breeding and developing new varieties has been done in Australia since 1987. In Australia, Sironaria is the most commonly grown cultivar followed by Saffola (S555 and S517) and Gila (Jochinke et al., 2008).

MOLECULAR BREEDING

Traditional breeding methods have contributed much to crop improvement of safflower in particular with the development of disease resistant cultivars, spineless cultivars and high oil content varieties but these methods do have several limitations. Breeding programs have been hampered by limited information on genetic variability in *C. tinctorius* and lack of genomics tools for trait breeding (Mayerhofer et al., 2010). In this respect, molecular tools such as linkage maps, gene identification, genetic engineering and genetic/genome information will be important in order to improve productivity/yield and develop resistance to other stresses such as drought, salinity and insect pests.

Gene discovery, development of techniques for comparison of DNA, and linkage maps are needed to understand relationships within and between *C. tinctorius* and its wild relatives. Such comparisons would help to identify homologous genes/alleles in wild species or homeologous loci within polyploidy taxa for trait improvement (Sehgal and Raina, 2011). Identifying genes important to certain traits will help to identify functional markers within the genes and these markers will allow high throughput selection for such traits as yield and flowering time (Mayerhofer et al., 2010; Sehgal and Raina, 2011).

2.4.2 Genetic modification

Efficient transformation and stable integration of transgenes in safflower using an Agrobacterium-mediated approach has been developed, see for example Belide et al. (2011), where the efficient recovery of transgenic plants was achieved by grafting a transgenic shoot into a non-transgenic rootstock.

GENETICALLY MODIFIED SAFFLOWER

GLA is an important essential fatty acid synthesised from linoleic acid by delta-6-desaturase in the endoplasmic reticulum. High GLA lines have been developed which are stable and heritable across generations and show no penalty in oil content, viability or fitness. The US-FDA approved the use of GLA derived from GM safflower, as a dietary supplement called SONOVATM 400. Clinical trials have shown GLA is effective in treatment of eczema, viral infections and some types of cancer (Nykiforuk et al., 2012).

METABOLIC ENGINEERING

In the oil seed industry there is a growing trend towards developing oils that are nutritionally beneficial. These oils would be low in saturated fatty acids, high in MUFAs such as oleic acid and have functional stability without the need for hydrogenation, and/or enriched with long-chain PUFAs (Liu et al., 2002).

The high level of oleic acid (75–85%) found in some safflower cultivars is ideal for food use but not ideal for industrial uses because of the very high level purity required. Potential industrial uses of HO vegetable oils with high oxidative stability include uses in biodiesel, lubricants, hydraulic oils and oleochemical applications. The oxidative stability was significantly improved in the oil extracted from super high oleic (SHO) safflower compared to the high oleic acid cultivar S317, composed of over 93% and 75.4% oleic acid, respectively (Wood et al., 2018). The SHO safflower was produced through seed-specific RNAi-silencing of FATB and FAD2.2 genes, which are responsible for the release of saturated medium-chain fatty acids and the desaturation of oleic acid to linoleic acid, respectively (Wood et al., 2018).

MOLECULAR PHARMING

Safflower has been developed as a host platform for the production of proteins such as pharmaceuticals and industrial enzymes in GM seed (Mündel et al., 2004; Mayerhofer et al., 2010). A Canadian-based company, SemBioSys Genetics Inc., genetically modified safflower to accumulate human insulin in the mature seeds. The insulin was readily purified along with the oil-bodies fraction of the seed (Mündel et al., 2004). This system was used for the transgenic expression and isolation of Apolipprotein A1 Milano and high levels of gamma-linoleic acid (over 70% (v/v) from seed oil (Nykiforuk et al., 2012).

SECTION 3 MORPHOLOGY

3.1 Plant morphology

Safflower is an erect, thistle-like plant that grows from 30 to 150 cm in height and from sowing to harvest can take 26 to 31 weeks depending on variety, management and growing conditions. Safflower emerges 1 to 3 weeks after sowing and the first leaves emerge forming a rosette. The rosette stage is slow and can last several weeks. As temperature and day length increase the stem begins to elongate and branch. Lateral branches develop on stems that are about 20 to 40 cm high and these lateral branches may branch to produce secondary and tertiary branches. The more branches that grow the higher the yield as each branch ends in a flower head.

Leaves are arranged on both sides of the stem. Leaf size varies with variety and position on the plant; leaves are typically 2.5–5 cm wide and 10–15 cm long. Upper leaves often develop hard spines, while those lower on the stem are usually spineless. These spines make the crop difficult to walk through but act as a deterrent to larger animals such as pigs and kangaroos (GRDC, 2010). As plants mature they become stiff and woody and resistant to some stresses such as hail or wind. The period from flowering to maturity takes around four weeks. Plants produce a strong taproot that, in the right soils, can elongate up to three meters, with numerous thin horizontal roots. This deep root system allows the plant to extract water and nutrients from deeper layers of soil than many other crop plants (Li and Mündel, 1996; GRDC, 2010).

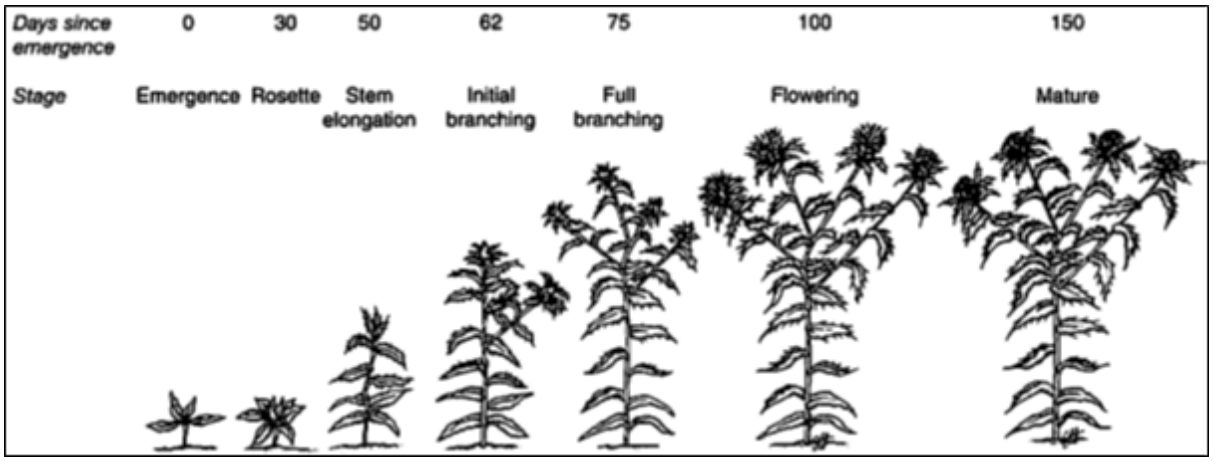

Figure 2 Development of a safflower plant.

(Kaffka and Kearney, 1998; as adapted by GRDC, 2010).

3.2 Reproductive morphology

Safflower flowers are typically brilliant orange, yellow or red, or more rarely white. The inflorescence is of the composite type characteristic of the family Asteraceae, with each plant producing 3–50 or more flowering heads called capitula on the ends of the branches. Each head contains between 20 and 180 individual florets (GRDC, 2010).

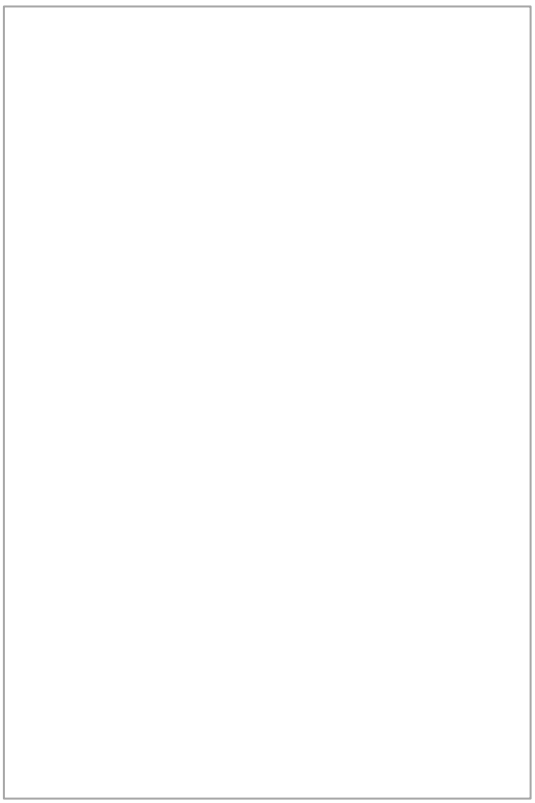

Figure 3 Safflower flowering head.

Photo: CSIRO. Reproduced under Creative Commons 3.0

SECTION 4 DEVELOPMENT

4.1 Reproduction

Safflower reproduces by seed and is not known to reproduce vegetatively (USDA-APHIS, 2008). The flowering period in safflower generally lasts from 10 days to a month. Capitula on the primary branches flower first, followed by those on secondary and tertiary branches. Flowering of the individual florets in each capitulum starts at the margin of the head and proceeds inward over 3–5 days. It may take from 10 to 45 days for all flowers on a plant to reach anthesis (Li and Mündel, 1996).

4.2 Pollination and pollen dispersal

4.2.1 Pollination

Safflower is primarily self-pollinating and cross-pollination rates or outcrossing rates are thought to be on average around 10% (Knowles, 1969). Self-pollination is predominant because the style and stigma grow through the surrounding anther column; after elongation, the stigma is usually covered with pollen from the same floret (Claassen, 1950). Individual safflower florets are largely self-pollinating, as safflower florets produce pollen that will outcompete with adjacent florets. However, an un-pollinated elongated stigma can remain receptive for several days, and outcrossing rates and seed set can be increased by insect pollinators (Claassen, 1950; Dajue and Mündel, 1996; GRDC, 2010). Outcrossing rates vary depending mainly on insect pollinators but also on variety, pollen source size and environment. Intra- and interspecific cross-pollination are considered in greater detail in Section 9.1.

4.2.2 Pollen movement

WIND

Safflower pollen is yellow and relatively large with a mean diameter of 53–56 µm (USDA-APHIS, 2008) and it is not transferred significantly by wind (Claassen, 1950; Li and Mündel, 1996). Claassen (1950) examined outcrossing rates for safflower plants grown either with or without insect exclusion cages. Depending on the cultivar, uncaged plants had outcrossing rates averaging 8.2–35% (range 6.3–58%), whereas the caged plants averaged 0.4–1.2% outcrossing (range 0–3.2%). The author acknowledged that the outcrossing observed in the caged plants could have been due to wind or to insect pollination of a few stigmas that had grown through the cage. In a glasshouse study, which excluded insects, no outcrossing was detected among the safflower plants (Claassen, 1950).

In the same study, pollen traps were placed at heights of 46, 76 and 122 cm above ground level while the safflower plants were in full flower. Safflower pollen was only detected at 46 cm, which was below the level of some of the flowers (Claassen, 1950). The height of the safflower plants was not given. Based on the assumption that some flowers were at or near the 46 cm height, there was no wind-dispersed pollen detected at distances of about 30 and 76 cm from the flowers (i.e. on the traps located 76 and 122 cm above ground). The results of these studies suggest that wind does not facilitate significant outcrossing or transport of safflower pollen and outcrossing is primarily due to insect-mediated pollen movement.

INSECT POLLINATORS

Safflower florets are largely self-pollinating but outcrossing rates and seed set can be increased by insect pollinators (Claassen, 1950; Li and Mündel, 1996; GRDC, 2010). Cross pollination is thought to occur in safflower at approximately 10% but this is highly variable and honey bees, bumblebees, beetles and other insects can increase the level of cross pollination (Emongor, 2010). Honey bees are the primary insect pollinators of safflower but other insects such as other species of bees and non-hymenopterous insects do forage in safflower (AOSCA, 2012). In studies in the United States, 80–90% of insects observed visiting safflower plants were honey bees and over 80% of observations occurred

between 8 am and noon (Boch, 1961; Levin and Butler, 1966). Bumblebees (*Bombus spp.*) play a role in the transfer of pollen in the Northern Hemisphere where they represent less than 10% of insect pollinators in safflower, but since bumblebees only occur in Tasmania (Cresswell, 1999, 2000), bumblebees do not play a major role in pollination of safflower in Australia.

In Australia, the most important insect pollinator in safflower are honey bees (*Apis mellifera*) which visit the flowers for both pollen and nectar, yet it has been suggested the presence of honeybees is unlikely to increase yield by more than 5% (GRDC, 2010). Langridge and Goodman (1980) examined insect visitors to the safflower variety Gila grown in Australia and found 75% of insect visitors were honey bees followed by a native species of halicitidae (21%), hoverflies (4%) and diptera species. Hoverflies and diptera species were not significant pollinators and other hymenopterous species are most effective in mediating cross pollination.

POLLINATORS

Safflower ranks highly among the commercial crops for honey bee preference. Chaney (1985, as cited by Van Deynze et al., 2005) found honey bee pollen collectors bypass cotton and fly five miles (8 km) to safflower while nectar collectors forage in nearby cotton. Conclusions from a Californian trial were that the population density of bees in trial crops (onion, carrot and safflower) were primarily a function of the quality and quantity of foraging resources and secondarily a function of competition from nearby colonies (Gary et al., 1977). Nectar gatherers were observed to be the predominant visitors in Australia on “Gila” safflower fields but many were well dusted with pollen (Langridge and Goodman, 1980). The distance of pollen dispersal or movement is dependent on pollinator behaviour but also on plant density and sparse areas of plants receive fewer pollinator visits (Kunin, 1997). Long distance bee foraging has been documented with one bee (of 2000 marked) found 7.1 km from the hive on safflower (Gary et al., 1977). Foraging distances of pollen-collecting honey bees is longer in simple sparse landscapes than complex landscapes with ample vegetation (AOSCA, 2012).

Studies of the foraging habits of honey bees on safflower fields in India observed honey bees made foraging trips that lasted 15 minutes, visiting 5 to 8 flowers per trip with an average of 15 seconds to two minutes spent per flower (Pandey and Kumari, 2008). In a study of safflower fields (variety Gila) in Australia, honey bees were observed to visit on average 9 flowers per head, usually visit one head per plant and spend 12.2 sec per plant. One bee visited 54 plants in 15 min while another visited 48 plants in under 8 min (Langridge and Goodman, 1980).

POLLEN VIABILITY

The likelihood of successful pollination or cross-pollination is both dependent on pollen dispersal and on how long the pollen grain remains viable. In general, pollen viability is dependent on a number of factors including temperature and humidity.

There is limited information on safflower pollen viability. Safflower is usually grown in dry conditions, where pollen is expected to desiccate rapidly (USDA-APHIS, 2006). Safflower anthers contain 150–300 pollen grains and pollen can be shed for 10–45 days (Pandey and Kumari, 2008). Safflower pollen has a short life, with no experimental evidence showing viability beyond the day pollen is released. However, anecdotally, breeders have reported viability extending into the second day after release (Knowles, 1980). The stigma is receptive for about two days after its exertion from the corolla tube (Knowles, 1980).

4.3 Fruit/seed development and seed dispersal

4.3.1 Fruit/seed development

Each safflower head or capitulum usually produces 15 to 60 seeds. Safflower seeds are contained within a thick hull, this type of fruit is known as an achene, which mature 4 to 5 weeks after flowering

(Li and Mündel, 1996; Singh and Nimbkar, 2006). The seeds are usually white but can be striped also and relatively large, 6–7 mm long with an average weight of 40 mg or 0.030–0.045 g (25,000 seeds/kg) (GRDC, 2010). The white hulled varieties are used for the birdseed and pet food market; seed with brown stipes or with mould or staining are not acceptable (Mündel et al., 2004). Seeds are typically smooth but some varieties have tufts of hairs (pappus) on the ends, which is not desirable in commercial cultivars (Li and Mündel, 1996). Therefore, most seeds of cultivated safflower lack a pappus or, if present, it is reduced (Bérvillé et al., 2005).

4.3.2 Seed dispersal

WIND

Safflower seed is not appreciably dispersed by wind. During domestication of safflower, traits that increased seed recovery at harvest were selected, and as a result cultivated safflower is highly shatter resistant compared to its wild relatives (Bérvillé et al., 2005; McPherson et al., 2009b). Safflower does not lodge readily but branches/flower heads could be dispersed by very strong winds, particularly if the plants or stems were weakened due to pathogen infections, or damaged through the activity of birds or other animals (McPherson et al., 2009b; GRDC, 2010).

WATER

No data is known on seed transport rates by water of safflower seed. It is likely that seed could be carried by heavy rains and flooding either shortly after planting or at harvest. If there were heavy rainfalls, transported seed is likely to germinate because safflower seed has little or no dormancy. However, safflower is very sensitive to excess moisture/water either as heavy rainfalls, standing water (waterlogging) or humidity. This is due to the increased chance of disease (e.g. *Phythophtora*) under these conditions which can lead to substantial yield losses (Nimbkar, 2008; GRDC, 2010).

HUMANS

Spillage during movement of seed on equipment for planting, harvest or post-harvest storage/shipping provides the greatest potential for dispersal of safflower seed. Seed could be spilled during transport but may also be dispersed if inadvertently transported on the machinery (e.g. on muddy wheels). It is also possible for small amounts of seed to be transported on or in clothing (e.g. pockets and cuffs) or boots (especially muddy boots) of workers.

ANIMALS

Primary loss of crop seeds is due to predation by insects, birds, mammals, pathogen attack and loss at harvest. Predation can result in large seed losses from the seed bank for crop seeds lost during harvest. Safflower seeds are a food source for a range of species including mammals, birds and invertebrates. Secondary seed dispersal may occur also and some seeds may be transported intact by ants, dung beetles or scatter-hoarding rodents (Vander Wall et al., 2005). Safflower seeds are firmly held within the seed heads and are highly shatter resistant, therefore limiting access by rodents. Post-harvest dispersal of seeds by small mammals, i.e. rodents, is most likely with predation of seeds present on the soil surface. Safflower seed may be dispersed (scattering) and hoarded by rodents.

For some larger animals such as cattle, foraging or grazing is minimal due to the spiny nature of mature safflower plants (Cummings et al., 2008), but sheep and goats are not irritated by the spines. Feral pigs or boars are very destructive and difficult to exclude from fields. Native animals may also feed on safflower. However, pests such as pigs and kangaroos are deterred from grazing safflower by its spines and unpalatability (GRDC, 2010). The viability of safflower seed after passing through the digestive gut of animals is poorly understood.

Safflower dispersal by birds is most likely as some safflower seed varieties are sold as birdseed. Small birds can feed on ripening safflower seed and larger birds such as cockatoos can chew safflower plants

at the base in order to access seeds (GRDC, 2010). Safflower seed dispersal by several bird species (blackbirds, mallard ducks, pigeons and pheasants) was examined and it was observed that viable seed did not pass through the digestive tract but did remain viable in the oesophagus/crop and gizzard regions for several hours. A few seeds were also transported externally on soil attached to feet or legs of pheasants and pigeons (Cummings et al., 2008). Seeds did not attach to plumage possibly due to the fact that safflower seeds are smooth. The researchers also mentioned other bird species that hoard or cache seeds such as ravens, jays and crows as potential transport vectors of safflower seeds.

There is limited information on predation by Australian bird species, such as cockatoos and galahs. These can be present in larger numbers but their ability to disperse viable safflower seed is unknown.

Figure 4 Safflower seed.

Photo: N. Wachsmann, (GRDC, 2010). Reproduced with permission.

4.4 Seed dormancy and germination

4.4.1 Dormancy and germination

Safflower seed has been selected for reduced dormancy during domestication (Bérvillé et al., 2005; McPherson et al., 2009b). Seeds of modern cultivars generally lack dormancy and can germinate in the head if rainfall occurs at harvest time (Zimmerman, 1972; Li and Mündel, 1996). A study was conducted to examine the germination of freshly harvested seed from 1973 accessions from over 50 countries, with seed germinated at 20°C. The average time to achieve at least 60% germination was 60 h for approximately 99% of the accessions. The remaining 1% required more than 120 h to reach at least 60% germination (Li et al., 1993, as cited by Li and Mündel, 1996). The little dormancy found in safflower appears to be cultivar dependent and is lost during storage, e.g. 24 weeks storage at room temperature (Kotecha and Zimmerman, 1978).

Safflower is ideally sown into moist soil at a depth of 2 to 3.5 cm; deeper sowing increases susceptibility of the seed to *Pythium* (GRDC, 2010). Germination can occur at temperatures as low as 2 to 5°C and takes between 3 and 8 days, depending on temperature (Li and Mündel, 1996; Emongor, 2010). However, germination is poor when soil temperatures are below 5°C. Safflower seedlings are

frost resistant to about -7°C. Sowing depth, light, temperature and moisture will all influence germination (McPherson et al., 2009b). Timing of emergence also depends on temperature but generally plants emerge 1–3 weeks after sowing (GRDC, 2010).

4.4.2 Seed banks/Persistence

Dormancy can affect the persistence of seeds in soil, but as discussed above, safflower generally has no or very little long-term seed dormancy which limits its persistence in seed banks.

In Australia, safflower seed loss during harvest is about 3–4% (GRDC, 2010). Similarly, harvest losses in California were estimated at 3–4%, or 192–384 seeds m^{-2} on yields of 2200 to 3400 kg/ha (Knowles et al., 1965). In one study conducted over 6 sites in Alberta, Canada, seed losses ranged from 230–1070 seeds m^{-2} with 80–520 viable seeds m^{-2}, representing a range of 26 to 84% viable seed depending on the site (McPherson et al., 2009a). It is not unusual that a large portion of seed lost during harvest is non-viable. Combine settings (e.g. sieve size, wind speed) are normally such that low weight and small sized seed are dispersed during harvest. Such seed is usually immature and is unlikely to be viable. However, these levels are relatively high and represent up to 5 times the recommended seeding rate for that region. The researchers did state that similar pre-harvest and harvest losses are found in wheat fields. Despite these large losses, safflower volunteers, emerging in spring ranged from 3–11 seedlings m^{-2}. Volunteers did not survive in fields under chemical fallow. In only three of ten cereal fields surveyed, a few volunteers (0.05–0.33 plants m^{-2}) survived the first year and generated viable seeds (1–4 seeds per plant). However, volunteer populations did not persist beyond two years (McPherson et al., 2009b).

Seed viability of safflower on soil surface and buried at two different depths was also examined (McPherson et al., 2009b). Viability of the seed was evaluated after burial in artificial seed banks or spreading the seed on the surface. Seeds did not persist beyond two years at the soil surface and beyond one year if buried at 2 cm or 15 cm. Thus, the authors recommended tillage to reduce persistence of the seed bank because the buried seed lost viability faster than the seed on the soil surface (McPherson et al., 2009b).

4.5 Vegetative growth

Safflower does not spread vegetatively and propagates only through seed germination (USDA-APHIS, 2008). After seed germination, safflower goes through a slow growing period called the rosette stage, during which several leaves are produced near the ground and taproots begin to develop but no stem is formed (Figures 2 and 5) (Li and Mündel, 1996). This stage generally lasts between 25 and 30 days, but the duration varies with variety and growing conditions and can be as long as several months. The rosette stage occurs in winter and is longer in southern than northern growing regions of Australia (GRDC, 2010).

The rosette stage is followed by rapid stem elongation and extensive branching (Figure 6), the degree of which depends on both variety and environment (Li and Mündel, 1996; Singh and Nimbkar, 2006). The number of branches is an important determinant of yield as each branch ends in a flower head (GRDC, 2010).

Figure 5 Rosette stage of safflower.

Photo: N. Wachsmann; (GRDC, 2010). Reproduced with permission.

Figure 6 Stem elongation stage of safflower.

Photo: N. Wachsmann; (GRDC, 2010). Reproduced with permission.

SECTION 5 BIOCHEMISTRY

Safflower plants have many uses, the seed oil is used as an edible oil and in industrial applications, the whole seed is used for the birdseed market, dehulled seed meal is used as feed for livestock and floral extracts have food and medicinal uses. Safflower plants contain many compounds including phenolic compounds, flavonoids, alkaloids and aromatic glucosides (Zhou et al., 2014). Many of these compounds are beneficial with antioxidant properties. Some compounds from safflower have reputed beneficial effects and this is reflected in the medicinal use of safflower plant, in China for treatment of a broad range of ailments including hypertension, coronary heart ailments, rheumatism and male and female fertility problems (Chengaiah et al., 2010). However, there is a lack of quality randomised control trials on safflower. There have been some reports of adverse effects of safflower use, primarily examining the effects of whole safflower flower extracts.

5.1 Toxins

5.1.1 Seeds

Safflower seed oil is generally not known to be toxic and has a long history of safe use. Safflower oil is used in clinical trials as a placebo and is well tolerated. Some of the toxic or anti-nutritive compounds present in low amounts in seeds include lignans, tannins, cyanidin and oxalates (Ingale and Shrivastava, 2011; Kuehnl et al., 2013). The main lignin compounds present in safflower seeds are trachelogenin, arctigenin and matairesinol and while having anti-nutritive properties these lignans may also have beneficial anti-inflammatory effects (Kuehnl et al., 2013).

Safflower meal, the by-product after oil extraction, can be used as a feed but there are natural toxins present. The chemical composition of two Indian hybrid safflower varieties was analysed and anti-nutritive or toxic compounds identified included hydrogen cyanide (3.5 mg/100 g), tannins (0.5 g/100 g) and oxalates (0.8 g/100 g). In animal feeding studies of these safflower seeds, the toxic compounds were present in such low amounts that they were non-toxic to rats. The safflower seeds showed comparable nutritive value to other oilseed crops (Ingale and Shrivastava, 2011). Fatty acid composition of safflower seed in presented in Table 3. High fibre content of the safflower seed or seed meal is the main factor limiting its use in livestock feed (see Section 2.2.1).

5.1.2 Flowers

Safflower petal extracts have been used in Chinese herbal medicine for centuries. The effect of safflower flowers or extracts from flowers has been examined and both harmful and beneficial effects have been reported.

The effect of safflower aqueous floral extract on mouse spermatogenesis was reported in a trial where mice were given doses of 200 mg/kg of extract for 35 days, resulting in damage to testicular tissue (Mirhoseini et al., 2012). However, other studies have examined the effect of safflower extract (dried safflower petal aqueous extract) in infertile rats and observed a positive effect with spermatogenesis and sperm count increased and researchers suggested safflower could improve fertility (Bahmanpour et al., 2012). Iranian researchers examined the potential teratogenic and cytotoxic effects of safflower extract in pregnant mice. The water extract of safflower was administered at 1.6 and 2 mg/kg/day to pregnant mice and elicited embryo abortion at lower doses and appeared to have negative effects on the mouse central nervous system (Nobakht et al., 2000).

Histological, ultra-structural and biochemical studies on the kidneys of mice treated with whole safflower methanol extracts revealed toxic effects. Exposure at doses of 1.4 and 2.8 mg/kg had harmful effects on the renal tissue of mice and therefore researchers recommended popular consumption of this plant should be reconsidered (Monfared, 2013). Previous work by the same researchers showed toxic impacts of safflower methanol extracts on mice embryo development and organogenesis.

The above consider the effects of whole safflower flower extracts, but it is not clear what compound or active ingredient may be causing adverse effects. Hydroxysafflor yellow A (HSYA) is thought to be one of the main active ingredients or components of floral pigments in safflower. Purified HSYA can cause slight nephrotoxicity in rats but not in mice (Liu et al., 2004). In another study, HSYA had a neuroprotective effect at doses as low as 6.0 mg/kg in rats (Zhu et al., 2003).

In a 90 day sub-chronic toxicity study using HSYA at 20, 60 or 180 mg/kg/day, researchers observed prolonged blood coagulation time at 60 and 180 mg/kg/day. HSYA at 180 mg/kg also increased the liver index without an obvious pathological change in liver histological analysis. There was no other organ injury found in this study (Liu et al., 2004). In a similar study, researchers observed histopathological kidney and liver abnormalities in sub-chronic toxicity studies of safflower floral

extracts (Mohseni et al., 2011). Extracts did not harm the acute toxicity system but HSYA did induce slight haematological, biochemical and pathological changes.

5.2 Allergens

Safflower oil is non-allergenic and suitable for use in injectable medications and cosmetics (Smith, 1996). Nonetheless, a recent study of adverse drug reactions reported in a hospital in China observed that some of these cases were due to safflower injections used as a traditional Chinese medicine. The manifestations included drug rash, shock, chest tightness and renal insufficiency (Shen and Chen, 2012). However, the adverse reactions may be due to other components of the injection. (Zhang et al., 2009) observed anaphylaxis induced by safflower injection of guinea pigs but safflower specific IgG antibody was not found in human blood samples and researchers indicated anaphylaxis may be due to liposoluble ingredients of the injection (Zhang et al., 2009). In a review of randomised clinical trials (RCTs) assessing the neuroprotective properties of safflower yellow as a treatment for ischemic stroke, of 39 RCTs only 7 RCTs were of an acceptable standard and skin rash was reported as an adverse reaction in one of the RCTs while it was unclear if any adverse reactions were observed in 4 of the RCTs (Fan et al., 2014).

Safflower flowers are used as a flavouring and food additive in Iran and India. In China, they have been used almost exclusively for medicinal purposes since 2nd century B.C. and are still widely used as a traditional medicine known as Hong Hua (Zhou et al., 2014). There is a safflower flower industry in some countries such as Japan and the United States (California). Rare cases of allergic reactions to safflower plants have been reported (Compes et al., 2006). An IgE-mediated immunological mechanism was responsible for occupational asthma in a single patient in response to dried safflowers.

5.3 Beneficial phytochemicals

Plants and seeds may contain many phytochemicals such as flavonoids, alkaloids, polyphenols, anthocyanins, phenols, terpenoids, glycosides, sterols/oils. Many of these phytochemicals have beneficial attributes such as antioxidant activity, anti-inflammatory and neuro-protective properties.

5.3.1 Compositional analysis of safflower seed

Cultivated varieties of safflower can range in seed oil content from 20–45% (Li and Mündel, 1996), with many modern cultivars containing about 30–40% oil, as well as 20% protein and 35% fibre (Sehgal and Raina, 2011). Safflower seeds are also rich in minerals (Zn, Cu, Mn, Fe), vitamins (thiamine, β-carotene) and tocopherols (α, β and γ). The leaves and shoots are rich in vitamin A, phosphorus, iron and Ca, and young shoots are sold as a vegetable in India and other countries. While the primary use of safflower is its seed oil, the flowers are also used in many countries for food flavouring and as medicines in China, India and Iran (see review by Sehgal and Raina, 2011). The fatty acid composition of different seed oil crops is listed in Table 2.

Table 2 Fatty acid composition in two safflower lines compared to two other common oil seed crops.

Fatty Acid	Safflower[a]	Safflower[b,c]	Soybean[b,c]	Canola[b,c,d]
Myristic acid (C14:0)	0.15[e]			
Palmitic acid (C16:0)	6.69	11.07± 0.10	16.29 ±0.54	8.23 ± 1.01
Palmitoleic acid (C16:1)	0.13	–	–	0.32 ± 0.08
Stearic acid (C18:0)	2.06	4.37 ± 0.22	6.66 ± 0	2.92 ± 0.79
Oleic acid (C18:1)	12.71	12.76 ± 0.24	22.70 ± 0.07	53.84 ± 0.97
Linoleic acid (C18:2)	77.74	69.65 ± 1.15	44.13 ± 0.60	23.38 ± 0.53
Linolenic acid (C18:3)	0.08	0.49 ± 0.05	8.97 ± 0.52	9.82 ± 0.87

Fatty Acid	Safflower[a]	Safflower[b,c]	Soybean[b,c]	Canola[b,c,d]
Arachidic acid (C20:0)	0.27	0.78 ± 0.09	0.62 ± 0.11	0.99 ± 0.03
Eicosanoic acid (C20:1)	0.13			
Behenic acid (C22:0)		0.59 ± 0.09	0.63 ± 0.02	0.52 ± 0.10
Lignoceric acid (C24:0)		0.29 ± 0.13	–	–

[a] high linoleic acid safflower cultivar (Cosge et al., 2007).

[b] medium linoleic acid safflower cultivar, soybean and rapeseed (Patrascoiu et al., 2013).

[c] "-" indicates below detection level; no entry - not measured

[d] referred to as rapeseed in reference

[e] all data expressed as g/100 g oil (= % weight)

5.3.2 Beneficial phytochemicals - Fatty acids

Safflower can have a high content of linoleic acid of up to 90% of total seed oil fatty acids. Linoleic acid is an essential omega-6-PUFA required in the human diet. GLA is an important essential omega-6-PUFA synthesised from linoleic acid (LA). Other safflower varieties contain high levels of oleic acid (OA), an omega-9-MUFA. MUFAs such as oleic acid tend to lower blood levels of low density lipoproteins ("bad" cholesterol) without affecting high density lipoproteins ("good" cholesterol). Increased oleic acid intake, particularly when used as a replacement for saturated fatty acids, has been shown to be beneficial to human health (Sales-Campos et al., 2013; Calder, 2015).

While some fatty acids, such as linoleic acid, are associated with lowering blood cholesterol, recent research has shown that this does not always translate to the expected benefits such as reduced risk of cardio vascular disease (OGTR, DIR 158). Recent literature suggests the clinical benefits of n-6-PUFAs, the most abundant being LA, are not established and that omega-3-PUFAs may instead be responsible for any clinical benefits (Ramsden et al., 2011; Ramsden et al., 2013; Chilton et al., 2014).

5.3.3 Beneficial phytochemicals - Antioxidants

Safflower has been used as a medicine for centuries especially in China. The safflower petals are used usually in the form of an aqueous concoction. Floral extracts are used in the form of infusions for circulatory system related diseases. In addition, extracts have been used to treat several chronic conditions including hypertension, coronary heart ailments, rheumatism, male and female infertility problems. One of the active ingredients from the petals is thought to be carthamin or HSYA a glucoside. Its active ingredients possess many reported biological activities including modulating the immune system, anticoagulation and anti-thrombosis, antioxidant, and anti-fatigue (Chengaiah et al., 2010).

Other researchers have reported anti-tumour activity and cardio-protective and neuro-protective properties (Zhou et al., 2014). Safflower extract may also have anti-diabetic properties (Asgary et al., 2012). In contrast, some researchers have observed no changes in these parameters, but have observed a level of toxicity when other parameters are examined such as embryo development or liver and kidney indices (Mohseni et al., 2011). See Section 5.1.2 for more detail.

Tocopherols are naturally occurring antioxidants in vegetable oils and have a role in reducing cardiovascular disease (ODS, 2019). There are four natural tocopherol isomers (all found in safflower) with differing antioxidant activities. In safflower, α-tocopherol (> 95% of total tocopherols) is the main tocopherol in seed (Velasco et al., 2005). The four tocopherol isomers together with four corresponding tocotrienols make up the eight vitamers that constitute vitamin E (Chester et al., 2001). The term vitamin E is used as a generic descriptor for tocopherol and tocotrienol derivatives exhibiting α-tocopherol activity (IUPAC-IUB, 1982).

5.3.4 Beneficial phytochemicals - Flavanoids

Safflower florets contain yellow and red quinochalcone natural dyes specifically safflower yellow A and B, safflomim C, precarthamin, and carthamin. These chalcones are the main constituents of a number of glycosylated flavonoids present in safflower petals that are known to have antioxidant activity (Salem et al., 2014). Variation in environment and region can affect composition of the natural dyes/pigments and their antioxidant ability (Salem et al., 2014). In addition to the glucosylquinochalcones, safflower petals also contain flavonoid glycosides. These naturally occurring flavonoids are polyphenolics with antioxidant activities (Lim et al., 2007).

Antioxidant activity is thought to be due to the presence of α and β unsaturated keto groups in the chalcone structure that act as metal chelators, and can play a role in bioavailability and toxicity of metals. It has been suggested that safflower dyes should be used as food additives /natural food colorants. HSYA can have a neuro-protective effect at doses as low as 6.0 mg/kg in rats (Zhu et al., 2003). Kinobeon A, an antioxidant isolated from cultured safflower cells was compared to two natural antioxidants, lignin and quercitin and found to exhibit stronger anti-oxidative effects and may be useful as a cryo-protective agent (Kanehira et al., 2003). Nicotiflorin, an antioxidant isolated from safflower has been shown to have a neuro-protective effect on memory/dementia in rats (Huang et al., 2007).

SECTION 6 ABIOTIC INTERACTIONS

6.1 Abiotic stresses

6.1.1 Nutrient stress

Safflower can be grown in a range of soil types but prefers alkaline soils that are well drained. Fertile deep black or grey self-mulching or cracking soils that allow full development of the root system are ideal, but alluvial and loam soils are also suitable.

Safflower has similar nutrient requirements to cereals, requiring similar amounts of nitrogen (25 kg/t seed) but more phosphorous (4.3 kg/t seed) and sulphur (4 kg/t seed). Surface applied fertilisers are not always effective while foliar fertilisers may be more suitable in allowing nutrients to be absorbed directly by leaves. The deep taproot of safflower can extract nutrients such as nitrates from deep in the soil that are beyond the reach of most other crops (GRDC, 2010). Nitrogen is generally the most limiting nutrient to safflower production, the application rate depending on soil moisture (Mündel et al., 2004).

On certain soil types in northern NSW and SA, safflower does respond to manganese, iron and/or zinc. These micronutrients are best applied 6 weeks after sowing as a foliar application (GRDC, 2010).

6.1.2 Temperature stress

Seedlings will emerge at soil temperatures above 4°C, but 15°C is considered optimal. The rosette stage of young safflower plants is resistant to cold and frosts as low as -7°C, as the growing point is protected by leaves. During the stem elongation phase, even a light -4°C frost can cause substantial damage to the stem and growing point (GRDC, 2010), and a frost just after flowering can dramatically lower yields and oil levels or kill the seed completely (Li and Mündel, 1996).

Safflower needs long days to flower, so flowering and seed growth occur in late spring and summer. Safflower can tolerate the hot dry conditions at this time of year as long as adequate water is supplied; hotter/drier conditions can hasten plant development. In Australia, mean daily temperatures above 26°C during flowering and seed growth can depress yield and oil content (GRDC, 2010). Pollination and seed set are reduced by high temperatures (>32°C) during pollen shedding (Li and

Mündel, 1996). In the US, research has shown that safflower can tolerate up to 46°C but that yields tend to be highest when temperatures during flowering remain below 32°C (GRDC, 2010).

6.1.3 Water stress

DROUGHT

Safflower is considered a moderately drought resistant crop due to its ability to access deep water due to its taproot system; it can access a larger area to retrieve water compared to other crops. It actually has a relatively high water requirement, preforming best (yields approaching 4t/ha) in regions receiving more than 450 mm annually. However, yields exceeding 1 t/ha can be expected on clay soils that are wet to 1 m depth at sowing, providing at least 50 mm post-sowing rainfall is received (GRDC, 2010).

RAINFALL AND WATERLOGGING

Despite a relatively high water requirement, safflower is not tolerant of waterlogging, especially when air temperatures exceed 20°C. Waterlogging for more than 48 hours can starve roots of oxygen and kill crops and such conditions favour the development of *Phytophthora* root rot. Older crops are more susceptible to waterlogging than younger crops. Pollination can be inhibited (Li and Mündel, 1996), diseases encouraged, seeds discoloured and sprouting can occur due to heavy rains and high humidity during flowering and seed maturation (Nimbkar, 2008; GRDC, 2010).

6.1.4 Other stresses

HERBICIDE RESISTANCE

Safflower has limited tolerance to herbicides. Plants are easily controlled by cultivation and a wide range of hormone and other herbicides (GRDC, 2010).

TOLERANCE TO WIND AND HAIL

Safflower has better tolerance of both wind and hail than cereals (GRDC, 2017). Hail can severely damage young/succulent plants, but as they mature, plants become stiff and woody and therefore develop more tolerance. Safflower resists lodging and mature plants are not prone to shattering (GRDC, 2010).

SALINITY STRESS

The salinity stress of safflower is considered moderate to high, being similar to that of barley or cotton. It is more tolerant of sodium than calcium or magnesium salts, with the later growth stages more tolerant than seedlings. Tolerance is cultivar dependent, with little information available on the Australian safflower cultivars (GRDC, 2010).

SECTION 7 BIOTIC INTERACTIONS

7.1 Weeds

Weeds that compete with safflower include grass and broadleaf weeds. Control of weeds in safflower is essential for optimum yields. Safflower is a poor competitor with weeds, due to slow growth at the rosette stage early in the season (GRDC, 2010). Later in the season many weeds can outgrow safflower in height and the resulting shading can reduce crop yields significantly (Li and Mündel, 1996).

Safflower can be sown later than other winter crops which enables more time for control of weeds prior to sowing. Harrowing when the safflower plants are 7 to 15 cm tall can give satisfactory control of small, later germinating weeds, but it is not clear if this approach is regularly used in Australia. Safflower is tolerant of some herbicides, but as a minor crop in Australia fewer herbicides are registered for use. Several pre-emergent herbicides are registered for control of broadleaf and grass

weeds such as ethyl dipropylthiocarbamate, trifluralin and pendimethalin. Post-emergent herbicides, dicloflop-methyl and propaquizafop are used for control of grass weeds while methosulfuron is used for control of broadleaf weeds (GRDC, 2010).

7.2 Pests and diseases

Safflower is usually grown as a rain-fed crop which means the incidence of diseases and pests are relatively low. However, safflower has developed from wild species growing in arid desert environments and is particularly susceptible to foliar diseases (favoured by moist environments), root-rot organisms (favoured by irrigation) and a large number of insects (especially in regions where it evolved) ((Li and Mündel, 1996). If grown under irrigation, humid conditions and waterlogging, favour the development of disease (GRDC, 2010).

7.2.1 Pests

INSECTS

In Australia, the main insect pests of safflower are aphids (plum, green peach, leaf curl), cutworms (*Agrotis spp.*), native budworm or heliothis (*Helicoverpa spp*.), rutherglen bugs (*Nysius vinitor*), red-legged earth mites (*Halotydeaes destructor*) and blue oat mite (*Penthaleus major*) all of which can be readily controlled with insecticides and some with biological controls (GRDC, 2010). Aphids are a major pest in many countries (e.g. Spain, India) (Li and Mündel, 1996) and infestations have caused losses of up to 74% (Nimbkar, 2008).

Other pests known to infest safflower crops in Australia include thrips, lucerne flea, black field crickets, grasshoppers, locusts, wireworms, false wireworms, jassids and myrids (GRDC, 2010).

Safflower often requires less pest management than other crops. In Australia, growers have found large numbers of beneficial insects such as ladybirds and spiders, in safflower fields (GRDC, 2010).

Safflower fly (*Acanthiophilus helianthi* Rossi) is one of the main limiting factors on production of the crop in several countries. The safflower fly is confined to Africa, Asia and Europe so is not a major pest in Australia. Resistance to safflower fly has been found in wild accessions of *C. oxyacanthus* and may be used in breeding programs to develop fly-resistant safflower cultivars (Sabzailian et al., 2010).

VERTEBRATES

Pests such as pigs and kangaroos are deterred from grazing safflower by its spines and unpalatability. Bird damage can be an issue especially near timbered areas which harbour birds (GRDC, 2010).

7.2.2 Diseases

Cultivation and crop rotation practices in Australia limit the prevalence of disease in safflower crops (see Section 2.4.1).

At present there are no fungicides registered for disease control in safflower in Australia (GRDC, 2010)[1]. Control of disease in Australia relies on using appropriate crop rotations, selecting resistant varieties, using clean seed, controlling volunteer and weed hosts, sound irrigation practices and selecting appropriate soils. Safflower diseases can be hosted on stubble, volunteer plants, other *Carthamus* species such as saffron thistle and some broadleaf crops (GRDC, 2010).

The three main diseases of safflower in Australia are the fungal diseases *Alternaria* blight (*Alternaria carthami*), *Phytophthora* root rot (*Phytophthora cryptogea*) and rust (*Puccinia carthami*). Other less

[1] The Australian Oilseeds Federation and NSW-DPI both indicated that as of Nov 2014, there were no permits from Australian Pesticides and Veterinary Medicine Authority (APVMA) for use of fungicides on safflower. A search of the APVMA Chemical Registration Information System (PUBCRIS) website did not list any fungicides when safflower was used as the search term (APVMA PUBCRIS; accessed September 2019).

prevalent diseases in Australia include seedling damping off, grey mould, charcoal rot, leaf spot and sclerotinia (GRDC, 2010).

SECTION 8 WEEDINESS

8.1 Weediness status on a global scale

As with all crops cultivated and harvested at the field scale, some seed may escape harvest and remain in the soil until the following season when it germinates either before or following seeding of the succeeding crop. In some instances the volunteers may provide competition to the seeded crop and warrant chemical and/or mechanical control. Volunteers can also be expected away from the planting site (e.g. along roadsides and around storage facilities) as a result of transportation of seed out of fields (e.g. in farm equipment) and spillage during transport.

Safflower lacks characteristics that are common to weeds, such as very high seed output, high seed dispersal, long-distance seed dispersal and seed shattering, persistent seed banks, and rapid growth to flowering. During the early stages of growth, safflower is slow growing and a poor competitor with fast growing weeds (Li and Mündel, 1996). However, it is considered a minor weed of agricultural and natural ecosystems in Australia (Groves et al., 2003). Primarily it is an agricultural or ruderal weed found in disturbed land use areas such as debris, roadside or disused fields (Groves et al., 2003).

Lack of seed dormancy in safflower (see Section 4.4.1) reduces the weediness potential and volunteers after harvest are uncommon (USDA-APHIS, 2008). However, some feral populations of safflower have become established in agro-ecosystems in the United States in several states (California, Iowa, Illinois, Kansa, New Mexico, Ohio, and Utah) (Bérvillé et al., 2005). There is little information on how long these populations persist, but anecdotal reports suggest safflower does not become established outside of agricultural areas (Bérvillé et al., 2005).

Studies over several years in Canada (see Section 4.4.2) suggest that safflower seed and volunteers would not persist beyond two years and that common herbicide and tillage practices would control any volunteer safflower (McPherson et al., 2009b). Moreover, experienced growers in the areas surveyed were not concerned with control of safflower in volunteers (McPherson et al., 2009b). This is likely the case in Australia (see Section 8.3)

8.2 Weediness status in Australia

In 2000/2001 a rating system was applied to weeds of natural and agricultural ecosystems in Australia (Groves et al., 2003). The weeds or naturalised non-native flora of Australia, were categorised on a scale from 0 (indicated naturalised but the population no longer exists or removed) to 5 (indicating naturalised and a major problem at four or more locations within a State or Territory). Safflower was classified as a category 1 weed of agricultural ecosystems and as a category 3 weed of natural ecosystems in Australia (Groves et al., 2003). Wheat, which is grown in rotation with safflower, is a category 2 weed in natural ecosystems and a category 3 weed in agricultural ecosystems (Groves et al., 2003).

There are no studies on percentages of safflower volunteers in crops in Australia. In Australia, like Canada, safflower is still a minor crop with less than 10,000 ha grown annually (ABARES, 2014; FAOSTAT, 2019). In Canada volunteer densities were low at 3–11 plants m^{-2} (McPherson et al., 2009a). However, Canada and Australia may not be directly comparable. For example, the ecology of the related safflower weedy species, *C. lanatus* was compared in France and Australia. Australia had much larger soil seedbanks than France and that was thought to be due to greater seed production per plant in Australia and due to the different types of herbivores present in Australia compared to Europe (Grace et al., 2002).

There are three related species which have naturalised in Australia: *C. lanatus*, *C. leucocaulos*, and *C. dentatus* (Atlas of Living Australia, accessed September 2019). Both *C. lanatus* and *C. leucocaulos* have been declared noxious weeds in some states or territories (Weeds Australia; accessed September 2019). There are doubts about the existence of *C. glaucus* in Australia; the two specimens that formed the basis of the record of this species in the 1986 Flora of SA have now been re-determined as *C. leucocaulos*, and the same may have happened in other States (personal communication: Micheala Heinson, Primary Industries Research South Australia).

8.3 Weediness in agricultural ecosystems

Safflower is unlikely to become a weed under most agricultural conditions. It is considered a category 1 weed of agricultural ecosystems in Australia, specifically in Qld, SA and the Northern Territory. A category 1 weed denotes it is naturalised and may be a minor problem but not considered important enough to warrant control (Groves et al., 2003). In New South Wales, Tasmania, Western Australia and Victoria, safflower is not considered an agricultural weed because it is not considered to be a problem (Groves et al., 2003). However, Randall (2017) considers it a high risk for weediness in Australia.

Safflower seed may be inadvertently dispersed into neighbouring fields or non-agricultural areas by water, wind, animals and insects (see Section 4.3.2). It is also deliberately and inadvertently spread by humans during transport and on farming equipment. If dispersed seed were to germinate it is unlikely to persist as safflower is a poor competitor and is easily controlled by standard agricultural practices and standard road side weed control measures. Overseas data suggests safflower plants/populations are unlikely to persist in an agricultural setting (see Section 8.1 above).

In an agricultural setting, safflower may impact follow on crops. As noted previously, safflower can extract water from deep in the soil profile at a greater depth than many other crops due to its deep tap root system and as such is effective at lowering the water table where drainage is required. Some growers use safflower to dry soil profiles (e.g. after irrigated cotton) to reduce waterlogging in subsequent crops. It may also have impacts on subsequent crop yields in areas where less water is available, as it is able to access (and remove) from areas deeper in the soil profile , and it takes time for the water profile to replenish (GRDC, 2010). However, safflower's ability to penetrate and break up hardpans and create channels in the soil profile can also facilitate air and water movement, thus benefiting following crops (GRDC, 2017).

8.4 Weediness in natural ecosystems

In Australia, safflower is classified as a category 3 weed in natural ecosystems, meaning it is naturalised and known to be a minor problem warranting control at four or more locations within a state or territory. However, (Groves et al., 2003) emphasises that safflower is primarily an agricultural or ruderal weed. Anecdotal evidence from weed risk experts in the different states in Australia indicate that *C. tinctorius* is not a significant weed in natural ecosystems in Australia (personal communication: Stephen Johnson, NSW Department of Primary Industries).

8.5 Control measures

Safflower is a poor competitor with weeds so safflower volunteer survival and fecundity is expected to be low in competitive following crops such as barley or wheat. Safflower is also not tolerant of many herbicides, so those commonly used on following crops are likely to further reduce safflower volunteers (Li and Mündel, 1996; McPherson et al., 2009b).

Tillage following harvest is also recommended as a means to reduce persistence of the safflower seed, because seed on the soil surface remained viable longer than seed buried to depths of 2 or 15 cm (see Section 4.4.2).

SECTION 9 POTENTIAL FOR VERTICAL GENE TRANSFER

9.1 Intraspecific crossing

Vertical gene transfer is the transfer of genetic information from an individual organism to its progeny. In flowering plants vertical gene transfer mainly occurs via pollen dispersal and cross pollination between related sexually compatible plants. Intraspecific crossing refers to fertilisation between *C. tinctorius* safflower plants. Outcrossing in safflower is mainly insect-mediated with wind-mediated outcrossing playing a minor role (see Section 4.2.2). Honeybees and bumble bees are the main pollinators of safflower. Bumble bees only occur in Tasmania so would not contribute to outcrossing in the safflower growing regions of the Australian mainland.

There is no information on intraspecific crossing of safflower in Australia. Worldwide, studies show that outcrossing rates appear to be quite variable (Table 3) and may depend on a number of factors, such as pollen source size and shape, environmental climatic conditions, insect numbers and type and variety/cultivar. Summaries of these studies are provided below.

One of the earliest studies to examine outcrossing in a number of safflower cultivars, using corolla colour as a marker was conducted in the United States (Claassen, 1950). Outcrossing levels between rows 1–1.5 m apart, ranged from 0 to over 50% for some cultivars, but most had rates of less than 10%. Individual plants varied considerably with outcrossing frequencies ranging from 0–100% at the 1m spacing (Claassen, 1950). In inbred varieties selected for high yield and high oil content, the average outcrossing between rows was less than 5%. Researchers also measured outcrossing rates in different regions within Nebraska, but didn't find any significant difference. These results indicate outcrossing rates were more dependent on variety than topography however other studies have not supported this.

In another early study conducted in India also using corolla colour as a marker, cross-pollination rates ranged from 1–28%, with an average of 10%, between safflower plants in close proximity (exact distance not given). At a distance of 13.7 m, the average outcrossing rate ranged from 0.8–5.9%, with an average of 1.9% (Kadam and Patankar, 1942).

Table 3 Outcrossing rates in safflower

Study	Outcrossing range % (average %)	Distance
Kadam and Patankar (1942) India	1–28 (10)	Close proximity
	0.8–5.9 (1.9)	13.7 m
Claassen (1950) United States	8.3–100 (34.2)	1 m
	0–26 (14.9) low outcrossing lines	1 m
	31.8–93.6 (57.3) high outcrossing lines	1 m
Rudolphi et al. (2008) Germany	6–33 (9.7–18)	Close proximity
	0–11.5 (6.5)	At least 5 m
McPherson et al. (2009a) Canada & Chile	0.48–1.7	0.3–3 m
	0–0.86	~10m
	0–0.26	~ 20m
	0–0.10	~ 30m
	0.03–0.16	~ 40m
	0.0024–0.04	~50m
	0.01	~100m
	nil	~300m

Study	Outcrossing range % (average %)	Distance
Cresswell (2010)	0.005–0.05 (mathematical model)	Field to field
Velasco et al. (2012) Spain	0.5–35.9 (10.3)	1–1.5 m
Nabloussi et al. (2013) Morocco	8–53 (26.6)	1–1.5 m

More recently, a small study in Germany found the level of outcrossing between plots of safflower ranged from 0–33%, with averages of 6.5–18% depending on the location of the sampled plant (Rudolphi et al., 2008). Outcrossing rates were also measured between plants grown together in the same plot and dropped from 63% in 2004 to 30% in 2005. The large variation between the two years of the study may have been due to different environmental conditions (Rudolphi et al., 2008).

A study in Spain examined outcrossing from a high oleic content cultivar (CR-6) to a low oleic content cultivar (Rancho) separated by 1 to 1.5 m. The CR-6 plants were surrounded by Rancho plants and high oleic acid was used as a biochemical marker to estimate outcrossing. The experimental crops were grown at three different times, winter sowing in 2009, winter sowing in 2010 and spring sowing in 2010. Average outcrossing rates of 5.7%, 12.1% and 13.2% were observed, respectively. Higher frequencies were detected at the single-plant level (35.9%) and at the single-head level (58.3%) (Velasco et al., 2012).

Nabloussi et al. (2013) used the same cultivars and field layout as Velasco et al. (2012) (above) to determine outcrossing rates under Moroccan conditions. The average outcrossing rate at 1–1.5 m was 26% with a range of 8.3–53% at the plant level. This rate was approximately twice that reported by Velasco et al. (2012). As the two studies used the same cultivars and field layout, this demonstrates the influence of the environment and possibly pollinators on outcrossing rates.

The frequency of natural outcrossing from GM safflower to non-GM safflower was measured under field conditions in three different environments. Outcrossing experiments were conducted in the province of Santiago, Chile (2002) and the Canadian provinces of British Columbia (2002) and Alberta (2004) (McPherson et al., 2009a). The GM safflower contained the pat gene which confers tolerance to the herbicide glufosinate and this trait was used to confirm outcrossing to the non-GM safflower. The three trial sites varied in design layout including the distance from the GM safflower to the first rows of non-GM safflower (0.3–3.0 m), distance over which outcrossing was measured, and size of the GM pollen source (99–900 m^2) (McPherson et al., 2009a).

The highest rate of outcrossing of 1.67% was detected at the British Columbia site at a distance of 3 m, which was the nearest distance measured. Outcrossing was observed at each distance sampled at this site (from 3–101 m), except for a single measurement at 300 m where no outcrossing was detected. At the site in Santiago, outcrossing was observed at nearly every distance (0.7–60.5 m) with the highest outcrossing rate of 0.48% again observed in samples taken at the closest distance of 0.7 m. No outcrossing was detected at most distances measured at the Alberta site (from 0.3–49.5 m), the highest outcrossing rate observed was 0.62% at 0.3 m (McPherson et al., 2009a). Highest levels of outcrossing occurred closest to the pollen source and declined over distance for all three sites.

Outcrossing frequencies were as heterogeneous between the three sites as they were between blocks (replicates). Researchers indicated this variation may be due to non-random movement of pollen by insects, as wind is not a significant factor in safflower outcrossing (Claassen, 1950; McPherson et al., 2009a). In addition, the size of the pollen source may be a factor. The area of the British Columbia pollen source was about 9 times larger (900 m^2) than either of the other two sites (99 and 110 m^2) and outcrossing close to the pollen source at this site was four times greater. The larger site also demonstrated a slower decline in outcrossing with distance (McPherson et al., 2009a). Other differences in site design may have affected outcrossing rates. The Alberta site had a barren zone between the GM and non-GM safflower and this may have affected insect-mediated cross pollination.

Differing insect populations at the sites has been proposed as a possible cause for the lack of outcrossing observed at the Alberta site (McPherson et al., 2009a).

McPherson et al. (2009a) also considered directionality at the three sites and noted that there were predominately westerly winds during flowering. However, greater outcrossing was not found on the leeward side of the trial sites, which supports Claassen (1950) findings that wind-mediated pollination plays a minor role, if any, in outcrossing of safflower.

Outcrossing rates in the McPherson et al. (2009a) study over 0.3–3m ranged from 0–1.7% and this is at least an order of magnitude lower than the other studies for distances of 1–1.5 m (see Table 3). One reason for this is environmental differences which can influence outcrossing rates e.g. Velasco et al. (2012) and Nabloussi et al. (2013) used the same cultivars and field designs in different countries (Spain or Morocco) but had a two-fold difference in outcrossing rates. The different outcrossing rates would be influenced by the cultivars studied, e.g. Claassen (1950) demonstrated huge variability in outcrossing (14.9% and 57.3% in low and high outcrossing lines, respectively). In addition, outcrossing would be influenced by the type and number of pollinators at the trial site.

McPherson et al. (2009a) did point out that this work cannot predict maximum distances of pollen movement by pollinators due to long distance foraging by bees, pollen can potentially be dispersed by bees foraging over a range of kilometres. In addition, the researchers found that the outcrossing rate in safflower was spatially heterogenous as was the case observed by Nabloussi et al. (2013), indicating that bee and other insect visitations occur in a random and unbalanced way. Cross pollination of safflower plants is predominantly insect-mediated, wind can only facilitate pollen movement over short distances (< 1 m) between plants grown close together (Claassen, 1950). There is evidence of long-distance insect-mediated pollen transfer in other predominantly self-pollinated crops, such as cotton and oilseed rape, due to the long-distance foraging capability of honey bees and bumble bees (AOSCA, 2012).

Bumblebees have been suggested as being more effective at field-to-field pollination of safflower than honeybees. Using a mathematical model of field-to-field gene flow due to insect pollination, the maximum level of bee-mediated gene flow between large fields was estimated at 0.005–0.05% (Cresswell, 2010). The highest value occurred when it was assumed that fields were pollinated exclusively by bumble bees. Values for the model were determined using observations of honey bee and bumble bee behaviour on a 40 ha field of safflower in Canada. Bees made long foraging bouts within the field, making between field pollinations rare. This factor, as well as safflower's high capacity for self-pollination, resulted in the very low estimates of pollinator mediated gene flow between fields (Cresswell, 2010). In Australia outcrossing rates over long distances may therefore be reduced due to the lack of bumblebees. The predominant insect pollinator of safflower is the honeybee (see Section 4.2) and long distance bee foraging has been documented in crops, including safflower (Gary et al., 1977).

It has also been suggested from safflower growers' observations that safflower varieties grown in Australia have less than 10% outcrossing rates unless hives are brought in for the purpose of cross-pollination (GRDC, 2010).

9.2 Natural interspecific crossing

Hybridisation between safflower and wild *Carthamus* species has probably played a role in the evolution of *C. tinctorius* in the Mediterranean and Asia where they are sympatric (McPherson et al., 2004). The information below includes literature regarding natural hybrid formation between *C. tinctorius* and species within the sections of this genus. Although Section 9.3 provides details related to artificial hybridisation, the results from artificial crosses are also mentioned here as indicative of the possibility of natural hybridisation.

Natural interspecific hybridisation between safflower and its wild relatives can only occur if there is synchronous flowering - temporal sympatry - and proximity - spatial sympatry (Ellstrand et al., 1999). Hybridisation between safflower and wild *Carthamus* species has probably played a role in the evolution of *C. tinctorius* in the Mediterranean and Asia where they are sympatric (McPherson et al., 2004). Spatial sympatry can be seen in Table 4, which summarises the geographical distribution of *Carthamus* species (McPherson et al., 2004; GBIF Backbone Taxonomy, 2017). The self-compatibility of *Carthamus* species and their compatibility with *C. tinctorius* is summarised in Table 5.

Table 4 Geographical distribution of *C. tinctorius* L. (cultivated safflower) and related species.

Taxon	Geographical Distribution
Section *Carthamus* (2n = 24)	
C. curdicus Hanelt	Iran only
C. gypsicolus Iljin	Iran, Iraq, Kazakhstan, Azerbaijan, Armenia, Lebanon, Turkey, Syria and Uzbekistan
C. oxyacanthus Bieb.	Pakistan, Iran, Afghanistan, Iraq, Turkey, India, Uzbekistan, Azerbaijan, Armenia and **Australia**
C. palaestinus Eig.	Israel and Iraq
C. persicus Willd. (syn. *C. flavescens* Spreng.)	Israel, Turkey, Iraq, Syria, Ethiopia, Lebanon, Jordan and Iran
C. tinctorius L.	Widely cultivated (safflower)
Section *Odonthagnathis* (DC.) Hanelt (2n = 20, 22)	
C. boissieri Halácsy	Greece, France and Cyprus
C. dentatus Vahl	**Australia**, Greece, Turkey, Bulgaria, Cyprus, Hungary, Iran and Macedonia
C. divaricatus Beguinot & Vacc.	Libya
C. glaucus Bieb.	Israel, Palestine, Turkey, Syria, Lebanon, Greece, Azerbaijan, Afghanistan, Egypt, Ukraine, Armenia, Jordan, Iraq, Russia and **Australia**
C. leucocaulos Sm.	Greece, **Australia**, America, Germany, Turkey and Argentina
C. tenuis (Boiss. & Bl.) Bornm.	Israel, Palestine, Lebanon, Greece, Cyprus, Jordan, Egypt, Syria and Turkey
Section *Atractylis* Reichenb. (2n = 44, 64)	
C. creticus L.	Greece, Spain, America, Portugal, Denmark, Morocco, New Zealand, **Australia**, France, Egypt, Iraq and Turkey
C. lanatus L.	Spain, France, Italy, Portugal, America, Greece, Argentina, Ethiopia, Morocco, Turkey, Germany, Brazil, Netherlands, India, Pakistan and **Australia**
C. turkestanicus Popov	Afghanistan, Iran, Armenia, Turkey, Uzbekistan and Pakistan
Uncertain placement (2n = 24)	
C. nitidus Boiss	Palestine, Israel, Jordan, Syria, Saudi Arabia, Lebanon and Egypt

Source: (McPherson et al., 2004; GBIF Backbone Taxonomy, 2017).

Table 5 Assessment of self-compatibility, compatibility with *C. tinctorius* L. and genomic formulae for *Carthamus* spp.

Taxon	Self-compatibility	Compatibility with *C. tinctorius*	Fertility Comments	Genomic formula
Section *Carthamus* (2n = 24)				
C. curdicus Hanelt	Compatible	Unknown	–	–
C. gypsicolus Iljin	Compatible	Unknown	–	–
C. oxyacanthus Bieb.	Both known	Yes	Fertile	BB
C. palaestinus Eig.	Compatible	Yes	Fertile	B_1B_1
C. persicus Willd. (syn. *C. flavescens* Spreng.)	Incompatible	Yes	Fertile	B_1B_1
C. tinctorius L.	Compatible	Yes	Fertile	BB
Section *Odonthagnathis* (DC.) Hanelt (2n = 20, 22)				
C. boissieri Halácsy	Unknown	Unknown	–	–
C. dentatus Vahl	Incompatible	No	–	A_1A_1
C. divaricatus Beguinot & Vacc.	Incompatible	Yes	Fertile self-incompatible hybrids	–
C. glaucus Bieb.	Unknown	Yes	Infertile hybrids	AAA_3A_3
C. leucocaulos Sm.	Compatible	Yes	Infertile hybrids	A_2A_2
C. tenuis (Boiss. & Bl.) Bornm.	Unknown	Unknown	–	–
Section *Atractylis* Reichenb. (2n = 44, 64)				
C. creticus L.	Compatible	Yes	Fertile	$A_1A_1B_1B_1A_2A_2$
C. lanatus L.	Compatible	Yes	Infertile hybrids	$A_1A_1B_1B_1$
C. turkestanicus Popov	Compatible	Yes	–	$A_1A_1B_1B_1A_3A_3$
Uncertain placement (2n = 24)				
C. nitidus Boiss	Compatible	Yes	Infertile hybrids	–

Source: McPherson et al. (2004)

SECTION CARTHAMUS (N=12)

Natural hybrids have been identified between *C. tinctorius* and *C. oxyacanthus* and *C. palaestinus*, which are all members of the *Carthamus* section (see Table 1) (Ashri and Knowles, 1960). *C. oxyacanthus* and *C. tintorius* have a relatively high rate of natural hybridising when grown side by side and the F_1 plants showed hybrid vigour (Deshpande, 1952). Natural hybrids between these species have been identified in both Pakistan and India where they are sympatric. In contrast, hybrids between *C. tinctorius* and either *C. oxyacanthus* or *C. palaestinus* did not demonstrate any hybrid vigour, increased fitness or weediness (Mayerhofer et al., 2011).

A review by Knowles and Ashri (1995) indicates that *C. flavescens* (= *C. persicus*), *C. oxyacanthus* and *C. palaestinus* will occasionally form natural hybrids. Hybrids of *C. tinctorius* and *C. oxyacanthus* have been documented in greenhouses and in the field in Pakistan and India where they are sympatric (McPherson et al., 2004 and references cited therein). *C. oxycanthus* is rated as one of the top ten weeds in Pakistan. Hybrids of these two species were also found where alternate rows of *C. tinctorius* and *C. palaestinus* were planted in field trials. Seeds from the plants were collected and planted in the field in the following seasons and hybrids with either species as the female parent were identified morphologically (Ashri and Rudich, 1965). The possibility of natural hybrids occurring between *C. tinctorius* and *C. gypsicolus* or *C. curdicus* has not yet been determined (Knowles and Ashri, 1995).

Although safflower can naturally hybridise or be artificially crossed and produce a fertile hybrid with some of the other *Carthamus* species, *C. tintorius* is the only species within the Section *Carthamus* which is present in Australia (Atlas of Living Australia; accessed September 2019). Thus there is no potential for natural interspecific crosses between *C. tinctorius* and other members of this section in Australia.

SECTION ODONTHAGNATHIS (N=10, 11)

A few species from this section are present in Australia. Naturalised populations of wild safflower species, specifically, *C. leucocaulos* and *C. dentatus*, have been reported in most states and territories in Australia (Groves et al., 2003; GBIF Backbone Taxonomy, 2017) but few studies have examined interspecific crosses. Although some reports indicate *C. glaucus* is present, previous samples have been re-classified as *C. leucocaulos* (see Section 8.2). *C. leucocaulos* is a noxious weed in Australia and California (Mayerhofer et al., 2011). The potential for natural crossing between *C. tinctorius* and *C. tenuis* or *C. boissieri* (both n=10) has not been determined. However, there are no reports of species within this section crossing with *C. tinctorius* under natural conditions.

SECTION ATRACTYLIS (N=22, 32)

Naturalised populations *C. lanatus* (n=22) have been reported in many states and territories in Australia (Groves et al., 2003). *C. lanatus* is a noxious weed in Australia and California (Mayerhofer et al., 2011). Hybridisation between species with either n = 10 or n = 12 with *C. lanatus* all produce infertile hybrids as a result of irregular pairing of chromosomes during meiosis (McPherson et al., 2004 and references cited therein), hence the probability of a fertile hybrid occurring naturally is highly unlikely.

Artificial crosses between *C. tinctorius* and *C. creticus* have resulted in the production of fertile F_1 hybrids, thus it is likely that natural interspecific crossing could occur between these two species provided that both temporal and spatial sympatry exists (McPherson et al., 2004). However, there is no potential for natural interspecific crosses between *C. tinctorius* and *C. creticus* or *C. turkestanicus* as the latter two are not known to occur in Australia.

SPECIES OF UNCERTAIN PLACEMENT (N=12)

There is no potential for natural interspecific crosses between *C. tinctorius* and *C. nitidus* or *Femeniasia balearica* as the latter two are not known to occur in Australia. Additionally, crosses

between *C. tinctorius* and *C. nitidus* have resulted in the production of F_1 hybrids which are infertile (Knowles and Schank, 1964), thus it unlikely that natural hybridisation between these species could occur.

9.3 Crossing under experimental conditions

Successful experimental (artificial) hybridisation of any two species is not an accurate measure of success in nature, although it does describe the potential for cross-compatibility. Cross-compatibility of safflower has been demonstrated with some of its weedy and wild relatives, both experimentally and theoretically (McPherson et al., 2004; Garnatje et al., 2006; Mayerhofer et al., 2011). Self-compatibility and outcrossing potential of safflower with its related species is summarised in Table 6 (Ashri and Efron, 1964; Knowles and Schank, 1964; Imrie and Knowles, 1970; Estilai and Knowles, 1976; Heaton and Klisiewicz, 1981; McPherson et al., 2004; Garnatje et al., 2006; McPherson et al., 2009a; Mayerhofer et al., 2011). Typically experimental crosses are performed by using emasculation and hand-pollination (Mayerhofer et al., 2011). Although hand-pollination is not an appropriate technique for investigating the potential for outcrossing, since the process does not simulate natural pollination and seed production (Ellstrand et al., 1999), it does provide information on cross-compatibility.

SECTION CARTHAMUS (N=12)

Most *Carthamus* species with n=12 chromosomes (*C. tinctorius*, *C. oxyacanthus* and *C. palaestinus*) can be crossed successfully to produce fertile progeny (Ashri and Knowles, 1960; Mayerhofer et al., 2011).

The success rate of these interspecific hybridisations occurring under artificial conditions was 30% with *C. palaestinus* and 56% with *C. oxyacanthus*. In comparison, *C. tinctorius* x *C. tinctorius* control crosses occurred at a rate of 40% (Mayerhofer et al., 2011).

Crosses between *C. tinctorius* and *C. flavescens* (= *C. persicus*) produced fertile F_1 and F_2 progeny (Imrie and Knowles, 1970) and a review by Knowles and Ashri (1995) indicates that *C. flavescens* (*C. persicus*), *C. oxyacanthus* and *C. palaestinus* can easily be artificially crossed with *C. tinctorius*. The possibility of artificial hybrids occurring between *C. tinctorius* and *C. gypsicolus* or *C. curdicus* has not yet been determined (Knowles and Ashri, 1995).

SECTION ODONTHAGNATHIS (N=10, 11)

Safflower has also been crossed with four species outside the section *Carthamus*, to produce viable hybrids. *C. tinctorius* has been artificially crossed with *C. divaricatus* (n=11) and produced self-sterile F1 hybrids which show some female fertility in backcrosses with *C. tinctorius* ((Knowles and Ashri, 1995). However, backcrossing these hybrids with *C. tinctorius* results in offspring with low fertility (Estilai and Knowles, 1976).

Artificial crosses between *C. tinctorius* and other members of the species with n=10, are reported to be difficult to achieve and the F_1 hybrids are highly sterile (Knowles and Ashri, 1995; McPherson et al., 2004). Ashri and Knowles (1960) crossed *C. tinctorius* with *C. tenuis* and *C. glaucus*, obtaining sterile hybrids in both cases. Crosses of *C. tinctorius* with *C. leucocaulos* or *C. glaucus* were performed (Mayerhofer et al., 2011). The cross with *C. leucocaulos* resulted in sterile offspring (seed was produced but would not germinate). Although the cross with *C. glaucus* produced fertile F_1 plants, the authors noted that there was some uncertainty about the identity of the *C. glaucus* seeds used. Different regional variants of *C. glaucus* behave differently in interspecific crosses, therefore it is possible that some subspecies or varieties may produce viable hybrids with *C. tinctorius* (McPherson et al., 2004). Hybrid vigour or increased fitness or weediness was not observed in the F_1 hybrids (Mayerhofer et al., 2011).

Artificial crosses were performed to investigate the potential for outcrossing between GM safflower, containing resistance to glyphosate (*pat* gene), and wild relatives. All experimental crosses produced F_1 hybrids that retained the intact transgene, with the exception of one species, and demonstrated that hybrid fitness was equal to or greater than the respective parents involved (Ellstrand et al., 1999; Mayerhofer et al., 2011). The transgene was completely deleted in approximately 21% of the F_1 progeny resulting from crosses between transgenic *C. tinctorius* and *C. glaucus*, which suggests that some *Carthamus* species possess a negative selection mechanism against foreign DNA (Mayerhofer et al., 2011). The transfer of any gene in nature is typically controlled by selective advantage, a trait that promotes a better chance of both selection and survival (Haygood et al., 2003; Chapman and Burke, 2006).

The potential for artificial or natural crossing between *C. tinctorius* and *C. dentatus* or *C. boissierei* (both n=10) have not been determined. However, cytogenetic analysis of the interspecific hybrids within this section showed a high frequency of chromosome pairing at meiosis, indicating the close relationship among them (see review by Kumar, 1991). In contrast, analysis of crosses between *C. leucocaulos or C. tenius* (both n = 10) with *C. tinctorius* (n = 12) showed very low chromosome pairing at meiosis, poor pollen stainability and a failure of the hybrids to produce seeds. A review of the potential for safflower to hybridise with other *Carthamus* species indicated that crosses between species with n=10 and *C. tinctorius* produced sterile hybrids (McPherson et al., 2004). Similarly, (Knowles, 1980) indicated that most n = 10 species will cross *C. tinctorius*, but the hybrids are highly sterile. Thus, it is highly likely that crosses between *C. tinctorius* and *C. dentatus* or *C. boissierei* will also have very low levels of chromosome pairing at meiosis and generate sterile offspring.

SECTION ATRACTYLIS (N=22, 32)

Successful crosses between *C. tinctorius* and *C. lanatus* (n=22) have been achieved, especially with *C. tinctorius* as the female parent, but all resulting F_1 plants are sterile (Ashri and Knowles, 1960; Heaton and Klisiewicz, 1981; Mayerhofer et al., 2011). Fertile hybrid plants could only be achieved by treating rescued embryos with colchicine (Heaton and Klisiewicz, 1981). F_1 hybrids did not demonstrate any hybrid vigour or increased fitness or weediness (Mayerhofer et al., 2011).

Crosses between *C. tinctorius* and two other members of section *Atractylis*, *C. creticus* or *C. turkestanicus* (both n=32) produced viable fertile offspring (McPherson et al., 2004; Bérvillé et al., 2005) but with very low success rates (< 2% and 0.3%, respectively) (Mayerhofer et al., 2011).

SPECIES OF UNCERTAIN PLACEMENT (N=12)

C. nitidus (n=12) has been artificially crossed with *C. tinctorius* with the F_1 hybrid showing low meiotic pairing and is sterile (Knowles and Ashri, 1995). Attempts to cross *C. nitidus* with other *Carthamus* species produced viable but sterile hybrids (Knowles and Schank, 1964; Knowles, 1989). There is no information on the potential for crossing between *C. tinctorius* and *Femeniasia balearica*.

ACKNOWLEDGEMENTS

Thank you to Nick Wachsmann for providing detailed comments during the preparation of this document.

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APPENDIX WEED RISK ASSESSMENT

Species: *Carthamus tinctorius* L. (Safflower)

Relevant land uses:

1. Intensive[2] uses (ALUM[3] classification 5),

2. Production from dryland agriculture (ALUM classification 3.3.4 Oilseeds)

3. Production from irrigated agriculture (ALUM classification 4.3.4 Irrigated Oilseeds)

4. Nature conservation[4] (ALUM classification 1.1)

Background: In Australia, safflower occurs in a limited range of environments, as deliberate plantings and volunteer populations. Safflower is cultivated on dryland and irrigated farms where domesticated cultivars are grown to produce safflower seed for culinary and industrial oil, and bird and animal feed. Historically, safflower was introduced to Australia for industrial oil production, and production area in Australia has fluctuated in response to different economic pressures and competition from other oilseed crops. Safflower becomes a weed when its range of growth extends beyond the boundaries of areas of deliberate plantings, which is facilitated by its propensity for seed dispersal - primarily by humans. **This WRA is for non-GM safflower volunteers in the land use areas identified above.** Reference is made to safflower as a cultivated crop only to inform its assessment as a volunteer.

The Weed Risk Assessment (WRA) methodology is adapted from the Australian/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol. The questions and ratings (see table) used in this assessment are based on the South Australian Weed Risk Management Guide (Virtue, 2008). The terminology is modified to encompass all plants, including crop plants.

Weeds are usually characterised by one or more of a number of traits, these including rapid growth to flowering, high seed output, and tolerance of a range environmental conditions. Further, they cause one or more harms to human health, safety and/or the environment.

[2] Intensive use includes areas of intensive horticulture or animal production, areas of manufacture or industry, residential areas, service areas (e.g. shops, sportsgrounds), utilities (e.g. facilities that generate electricity, electrical substations, along powerlines) areas of transportation and communication (e.g. along roads, railways, ports, radar stations), mine sites and areas used for waste treatment and disposal.

[3] ALUM refers to the Australian Land Use and Management classification system version 8 published October 2016 (ABARES, 2016).

[4] Nature conservation refers to land use areas that have relatively low level of human intervention, with nature conservation the prime use. This class of land use includes nature reserves, wilderness areas, national parks and other protected or conserved areas.

Safflower has been grown globally for centuries, without any reports that it is been become a serious weed. It lacks many common weedy characteristics. In Australia, Groves et al. (2003) consider safflower to be a category 3 weed of natural ecosystems[5], but primarily a ruderal or agricultural weed. Safflower is a recognised as a naturalised weed of agricultural systems in all Australian states, and reaches a category 1 classification in SA, NT, and Qld[6] (Groves et al., 2003), whereas (Randall, 2017) assessed safflower to be a high risk organism in Australia.

Unless cited, information in this weed assessment is taken from the document *The Biology of* Carthamus tinctorius *L. (safflower)*.

[5] Category 3 weeds are characterised as naturalised and known to be a minor problem warranting control at 4 or more locations within a State or Territory.
[6] Category 1 weeds are characterised as naturalised and may be a minor problem but not considered important enough to warrant control at any location.

Invasiveness Questions	***Carthamus tinctorius* L. (Safflower)**
1. What is safflower's ability to establish amongst existing plants?	**Rating: Low in all relevant land uses** Safflower is a domesticated crop plant that is poorly competitive. Volunteers require bare ground to effectively establish, and are not competitive against most other plants due to slow growth rate. Safflower is not shade tolerant, further reducing its competitiveness amongst existing plants. Naturalised populations of safflower have been found in natural ecosystems in Australia, indicating that it is possible for these species to establish outside agricultural cultivation. However, safflower seems to have a limited ability to invade and establish in undisturbed nature conservation areas.
2. What is safflower's tolerance to average weed management practices in the land use?	**Rating: Low in all relevant land uses** In agricultural land uses, safflower volunteers are well controlled in subsequent crops or along field margins by control methods such as tilling combined with herbicide application. Safflower volunteers in intensive use areas are not known to sponsor self-perpetuating feral populations. Typically, such volunteers are killed by roadside management practices (e.g. herbicide treatment or slashing/mowing), thereby limiting their potential to reproduce.
3. Reproductive ability of safflower in the land use:	
3a. What is the time to seeding in the land uses?	**Rating: < 1 year in all relevant land use**s Safflower grows to maturity, with harvestable seed, in 26–31 weeks in agricultural systems. Volunteer safflower might be expected to achieve maturity slightly later, due to sub-optimal growth conditions, however this is still within a single year.
3b. What is the annual seed production in the land use per square metre?	**Rating: Low in all relevant land uses** In a Canadian study volunteer densities have been reported as high as 11 plants m^{-2}, however viable seed production was very low (McPherson et al., 2009b). In Australia[7], likely seed densities after harvest losses would be 43 – 58 seeds m^{-2}, which are within the ranges of recommended seeding rates for Australia (GRDC, 2017). However it is likely that the seed viability is low as seed material lost at harvest may not be mature and conditions for germination are less than ideal. It is likely that annual seed production m^{-2} would be low in all relevant land uses.
3c. Can safflower reproduce vegetatively?	Under natural conditions, safflower cannot reproduce by vegetative propagation.

[7] Based on seed losses at harvest are generally 3-4 %, with an average seed weight of 40 mg (GRDC, 2010) and average yield of 0.58 t/ha (ABARES, 2014; FAOSTAT, 2019).

Invasiveness Questions	***Carthamus tinctorius* L. (Safflower)**
4. Long distance seed dispersal (more than 100m) by natural means in land uses	
4a. Are viable plant parts dispersed by flying animals (birds and bats)?	**Rating: Unlikely in all relevant land uses** Safflower seed is not likely to be dispersed by flying animals, where it has been shown that viable seed does not pass through the digestive tract of ducks, pheasants, blackbirds, and pigeons (Cummings et al., 2008; USDA-APHIS, 2008). Some seed can remain viable in the oesophagus, crop, and gizzard regions for several hours, during which it could be regurgitated, but safflower seed viability is poor after ingestion for any length of time (Cummings et al., 2008). Large birds such as cockatoos are known to cut through safflower stalks to access the seed, and could transport seed further than 100m. As above, the seed is likely to be digested beyond viability. Safflower seeds could be transported externally attached to feet or legs of birds via clay or heavy soil - as has been observed under experimental conditions (Cummings et al., 2008; USDA-APHIS, 2008). Domesticated safflower seeds are smooth (Cummings et al., 2008) and do not possess adaptations for dispersal on the exterior of animals such as hooks or spines (Mayerhofer et al., 2011).
4b. Are viable plant parts dispersed by wild land based animals?	**Rating: Unlikely to Occasional in all relevant land uses** Safflower is highly shatter resistant, limiting access to seed by small animals. Nonetheless, seed can be dispersed by small land-based animals such as rodents. Larger animal foraging or predation is limited due to the spiny nature of mature safflower plants (Cummings et al., 2008), and domesticated safflower seeds do not possess adaptations for dispersal on the exterior of animals such as hooks or spines (Mayerhofer et al., 2011). As described in section 4a, the smooth seed lacks adaptation for dispersal on the exterior of animals, but may be dispersed on wild animals interspersed with attached mud. Dispersal of viable seed by ingestion and later excretion has not been reported for safflower, and although no primary research is available, endozoochory is unlikely because safflower seed is probably digested beyond viability by wild land-based animals (USDA-APHIS, 2008). As mentioned above, grazing of domesticated safflower cultivars is probably deterred by spininess.
4c. Are viable plant parts dispersed by water?	**Rating: Occasional in all relevant land uses** Dispersal of viable seed by water is possible, for example through flooding or irrigation run-off, but no data is available. Domesticated safflower lacks the papus structure mediating water dispersal found in its wild relatives (Mayerhofer et al., 2011). Safflower is very sensitive to excess moisture/water due to the increased chance of disease which could reduce viability and persistence of volunteers.

Invasiveness Questions	***Carthamus tinctorius* L. (Safflower)**
4d. Are viable parts dispersed by wind?	**Rating: Unlikely in all relevant land uses** Safflower pollen and seed are not appreciably dispersed by wind due to the large size of pollen (53-56 µm) and seeds (6–7 mm). Pollen is not likely to be transported long distances by wind, and in pollen trap experiments, pollen is only detected at traps below flower height. Similarly, seeds are likely to fall immediately adjacent to the plant due to their size and weight. Domesticated safflower lacks the physical papus structure mediating wind dispersal found in its wild relatives (Mayerhofer et al., 2011).
5. Long distance seed dispersal (more than 100m) by human means in land uses:	
5a. How likely is deliberate spread via people?	**Rating: Common in/from dryland and irrigated cropping and intensive land uses** **Highly unlikely in nature conservation land use** Safflower is a crop species that is purposely cultivated for the production of seeds. Safflower seeds are an important oilseed crop and bird food, and are distributed commercially for these purposes. Whereas larger birds are able to digest safflower seed whole, low digestibility of whole safflower seeds by animals means that safflower seed is normally ground to meal before use as animal feed, eliminating viable seed and the possibility of seed spread by animals. Safflower seeds are deliberately transported for cultivation in dryland and irrigated cropping areas and to intensive land use areas for processing and use in feed lots and dairy farms. Safflower seed is not deliberately dispersed within/into nature conservation land use areas.
5b. How likely is accidental spread via people, machinery and vehicles?	**Rating: Occasional in dryland and irrigated cropping areas and intensive land uses** **Unlikely in nature conservation land use** There is no likelihood of spread of vegetative propagules by people, machinery, and vehicles, as safflower has no capacity for vegetative propagation in relevant land uses. Safflower seed may be accidently dispersed by people, machinery, and vehicles. For instance, seed may be spilled as part of the agricultural supply chain - leading to volunteers on roadsides, around seed stores, or areas adjacent to seed handling facilities. Safflower seeds are large and smooth, meaning they may easily be cleaned from machinery and limiting the spread of seed by people, machinery, and vehicles. In nature conservation areas, human activity is relatively low so dispersal of safflower seed to and from these areas is considered unlikely.

Invasiveness Questions	***Carthamus tinctorius* L. (Safflower)**
5c. How likely is spread via contaminated produce?	**Rating: Unlikely in/from all relevant land use areas** Safflower farming in dryland and irrigated cropping areas is often characterised by rotation with other crops, such as wheat. The amount of safflower seed left in the field prior to the planting of a rotation crop depends upon the efficiency of harvesting, but in general seed loss is 3–4% of total seed. The persistence of safflower seed in the seed bank is poor, so volunteer populations are transient with accepted control practices. Safflower can be effectively controlled in subsequent rotation crops and volunteer safflower is unlikely to flower and set seed at the same time as the following crop. Thus, there is very low risk of contamination of subsequent crops.
5d. How likely is spread via domestic/farm animals?	**Rating: Unlikely in nature conservation areas** **Occasional in all other relevant land uses** Safflower does not propagate vegetatively, so animal-mediated spread is limited to the spread of safflower seed. Safflower seeds do not possess adaptations for dispersal on the exterior (fur) of animals (e.g. hooks or spines). Further, domesticated varieties of safflower are spiny, deterring grazing by livestock or large animals (Cummings et al., 2008). Safflower is indicated as a rare fodder crop for some ruminants, especially as sheep and goats are not deterred by safflower spines. This may mediate spread by endozoochory; the viability of safflower seed after ingestion by ruminants is poorly understood. Safflower seed may be transported from field to field within cloven hooves, or attached to hooves by mud.

Impact questions	***Carthamus tinctorius* L. (Safflower)**
6. Does safflower reduce the establishment of desired plants?	**Rating: Reduces establishment by < 10% in all relevant land uses** Some evidence suggests that aqueous extracts of safflower are allelopathic (Machado, 2007). Safflower is a poor competitor with other plants, so volunteer safflower survival and fecundity is expected to be low in following crops such as barley or wheat. Safflower is also not widely tolerant to herbicides, so those commonly used on following crops are likely to further reduce safflower volunteers (McPherson et al., 2009b). Safflower is a cultivated plant that may establish in disturbed land. However, the ability of safflower to establish in relevant land use areas is low. These areas are subject to standard weed management practices that would minimise the impact of any volunteers on the establishment of desired crop plants.

Impact questions	***Carthamus tinctorius* L. (Safflower)**
	In intensive use areas, such as along roadsides, desired species may range from native flora to introduced trees, bushes and shrubs. Such areas are often managed, for either aesthetic or practical reasons (e.g. maintaining driver visibility) by the removal of larger trees and invasive weeds. Safflower would be treated as a weed and managed accordingly. In nature conservation areas, the ability of safflower to establish is rare and unlikely to affect the establishment of native plants.
7. Does safflower reduce the yield or amount of desired plants?	**Rating: Reduces yield/amount by < 10% in all relevant land uses** Safflower is considered a minor weed in Australia and is not considered to threaten agricultural productivity or native biodiversity. The density of safflower volunteers is likely to be low in all relevant land uses and the poor competitiveness of safflower would likely lead to negligible reduction of yield or desired plants in relevant land uses.
8. Does safflower reduce the quality of products or services obtained from the land use?	**Rating: Low in all relevant land uses** Safflower has a low impact on both the establishment and yield/amount of desired species and thus there is no expectation that safflower would reduce the quality or characteristics of products, diversity or services available from the relevant land use areas.
9. What is the potential of safflower to restrict the physical movement of people, animals, vehicles, machinery and/or water?	**Rating: Low in all relevant land uses** Commercial safflower cultivars are ordinarily spiny, but they are unlikely to establish to high densities in all relevant land uses. Standard management practices would keep the density of the safflower volunteers very low. Thus, the potential for safflower to restrict the physical movement of people, animals or water would be low.
10. What is the potential of safflower to negatively affect the health of animals and/or people?	**Rating: Low in all relevant land uses** Safflower seeds and oil are non-allergenic and contain trace amounts of toxic compounds. Animal feeding experiments have shown the seed to be essentially non-toxic. Safflower flowers contain a variety of compounds which might be toxic in sufficient quantity, but are unlikely to be ingested by animals due to the plant's spininess. Furthermore, the low expected density of volunteer safflower limits the amount that may be ingested.
11. Major positive and negative effects of safflower on environmental health in the land use	
11a. Does safflower provide food and/or shelter for pathogens, pests and/or diseases in the land use?	**Rating: Minor negative effects in all relevant land use areas** Safflower is susceptible to a range of fungal pathogens and insect pests. However, being an arid-land crop, the fungal burden is normally quite low. While infected safflower volunteers in dryland or irrigated

Impact questions	***Carthamus tinctorius* L. (Safflower)**
	cropping use areas could act as a reservoir of these pathogens and pests, they are unlikely to create reservoirs leading to infection of adjacent or subsequent crops. In crop rotation regimes, safflower can provide a disease break for other crops and this would constitute a major positive effect. It is unlikely that safflower volunteers would have this major positive effect because volunteer densities are expected to be low due to standard weed management practices. In intensive or nature conservation use areas the density of safflower volunteers is expected to be low and thus may have only minor or no effect.
11b. Does safflower change the fire regime in the land use?	**Rating: Minor or no effect in all relevant land uses** Primary research is lacking, but the number and density of safflower volunteers is expected to be low for all relevant land uses, and would not be expected to affect fire regimes.
11c. Does safflower change the nutrient levels in the land use?	**Rating: Minor or no effect in all relevant land uses** Safflower roots can extract nutrients e.g. nitrates from deep in the soil that are beyond the reach of most other crops (GRDC, 2010). The number and density of safflower volunteers is expected to be low for all relevant land uses, and would not be expected to affect nutrient levels substantially.
11d. Does the species affect the degree of soil salinity in the land use?	**Rating: Minor or no effect in all relevant land uses** Safflower is salt tolerant but the number and density of safflower volunteers is expected to be low for all relevant land uses, and would not be expected to affect soil salinity.
11e. Does the species affect the soil stability in the land use?	**Rating: Minor or no effect in all relevant land uses** The number and density of safflower volunteers is expected to be low for all relevant land uses, and would not be expected to affect soil stability. Recently disturbed land is the only area in which large volunteer populations might arise, and there they could possibly have a positive soil-stabilization effect.
11f. Does the species affect the soil water table in the land use	**Rating: Minor or no effect in all relevant land uses** Safflower can use its deep tap-root to access water from deep in the soil. Some growers use safflower to dry soil profiles (e.g. after irrigated cotton) to reduce waterlogging in subsequent crops. Depletion of water from the soil can result in less water being available for subsequent crops. However, the number and density of safflower volunteers is expected to be low for all relevant land uses, and would not be expected to affect the soil water table.

Impact questions	***Carthamus tinctorius* L. (Safflower)**
11g. Does the species alter the structure of nature conservation by adding a new strata level?	**Rating: Minor or no effect in all relevant land uses** The number and density of safflower volunteers is expected to be low for all relevant land uses, and would not be expected to add a new strata level.