Australian Government

Department of Health

Office of the Gene Technology Regulator

The Biology of *Dianthus caryophyllus* L. (Carnation)

Photo by James Demers, via Pixabay

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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.

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PREAMBLE

This document addresses the biology of *Dianthus caryophyllus* (carnation), with particular reference to the Australian environment, production and use. Information included relates to the taxonomy and origins of cultivated carnation, general descriptions of its morphology, reproductive biology, physiology, biochemistry, biotic interactions, toxicity, allergenic potential, and weediness. 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 analysis of genetically modified carnation that may be released into the Australian environment.

In this document, we refer to the floriculture cultivars as ‘carnation’, and to the wild parent ‘as wild carnation’, as per common usage of the term. The wild carnation (*D. caryophyllus*) has an extremely limited distribution in parts of the northern Mediterranean. The floriculture cultivars are grown globally, often in controlled glasshouse environments and mostly for commercial floristry markets. Carnations can be grown as ornamentals in gardens, but are not as popular now as they have been in the past. The differences in morphology between floriculture carnations and their wild ancestors is the result of hundreds of years of selective breeding.

SECTION 1 TAXONOMY

The genus *Dianthus* belongs to the family Caryophyllaceae (Order Caryophyllales). The Caryophyllaceae comprises over 80 genera and 3000 species, in a mostly Holarctic (i.e., temperate to arctic portions of Eurasia and North America) distribution (Harbaugh et al. 2010). Over 300 species of *Dianthus* are described, and they are commonly known as carnations or pinks (Galbally & Galbally 1997; Jurgens et al. 2003a). Some carnations (wild but not floriculture) and other *Dianthus* species have a fragrance similar to cloves.

In this document, the common name ‘carnation’ is used to refer to *D. caryophyllus* and its cultivars. It also applies to hybrids between *D. caryophyllus* and other species of *Dianthus*, which are also commonly referred to as carnations in trade, botanical and horticultural literature. Most other species in the genus *Dianthus* are known as pinks.

Of the several kinds of carnations, the three most common are annual, border, and perpetual-flowering carnations (see figure 1).

Border carnations are the oldest form of carnation still in cultivation. The ancestor of the border carnations is the wild carnation *D. caryophyllus* and selective breeding for centuries has resulted many cultivated varieties and hybrids (Galbally & Galbally 1997). Carnations grown in home gardens are usually border carnations because they are hardier than perpetual flowering cultivars (below).

Annual carnations are the result of hybridisation between *D. chinensis* and border carnations (Galbally & Galbally 1997). Despite being perennial, these carnations behave more like annuals because, as a result of hybridisation, a perpetual flowering habit has developed that causes the plants to virtually exhaust themselves in a season producing a continual succession of blooms. When winter arrives, the plants in cold climates seldom have sufficient reserves left to cope with the damp and cold (Sitch 1975).

Perpetual flowering carnations are the newest form of carnation, originating in Europe around 1850 (Galbally & Galbally 1997). They are thought to be derived from crosses between *D. caryophyllus* and *D. chinensis* and were propagated for the cut flower market (see Hughes 1993). The thick-stemmed, tall, heavy-flowered, scentless carnations known as the ‘Sim’ form became the basis of varieties used in the international cut flower market (Hughes 1993; Sitch 1975). These cultivars are mainly grown in glasshouses or polytunnels by commercial flower farms and do not survive well outdoors in home gardens.

Figure 1: The main kinds of carnations. A: wild *D. caryophyllus* from Turkey, B: annual carnation, C: border carnation, D: perpetual flowering carnation[1].

Carnations are generally diploid (2n = 30) plants (Carolin 1957). Tetraploid forms (4n = 60) have also been identified. Triploid carnations were produced for commercial purposes, but the resulting plants were mostly aneuploid (Brooks 1960). The majority of available cultivars in Australia and Europe are diploid (Galbally & Galbally 1997).

SECTION 2 ORIGIN AND CULTIVATION

2.1 Centres of diversity and domestication

Wild *D. caryophyllus* is likely to have originated from the Mediterranean regions of Greece and Italy (including Sicily and Sardinia), but the long time in cultivation makes it difficult to confirm its precise origin (Tutin & Walters 1993). The genus *Dianthus* contains several species that have been cultivated for hundreds of years for ornamental purposes (Ingwerson 1949). Table 1 summarises the origins of popular, commercially grown *Dianthus* species for ornamental or home gardens, and to a lesser extent, floriculture.

[1] Image sources: A) Wild *D. caryophyllus*: Photo by Zeynel Cebeci at Wikimedia Commons
B) Orange annual: image from Rare Seeds
C) 'Anne S. Moore' border: image from British National Carnation Society
D)'Joanne' perpetual flowering: image from British National Carnation Society

Table 1. Commercially popular species of the genus *Dianthus*.

Botanical name	Common name	Origins
D. alpinus L.[a]		Austrian Alps
D. arenarius L.[ab]		Northern and eastern mountains of Europe
D. armeria L.[b]	'Deptford pink'	
D. arvernensis[a]	'Finnish Pink'	Auvergne mountains of France
D. barbatus L.[ab]	'Sweet William'	Grown in Britain as early as 1573
D. carthusianorum L.[b]	'Cluster-head pink'	
D. caryophyllus L.[ab]	'Carnation'	Mediterranean
D. chinensis L.[ab]	'Indian Pink' or 'Rainbow Pink'	Hills of eastern Asia
D. deltoides L.[ab]	'Maiden Pink'	Europe and Asia. Was reported in Britain in 1581.
D. erinaceus Boiss.[a]		Dwarf, alpine form, mountains of the Middle East
D. fragrans M.F. Adams[b]	'Fragrant Pink'	
D. freynii Vandas [a]		Native of Hungary and Bosnia.
D. gratianopolitanus Vill.[ab]		Southwestern France, introduced to Britain in 1792
D. haematocalyx Boiss. & Heldr.[a]		Greece
D. knappii Asch. & Kanitz[a]		Hungary
D. microlepis Boiss.[a]		Mountains of Bulgaria
D. myrtinervius Grisch[a]		Alpine meadows of Macedonia
D. neglectus Loisel.[abc]		Swiss and Italian Alps
D. nitidus Waldst & Kit.[ab]		Mountains of Macedonia
D. plumarius L.[ab]	'Feathered pink'	Southern Russia
D. repens Willd.[b]		Eastern European grasslands [d]
D. seguieri Vill.[b]		Temperate regions of the eastern Mediterranean [d]
D. squarrosus M. Bieb[a]		Southern Russia
D. superbus L.[ab]		Central Europe and northern Asia
D. sylvestris Wulfen[ab]	'Woodland pink'	Alpine plant of southern Europe

[a] from Galbally and Galbally (1997).

[b] Listed in GBIF (2015).

[c] *D. neglectus* is identified by Galbally and Galbally (1997) and its alternative name *D. pavonius* is also mentioned. The Global Biodiversity Information Facility (GBIF) portal lists it as *D. pavonius*.

[d] Source: Slabý (2015).

Confusion associated with the common name 'carnation' has led to speculation about when and where *D. caryophyllus* was first grown outside its endemic areas. Prior to the 16th century, the common name for all carnations was 'gillofloure' or 'gillyflower' (McGeorge & Hammett 2002), and gillyflowers were described as 'clove-scented'. However, this name may also have been applied to the culinary clove *Eugenia caryophyllata* which was commonly known by the French as *clou de girofle* (*girofle* is similar sounding to gillyflower and is also related to the Greek *karyophyllon*). This confusion between the culinary clove and the clove-scented wild carnations exists in many forms, perpetuated over time. Texts refer to Chaucer's "*clove-gilofre… to putte in ale, whether it be moyste or stale*", and other references to "sops in wine" as the carnation, when there is little or no evidence for these relating to carnation (Galbally & Galbally 1997), and it is more likely that this term was actually referring to the culinary clove (McGeorge & Hammett 2002). While carnations were known in Turkey, the Middle East, and parts of western Europe in the Middle Ages, there is little evidence to suggest that they were grown in England at that time (Galbally & Galbally 1997).

Modern cut-flower varieties of carnation have been selected for flower size, petal number, stem length and disease resistance. The carnations grown and sold in floriculture today are very different from the wild *D. caryophyllus* growing in Mediterranean regions. Flowers of wild type *D. caryophyllus* are single and five-petaled (see Fig. 1; Galbally & Galbally 1997).

2.2 Commercial use

Carnations have been extremely popular cut flowers since the 18th century, with large-scale production of flowers beginning in the mid-1800s (Galbally & Galbally 1997). Popularity of carnations can be assessed using sales statistics from Dutch flower markets, FloraHolland, the largest flower markets in the world. In 2013, carnations were ranked 16th most popular cut flower, with a turnover of €24 million (compared with the top-ranked flower, rose, at €780 million) (FloraHolland 2014).

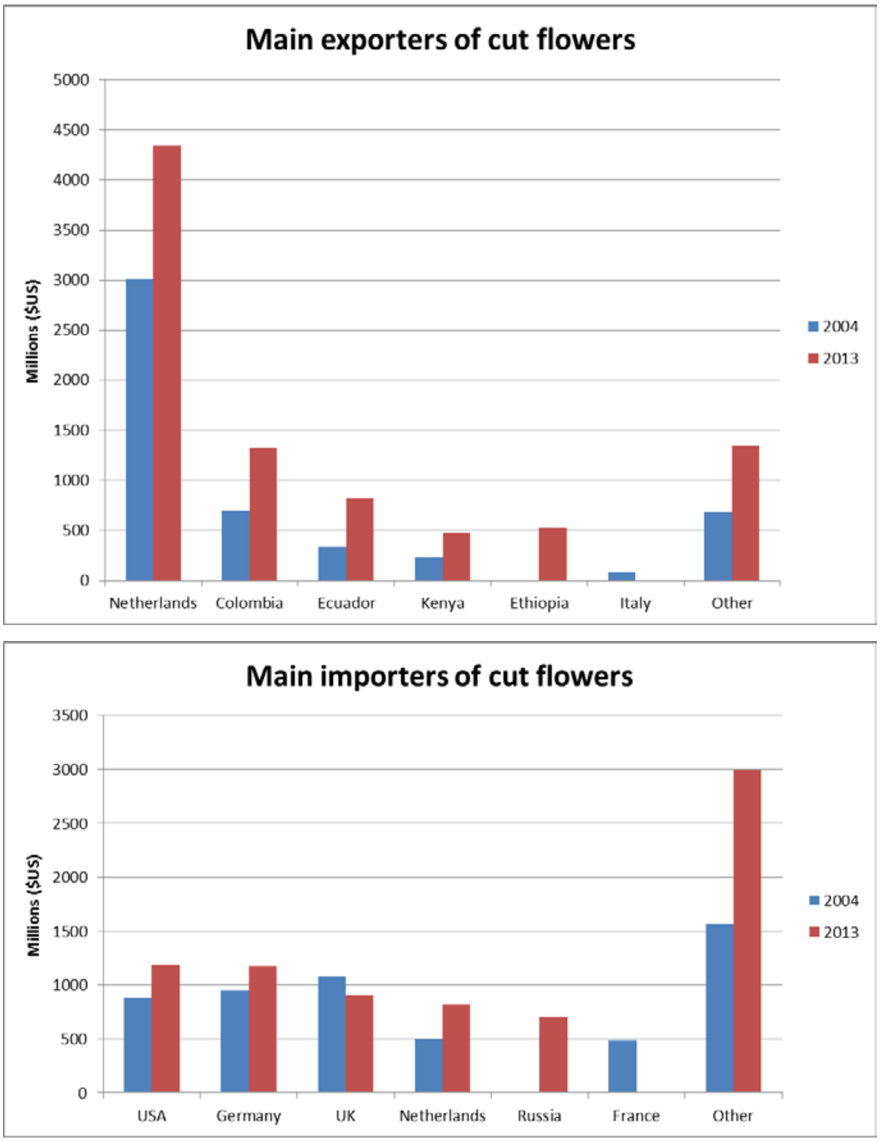

Figure 2: Comparisons of top five exporter (top panel) and importer (bottom panel) countries of cut flowers in 2003 and 2014 (UN Comtrade 2015).

Carnations comprise one part of an extremely broad market in cut flowers globally. In 2013, the United States was the largest importer of cut flowers, compared with the UK in 2004 (UN Comtrade 2015) (figure 2). The Netherlands is the largest exporter of cut flowers. While the amount (in dollars) exported is still highest, the percentage of Dutch flowers in the export market has decreased from around 60% of the market in 2004, to just over 49% in 2013 (UN Comtrade 2015) (see figure 2).

2.2.1 Other uses

While not a food, carnation can be used as a garnish. Wild-type *D. caryophyllus* (and other members of the genus) may have a clove scent, and can be crystallised or used as a garnish in salads or for flavouring many foods including fruit, fruit salads, butter, lemonade, vinegars, conserves and syrups (Facciola 1990; Hughes 1993). However, much of this reported usage of carnations having the scent and taste of cloves relies on historical records, such as those from the 17th century quoted by Hughes (1993). Modern floriculture carnations have little or no scent, and scent loss is often correlated with increased vase-life in cut flowers (e.g., roses) (Chandler & Brugliera 2011). It is said that the Spaniards and Romans used carnation flowers as a spicy flavouring in wine (Cornett 1998), but it is more likely that they used culinary cloves, not carnation petals (Galbally & Galbally 1997). Carnation petals can be used as an ingredient for a tonic to perfume the skin (Pieroni et al. 2004), or can be crushed for oil used in perfumery (Lim 2014).

Carnation has been used in European traditional herbal medicine for coronary and nervous disorders (McGeorge & Hammett 2002) and previously used to treat fevers (Bown 1995; Lim 2014). Carnation flowers are considered to be alexiteric (counteracting the effects of poison), antispasmodic (counteracting spasms of smooth muscle, usually in the gastrointestinal tract), cardiotonic (having a favourable effect on the heart), diaphoretic (promoting sweating) and nervine (acting therapeutically on the nerves) (Chopra et al. 1956). Compounds from carnation buds have exhibited *in vitro* activity against several bacteria, including *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* (Lim 2014). Furthermore, antiviral compounds have been isolated from the leaves and seeds of carnation (Lim 2014).

2.3 Cultivation in Australia

Carnation seeds are commercially available in Australia for cultivation in gardens. Commercial information does not specify particular regions within Australia to cultivate carnations. However, border carnations are hardy and can survive in cold areas (minimum temperatures as low as -28ºC) (Galbally & Galbally 1997). Perpetual flowering carnations are typically grown in glasshouses.

Carnations are exotic to Australia but have been grown commercially as a flower crop since 1954. The carnation industry produces approximately 140 million cut flowers per annum across a total of 100 ha in Victoria, South Australia, Western Australia and New South Wales. Victoria is the largest production centre and also has a significant emphasis on hydroponic production (Carruthers 2002). The hydroponic system helps particularly to prevent losses from wilt disease (*Fusarium oxysporum* – see Section 7.3) which can be a problem in untreated soil.

Currently, carnations are one of only three GM crops that are grown commercially in Australia (the others being cotton and canola, see OGTR website for more information). In 1995, four carnation varieties genetically modified for flower colour were approved for commercial release. Around 4.5 million of these GM cut flower carnations were sold within Australia between 1995 and 2006. In 2007, these GM carnations were placed on the GMO Register (registration number: 001/2004, see: OGTR website).

2.3.1 Import of carnations and cut flowers

Australia imports around $50 million of cut flowers per year, mostly from Kenya (~$22 million in 2014) (UN Comtrade 2015). Carnations are imported to supplement the domestic market, and this includes carnations (both non-GM and GM) imported from Colombia. Regardless of where cut flowers are imported from, the Australian Department of Agriculture requires that all propagatable cut flowers and foliage must be treated to devitalise plant tissue to prevent vegetative reproduction (Department of Agriculture 2012). This process usually involves application of glyphosate herbicide as they enter Australia.

2.4 Plant improvement

2.4.1 Breeding

Carnation breeding is directed to outcomes such as:

- product quality
- improved productivity, more rapid flowering, better yield distribution
- new varieties to increase diversity and sustain market demand and
- disease resistance e.g., to *Fusarium* wilt (Ben-Yephet & Shtienberg 1997; Segers 1987).

The intraspecific breeding procedure typically comprises hybridisation, self-pollination and selection (Holley & Baker 1992). If the desired trait is recessive, it may not be expressed in the F_1 progeny. By self-pollinating the F_1 generation and growing a large population of F_2, selection of one or more individuals with desirable traits is possible. The process of inbreeding (self-pollination) may, however, hinder the breeding objectives by generating recessive homozygotes expressing undesirable traits. A variation of the above breeding method has been described as pedigree breeding which entails selecting F_2 individuals by continual soft-cut propagation (Galbally & Galbally 1997). While inbred parental lines are necessary to breed homogeneous F_1 hybrid varieties, inbreeding detrimentally affects the plants (Galbally & Galbally 1997). Inbreeding depression appears in the third selfed generation (S_3) and therefore, it is almost impossible to produce S_4 seeds (Sato et al. 2000).

In the absence of self-pollination, continuous hybridisation has inadvertently resulted in highly heterozygous carnation varieties. This may, on the one hand, provide benefits in that it promotes recombination resulting in further new varieties (Holley & Baker 1992). On the other hand, it means that neither pure-bred varieties nor F_1 hybrids are produced and that most of the commercially important varieties are clones of selected individuals.

Mutation breeding is used to create new colour mutants. The development of double haploidy techniques has permitted breeders to accelerate breeding and selection (Holley & Baker 1992). Dwarf carnations, which have been commercialised as alternatives to potted chrysanthemums, have also been generated by breeding programs (Holley & Baker 1992).

Post-harvest flower longevity is a trait of interest in carnation breeding. De Benedetti et al. (2001) used randomly amplified polymorphic DNA (RAPD) analysis on two cultivars, their F_1 progeny and subsequent backcross progeny to identify molecular markers associated with flower vase life. However, flower vase life in carnations appears to be a complex quantitative trait involving multiple genes with additive effects.

Our understanding of the genetics of carnations is improving. In 2012, the carnation transcriptome was published. Over 300,000 unique sequences were obtained, including genes involved in flower chlorophyll and carotenoid metabolism, anthocyanin biosynthesis, and ethylene biosynthesis (Tanase et al. 2012). The carnation genome was published in 2014, which provides novel opportunities to explore similarities in genetic structure of carnations and other ornamental plants (Yagi et al. 2014).

Interspecific hybrids between carnations and *D. capitus* have resulted in plants that are highly resistant to bacterial wilt caused by *Pseudomonas caryophylli*. However, the flower quality was adversely affected and further improvement through backcrossing was necessary before commercial production (Onozaki et al. 1998a). Hybrids between *D. caryophyllus* and *D. japonicus* have expressed traits that may prove useful in breeding programmes specific for the Japanese climate (Nimura et al. 2003). Crosses between carnations and other *Dianthus* species to generate progeny with desirable floral characteristics such as colour patterns, bud number, flower arrangement; and improving year-round flowering (for northern Europe) have also been successful (Sparnaaij & Koehorst-van Putten 1990; Umiel et al. 1987).

2.4.2 Genetic modification

Early experiments with carnation established plant tissue culture regeneration systems – a necessary precursor to successful transformation. Efficient direct plant regeneration via adventitious shoot initiation has been obtained from petals, receptacles, stems, hypocotyl callus tissues, calyces, nodes, internodes and leaves (Frey & Janick 1991; Nugent et al. 1991). *Agrobacterium*-mediated transformation systems were also developed (see Lu et al. 1991and references in Tanaka et al. 2005) and have become the standard method for gene transfer in carnation and in other plants.

For carnations, the main targets for genetic modification research have been manipulation of flower colour and reduced ethylene synthesis (Auer 2008). The research focus is on aesthetic traits as cut flowers are purchased as discretionary items (Potera 2007). Consumers expect good quality flowers in a range of colours that last well in vases.

Carnations do not naturally produce delphinidin-based anthocyanin pigments responsible for blue and purple coloured petals (see Section 5.1 for discussion of anthocyanins in carnation). By inserting genes involved with the biochemical pathway for production of the pigment delphinidin into white carnations, purple carnations can be produced (Tanaka et al. 2009). Carnations in different shades of purple have been generated using different techniques, including co-expression of the *F3'5'H* gene from petunia alongside a petunia cytochrome b_5 gene; and by down-regulate endogenous DFR in carnations using RNA interference (RNAi) (Tanaka & Brugliera 2014). Less successful techniques have included using antisense suppression to block the expression of a gene encoding flavanone 3-hydroxylase, another key enzyme in the anthocyanin pathway (Zuker et al. 2002).

Other traits targeted for genetic modification include longer vase life and improved flower fragrance. Vase life longevity was achieved by down-regulating ethylene production (Bovy et al. 1999; Iwazaki et al. 2004; Kinouchi et al. 2006; Kosugi et al. 2000; Savin et al. 1995). However, despite the research into cut flower senescence, and the potential for consumer support for longer vase life of flowers, there has not been large commercial uptake of plants with reduced ethylene synthesis (Scariot et al. 2014). Flower fragrance is often lost in floriculture cultivars, as it is correlated with poor vase life-span. There are no carnation cultivars that have yet had fragrance manipulated to commercially acceptable standards (Chandler & Brugliera 2011). Resistance against *Fusarium* disease was attempted, with combinations of osmotin, PR-1 and/or chitinase genes inserted into carnations (Zuker et al. 2001). However, carnations with GM disease resistance are not grown commercially (Hammond et al. 2006).

SECTION 3 MORPHOLOGY

3.1 Plant morphology

Plant morphological characteristics vary considerably between the three carnation types (annual, border, perpetual flowering). Generally, *D. caryophyllus* is a perennial, growing up to 80cm (Tutin & Walters 1993). Approximately 10-15 side-shoots cluster together around the base of the plant. Young outdoor plants send up between one to five stems that can each produce up to six flowers (Galbally & Galbally 1997). Stems are woody at the base but have herbaceous branches. Leaves are opposite, linear, flat and soft in texture and their colour varies from green to grey-blue or purple, with conspicuous sheaths. The flowering stems are often swollen and brittle at the nodes (Bird 1994).

3.2 Reproductive morphology

Some morphological characteristics of wild-type *D. caryophyllus* are given in Figure 3. The flowers of wild *D. caryophyllus* are single, have 5 petals and vary from white to pink in colour (Galbally & Galbally 1997). Flowers are hermaphrodite and have 10 stamens (in one or two whorls) and two fused carpels with two separate styles. In highly bred cultivars, the reproductive organs may be completely enclosed in the petals thus restricting the access for insect pollinators, especially those without a long proboscis. Nectaries are located at the base of the flower. Flowers bloom simply or in a branched or forked cluster. The fruit is a short-stalked capsule and contains many small seeds.

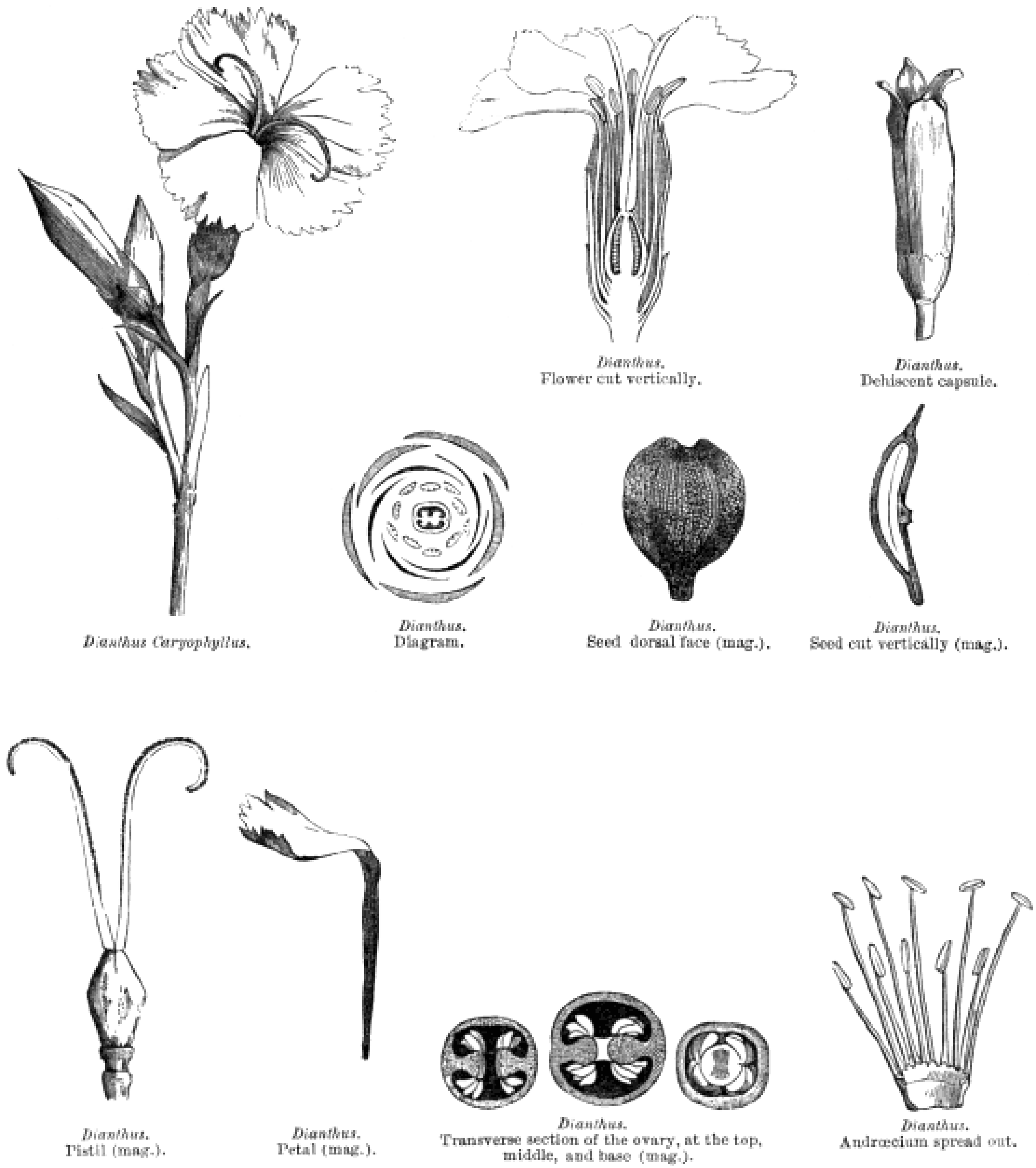

Figure 3: Floral characteristics of *Dianthus caryophyllus* (single flower) (Watson & Dallwitz 2000).

Over 500 years of cultivation has resulted in highly modified morphology of the border and perpetual-flowering cultivars. Border carnation cultivars may have double flowers with as many as 40 petals (Bird 1994). Breeding in the perpetual-flowering floriculture carnations has similarly resulted in large flowers with many petals. When grown in gardens, flowers grow to between 6 and 8.5 cm in diameter. Some greenhouse-grown plants, disbudded for exhibition, have flowers up to 10 cm in diameter (Galbally & Galbally 1997).

There are many cultivars of border and perpetual flowering carnation. These are divided into groups based on plant form, flower size and flower type: standards (Sim), sprays (minis or miniatures), and midis (chinensii). Standards or Sim flowers have a single large flower per stem, whereas sprays have a larger number of smaller flowers. The flowers of midis are smaller and the stem is shorter than the standard type, and there are twice as many flowers (per plant per annum) as standards. Midis can produce either a single flower per stem, or have multiple side branches with flowers (Galbally & Galbally 1997). Petal colours are described as self (single colour), bizarre (two or three colours), flaked (two colours, in a striped pattern), or picotee (petals edged in a different colour to the rest of the petal) (Jarrat 1988).

SECTION 4 REPRODUCTION

4.1 Reproductive development

Carnation cultivars are highly heterozygous to avoid inbreeding depression (Tanase et al. 2012). Consequently, vegetative propagation is preferred to produce plants with the same phenotype as their parents, with plants only grown from seed for selection of new varieties (Galbally & Galbally 1997).

All carnations, but particularly the perpetual flowering varieties, can be propagated by cuttings with best success being achieved in late summer from short, sturdy, non-flowering side shoots (less than 10 cm long) (Jarrat 1988; McGeorge & Hammett 2002). Commercial growers of perpetual flowering varieties reserve stock plants exclusively for the production of cuttings and will continually rogue out poor performers and/or diseased plants (Hughes 1993).

Perpetual flowering carnations, as the name implies, are capable of flowering all year round but good quality plants and flowers are achieved only in a protected environment (Huxley et al. 1992). They are technically categorised as being facultative long day plants (plants whose flowering is promoted by a long-day condition but can flower under short days, although flowering is delayed). However, day length, light intensity, temperature and stage of development all interact to influence flowering and flower quality (Beisland & Kristoffersen 1969).

In Dutch commercial carnation nurseries, stem and spray carnations are lit for 14 consecutive nights during winter to accelerate flowering (van der Hoeven 1987). Very low light intensity can be a problem in flower production and some authors have suggested that interspecific hybridisation could lead to the development of carnation genotypes that could flower under low light intensity in winter (Demmink et al. 1987; Sparnaaij & Koehorst-van Putten 1990).

Carnations have also been propagated using plant tissue culture techniques (see citations in George (1996) ranging from micropropagation to regeneration from differentiated explants. Techniques for the latter have played an important role in the success of genetic modification. George (1996) concluded that the relatively high cost of tissue culture, compared with ‘conventional’ propagation, has excluded it as a commercially viable propagation option for carnation.

For carnations grown in gardens, annual carnations have the potential for a perpetual flowering habit but tend to put all their reserves into summer growth and may not have sufficient left to be able to survive in areas where there are cold winters. In warmer climates or under glass, plants can survive over winter and the flowering period can be extended appreciably (Sitch 1975). Border carnations have one main flowering period in summer and plants will be in bloom for about 4 weeks after which they become vegetative until the following year (Sitch 1975). Border carnations are traditionally propagated by layering (McGeorge & Hammett 2002; Sitch 1975), which involves making a shallow cut in the stem and covering this with soil. Roots will then form at this cut and once established the new plant can be severed from the parent plant. The best time for layering is mid-summer, or as soon as the plants have finished flowering.

4.2 Pollination and pollen dispersal

Carnations are protandrous (male gametes mature and are shed before the female gametes mature) and typically outcross because of the temporal separation of anther dehiscence and pistil receptivity. The stigma is not receptive to pollen grains until one week or more after anthers have shed them. Floriculture carnations require pollination by hand to set seed because they have too many petals for pollinators to access pollen, and because they are mostly housed in greenhouses or polytunnels that preclude access by potential pollinators (Bird 1994). Technicians remove petals to expose the reproductive parts of the flower, and then dip the stigma in pre-collected pollen from other flowers (Sparnaaij & Beeger 1973). The optimal temperature for pollen production in glasshouse plants is approximately 23°C, but temperatures lower than 17^{o}C suppress stamen production completely (Kho & Baer 1973).

Wind plays little role in pollen dispersal. Carnation pollen is heavy and sticky and has low viability (germination for some lines is less than 10%) although this is somewhat cultivar dependent. Floriculture carnations do not produce much pollen, as a result of the long history of use of vegetative propagation and selection for flower characteristics (Galbally & Galbally 1997). Seed set is low or absent compared with wild carnations and the quantity and quality of pollen varies according to the cultivar (Galbally & Galbally 1997; Kho & Baer 1973). Non-compatible pollen may germinate and even grow a tube down the length of the style but fail to bring about petal ethylene production and fertilization (Larsen et al. 1995).

In the wild, pollination of carnation relies on insect pollinators. Moths and butterflies (phylum Arthropoda, order Lepidoptera) pollinate wild *Dianthus* species (see Table 2). Carnations have nectaries located at the base of the flower, and only moths and butterflies have proboscides long enough (up to 2.5 cm) to reach them (Kephart et al. 2006). Presence of lepidopteran pollinators in glasshouses, however, is not common. Within the cut flower industry, the method of shipment and normal handling of the flower stems severely restricts the potential for visits by any insects. Moreover, the morphological changes to flower structure of the cut flower varieties compared with wild *D. caryophyllus* (increased petal number, and enclosure of stamens, anthers and nectaries within the petals) means that access by lepidopteran pollinators is almost entirely precluded (Tanaka et al. 2009).

Table 2: Lepidopteran pollinators of species of *Dianthus*. References: (Kephart 2006; Kephart et al. 2006; Pitkin & Jenkins 2014).

Family	Genus	Distribution
Hesperiidae	*Ochlodes* *Thymelicus*	Palearctic[a], Nearctic[b] Palearctic
Noctuidae	*Autographa* *Euchalcia* *Hadena*	Palearctic, Nearctic Palearctic Palearctic, Nearctic
Nymphalidae	*Melanargia* *Satyrus*	Palearctic Palearctic
Papilionidae	*Papilio**	Global
Sphingidae	*Hyles** (*Celerio* = junior syn.) *Macroglossum** *Sphinx* (*Herse* = junior syn.)	Global Global Palearctic, Nearctic
Zygaenidae	*Zygaena*	Palearctic

[a] Eurasia, North Africa
[b] North America
* Recorded in Australia

While all the lepidopteran families listed in Table 2 are present in Australia, only the genera *Papilio*, *Hyles* and *Macroglossum* have been recorded in Australia (Nielsen & Common 1991). *Hyles* is represented by the single species *H. lineata*, from inland Australia, which feeds on *Boerhavia* and *Tribulus*; *Macroglossum* spp. larve feed on plants of the Rubiaceae (Nielsen & Common 1991).

4.3 Seed development, dispersal and dormancy

Under horticultural conditions, erratic and inadequate seed production has been a problem for carnation breeders. It was common practice to remove lateral shoots and buds from the flowering stems to stimulate the development of the central flower but this actually causes a reduction in fruit setting and seeds/fruit (Sparnaaij & Beeger 1973).

The carnation fruit ripens in approximately six weeks following pollination (Sparnaaij & Beeger 1973). Up to 100 seeds can develop in each fruit, although the average is 40 seeds each (Sparnaaij & Beeger 1973). Once the seed has matured, it is contained within a tubular capsule with a single compartment, which opens from the top. The wind facilitates seed dispersal by causing a back and forth movement of the capsule which is located on the tip of a long flowering stalk (Bird 1994). If carnation seeds are stored in a cool and dry place, they will remain viable for several years (Sparnaaij & Beeger 1973).

4.4 Germination, growth and development

For ornamental varieties, best germination rates are achieved if seed are fresh. No special treatment is required before sowing. Seeds sown into trays containing a standard seed-raising mix and kept warm and moist will germinate within 4-10 days. Carnation seeds germinate better in the dark (Ingwerson 1949). The cotyledons are broad and rounded. When plants reach about 5 cm in height they can be transferred to small individual pots. Transplanting into permanent positions can be done when the plants are about 15 cm high and have developed a good root system (McGeorge & Hammett 2002).

Carnations do not grow efficiently in acidic soil but will tolerate a range of pH 6-8. Addition of lime to soil at a rate of 60 – 120 g/m^2 is sufficient to allow healthy growth (Bird 1994). Carnation plants, particularly the perpetual flowering varieties, are often ‘stopped’ to produce a compact plant (Jarrat 1988). This procedure entails pinching out the centre top leaves of a shoot so that it will branch. When these new shoots are long enough they can also be stopped (‘second stopping’). Second stopping extends the flowering season (Huxley et al. 1992).

SECTION 5 PHYSIOLOGY AND BIOCHEMISTRY

5.1 Biochemistry of carnation flower colour and scent

Flower colour in carnations is produced by two different pigment types: carotenoids and flavonoids. The carotenoids, where present, are responsible for colours ranging from yellow to orange. Anthocyanins are water-soluble pigments derived from flavonoids (Zuker et al. 2002). Wild type carnations have the anthocyanins pelargonidin (orange, pink, red) and/or cyanidin (red, magenta), but do not naturally synthesise delphinidin (blue or purple) (Fukui et al. 2003).

Flower fragrance in carnations is predominantly due to eugenol, beta-caryophyllene and benzoic acid derivatives. Observations in the cultivar ‘Eliat’ show that the level of these compounds increases during flower development and coincides with an increase in flower fragrance (Zuker et al. 2002). There are significant differences in the chemical composition of scents in different cultivars of carnations. A study on five perpetual-flowering carnations and one malmaison (border) carnation showed that the proportions of eugenol caused differences in scent (Clery et al. 1999).

Similarities in floral scent composition may be associated with adaptation to different pollinators. A study on seven non-carnation *Dianthus* species and *Saponaria officinalis* (members of the Caryophyllaceae) found that diurnal *Dianthus* spp. (*D. armeria*, *D. barbatus*, *D. sylvestris*, *D. deltoides*) were pollinated by day-active butterflies. Their flowers contained higher amounts of fatty acid-derived hydrocarbons than those pollinated by night-active species (*D. superbus*, *D. arenarius*, *S. officinalis*) (Jurgens et al. 2003b).

5.2 Toxicity and allergenic potential

Despite carnation having a long history of floriculture, there are few reports of occupational allergy within the floral industry and no reports of toxicity in humans. The petals and leaves are reportedly mildly toxic to dogs and cats, causing gastrointestinal upset if ingested (ASPCA 2015). Petals may also cause contact dermatitis in pets, but it is not known if it occurs spontaneously following exposure, or whether it develops following a latency period and repeated contact (ASPCA 2015). No toxicity or ill-effects were observed in mice fed extracts of carnation petals in an acute toxicity test (Chandler et al. 2013).

Many ornamental or floriculture flowers can cause dermatological symptoms (e.g., eczema, urticaria and contact dermatitis) and may or may not be associated with respiratory responses (Sanchez-Guerrero et al. 1999). Contact dermatitis and respiratory allergy associated with carnation-handling generally develops following a latency period (Lamminpaa et al. 1996; Sanchez-Guerrero et al. 1999). Contact dermatitis has been observed in workers who had previously handled carnations for four (Stefanaki & Pitsios 2008) and eight years (Vidal & Polo 1998). Allergy to carnation without prior occupational exposure is extremely rare, with only one case known. An individual, having developed asthma and rhinitis symptoms when in contact with the flowers as part of recreational activities, was found to be allergic to petals, stamens and stems of carnations (Brinia et al. 2013).

Allergy to carnation may be associated with IgE-mediated reactions in rhinitis and occupational asthma. Causal relationships between carnation allergen exposure and asthma symptoms in carnation workers have been observed (Sanchez-Guerrero et al. 1999). Sensitivity to other pathogens must be considered when allergy to carnations is suspected. The two-spotted mite, *Tetranychus urticae* (Acari: Prostigmata: Tetranychidae), is a parasite of flowers and a well-known allergen itself, and allergy to both carnations and *T. urticae* have been recorded (Cistero-Bahima et al. 2000; Sánchez-Fernández et al. 2004).

SECTION 6 ABIOTIC INTERACTIONS

6.1 Temperature

Dianthus species in general may be propagated by seed in spring and by cuttings in late summer (Galbally & Galbally 1997). In the glasshouse environment, propagation may be conducted throughout the year. Carnation seeds germinate better in the dark (Ingwerson 1949). Seeds normally germinate in seven to ten days at 21°C.

Border carnations are annuals or evergreen perennials, flowering prolifically in midsummer. They prefer a cool-temperate climate and thrive in light and sunshine in summer (Huxley et al. 1992). Perpetual flowering carnations are grown mainly for cut flowers and good quality plants are best achieved by growing them under protection such as a glasshouse which should provide a minimum temperature of 7-10^{o} C so that flowering will continue throughout winter (Huxley et al. 1992).

Low temperature stimulates the initiation of flowers whereas higher temperature results in rapid vegetative growth, more leaf pairs initiated prior to flower initiation, shorter internodes, weaker stems, reduced flower size, reduced cut flower life, and reduced branching (see citations in Beisland & Kristoffersen (1969).

6.2 Water and nutrients

Limiting water to carnations can inhibit their growth. Significant differences in shoot weight, plant height and foliage width were observed in plants that received only 35% of a regular watering regime (Alvarez et al. 2009). However, plants that received 70% regular watering had less obvious reductions in growth. Flower colour, chroma and hue angle were all the same as in plants with 100% regular watering as those with 70% of the regular watering regime, indicating that carnations can produce good-quality flowers in reduced water scenarios (Alvarez et al. 2009). This may be potentially useful for managing carnation floriculture in times of water scarcity, for example, during droughts.

Deficiencies in soil micronutrients can affect carnation growth and flower yield. Nitrogen is the most commonly deficient element and is characterised by yellowing of leaves and delayed bud growth (Galbally & Galbally 1997). Potassium deficiency is identified by plants having scorched leaf tips with necrotic spots and poor flower yield (Galbally & Galbally 1997).

SECTION 7 BIOTIC INTERACTIONS

7.1 Weeds

Commercial production of carnations is for cut flowers. Commercial growers cultivate carnations in closed greenhouses or polytunnels using sterile soil in concrete channels, rather than planting in untreated garden soil (Galbally & Galbally 1997). This method reduces the risk of weeds and diseases entering the carnation-growing system. Amateur growers generally plant carnations in their gardens. They are advised to monitor and physically remove common weeds. Grass can also grow among carnations and its early growth can go unnoticed as its leaves resemble those of young carnations (Bird 1994).

7.2 Invertebrate pests

Invertebrate pests in carnation floriculture crops include aphids (*Myzus persicae*), two spotted mite (*Tetranychus urticae*), carnation shoot mite (*Eriophyes paradianthi*), plague thrips (*Thrips imagines*) and budworms (*Heliothis* spp.) (Williams 2000). Pests cause a range of problems including spoilage of the flowers (Seaton et al. 1997) and allergy in workers handling the flowers (Cistero-Bahima et al. 2000). Some arthropods are both beneficial and detrimental to *Dianthus* species.

Several moth pollinators of *Dianthus* species have an antagonistic and/or mutualistic relationship with the plants, because they also oviposit in flowers and their caterpillars feed on the plants (Collin et al. 2002). For example, moths of the genus *Hadena* (family Noctuidae) will lay eggs on the plants they feed from, and their caterpillars depredate the flower's seeds (Gargano et al. 2009). Other lepidopteran families that are predators of *Dianthus* spp. include (with localities in parentheses): Arctiidae (Nearctic), Coleophoridae (Palearctic), Gelechiidae (Palearctic), Geometridae (Nearctic, Palearctic), Lymantriidae (East Africa), and Tortricidae (Nearctic, Palearctic) (Pitkin & Jenkins 2014).

Thrips are common pests of vegetable crops and floriculture crops such as carnations (Seaton et al. 1997). The western flower thrips (*Frankliniella occidentalis*) is an introduced pest from western USA and now infests crops throughout North America, Mexico, New Zealand and Australia (Seaton et al. 1997). Thrips can be difficult to manage in carnations because some species have developed resistance against common insecticides (Trujillo et al. 1989). Further, thrips hide within flowers or in the soil and can avoid contact with insecticides (Seaton *et al.* 1997). Mites can establish themselves in pockets, relatively unnoticed, in protected crops. The most common mite pest of carnations is the two-spotted spider mite (*T. urticae*), which has a cosmopolitan distribution on horticulture crops and thrives in warm greenhouse conditions (British Columbia Ministry of Agriculture 2015). Two-spotted spider mites damage plants by piercing leaf cells and consuming the contents. This causes the cells to collapse and die, making leaves shrivel and turn yellow (Williams 2000). The first symptom of mite infection of the plant is a silvery appearance of leaves.

Other pests of floriculture carnations include moths and butterflies. Larvae of *Heliothis* moths are one of the most important agricultural pests in Australia (Matthews 1999). These caterpillars can cause severe damage to unopened flower buds particularly during the late spring and summer period.

Non-arthropod invertebrate pests of carnations include plant-parasitic nematodes, which can cause stock losses in carnations. Root-knot nematodes (*Meloidogyne* spp.) are the most common nematode parasites of carnations (Stirling et al. 1992). They cause lesions on the roots that stunt the growth of plants due to root damage.

7.3 Bacterial, fungal and viral pathogens

Species of the bacteria genus *Erwinia* cause streak disease in carnations. Symptoms of the disease include purple or brown streaks along stems and on flowers (Texas A&M Extension Plant Pathologists 2012). It is usually controlled with chemical treatments. Other bacteria, e.g., *Pseudomonas* spp., also cause diseases in carnations, with symptoms including spots on leaves, wilting and rotting of roots.

Fusarium wilt (caused by the fungus *Fusarium oxysporum* f. sp. *dianthi*, principally race 2) is of particular concern to carnation growers and varieties are rated according to their susceptibility (Ben-Yephet & Shtienberg 1997). Fusarium wilt is characterised by wilting of shoots, discolouration of leaves, and brown streaks on vascular tissue in stems (Texas A&M Extension Plant Pathologists 2012). Other fusarium pathogens cause diseases such as stem rot (*F. graminearum*) and bud rot (*F. tricinctum*) (Moorman 2008). Hydroponic systems are favoured as a means of confining outbreaks, and effective methods of control include propagation of certified disease-free cuttings and sterilisation of all work surfaces, including soils (Tanaka et al. 2005; Texas A&M Extension Plant Pathologists 2012). *Alternaria dianthicola* is a fungus that causes purple spots on leaves and can rot branches (Moorman 2008). *Botrytis cinerea* causes grey mould disease. Symptoms of the disease include petals turning brown and decay, with woolly grey spores forming on the petals (Moorman 2008).

There are over 15 known viruses that affect carnations (Moorman 2008). The most widespread carnation virus in Australia is the carnation mottle virus, caused by carmovirus (Moran 1994). Plants infected may show yellow mottling on the leaves or be asymptomatic. It is highly contagious and spread by foliage contact or direct handling (Moran 1994). Several viruses are spread by aphid vectors, including caulimovirus (carnation etched ring virus), potyvirus (carnation vein mottle virus), and carlavirus (carnation latent virus) (Moran 1994). Symptoms of these viruses include brown flecks and yellowing on leaves, depressed yield and calyx splitting, and impaired flower quality respectively (Moran 1994).

SECTION 8 WEEDINESS OF CARNATION

Weeds are plants that spread and persist outside their intended land uses and cause harms, such as toxicity to people or a reduction in biodiversity. Weediness in Australia is often correlated with weediness of the plant, or a close relative, elsewhere in the world (Panetta 1993; Pheloung et al. 1999). The likelihood of weediness is increased by repeated intentional introductions of plants outside their natural geographic range that increase the opportunity for plants to establish and spread into new environments, e.g., escapes of commonly used garden plants (Groves et al. 2005).

Although *D. caryophyllus* and its cultivars are widely grown as floriculture and ornamental plants, there are few records of their being found as naturalised plants even in Mediterranean countries. There are no records of naturalised *D. caryophyllus* in Australia. There are three species of *Dianthus* listed as weeds in Australia: *D. armeria* (found in NSW, Vic, Tas), *D. barbatus* (NSW) and *D. plumaris* (Tas), but each species is mainly ornamental/cultivated and all are considered to be low risk environmental weeds (Groves et al. 2003; Lazarides et al. 1997; NSW Royal Botanic Gardens and Domain Trust 2015; Rozefelds et al. 1999). Carnation is not closely related to any Weeds of National Significance in Australia (see: Weeds of National Significance list).

Cultivated carnation shares few life history strategies with plants that are classed as weeds or invasive species. It does not reproduce rapidly, is not dispersed widely by abiotic means, and is not a nitrogen-fixer or a climber. In addition, cultivated carnations generally do not produce much pollen and consequently seed set is low or absent (Galbally & Galbally 1997). Although cultivation of carnation is via vegetative reproduction, carnation does not naturally reproduce asexually and the cuttings used for propagation have to be struck under conducive conditions (see Section 2.5). Carnations have not been reported as weeds, invasive species or pest species in any of the countries where they are grown as floriculture crops, including Australia, Israel, Japan and parts of Europe and South America.

8.1 Weed risk assessment for carnation

A full weed risk assessment for carnation was conducted (see Appendix A). Carnation is considered to have a very low weed risk.

8.1.1 Potential to cause harm - adverse environmental effects

Carnation is a cultivated plant and only grown in highly managed areas: it is grown as a monoculture in horticultural contexts, and as a deliberately-planted ornamental in gardens. Carnation volunteers have not been observed outside of cultivation. Therefore, carnation does not adversely affect any land use or native biodiversity. Carnation is not known to affect the quality of products or services in any land use. Like other commonly cultivated flower plants, carnation has no adverse effect on soil salinity or nutrient levels.

Carnation is susceptible to a range of pathogens, such as Fusarium wilt, and insect pests such as *Heliothis* caterpillars, aphids, thrips and mites. However, it is mainly grown in glasshouses or polytunnels, and the likelihood of it harbouring these pests and pathogens is low because plants are monitored and pesticides applied as required.

8.1.2 Potential for spread and persistence (invasiveness)

<u>Establishment and management of carnation volunteers</u>

Dianthus caryophyllus (either cultivated varieties or wild-type) is not known as naturalised in Australia (Groves et al. 2003; Lazarides et al. 1997), despite having been commercially cultivated as a flower crop since 1954. This experience demonstrates that cultivated carnations have a limited ability to invade and establish in disturbed or undisturbed areas. Any carnation volunteers found in urban or rural residential areas could be killed by manual removal, or by herbicide treatment. In horticultural areas, plants are grown in glasshouses or polytunnels, and any volunteers would be removed using the same methods.

<u>Reproduction of carnation</u>

Carnation is a perennial and plants under horticulture conditions are grown for two to four years before being replaced. Cultivated carnation is rarely grown from seed, because its high heterozygosity reduces the reliability of offspring with the same phenotype. In cultivation, carnations are mainly reproduced by cuttings. However, no evidence has been found to suggest that carnation would reproduce vegetatively in the wild.

Within one year of planting, flowers develop and seed production can be initiated. When flowers are pollinated, up to 100 seeds can develop in each short-stalked capsule, although the average is 40 seeds each (Sparnaaij & Beeger 1973). It takes at least four weeks after pollination before a mature seed is formed (Gatt et al. 1998; Sparnaaij & Beeger 1973).

Any carnations grown as ornamentals in gardens are unlikely to set seed owing to a highly modified flower morphology that makes natural pollination difficult. Whereas the flowers of wild *D. caryophyllus* are single and accessible to pollinators, the reproductive organs of the flowers of cultivated carnation may be completely enclosed in the petals thus restricting access for potential pollinators, especially those without a long proboscis.

<u>Dispersal of carnation</u>

Carnation is deliberately spread by people and its seeds are sold for growing in gardens as ornamental plants. Although the seeds of carnation are small and might get caught in equipment and clothing, they are unlikely to be accidentally spread by humans as it requires considerable effort to produce seeds and as such they are treated as a desirable commodity.

There is no evidence that animals play a role in the dispersal of carnation seeds. Carnation seeds do not possess adaptations for dispersal via the fur or feathers of animals such as hooks or spines. Dispersal of viable seed by water may be possible, e.g. through flooding or irrigation run-off; however, the seeds are not specifically adapted for water dispersal. Wild carnation seed spread is facilitated by wind moving the seed heads to release the seeds (Bird 1994).

SECTION 9 POTENTIAL FOR GENE TRANSFER

Vertical gene transfer is the transfer of genetic material from parent to offspring by reproduction. This type of gene transfer can occur by sexual or asexual reproduction. This section deals with gene transfer from *D. caryophyllus* to other plants of the same species or closely related species by sexual reproduction.

Carnations generally produce only small quantities of pollen. The quantity and quality of pollen varies according to cultivar and species (Galbally & Galbally 1997; Kho & Baer 1973). The pollen of carnation is heavy and sticky, is not wind-dispersed, and has low viability. Due to these factors, the chance of natural hybridisation of cultivated carnations with wild relatives or even other cultivars is low. The likelihood of dissemination of genetic material through pollen or seeds is limited even further in the production of cut flowers because stems are cut before anthesis.

9.1 Intraspecific gene transfer

Dianthus caryophyllus is an obligate outcrosser because it is protandrous. There is no information on natural intraspecific gene transfer of ornamental carnations in Australia. Most gene transfer is performed in the context of generating novel phenotypes for flower display (see Section 2.4.1). Insect pollinators (Section 4.2) can contribute to gene transfer as they help outcrossing between individual plants. However, this is more relevant to wild *D. caryophyllus*, not its domesticated cultivars, because pollination in ornamental cultivars of carnations is difficult due to modified flower morphology.

9.2 Natural interspecific and intergeneric gene transfer

The flowers we know as carnations are interspecific hybrids. The perpetual flowering carnations used globally in floriculture are descended from hybridisation between *D. caryophyllus* and *D. chinensis* (Galbally & Galbally 1997). Little is known about how readily species of *Dianthus* interbreed naturally, because most focus is on the cultivated varieties in ornamental/horticultural contexts.

Three species of *Dianthus* (namely *D. armeria*, *D. barbatus* and *D. plumarius*) are present as weeds in parts of eastern Australia (Groves et al. 2003; Lazarides et al. 1997; Rozefelds et al. 1999). Carnation (*D. caryophyllus*) has been recorded as hybridising with *D. barbatus*, but this was under experimental conditions only, see Section 9.3 (Umiel et al. 1987). The likelihood of crossing with cultivated carnations is low, due to pollination reasons outlined in section 4.2.

There are 21 introduced genera from the family Caryophyllaceae present as weeds in Australia (NSW Royal Botanic Gardens and Domain Trust 2015). Within the Caryophyllaceae, the genus *Dianthus* is most closely related to the genera *Acanthophyllum*, *Gypsophila*, *Vaccaria*, *Petrorhagia* and *Saponaria* (see Greenberg & Donoghue 2011; Harbaugh et al. 2010). Each of those genera except *Acanthophyllum* has been recorded as present as weeds in Australia (Groves et al. 2003; Lazarides et al. 1997; Rozefelds et al. 1999). However, most hybridisation in the Caryophyllaceae happens within, not between, genera (Greenberg & Donoghue 2011), therefore the likelihood of gene transfer between carnations and species of related genera is low.

9.3 Crossing under experimental conditions

A large number of *Dianthus* species and cultivars are sexually compatible. Carolin (1957) made 108 different interspecific crosses within the genus and found that 22% produced fertile or sub-fertile offspring. Within these crosses, *D. caryophyllus x D. inodorus* (now a synonym of *D. sylvestris*) hybrids were able to produce viable seed only when *D. caryophyllus* provided the pollen; in the reciprocal cross using *D. caryophyllus* as the female parent, there was no fertilization. Carolin (1957) suggested that this was because *D. caryophyllus* has a style 4 times longer than *D. inodorus* and that the pollen produced by the short-style species is unable to grow the full length of the styles in long-style species.

Efforts to artificially hybridise cultivated carnation with other *Dianthus* species, with the aim of introducing useful traits into the cultivated cultivars, have met with some success (Table 3). However it must be stressed that most of the crosses were done under glasshouse conditions and with human intervention (e.g. petal removal, manual pollination, calyx opening).

In a horticultural setting, pollination between carnation and other *Dianthus* species rarely occurs without human intervention. This is because with continual breeding of carnation many cultivars have lost their ability for natural fertilisation. In addition, hand pollination can contribute in selecting desirable varieties. Selection within *D. caryophyllus* and propagation by soft cuttings has typified the breeding and commercialisation process.

In Australia, gene transfer from carnations to any other plant species, even the most closely related naturalised *Dianthus* species, is unlikely due to the very low fertility of carnations.

Table 3. *Dianthus* species that have been successfully hybridised with *D. caryophyllus*.

Species	Reference
D. allwoodii	Umiel et al. (1987)
D. arenarius	Holley & Baker (1963); Umiel et al. (1987)
D. barbatus	Pax & Hoffman (1934); Umiel et al. (1987)
D. capitatus	Onozaki et al. (Onozaki et al. 1998b)
D. carthusianorum	Mehlquist (1945); Demmink (1978);(Segers 1987); Sparnaaij & Koehorst–van Putten (1990)
D. chinensis	Mehlquist (1945); Demmink (1978);(Segers 1987); Sparnaaij & Koehorst-van Putten (1990)
D. deltoides	Umiel et al. (1987)
D. gallicus	Andersson-Kottö & Gairdner (1931); Holley & Baker (1963)
D. giganteus	Demmink (1978); Sparnaaij & Koehorst-van Putten (1990)
D. hungaricus	(Kishimoto et al. 2013)
D. japonicus	Nimura et al. (2003; 2008)
D. knappii	Holley & Baker (1963);(Segers 1987); Sparnaaij & Koehorst-van Putten (1990)
D. monspessulanus	Holley & Baker (1963)
D. seguieri	Holley & Baker (1963)
D. sinensis	Holley & Baker (1963); Umiel et al. (1987)
D. superbus (var.longicalycinus)	Onozaki et al. (1998) (Kishimoto et al. 2013; 2011; 2013)
D. sylvestris[1]	Carolin (1957); Holley & Baker (1963); Demmink (1978); Umiel et al. (1987)
D. versicolor	Sparnaaij & Koehorst-van Putten (1990)

[1] in Carolin (1957) *D. sylvestris* is referred to as *D. inodorus*.

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APPENDIX A WEED RISK ASSESSMENT FOR CARNATION.

Species: *Dianthus caryophyllus* (carnation)

Relevant land uses:
1. Intensive[2] uses (ALUM[3] classifications 5.1.1 shade houses, 5.1.2 glasshouses, 5.4.1 urban residential, 5.4.3 rural residential without agriculture);
2. Production from irrigated agriculture and plantations (ALUM classification 4.4.6 irrigated perennial flowers and bulbs).

Background: 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 2004). 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 to a range of environmental conditions. Further, they cause one or more harms to human health, safety and/or the environment. Carnations have been grown as an ornamental flower for centuries and as an intensively produced floriculture crop since the late 1800s. In Australia, carnations are mainly grown in Victoria and southern New South Wales, and are usually contained in glasshouses or polytunnels. Unless cited, information in this weed risk assessment is from the body of this document. This weed risk assessment is for non-GM carnation volunteers in the land use areas identified above. Reference to carnation as a cultivated crop is only made to inform its assessment as a volunteer.

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[2] *Intensive use* includes areas of intensive horticulture or animal production, areas of manufacture or industry, residential areas, service areas (eg shops, sportsgrounds), utilities (eg. facilities that generate electricity, electrical substations, along power lines) areas of transportation and communication (eg 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 7 published May 2010.

Invasiveness questions	**Carnation**
1. What is carnation's ability to establish amongst existing plants?	**Rating: Low in all relevant land uses** Carnation is a domesticated crop that grows best under horticultural conditions, although carnation seeds are sold for growing in gardens as ornamental plants. There are no naturalised populations of *D. caryophyllus* (either cultivated varieties or wild-type) known in Australia. Floriculture carnations produce little seed relative to wild-type, and it would appear that carnations have an extremely limited ability to invade and establish in disturbed or undisturbed areas.
2. What is carnation's tolerance to average weed management practices in the land use?	**Rating: Low in all relevant land uses** Weed management practices (preventive, cultural and chemical) aim at reducing the loss in yields due to weeds. Any carnation volunteers found in urban or rural residential areas would be killed by manual removal, or by herbicide treatment. In horticulture areas, plants are kept contained in glasshouses or polytunnels and any volunteers would be removed using the same methods.
3. Reproductive ability of carnation in the land use:	
3a. What is the time to seeding in the land uses?	**Rating: ≤ 1 year** Carnation is a perennial and plants under horticulture conditions are grown for 2-4 years before being replaced. Flowers appear within one year of planting, and theoretically seed production would occur within one year of planting.
3b. What is the annual seed production in the land use per square metre?	**Rating: Low in all relevant land uses** Horticulture carnation is rarely grown from seed, because high heterozygosity reduces reliability of plants to produce offspring of the same phenotype. Any carnations grown as ornamentals in gardens are unlikely to set seed owing to a highly modified flower morphology that makes pollination by insects extremely difficult.
3c. Can carnation reproduce vegetatively?	Under natural conditions, carnation **cannot** reproduce by vegetative propagation.
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** There is no evidence that flying animals play a role in the dispersal of carnation seeds.

Invasiveness questions	**Carnation**
4b. Are viable plant parts dispersed by wild land based animals?	**Rating: Unlikely in all relevant land uses** Carnation seeds do not possess adaptations for dispersal on the exterior (fur) of animals (e.g., hooks or spines).
4c. Are viable plant parts dispersed by water?	**Rating: Unlikely in all relevant land uses** Dispersal of viable seed by water is theoretically possible, for example through flooding or irrigation run-off, but production of seed to disperse is unlikely.
4d. Are viable parts dispersed by wind?	**Rating: Unlikely in all relevant land uses** Wild carnation seed spread is facilitated by wind moving the seed head to release the seeds.
5. Long distance seed dispersal (more than 100m) by human means in land uses:	
5a. How likely is deliberate spread via people?	**Rating: Occasional in all relevant land uses** Carnation seed is available for purchase to grow plants as ornamentals.
5b. How likely is accidental spread via people, machinery and vehicles?	**Rating: Unlikely in all relevant land uses** Carnation seed is unlikely to be *accidentally* spread. Seeds would be grown deliberately by people who would like the plants in their gardens, and likelihood of accidental spread is low.
5c. How likely is spread via contaminated produce?	**Rating: Unlikely in/from all relevant land use areas** Carnation grown in horticulture is not used for stock feed, or in rotation with other crops.
5d. How likely is spread via domestic/farm animals?	**Rating: Unlikely in all relevant land uses** Carnations rarely produce seeds in garden settings. Seeds do not possess adaptations for dispersal on the exterior (fur) of animals (e.g. hooks or spines).

Impact questions	**Carnation**
6. Does carnation reduce the establishment of desired plants?	**Rating: None** Carnation is a cultivated plant. It grows as a monoculture in horticultural contexts, and as a deliberately-planted ornamental in gardens. If carnation was to be found in undesirable areas, it would be treated as per item 2 above.

Impact questions	**Carnation**
7. Does carnation reduce the yield or amount of desired plants?	**Rating: None** Carnation is not considered a weed in Australia, and does not threaten agricultural productivity or native biodiversity.
8. Does carnation reduce the quality of products or services obtained from the land use?	**Rating: None** As discussed in questions 6 and 7 above, carnation has virtually no impact on either the establishment and/or yield/amount of desired species. Carnation would not affect the quality of products or services.
9. What is the potential of carnation to restrict the physical movement of people, animals, vehicles, machinery and/or water?	**Rating: None** Carnation would have no effect on physical movement.
10. What is the potential of carnation to negatively affect the health of animals and/or people?	**Rating: Low in all relevant land uses** Carnation has been known to cause allergic dermatitis in some people (Stefanaki & Pitsios 2008; Vidal & Polo 1998), and can also be associated with occupational asthma (Lamminpaa et al. 1996; Sanchez-Guerrero et al. 1999). Most cases of allergy to carnation occur with repeated exposure over a period of time, and are not associated with acute allergy from novel exposure. Thus, the potential of carnation to negatively affect the health of animals and/or people is low.
11. Major positive and negative effects of carnation on environmental health in the land use:	
11a. Does carnation provide food and/or shelter for pathogens, pests and/or diseases in the land use?	**Rating: Minor or no effect** Carnation is susceptible to a range of pathogens, such as Fusarium Wilt, and insect pests such as the *Heliothis* caterpillar, aphids, thrips, and mites. However, carnation is contained in horticulture scenarios, and the risk of carnation spreading these pathogens is low because of monitoring of plants and use of pesticides as required.
11b. Does carnation change the fire regime in the land use?	**Rating: Minor or no effect** Carnation is a plant that only grows in controlled conditions.
11c. Does carnation change the nutrient levels in the land use?	**Rating: Minor or no effect** Carnation does not have any properties that would cause it to affect soil nutrient levels if volunteers were able to grow unhindered.

Impact questions	**Carnation**
11d. Does the species affect the degree of soil salinity in the land use?	**Rating: Minor or no effect** Carnation does not cause high salinity in soils.
11e. Does the species affect the soil stability in the land use?	**Rating: Minor or no effect** Carnation is a plant that grows under managed conditions, and as such it does not grow widely in unprotected areas and would not be expected to affect soil characteristics.
11f. Does the species affect the soil water table in the land use	**Rating: Minor or no effect** See above.
11g. Does the species alter the structure of nature conservation by adding a new strata level?	**Rating: Minor or no effect** See above.