Australian Government
Department of Health and Ageing
Office of the Gene Technology Regulator

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

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PREAMBLE

This document addresses the biology and ecology 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, allergenicity and weediness. This document also addresses the potential for gene transfer to occur to closely related species. The purpose of this document is to inform risk assessments of genetically modified carnation that may be released into the Australian environment.

SECTION 1 TAXONOMY

The genus *Dianthus* belongs to the dicotyledonous Caryophyllaceae family (Order: *Caryophyllales*). The family consists of 80 genera and 2000 species which are either annual or perennial and occur mostly in the northern hemisphere. Over 300 *Dianthus* species have been identified (Galbally & Galbally 1997; Jurgens et al. 2003a). The genus is usually thought of as containing two broad groupings of plants – carnations and pinks.

Carnation is a term that is used for plants in the *Dianthus caryophyllus* group. The genus name, *Dianthus,* is derived from the Greek *dios* (of Zeus or Jove) + *anthos* (flower) ie flowers of Jove. The name *caryophyllus*, is from the Greek *karyon* (a nut) + *phyllon* (a leaf) ie nut leaved; the term comes from the old name of the Indian clove tree (*Eugenia caryophyllata*) and was transferred to the carnation because the flower was so strongly scented of cloves.

In this document, carnation is used to refer to *D. caryophyllus* and its cultivars, and to hybrids of *D. caryophyllus* with other species of *Dianthus*, which are commonly referred to in trade, botanical and horticultural literature as carnations.

Of the several kinds of carnations, the three most common are annual carnations, border carnations and perpetual-flowering carnations.

> **Annual carnations** are falsely named in that all carnations are perennial by nature. However the terminology has arisen because, as a result of hybridisation, a perpetual flowering habit has developed that causes the plants to virtually exhaust themselves in 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).
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> **Border carnations** are the oldest form of carnation still in cultivation. *D. caryophyllus*, which is also known as the wild carnation or clove pink, is the ancestor of the border carnations (McGeorge & Hammett 2002). *D. caryophyllus* has been used extensively by breeders for centuries and as a result many cultivated varieties and hybrids exist (Galbally & Galbally 1997).
>
> **Perpetual flowering carnations** are the newest form of carnation. Their precise origin is obscure but it is possible that they are the result of a series of natural crosses between *D. caryophyllus* and *D. sinensis* (Hughes 1993). The development of perpetual flowering carnations was particularly associated with William Sim a Scottish émigré to the US. 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 (Sitch 1975; Hughes 1993).

Carnations are generally diploid (2n = 30) plants (Carolin 1956). 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).

There are four types of pinks: cottage (mainly derived from *D. plumarius*), rockery (represented mainly by *D. alpinus and D. gratianopolitanus*), annual (including *D. chinensis*) and cluster-head (including *D. carthusianorum*). Other species commonly grown as pinks include, *D. sylvestris, D. deltoides, D. superbus and D. armeria.* Sweet Williams (*D. barbatus*) are included by some (eg Huxley et al. 1992) in the pinks group while others (eg McGeorge & Hammett 2002) consider them to be a group of their own.

SECTION 2 ORIGIN AND CULTIVATION

2.1 Centres of diversity

Members of the *Dianthus* genus are fairly diverse, as their origins range from southern Russia to Alpine Greece and the Auvergne mountains of France. The *Dianthus* species are adapted to the cooler Alpine regions of Europe and Asia, and are also found in Mediterranean coastal regions. Table 1 summarises the origins of popular, commercially grown *Dianthus* species.

Table 1. Commercially popular members of *Dianthus* spp.

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 and 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
D. sylvestris Wulfen[ab]	'Woodland pink'	Alpine plant of southern Europe.

[a] from Galbally and Galbally (1997).

[b] Listed in GBIF (2006)

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

[d] Source: Rock Garden Plants Database (2005)

D. caryophyllus is not seen in the wild except in some Mediterranean countries. This is consistent with Floras records (databases describing the plants of a region or regions) indicating that the natural distribution of carnations is restricted to the Mediterranean regions of Greece, Italy, Sicily, and Sardinia (Tutin et al. 1993).

2.2 Domestication and use

The genus *Dianthus* contains several species that have been cultivated for hundreds of years for ornamental purposes (Ingwerson 1949). Confusions associated with the names that have evolved for the genus have led to speculation about when and where the species was first grown outside its origins. 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'; the name is etymologically related to the Greek *karyophyllon* (See Section1). 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*).

Carnations are used as ornamental plants in gardens and in the cut-flower industry. Modern cut-flower varieties of carnation have been selected for flower size, petal number, stem length and disease resistance. In the 19th century, commercial growing was extensive in France and included both field production and glasshouse production. After germplasm was transferred to the USA, carnation breeding and growing for the cut-flower market became very popular in the USA.

In 2004 global trade in cut flowers was valued at around US$5.5 billion and it is predicted to steadily increase. Nearly 70% of this trade is with the EU and figures (Table 2) for the top cut flower species sold at the Netherlands auctions (which can be used as an indicator for the best sold cut flower species in the EU) place carnation (Dianthus) just out of the top 10 (listed 13th in 2004). A more detailed analysis of the European cut flower trade (EU Market Survey 2005) reveals that the major suppliers of carnations to Europe are Colombia, the Netherlands and Spain while the major importers are the United Kingdom, the Netherlands and Germany.

Table 2. Top 10 cut flowers from 2001 – 2005 (Netherlands auction turnover € 1,000,000)**

Species	2001	2002	2003	2004	2005
Rosa	653.0	699.8	681.3	705.9	728.6
Chrysanthemum (identified as 'raceme' from 2003)	289.1	307.1	299.1	285.3	293.1
Tulipa	177.3	171.2	185.9	185	191.5
Lilium	155.9	168.1	160.0	158.3	164.1
Gerbera	103.8	107.7	105.9	115.9	121.2
Cymbidium	66.6	66.2	65.7	65.2	70.2
Freesia	61.7	62.1	60.2	59.6	57.4
Dianthus	56.2	45.5	Not listed	Not listed	Not listed
Alstroemeria	44.6	44.5	40.0	38.4	39.1
Anthurium	Not listed	41.6	42.6	39.7	41.0
Gypsophila	42.0	Not listed	Not listed	Not listed	Not listed
Chrysanthemum	Not listed	Not listed	37.7	38.9	46.7

** Data compiled from Flower Council of Holland (2006).

Outside of Europe, the US and Japan are also major cut flower markets. In the US, domestic production of carnations, along with the other 'everyday' species such as roses, chrysanthemums, alstroemeria and gladioli, has decreased. However, imports from countries such as Colombia, Ecuador and the Netherlands have replaced local production. During 2004, about 36% of US cut-flower imports were fresh roses, followed by chrysanthemums (9.5 %) and carnations (9.4%) (cited in Southern Africa Development Community 2005). The major cut flower varieties in the Japanese market are chrysanthemums (32%), carnations (8.2%), roses (7.2%), gerbera (3.4%), lilies (3%) and orchids (1.7%) (cited in Southern Africa Development Community 2005).

In addition to their use as cut flowers, carnations have been, and are still, used for culinary purposes. The flower petals have a strong smell of cloves and can be crystallised or used as a garnish in salads or for flavouring fruit, fruit salads, butter lemonade, vinegars, conserves and syrups etc. (see eg Facciola 1990; Hughes 1993). The Spaniards and Romans used carnation flowers as a spicy flavouring in wine and it is claimed (eg Cornett 1998) that this culinary use led the English to call carnations "sops-in-wine" during the time of Chaucer. However, it is likely that this term was actually referring to the culinary clove as it is arguable that carnations grew in England in the 14th century (McGeorge & Hammett 2002) although it would appear that flowers of carnations were added to wine at some stage and were called sops-in-wine. Carnation petals are one of the ingredients that has been used to make the French liqueur, green Chartreuse, since the 17th century.

Essential oil is present in small amounts in petals of the carnation. About 500kg of flowers are required to produce 100g of oil which can then be used in perfumes. Modern perfumes containing carnation oil include Yves Saint Laurent Opium, Lauren by Ralph Lauren, Red Door by Elizabeth Arden, Gucci No. 1.

Carnation was traditionally prescribed in European herbal medicine to treat coronary and nervous disorders (McGeorge & Hammett 2002) and fevers (Bown, 1995). In Spain and North America, the flowers have been 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).

2.3 Cultivation in Australia

Carnations are exotic to Australia but have been grown commercially as a flower crop since 1954. In 2006, the carnation industry produced 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 (*Fusarium oxysporum* – see Section 6.3) which can be a problem in untreated soil.

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

Currently, carnations are one of only two GM crops that are grown commercially in Australia (the other being cotton). In 1995 the Australian company Calgene Pacific Ltd. (now Florigene Pty Ltd, a part of the Suntory Group of Companies), was approved under the voluntary system overseen by the Genetic Manipulation Advisory Committee (GMAC) to grow carnations, genetically modified for flower colour (see Section 2.4.2), for commercial purposes. In 2003, as required under the *Gene Technology Act (2000)*, these commercial dealings were reassessed and a licence DIR 030/2002 was issued by the Gene Technology Regulator (OGTR 2003). Around 4.5 million of these GM carnations have been sold within Australia since 1995.

2.4 Plant improvement

2.4.1 Breeding

Carnation breeding is directed to outcomes such as (Segers 1987):

- a qualitatively better product;
- improved productivity, more rapid flowering, better yield distribution;
- better utilization of planned production cycles;
- new varieties to increase diversity and sustain market demand; and
- disease resistance (eg to Fusarium wilt (Ben-Yephet & Shtienberg (1997))

The breeding procedure typically consists of 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 and growing a large population of F_2, selection of one or more individuals with desirable traits will be 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 inbred 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).

Hybrids between carnation and other *Dianthus* species can provide useful sources of genetic traits to achieve the above objectives (Segers 1987). For instance, interspecific hybrids have been obtained through crossing *D. caryophyllus* and *D. capitatus*. These hybrids are highly resistant to bacterial wilt caused by *Pseudomonas caryophylli*. However, the flower quality is adversely affected and further improvement through backcrossing is necessary before commercial production (Onozaki et al. 1998). 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). However utilising the hybrids would first require the production of amphidiploids to restore pollen fertility. Umiel et al. (1987) made a number of crosses to evaluate the possibility of using interspecific hybridisation as a means of increasing ranges in floral characteristics (eg colour patterns, bud number, flower arrangement). Sparnaaij & Koehorst-van Putten (1990) conducted a comparative trial of seedling progenies between a number of *Dianthus* species as a starting point in evaluating whether rates of growth and flower production could be improved in carnations growing in the low light intensity winter months in NW Europe; hybrids of *D. chinensis* x *D. caryophyllus* were able to flower relatively early.

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 F1 hybrids have been produced and that most of the commercially important varieties are clones of selected individuals.

Mutation breeding has been also employed to create new colour mutants. More recently, the development of doubled haploidy techniques has also 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 major 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. The cultivars used, each with different flower longevity, were 'Roland' and 'Milady'. Flower vase life in carnations appears to be a complex quantitative trait involving multiple genes with additive effects.

2.4.2 Genetically modified carnation

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, calyxes, nodes, internodes and leaves (Frey & Janick 1991; Nugent et al. 1991). Regeneration from stems is apparently preferred, as plants grow faster, look healthier, and do not flower prematurely. *Agrobacterium*-mediated transformation systems were also developed (see eg Lu et al. 1991 and references in Tanaka et al. 2005) and have become the standard method for gene transfer in carnation.

The major target for the genetic modification of carnations has been flower colour. Extensive research by Florigene (see OGTR 2003) resulted in the production of a number of GM lines with colours ranging from pale mauve to purple. These modifications were achieved by inserting genes, involved with the biochemical pathway for production of the anthocyanin pigment delphinidin, into a white 'parent'. Specifically these genes were flavonoid 3' 5' hydroxylase and dihydroflavanol 4 reductase (see Section 5.2). A different approach to colour modification was taken by Zuker et al (2002) who used antisense suppression to block the expression of a gene encoding flavanone 3-hyroxylase, another key enzyme in the anthocyanin pathway. Sensory evaluation tests of glasshouse grown plants demonstrated that flowers of these GM carnations were also more fragrant than those of control plants.

Production of transgenic plants with linalool production has also been investigated as a means of increasing the fragrance of carnations (Lavy et al. 2002). Linalool is a monoterpene fragrance compound that is not produced in carnation. The variety 'Eilat' was transformed with a S-linalool synthase gene from *Clarkia breweri* (a plant native to California that emits a strong, sweet scent of which S-linalool is a major component) but, while lines thus obtained emitted linalool, this did not lead to any increase in scent detection.

Zuker et al (2001) generated carnations with novel agronomic and ornamental traits by using the *rolC* gene from *Agrobacterium* rhizogenes to generate plants with improved rooting ability and production yield (in terms of both number of stem cuttings and number of flowering stalks per mother plant). They also transformed cultivar 'White Sim' with osmotin, PR-1 and/or chitinase genes to obtain lines that showed a high level of resistance, in a standard glasshouse trial, to a major carnation pathogen (*Fusarium oxysporum* f. sp. *dianthi*, race 2).

Genetic modification of carnation in order to down-regulate ethylene production or responsiveness to ethylene has resulted in flowers with prolonged vase life. A number of researchers have generated GM carnation lines that have altered ACC oxidase (eg Savin et al. 1995; Kosugi et al. 2000) or ACC synthase (eg Iwazaki, et al. 2004) expression. These two enzymes are important in the synthesis of ethylene in the plant and normally result in massive ethylene production during flower senescence. In 1993 the Australian company Calgene Pacific Ltd. (now Florigene Pty Ltd, a part of the Suntory Group of Companies), was approved under the voluntary system overseen by GMAC to field trial carnations, genetically modified for enhanced cutflower vase life (GMAC 1993).

A number of agencies have conducted risk assessments for the commercial release of GM carnations (Table 3).

Table 3. Details of commercial releases of GM carnations

Assessment Date	Country or Agency[1]	Applicant	Line	Identification No.	Type of Release	Modification	Reference
1995	GMAC	Florigene (Australia)	66	GR-1	Commercial: cultivation & marketing	Improved vase life	GMAC (1995a)
1995	GMAC	Florigene (Australia)	Moonlite Moonshade Moonshadow Moonvista	123.2.38 123.2.2 11363 123.8.8 } GR-2	Commercial: cultivation & marketing	Flower colour	GMAC (1995b)
1997	COGEM	Florigene (Australia)	4 11 15 16	C/NL/96/14-04 C/NL/96/14-11 C/NL/96/14-15 C/NL/96/14-16	Commercial: cultivation & marketing	Flower colour	COGEM (1997)
1998	COGEM	Florigene (Europe)	66	C/NL/97/12	Commercial: marketing	Improved vase life	COGEM (1998a)
1998	COGEM	Florigen (Europe)	959A 988A 1226A 1351A 1363A 1400A	C/NL/97/13-959A C/NL/97/13-988A C/NL/97/13-1226A C/NL/97/13-1351A C/NL/97/13-1363A C/NL/97/13-1400A	Commercial: cultivation & marketing	Flower colour	COGEM (1998b)
2003	OGTR	Florigene (Australia)	Moonlite Moonshade Moonshadow Moonvista	123.2.38 123.2.2 11363 123.8.8 } DIR 030/ 2002	Commercial: cultivation & marketing	Flower colour	OGTR (2003)
2004	Japan	Suntory Flowers	Moonvista Moonlite Moonshadow Moondust Moonshade	FLO-40685-1 FLO-40644-4 FLO-11363-1 FLO-07442-4 FLO-40619-7	Commercial: Type1 – cultivation & marketing	Flower Colour	JBCH (2004a,b,c,d ,e)
2004	COGEM	Florigene (Australia)	Moonlite	C/NL/04/02	Commercial: marketing	Flower Colour	COGEM (2005a,b), SNIF (2004)
2005	ACRE	Florigene (Australia)	Moonlite	C/NL/04/02	Commercial: marketing	Flower Colour	ACRE (2005)
2006	EFSA	Florigene (Australia)	Moonlite	C/NL/04/02	Commercial: marketing	Flower Colour	EFSA (2006)
In process	COGEM	Florigene (Australia)	Moonaqua	C/NL/06/01	Commercial: marketing	Flower Colour	SNIF (2006)

[1] ACRE = Advisory Committee on Releases to the Environment (Europe); COGEM = Netherlands Competent Authority; EFSA = European Food Safety Authority; GMAC = Genetic Manipulation Advisory Committee (Australia); SNIF = Summary Notification Information Format (Europe)

2.5 Commercial propagation

Due to its highly heterozygous nature, the carnation does not come true to its parent when grown from seed. Consequently, under horticultural conditions this method of propagation is only used for selection of new varieties (Galbally & Galbally 1997) although annual carnations tend to be propagated by seed (Sitch 1975). Furthermore, since most cultivars are highly heterozygous, vegetative propagation is used to maintain the selected characteristics.

If seeds are used, best germination rates are achieved if they 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. 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).

All carnations, but particularly the perpetual flowering varieties, can be propagated by cuttings with best success being achieved in late summer from short (less than 10 cm long), sturdy, non-flowering side shoots (Jarratt 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 (Hughes1993).

Border carnations are traditionally propagated by layering (see eg Sitch 1975; McGeorge & Hammett 2002) and plants will do this naturally themselves once mature. The best time for layering is mid-summer, or as soon as the plants have finished flowering.

Carnations have also been propagated using plant tissue culture techniques (see citations in e.g. 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) concludes that the relatively high cost of tissue culture, compared with 'conventional' propagation, has excluded it as a commercially viable propagation option for carnation.

SECTION 3 MORPHOLOGY

3.1 Plant morphology

Plant morphological characteristics vary considerable between the three types (annual, border, perpetual flowering). Various authors (eg Huxley et al. 1992; Bird 1994; Galbally & Galbally 1997) describe the general morphology of *D. caryophyllus* as follows: a perennial normally growing up to 60 cm in the open garden and which may grow a further 15 cm when cultivated in pots. Side-shoots cluster together around the base of the plant, usually about 10 – 15 in number. Young outdoor plants send up between one to five stems that can each produce up to six flowers. 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. They have conspicuous sheaths. The flowering stems are often swollen and brittle at the nodes.

3.2 Reproductive morphology

Some floral characteristics of *Dianthus* spp. are given in Figure 1. The single flowers of wild *D. caryophyllus* have 5 petals and vary from white to pink to purple in colour (Galbally & Galbally 1997). In contrast, border carnation cultivars may have double flowers with as many as 40 petals (Bird 1994). Breeding in the perpetual-flowering 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). Petals are generally clawed or serrated. The flower is bisexual and has 10 stamens (in one or two whorls) and 2 fused carpels with 2 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.

There are many flower varieties of 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.

Flower colour types are classified as follows (Jarratt 1988):

- **Bizarres** have a clear ground, marked and flaked with two or three colours and are characterised by the predominant colour.
- **Flakes** have a clear ground flaked with one colour.
- **Selfs** are of any one shade.
- **Fancies** are varieties not falling into any one of the above classes, having a yellow or white ground or mottled, flaked or spotted with various colours.
- **Picotees** have the colours confined to the margins of the petals.

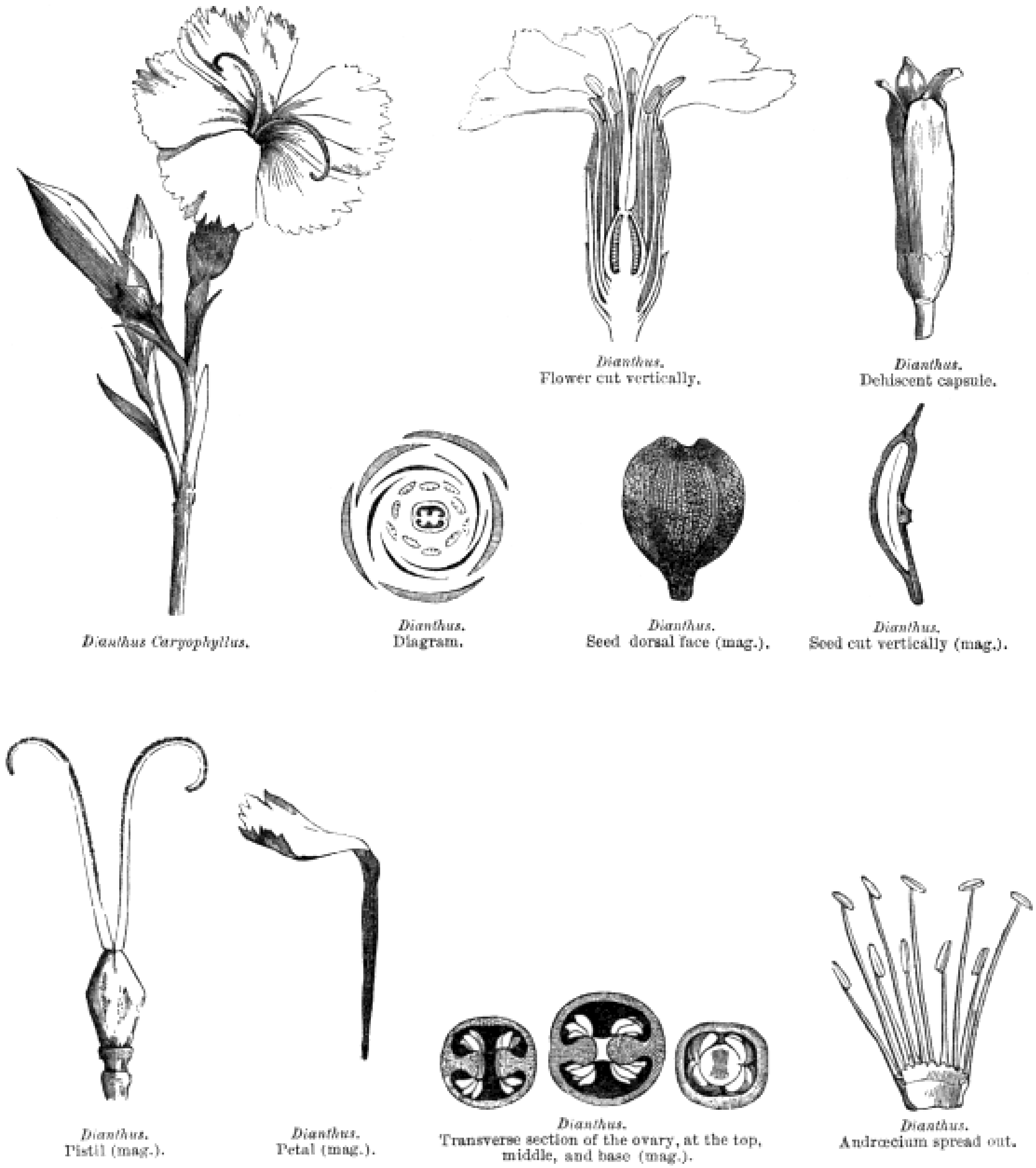


Figure 1. Floral characteristics of *Dianthus* spp. (single flower) (Watson & Dallwitz 2000).

SECTION 4 REPRODUCTION

4.1 Reproductive development

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) but daylength, light intensity, temperature and stage of development all interact to influence flowering and flower quality (Beisland & Kristoffersen 1969).

In Dutch carnation nurseries, stem and spray carnations are lit for 14 consecutive nights during winter to accelerate flowering (van der Hoeven 1987). There is some indication that intermittent illumination of high intensity given through the night may be more effective in promoting flower initiation than illumination of the same intensity given continuously as a night break; however, under low light intensity (but not so low as to reduce the flowering response) there is no difference between continuous and intermittent lighting (Harris 1972). Very low light intensity can be a problem in flower production and Demmink et al. (1987) and Sparnaaij & Koehorst-van Putten (1990) have suggested that interspecific hybridisation could lead to the development of carnation genotypes that could flower under low light intensity in winter.

Low temperature stimulates the initiation of flowers whereas increased 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).

The shoots are most sensitive to day length when 5 – 7 leaf pairs are visible. In long days, 8 – 10 leaf pairs and in short days 16 – 18 leaf pairs may be initiated prior to flower differentiation (Beisland & Kristoffersen 1969)..

Calyx splitting is a problem in cut-flower production. The split calyx is unsightly and devalues the flowers. It is caused by the formation of a large number of petals or by lateral buds inside the calyx at low temperatures (Holley & Baker 1992). The flower bud is most sensitive to calyx splitting at low temperatures when it is 3 – 6 mm in diameter (Kohl 1961).

Annual carnations, as a result of breeding, 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). The timing of the flowering period can be brought forward by a few weeks by growing plants under glass from early spring but the total length of the flowering period is not changed (Sitch 1975).

4.2 Pollination and pollen dispersal

Dianthus flowers are protandrous (male gametes mature and are shed before the female gametes mature) and typically outcross as a result 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. The cultivated carnations require pollination by hand to set seed (Bird 1994). The flower generally collapses within 24 hours of successful pollination (McGeorge & Hammett 2002). This is mediated by an increase in ethylene production in the petals in response to the sexual compatibility of the pollen; 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 addition, as a result of the long history of use of vegetative propagation and selection for flower characteristics, the carnation does not produce much pollen, and consequently seed set is low or absent (Galbally & Galbally 1997). The quantity and quality of pollen varies according to the cultivar (Kho & Baer 1973; Galbally & Galbally 1997). Carnation pollen is heavy and sticky and has low viability (percentage germination for some lines is less than 10%) although this is somewhat cultivar dependent. Wind plays little role in pollen dispersal.

The optimal temperature for pollen production in glasshouse plants is approximately 23°C. This temperature, however, results in smaller flowers when compared to those produced by plants incubated at 10°C (Kho & Baer 1973). Temperatures lower than 17^{o}C suppress stamen production completely.

For seed production, flowers on female plants are prepared (Sparnaaij & Beeger 1973) by cutting away the upper half of the calyces 7 – 10 days before anthesis to expose the pistil and any anthers. When the lobes of the stigma begin to bend outward, the stigma is dipped in pollen that has been previously collected and stored. The high humidity found in glasshouses reduces pollen longevity but pollen can be stored in a desiccator in the glasshouse for at least a week.

In the wild, cross-pollination of carnation relies on insect pollinators. There are no known reports of insect pollinators of *D. caryophyllus,* in particular. However, pollination is likely to be effected by lepidopteran insects (see e.g. Kephart et al. 2006) as these insects have probosci long enough (up to 2.5 cm) to reach the nectaries located at the base of the flower. Lepidopteran species of the genera *Macroglossum*, *Plusia*, *Pieris*, *Hesperia, Aphantopus, Aporia, Cyaniris, Ochlodes, Mesoacidalia, Polyommatus* and *Thymelicus* are documented pollinators of other *Dianthus* species. Lepidopteran pollinators, however, have not been reported by commercial carnation growers and certainly, 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. In addition, the morphological changes to flower structure of the cut flower varieties compared with wild *D. caryophyllus* (increased petal number, enclosure of stamens, anthers and nectaries in the petals) means that access by lepidopteran pollinators is virtually precluded.

Of the species mentioned above, only *Macroglossum, Plusia* and *Pieris* occur in Australia. *Pieris rapae* (family Pierinae) is an introduced lepidopteran and occurs in the south-east and south-west of mainland Australia and in Tasmania. The larvae damage cruciferous plants (e.g. mustard, radish, turnip etc). *Plusia argentifera* and *P. chalcites* are pests of dicotyledonous plants. Moths of the genus *Macroglossum* pollinate a number of different *Dianthus* species, including *D. barbatus* (Britton et al. 1979).

The HOSTS database of the World's lepidopteran hostplants (Robinson et al 2006) lists a large number of caterpillars (see Table 4 in Section 6.2) that feed on *D. caryophyllus*. These species may or may not have implications in pollination.

4.3 Seed development, dispersal and dormancy

Under horticultural conditions, erratic and inadequate seed production has been a chronic 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 within five weeks to two months of pollination. Up to 100 seeds can develop in each fruit. However, fruits contain on average 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).

SECTION 5 PHYSIOLOGY AND BIOCHEMISTRY

5.1 Germination, growth and development

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. The cotyledons are broad and rounded. Transplantation to pots must be done after the first true leaves appear.

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). Good soil drainage is important to prevent fungal diseases. While plants respond to fertilising, especially around blooming, too much nitrogen can lead to foliage growth at the expense of flowers (McGeorge & Hammett 2002). Perennials tend to lose their vigour and flower less prolifically after a few seasons.

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).

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).

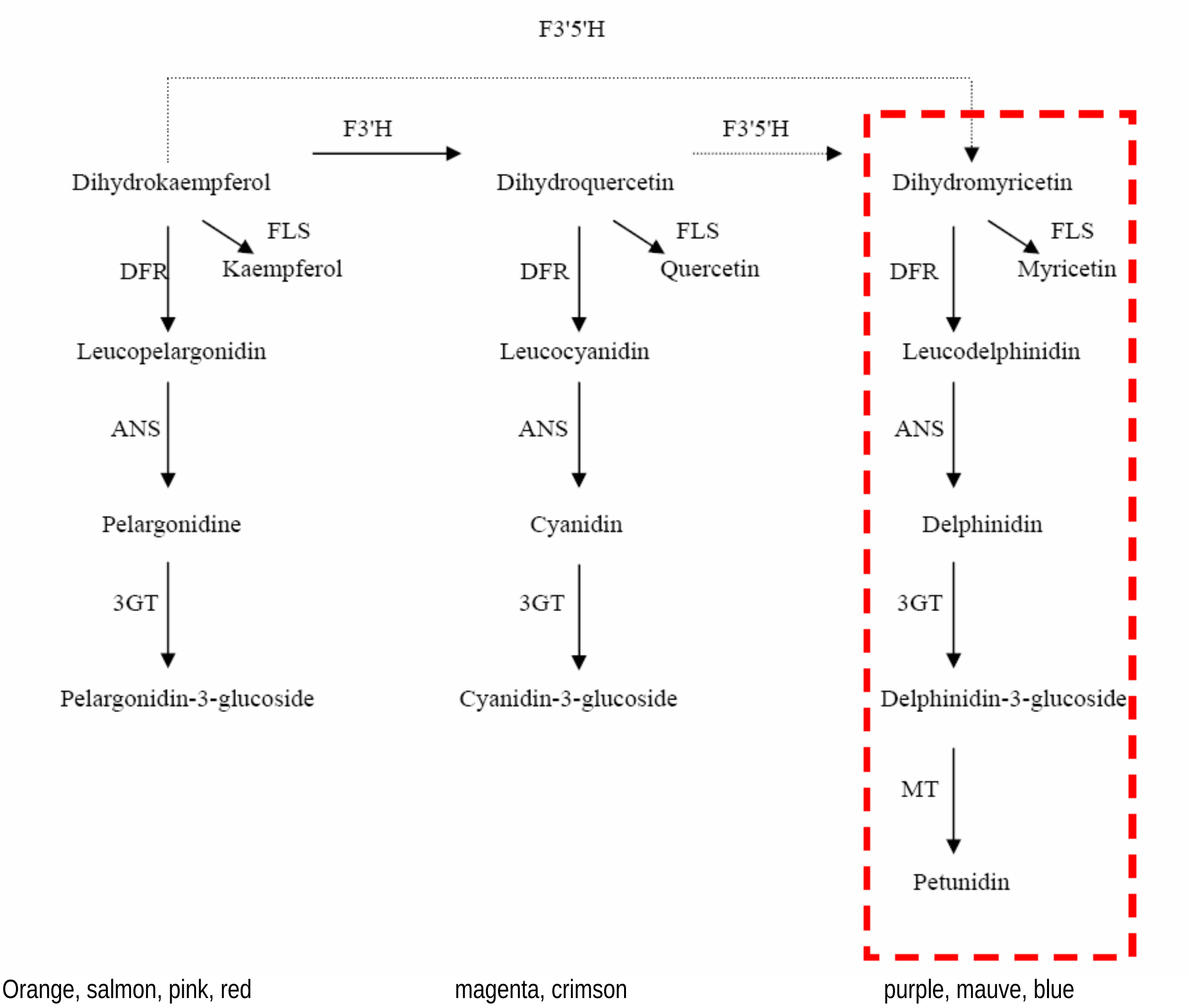
Carnation plants, particularly the perpetual flowering varieties, are often ‘stopped’ (Jarratt 1988) to produce a compact plant. This procedure entails pinching out the centre top leaves of a shoot so that it will branch. When these new shoots are long enough they can also be stopped (‘second stopping’). Second stopping extends the flowering season (Huxley et al. 1992).

5.2 Biochemistry of carnation flower colour and scent

Flower colour in carnations is attributed to the presence of two pigment types: carotenoids and flavonoids. The carotenoids are responsible for colours ranging from yellow to orange. However, many carnation plants do not contain carotenoid pigments. Flavonoids are water-soluble pigments such as anthocyanins which accumulate in the vacuoles. There are three major types of anthocyanins that contribute to flower colour (Zuker et al. 2002):

- delphinidins that produce blue or purple flower colour;
- cyanidins that produce red or magenta flower colour; and
- pelargonidins that produce orange, pink or brick red flower colour.

Synthesis of all anthocyanins follows a similar pathway until the colourless naringenin is converted to dihydrokaempferol (DHK) (Figure 2). In cultivated carnations, DHK is either converted to the colourless leucopelargonidin by the enzyme dihydroflavonol 4-reductase (DFR) or to dihydroquercetin (DHQ) by flavonoid 3'-hydroxylase. Pelargonidin or cyanidin is produced depending on whether DHK is first converted to leucopelargonidin or DHQ, respectively. Delphinidin synthesis requires the conversion of DHK or DHQ to dihydromyricetin (DHM) by flavonoid 3', 5' hydroxylase (F3'5'H). Carnations do not naturally have blue or mauve flowers because they lack this part of the anthocyanin biosynthetic pathway that produces delphinidins or blue pigments.



Key to enzymes:

F3'5'H: flavonoid 3',5' hydroxylase
F3'H: flavonoid 3' hydroxylase
FLS: flavonol synthase
DFR: dihydroflavonol 4-reductase
ANS: anthocyanidin synthase
3GT: Flavonoid 3-glucosyltransferase
MT: Methyltransferase

Figure 2. Anthocyanin biosynthetic pathway (taken from JBCH 2004a, adapted from Holton & Cornish 1995). NOTE: the formation of delphinidin-3-glucoside (shown within the dotted line) does not occur normally in carnation, Introduction of the F3'5'H gene through genetic modification enables biosynthesis of dihydromercitin and endogenous enzymes that then allow the pathway to proceed through to production of delphinidin-3-glucoside

The carnation flower fragrance is predominantly due to eugenol, *B*-caryophllene 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 carnations. One study on five perpetual-flowering carnations and one malmaison carnation showed that the proportion of eugenol (trace – 84%) and methyl salicylate (0.1 – 1.4%) caused differences in scent (Clery et al. 1999).

Another study on seven non-carnation *Dianthus* species and *Saponaria officinalis* (all members of the Caryophyllaceae) suggests that similarities in floral scent composition may be explained by adaptation to different pollinator groups (Jurgens et al. 2003b). Diurnal or day-active *Dianthus* spp. (*D. armeria*, *D. barbatus*, *D. sylvestris*, *D. deltoides*) were pollinated by day-active butterflies. Their flowers were brightly coloured and contained higher amounts of fatty acid-derived hydrocarbons than the night active (night opening), white-coloured species (*D. superbus*, *D. arenarius*, *S. officinalis*).

SECTION 6 BIOTIC INTERACTIONS

6.1 Weeds

Because carnations are generally short, they can be rapidly swamped by weeds which compete with the plants and can cause production problems.

Commercial production of carnations is for cut-flowers. Commercial growers cultivate carnations in greenhouses using sterile soil. This greatly improves weed and disease management (Galbally & Galbally 1997).

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).

6.2 Pests

Thrips including Western Flower thrips (*Frankliniella occidentalis*) are serious pests of carnations. White markings on the petals, particularly in red varieties, indicate the presence of thrips. Thrips can reinfest a crop from nearby vegetation. Peak infestation periods occur in spring when grass and bush dry out in surrounding areas. Thrips can be chemically controlled by registered products. Agricultural chemicals are regulated in Australia by the Australian Pesticides and Veterinary Medicine Authority (APVMA). The APVMA registers products and approves use patterns.

Aphids are seldom seen in well-managed crops. Chemicals used to control thrips should also control aphids.

Mites can establish themselves in pockets, relatively unnoticed, in protected crops. The first symptom of mite infection of the plant is a silvery appearance of leaves. Yellow flowering varieties are particularly attractive to mites. Early infestations may be found underneath curled leaves. Mites can be controlled with chemical treatment.

Helicoverpa caterpillars can cause severe damage to unopened flower buds particularly during the late spring and summer period.

Depending on the country, other arthropod pests including ants, earwigs and wireworm (*Agriotes lineatus*) may also damage carnations (Galbally & Galbally 1997).

Slugs (*Arion distinctus)* have also been reported as pests causing problems in garden carnations (Bird 1994).

The Victorian Department of Primary Industries (Williams 2000) lists aphids, two spotted mite (*Tetranychus urticae*), Plague thrips (*Thrips imagines*), Carnation shoot mite (*Eriophyes paradianthi*) and Budworms (*Heliothis* spp.) as the major pests of carnations in Australia.

The HOSTS database of the World's lepidopteran hostplants (Robinson et al 2006) lists a large number of caterpillars (Table 4) that feed on *D. caryophyllus*.

Table 4. Lepidopteran species that feed on *Dianthus caryophyllus*

Lepidoptera Family	Lepidoptera Name	Country
Arctiidae	*Epitoxis albicincta*	East Africa
Arctiidae	*Pyrrharctia isabella*	Nearctic
Coleophoridae	*Coleophora dianthi*	Palaearctic
Geometridae	*Gymnoscelis rufifasciata*	Libya
Geometridae	*Scopula fulminataria*	Libya
Lymantriidae	*Lacipa florida*	East Africa
Lymantriidae	*Lacipa florida*	East Africa
Lymantriidae	*Lacipa quadripunctata*	East Africa
Lymantriidae	*Lacipa quadripunctata*	East Africa
Noctuidae	*Agrochola lychnidis*	Europe
Noctuidae	*Chazaria incarnata*	Palaearctic
Noctuidae	*Copitarsia incommoda*	Colombia
Noctuidae	*Copitarsia incommoda*	Neotropical
Noctuidae	*Hadena bicruris*	Palaearctic
Noctuidae	*Hadena compta*	Europe
Noctuidae	*Hadena compta*	Finland
Noctuidae	*Hadena rivularis*	Palaearctic
Noctuidae	*Helicoverpa armigera*	East Africa
Noctuidae	*Helicoverpa armigera*	India
Noctuidae	*Helicoverpa armigera*	Old World
Noctuidae	*Helicoverpa armigera*	Southern Africa
Noctuidae	*Helicoverpa zea*	East Africa
Noctuidae	*Helicoverpa zea*	Nearctic
Noctuidae	*Heliothis maritima*	Palaearctic
Noctuidae	*Lacanobia oleracea*	Palaearctic
Noctuidae	*Lacanobia suasa*	Europe
Noctuidae	*Mamestra brassicae*	Palaearctic
Noctuidae	*Noctua pronuba*	Holarctic
Noctuidae	*Papaipema nebris*	Nearctic
Noctuidae	*Peridroma saucia*	Nearctic
Noctuidae	*Spodoptera eridania*	Brazil
Noctuidae	*Trichoplusia ni*	Nearctic
Tortricidae	*Argyrotaenia citrana*	Nearctic
Tortricidae	*Cacoecimorpha pronubana*	Holarctic
Tortricidae	*Choristoneura rosaceana*	Nearctic
Tortricidae	*Epichorista galeata*	Kenya
Tortricidae	*Epichorista sp.*	East Africa

Lepidoptera Family	Lepidoptera Name	Country
Tortricidae	*Epichoristodes acerbella*	Old World
Tortricidae	*Epichoristodes acerbella*	Southern Africa
Tortricidae	*Epichoristodes acerbella*	Spain
Tortricidae	*Platynota flavedana*	Nearctic
Tortricidae	*Platynota stultana*	USA

6.3 Pathogens and diseases

A list of common pathogens and diseases affecting carnations is given in Table 5. Fusarium wilt (caused by the fungus *Fusarium oxysporum f. sp. dianthi,* principally race 2) is of particular concern (Ben-Yephet & Shtienberg 1997) and carnation varieties are rated according to their susceptibility. Hydroponic systems are favoured as a means of confining outbreaks (Tanaka et al. 2005).

Table 5. Common pathogens and diseases of carnation†

Type of organism	Taxonomic name	Disease	Symptoms
Bacterium			
	Pseudomonas andropogonis *P. woodsii*	Bacterial Leaf Spot	Leaves develop spots similar to those caused by fungal infections. Spots, however, are translucent.
	P. caryophylli	Bacterial wilt	Sudden wilting of tops or individual branches. Basal stem cracks. Roots may be rotted. Vascular discoloration in stems is yellowish to brown. The outer layer (epidermis) separates easily from the stem, which is sticky to the touch.
	Erwinia chrysanthemi	Bacterial slow wilt	Wilting, twisting, curling, and stunting of lower leaves and side shoots followed by death of lower leaves. Wilted plants may recover only to wilt again. Stunted plants have narrow, yellow-green leaves. Symptoms require several weeks to develop. If plant wounds are infected, the wounded area becomes soft and slimy.
	Agrobacterium tumefaciens	Crown gall	Tumour-like swellings (galls) that occur at the crown of the plant, just above soil level. Although it reduces the marketability, it usually does not cause serious damage to older plants.
	Corynebacterium fascians	Fasciation	Clusters of short, spindly or swollen, fleshy shoots develop at a node on the main stem. These shoots are dwarfed, with misshapen leaves and may be at, below, or near the soil line. The main stem of an affected plant sometimes appears to grow normally, but may be stunted. Blossoming is reduced. The roots on a diseased plant are sometimes short with swollen areas.
Fungus			
	Alternaria dianthicola	Alternaria leaf spot	Gray-brown leaf or petal spots with purple margins. Black spore masses form in spots. Branch rot starts at nodes and girdles stem.
	Uromyces dianthi Niessl	Rust	Small blisters containing rust-red spores form on leaves.
	Ustilago violacea	Anther smut	The fungus completely takes over the anthers of the host, which burst open to release the purple, powdery spores instead of pollen.
	Stemphylium botryosum	Calyx rot	Rot starts at tip of calyx and progresses towards base.
	Macrophomina phaseolina	Charcoal rot	Microsclerotia, small black structures, develop under the cortical tissue of roots or on the surface of stem lesions which enable the fungus to survive in the soil. If lesions continue to develop, usually only under very moist conditions, plants may die. Fruit may also become infected, resulting in a firm, dark-coloured decay.
	Peronospora dianthicola	Downy mildew	Downy mildew appears as white to purplish-gray "down" on leaf undersides when the fungus sporulates.

Type of organism	Taxonomic name	Disease	Symptoms
	Cladosporium echinulatum	Fairy-ring leaf spot	Conspicuous tan spots with concentric rings. Margin of spot may be red. Dark spores form in spots.
	Fusarium tricinctum	Fusarium bud rot	Outwardly normal buds are brown and decayed inside. Fungus spores are carried to the buds by grass mites. Petals inside bud decay first.
	Fusarium graminearum	Fusarium stem rot	Stem rotted at soil line and high up on plant. Roots and base of stem rotted. Tops wilt and die. Pink cushions of spores may form at base of plant on decayed tissues. Common as a cutting rot.
	Fusarium oxysporum	Fusarium wilt	Yellow, wilted branches frequently occur on one side at first. Vascular discoloration is dark brown. Root system usually remains intact. In late stages, stem develops a dry, shredded rot. Infected parts die.
	Botrytis cinerea	Grey mold (*Botrytis* blight)	Woolly grey fungal spores form on soft, brown, decayed blossoms and can move into plant parts wherever blossoms touch them.
	Zygophiala jamaicensis *Schizothyrium pomi*	Greasy blotch	Greasy-appearing spots on leaves with radiating weblike margins. Pimpling of infected areas. Leaves yellow and die.
	Phialophora cinerescens	Phialophora wilt	Gradual wilting of plants; leaves become straw coloured. Not one-sided like in Fusarium wilt. Brown discoloration of vascular system. Little or no tissue rotting in late stages. Uncommon.
	Rhizoctonia solani *Thanatephorus cucumeris*	Rhizoctonia stem rot	Stems at the soil level have a lesion with a brown border. Rot progresses from the outside. Stems have dry, shredded appearance. Entire plant wilts and dies. Dark fungal strands and sclerotia may be visible.
	Sclerotinia sclerotiorum	Sclerotinia flower rot	A cottony, white, dense mat of mycelial growth on the surface of the host and on the adjacent soil surface. Within this white mass, dense white bodies of fungus form which become black and hard (sclerotia) as they mature.
	Septoria dianthi	Septoria leaf spot	Yellowish brown, withered spots surrounded by a purplish margin on leaves. As the disease progresses, entire leaves and stems become necrotic.
	Sclerotium rolfsii	Southern blight	Lower leaves yellow and wilt first. Leaves will also die back from the tips and stems will fall over. The fungus typically attacks the plant just under or at the soil line. Sometimes a dark brown lesion can be seen on the stem before other symptoms are visible.
Oomycete			
	Phytophthora parasitica	Phytophthora stem rot	Stem rotted at soil line. May be mistaken for Rhizoctonia stem rot.
	Pythium spp.	Pythium root rot	Plants are stunted, particularly in lower, poorly drained areas. Rootlets rotted.
Nematode			
	Heterodera trifolii	Cyst	Multiple infections may kill root tips of the plant.
	Hoplolaimus spp.	Lance	Lance nematodes feed externally along root surfaces but may also feed with at least part of the body embedded in the root. Roots damaged by lance nematodes may be darkened and restricted in their development. Above-ground symptoms include yellowing, stunting, and sometimes death of plants.
	Pratylenchus spp.	Lesion	Above-ground symptoms caused by high populations of root lesion nematodes are sometimes falsely attributed to lack of water or nutrients.
	Paratylenchus hamatus	Pin	Pin nematodes do not cause any particular symptoms but can slow down the growth and reduce crop production.
	Mesocriconema spp.	Ring	At high population densities, ring nematodes can cause extensive root pruning which stresses the plant and reduces yields.
	Meloidogyne spp.	Root-knot	Above-ground symptoms of root-knot nematode damage may be mistaken for nutrient deficiency or water stress. Roots usually develop galls or knots, and tubers can be invaded when root-knot nematode populations are high.

Type of organism	Taxonomic name	Disease	Symptoms
Virus			
Cauliovirus	*Carnation etched ring virus* (CERV)	Carnation etched ring	Infection by CERV is sometimes mixed with infection by CarMV. CERV causes more severe symptoms such as leaf yellowing, brown spots and rings on leaves, and streaking and flecking of the stems. Plants flower at a later date and the flower quality is reduced. CERV is spread from plant to plant by aphids. Symptoms may be slight in young cuttings. No obvious effect on plant vigour.
Carlavirus	*Carnation latent virus* (CLV)	Carnation latent	No distinct symptoms on carnation plants; however, it can affect crop production and has been demonstrated to impair flower quality. It is transmitted from plant to plant by aphids.
Carmovirus	*Carnation mottle virus* (CarMV)	Carnation mottle	The most common and widespread virus disease of carnations. Usually there are no symptoms. However, when the symptom is expressed, the infected plant may have a yellow mottle on the leaves. Flower quality and yield are reduced. Virus free plants have broader leaves and more vigorous growth. CarMV is highly infectious and is rapidly spread from plant to plant by foliage contact or during handling.
Closterovirus	*Carnation necrotic fleck virus* (CNFV)	Carnation necrotic fleck Carnation streak	Yellow or brown flecks and streaks on the leaves, sometimes followed by reddening and necrosis. Flower quality and yield are affected. Symptoms are masked at low temperatures.
Dianthovirus	*Carnation ringspot virus* (CRSV)	Carnation ring spot	Small (1 - 2 cm) rings, sometimes concentric, appear on leaves. Chlorosis, mottling, and distortion of young leaves. Plants stunt.
Potyvirus	*Carnation vein mottle virus* (CVMV)	Carnation vein mottle	Yellow spotting and mottle patterns on the leaves. Young leaves tend to exhibit spots and flecks of a darker green colour on the veins. Infected plants have depressed yields and the incidence of "colour breaks" and calyx splitting are greater. Symptoms tend to disappear on old leaves. CVMV is spread from plant to plant by aphids and is found wherever carnations are grown.

† references: Moran (1994); Wick (2000); Raabe et al. (2002); Government of New Brunswick (2006); Moorman (2006)

SECTION 7 TOXICITY AND ALLERGENICITY OF CARNATION

Despite carnation having a long history of floriculture, there are few reports of occupational respiratory allergy within the floral industry and no reports of toxicity. Allergic disorders induced by ornamental flower exposure are usually manifested as dermatological symptoms (eczema, urticaria and contact dermatitis) that may or may not be associated with respiratory manifestations. However, respiratory symptoms are sometimes observed exclusively (Sanchez-Guerrero et al. 1999).

Reports of allergic reactions to carnations include the following:

- Twelve cases of occupational allergic contact dermatitis were diagnosed over a 14-year period among workers involved with decorative plants. The plant families and plants causing occupational contact dermatitis were Caryophyllaceae (two patients- carnation, cauzeflower), Compositae (five patients- chrysanthemum, elecampane, gerbera, feverfew), Alstroemeriaceae (five patients- Alstroemeria), Liliaceae (four patients- tulip, hyacinth) and Amaryllidaceae (two patients- narcissus). The patients were middle-aged, and their average exposure time was 13 years (Lamminpaa et al. 1996).
- A commercial flower seller/distributor developed severe dermatitis and rhino conjunctivitis from dermal and pulmonary exposure to *D. caryophyllus, Gypsophila paniculata* and *Lilium longiflorum* after eight years working with these flowers. The

patient showed positive skin prick responses to all three species, and serum specific IgE for *D. caryophyllus* and *G. paniculata* (Vidal & Polo 1998).

- The relationship between the allergic symptoms induced by carnations and IgE-mediated reactions was investigated in carnation cultivation workers exhibiting exposure related rhinitis and asthma. Skin prick test responses with carnation extract were positive in 15 of 16 patients, and negative in all control subjects. Nasal provocation response to carnation extract was positive in 13 of 16 patients. Immunoblotting of sera from 13 patients showed 2 major IgE-binding fractions in most of the patients, which could constitute the major allergens. This study suggests the involvement of carnations in occupational allergy, mediated by an IgE-dependent mechanism (Sánchez-Guerrero et al. 1999).
- Recent work (Sánchez-Fernández et al 2004) has indicated that the respiratory reactions shown by some carnation workers may, in fact, be caused by both the flowers and/or the presence of the two spotted mite *Tetranychus urticae* that parasitises the flowers and is itself a well-known allergen.

SECTION 8 WEEDINESS OF CARNATION

Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens. 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 cultivated as ornamental plants, there are few records of their being found as naturalised plants even in Mediterranean countries, and there are no records of naturalised *D. caryophyllus* in Australia. However, two other species of *Dianthus* are recorded as weeds in Australia (Lazarides et al. 1997). Deptford Pink (*D. armeria*) became a weed through garden escape and is currently identified as a weed in New South Wales (NSW), Victoria and Tasmania. Its habit is either biennial or annual. *D. barbatus*, another garden escapee, is described as a perennial weed in NSW.

Carnations are grown in many countries including Australia, European countries, Israel, Japan and South American countries. They have not been reported as weeds, invasive species or pest species in any of these countries.

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 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 (see Section 2.5) have to be struck under optimised conditions.

The Co-operative Research Centre for Weed Management systems reviewed recent incursions of weeds between 1971 and 1995 and identified four members of the Caryophyllaceae (Groves 1998). These included:

- *D. plumarius* ssp. *vulgare*: recorded as naturalised in northern Tasmania in 1991;
- *Silene dioica*: recorded as naturalised in NSW in 1982, originally from Mediterranean Europe;

- *Silene tridentata*: recorded as naturalised in NSW in 1986, originally a native of Algeria; and
- *Euonymus* sp.: Spindle Tree which is thought to be a native of Japan, recorded as naturalised in Victoria in 1988.

Southern Weed Science Society, USA (2001) has described *D. armeria* as an invasive weed which has spread across North America. Other members of the Caryophyllaceae that are identified as wildflowers of south-eastern USA are species of *Silene, Cerastium, Stellaria, Saponaria* and *Minuartia.*

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.

Successful gene transfer requires that three criteria are satisfied. The plant populations must:

- overlap spatially;
- overlap temporally (including flowering duration within a year and flowering time within a day); and
- be biological relatives close enough to result in fertile hybrids and facilitate introgression into a new population (den Nijs et al. 2004).

Carnation has been cultivated for over 2000 years and new varieties have been developed mainly by the selection of desirable individuals from inter- and intra-specific crosses.

Carnations generally produce only small quantities of pollen. The quantity and quality of pollen varies according to cultivar and species (Kho & Baer 1973; Galbally & Galbally 1997). The pollen of carnation is heavy and sticky, is not wind-dispersed, and has low viability (percentage germination for some lines is less than 10%). 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

Many *Dianthus* species are obligate outcrossers because they are protandrous (i.e. the anthers and pollen mature before the pistils) thereby preventing self-pollination.

Insect pollinators can contribute to gene transfer as they help outcrossing between individual plants. These pollinators are described in Section 4.2.

9.2 Interspecific gene transfer

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 *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 6). However it must be stressed that most of the crosses were done under glasshouse conditions and with human intervention (eg petal removal, manual pollination, calyx opening). Of the plants in Table 6, only *D. barbatus* is recorded as a weed in Australia (NSW) (Lazarides et al. 1997).

Table 6. *Dianthus* species that have successfully hybridised with *D. caryophyllus*[**]

Species	Reference
allwoodii	Umiel et al. (1987)
arenarius	Holley & Baker (1963); Umiel et al. (1987)
barbatus	Pax & Hoffman (1934); Umiel et al. (1987)
capitatus	Onozaki et al. (1998)
carthusianorum	Mehlquist (1945); Demmink (1978); Segers (1987); Sparnaaij & Koehorst–van Putten (1990)
chinensis	Mehlquist (1945); Demmink (1978); Segers (1987); Sparnaaij & Koehorst-van Putten (1990)
deltoides	Umiel et al. (1987)
gallicus	Holley & Baker (1963)
giganteus	Demmink (1978); Sparnaaij & Koehorst-van Putten (1990)
japonicus	Nimura et al. (2003)
knappii	Holley & Baker (1963); Segers (1987); Sparnaaij & Koehorst-van Putten (1990)
monspessulanus	Holley & Baker (1963)
seguieri	Holley & Baker (1963)
sinensis	Holley & Baker (1963); Umiel et al. (1987)
sylvestris[1]	Carolin (1956); Holley & Baker (1963); Umiel et al. (1987); Demmink (1987)
versicolor	Sparnaaij & Koehorst-van Putten (1990)

[**] Taken largely from COGEM (1998b) – Annex 3 ‘An assessment of the probability of gene dispersal from cut-flower varieties of the cultivated carnation (*Dianthus caryophyllus*) in Europe’.

[1] in Carolin (1956) *D. sylvestris* is referred to as *D. inodorus*.

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.

9.3 Intergeneric gene transfer

Carnation is not closely related to any important weed species in Australia. There are a number of major weeds in Australia in the Caryophyllaceae family (Table 7) but none of these is taxonomically closely related to *D. caryophyllus* and interbreeding would therefore be highly unlikely, if not, impossible.

Table 7. Weeds of the Caryophyllaceae family in Australia[1]

Species	Common name	Distribution
Agrostemma githago	corn cockle	Qld, NSW, Tas, SA
Arenaria leptocladus	lesser thyme-leaved sandwort	WA, SA, NSW, Vic, Tas
Arenaria serpyllifolia	thyme-leaved sandwort	SA, NSW, Vic, Tas
Cerastium spp. – 6 species	mouse-eared chickweed	various
Corrigiola litoralis	strapwort	WA, Vic
Drymaria cordata	tropical chickweed	Qld, NSW
Gypsophila paniculate		SA
Gypsophila tubulosa	chalkwort	WA, NT, Vic, Tas
Herniaria cinerea	hairy rupturewort	WA, SA, NSW, Vic
Lychnis chalcedonica	maltese-cross campion	NSW
Lychnis coronaria	rose campion	SA, NSW
Minuartia mediterranea	slender sandwort	WA, SA, Vic, Tas
Moenchia erecta	erect chickweed	WA, SA, NSW, Vic, Tas
Paronychia argentea	whitlowwort	SA
Paronychia brasiliana	brazilian whitlow	Qld, NSW, Vic, SA
Paronychia franciscana	whitlowwort	Vic
Petrorhagia nanteuilii	proliferous pink	WA, SA, Qld, NSW, Vic, Tas
Petrorhagia velutina	velvet pink	WA, SA, Qld, NSW, Vic, Tas
Polycarpon tetraphyllum	four-leaved allseed	all states
Sagina apetala	annual pearlwort	all states
Sagina maritima		WA, SA, Vic, Tas
Sagina procumbens	spreading pearlwort	WA, SA, Qld, NSW, Vic, Tas
Saponaria calabrica	Adriatic soapwort	NSW
Saponaria officinalis	soapwort	NSW, Vic, Tas
Scleranthus annuus	knawel	NSW, Vic, Tas
Silene spp. – 16 species	catchfly	various

Species	Common name	Distribution
Spergularia spp. – 6 species	sandspurrey	various
Stellaria spp – 5 species	starwort	various
Vaccaria hispanica	bladder soapwort	all states

[1] compiled from Lazarides et al. (1997)

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