

SUNTORY

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NO CBI

Dear Dr. Firko

I am writing as the United States (USA) entity of Suntory Flowers Limited (SFL), 4-17-5 Shiba, Minato-ku, Tokyo 108-0014, Japan. Suntory flowers (<http://suntoryflowers.com/>) has developed blue-flower color transgenic cut-flower varieties of the plant species *Chrysanthemum morifolium* syn. *Dendranthema x grandiflora* Tzvelev (chrysanthemum), an ornamental plant used for cut flower production. Through this "Am I regulated?" query letter, SFL would like to request an opinion on the regulatory status of imported cut flowers from these varieties under 7 CFR Part 340. To support this query, we have compiled details of the phenotypic change, construct, donor organisms and biology of the recipient plant, chrysanthemum. These details are shown from page 4 of this letter.

The intended activity associated with the transgenic chrysanthemum events described in this letter is release, as import into the USA of cut-flowers only. There is no intention to grow these events in the USA. The intended site of production of the flowers from the transgenic chrysanthemum events will be Colombia. Cut flowers of non-transgenic chrysanthemum are exported daily from Colombia to the USA and it is intended that the flowers of the transgenic events will be harvested, treated, packed and exported in the same way as flowers from non-transgenic chrysanthemum plants. It is intended that once in the USA the transgenic flowers will be imported, distributed, retailed and used in the same way as non-transgenic chrysanthemum flowers.

The transgenic chrysanthemum varieties have a novel flower color because of re-direction of the anthocyanin biosynthesis pathway, resulting in the accumulation of delphinidin-related anthocyanins in flowers. The flowers have a novel flower color for the chrysanthemum plant.

The same type of anthocyanin biosynthesis pathway manipulation has been made in the transgenic carnation¹ and rose² varieties already imported as cut-flowers into the USA. Genetically-modified carnation flowers containing a pansy or petunia flavonoid 3', 5' hydroxylase have been traded in the USA for nearly 18 years, with no reports of adverse effects³. The phenotypic change in chrysanthemum is due to expression of two plant genes; a cDNA clone of flavonoid 3', 5' -hydroxylase from campanula and a cDNA clone of UDP-glucose: anthocyanin 3',5'-O-glucosyltransferase gene from butterfly pea⁴. The selectable marker is the nptII(neo) gene from *Escherichia coli*. We are seeking an opinion on multiple chrysanthemum events, all of which have been generated by the same construct, pB423.

The modified phenotype is innocuous and does not pose a risk to human or animal health as delphinidin is a common anthocyanin, present in many other flower species, and at high concentrations in commonly consumed foods⁵. The introduced flavonoid 3',5'-hydroxylase occurs in all plants and foods containing delphinidin.

In 1991, USDA-APHIS issued a field test permit for transgenic chrysanthemum, carrying a kanamycin resistance gene⁶.

Though the transformation method used was disarmed *Agrobacterium* co-cultivation and some genetic elements of the construct pB423 (borders, promoter and terminator) are derived from the *Agrobacterium tumefaciens* (a designated plant pest in 7 CFR sub-section 340.2) we would like to query whether the transgenic chrysanthemum events described in this letter meet the definition of a regulated article as described in 7 CFR part 340. Our grounds for enquiry are that USDA-APHIS has stated that transgenic cut flowers grown in other countries pose little if any risk and may not require permits⁷. This consideration was included in advice applied to import of cut flowers of transgenic gypsophila⁸ and transgenic rose⁹. Additionally, USDA-APHIS has provided opinion that permits are not required for pineapple fruit import, on the basis that imported fruits do not have an ability to persist in the environment¹⁰.

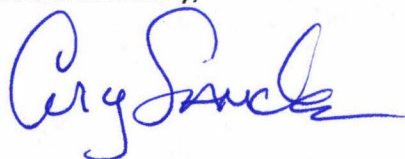
Regarding the transgenic chrysanthemum flowers described in this letter;

- As the intended activity with the transgenic chrysanthemum is import of cut-flowers only there is no realistic avenue for gene flow and the cut flowers would therefore pose no risk to plant health.
- Imported cut flowers of chrysanthemum are not capable of self-propagation or forming seeds.
- Transfer of pollen from the imported cut flowers to potential recipient plants is extremely unlikely.

- No *Chrysanthemum* species is currently¹¹ listed as a Federal Noxious Weed or listed in any U.S. State as a noxious weed.

Thank you for your attention to this matter.

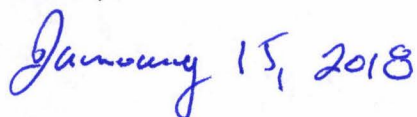
Yours sincerely,



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Dated;



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¹ <http://www.florigene.com/index.html>

² https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/15-324-02_air_response_signed.pdf

³ Chandler, S. F., Senior, M., Nakamura, N., Tsuda, S. and Tanaka, Y. (2013). Expression of flavonoid 3',5'-hydroxylase and acetolactate synthase genes in transgenic carnation: assessing the safety of a non-food plant. *Journal of agricultural and food chemistry* **61**;11711-11720

⁴ Noda, N., Yoshioka, S., Kishimoto, S., Nakayama, M., Douzono, M., Tanaka, Y., & Aida, R. (2017). Generation of blue chrysanthemums by anthocyanin B-ring hydroxylation and glucosylation and its coloration mechanism. *Science Advances*, 3(7), e1602785

⁵ Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E. and Prior, R.L. (2006). Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal of Agricultural Food Chemistry* **54**; 4069-4075.

⁶ <http://www.isb.vt.edu/getRelDetail.aspx?bp=91-106-01r>

⁷ Federal Register /Vol. 73, No. 197 /Thursday, October 9, 2008 / Proposed Rules; Department of Agriculture, Animal and Plant Health Inspection Service 7 CFR Part 340 [Docket No. APHIS-2008-0023]

⁸ https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/Response_Babysbreath_122011.pdf

⁹ https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/15-324-02_air_response_signed.pdf

¹⁰ https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/aphis_response_del_monte.pdf

¹¹ <https://plants.usda.gov/java/noxious?rptType=Federal> accessed Aug 28 2017

**Technical information to support “Am I regulated?” query to the US Department of
Agriculture Animal and Plant Health Inspection Service Biotechnology Regulatory Services
enquiring whether imported transgenic chrysanthemum flowers are regulated articles under
7 CFR part 340**

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1. Description of intended genetic change in final product

The intended genetic change is gene insertion. In the transgenic chrysanthemum events described in section 2, three genes have been inserted, contained in construct pB423;

- The nptII (neo) antibiotic resistance selectable marker gene from *Escherichia coli*.
- The flavonoid 3',5'-hydroxylase (F3'5'H) coding sequence (cDNA) from campanula (*Campanula medium*).
- The UDP-glucose: anthocyanin 3',5'-O-glucosyltransferase (UDPG) coding sequence (cDNA) from butterfly pea (*Clitoria ternatea*).

Further details of the construct used, and function of these genes are provided in section 2.3 and section 3. Co-expression of the F3'5'H and UDPG genes is required for development of blue colored chrysanthemum flowers (Noda et al., 2017). Multiple transgenic events have been selected after transformation (Noda et al., 2017), from parental varieties representing several different chrysanthemum cut-flower forms (refer to section 4). Southern analysis has been carried out on 28 transgenic events, using a probe for the F3'5'H gene, a gene in the T-DNA, to estimate copy number. Copy estimate per event varies from single copy to six inserted copies of the gene.

2. Description of intended phenotype

The intended phenotype is a change in flower color after manipulation of the anthocyanin biosynthesis pathway in flowers. There are multiple transgenic chrysanthemum events with the intended phenotype. These have all been generated using construct pB423 (details in section 3).

2.1 The anthocyanin biosynthesis pathway

Flower color is generally the result of the relative concentration and type of two pigment types - carotenoids and flavonoids. Carotenoids are responsible for yellow through orange colors. Anthocyanins are flavonoid based colored pigments. There are three groups of anthocyanins, the delphinidin-based anthocyanins that generally produce purple-blue flower

color, cyanidin-based anthocyanins that produce red or pink flower color and pelargonidin-based anthocyanins that produce orange or brick red flower color (Yoshida et al., 2009). The anthocyanin biosynthesis pathway is shown in Figure 1 at page 28.

The key enzyme at the start of the flavonoid pathway is chalcone synthase, from which the dihydroflavonol dihydrokaempferol is produced after enzymatic reactions catalysed by chalcone isomerase (CHI) and flavanone 3 β -hydroxylase (F3H) (Figure 1). The dihydroflavonols are substrates for the biosynthesis of the colored anthocyanin pigments, which are produced in three steps; firstly, a reduction, catalysed by the enzyme dihydroflavonol 4-reductase (DFR); secondly the production of colorless anthocyanidin through the action of the enzyme anthocyanidin synthase (ANS) and thirdly addition of a glucose residue to produce a colored anthocyanin molecule. Anthocyanins can be further modified in many ways, by addition of glycosyl, acyl and methyl molecules. These reactions are not shown in Figure 1. The enzyme flavonoid 3'5'-hydroxylase converts dihydrokaempferol (DHK) and dihydroquercetin (DHQ) to dihydromyricetin (DHM) (Figure 1) and is a pre-requisite for the biosynthesis of delphinidin.

2.2 The anthocyanin biosynthesis pathway in chrysanthemum

Non-transgenic chrysanthemums lack the part of the anthocyanin biosynthetic pathway that is responsible for the production of delphinidin, as the gene for the enzyme flavonoid 3'5'-hydroxylase (F3'5'H; see Figure 1 and Figure 2) is missing in this species. Non-transgenic chrysanthemum flowers accumulate cyanidin-related anthocyanins (Brugliera et al., 2013; Noda et al., 2013, 2017).

2.3 The anthocyanin biosynthesis pathway in transgenic chrysanthemum

In the genetically modified chrysanthemum lines described in this document a campanula gene encoding F3'5'H has been introduced (Noda et al., 2013, 2017; Tanaka and Brugliera, 2014) and delphinidin is produced because of the expression of this gene. The change to the biosynthetic pathway is illustrated schematically in Figure 2.

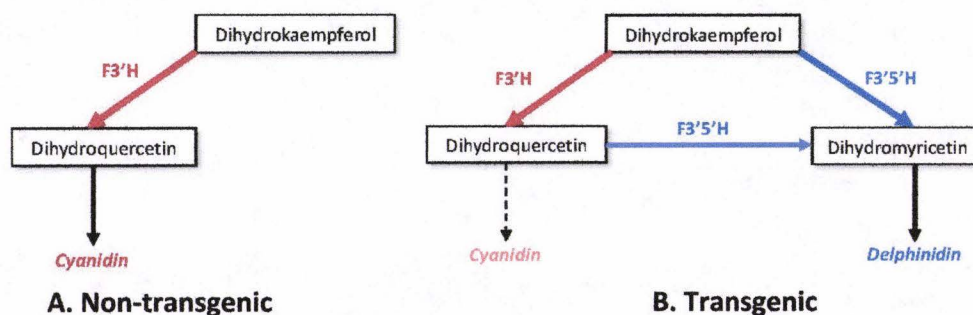


Figure 2. Modification of the anthocyanin pathway in chrysanthemum. A. Untransformed chrysanthemum plants, expressing flavonoid 3' hydroxylase (F3'H). B. The anthocyanin pathway in transgenic chrysanthemum after addition of a gene for flavonoid 3'5' hydroxylase (F3'5'H).

A second gene, the UDP-glucose: anthocyanin 3',5'-O-glucosyltransferase gene from butterfly pea, functions to add two glucosyl moieties to the delphinidin anthocyanin structure (Kogawa et al., 2007). The addition of these side groups confers a bluer hue to the anthocyanin (Noda et

al., 2017). It has been determined the delphinidin-related anthocyanins in the transgenic chrysanthemum is mainly delphinidin 3-*O*-(6''-*O*-malonyl) glucoside-3',5'-di-*O*-glucoside (Noda et al., 2013, 2017).

2.4 Modified phenotype

The modified phenotype is production in the flowers of an anthocyanin derived from the anthocyanidin delphinidin, leading to a change in anthocyanin profile and a modified flower color (Noda et al., 2017). Figure 3 illustrates some typical flower color changes in flowers of the transgenic lines. Transgenic events have been generated from parental varieties with different chrysanthemum cut-flower forms and three different types are shown in figure 3.



Figure 3. Typical flower color phenotypes of transgenic chrysanthemum transformed with pB423. Each photograph is of a different transgenic line and in all three photographs the flower from the transgenic line is on the right and a flower from variety used for transformation is on the left.

Depending on the transgenic line, the amount of delphinidin-based anthocyanin in the petals of the transgenic flowers ranges from 0.2 to 5.8 nmol mg⁻¹ and represents 40 - 100% of total anthocyanins (Noda et al., 2017). The color of the flowers from the transgenic chrysanthemum lines fall in the range of Royal Horticultural Society color groups violet (85D), violet-blue (94B) to blue (100C) (Noda et al., 2017).

Noda et al. (2013) reported no delphinidin-related anthocyanins to be present in the leaves and stems of transformed lines with high concentrations of delphinidin in flowers. This is because both the F3'5'H and UDPG genes are driven by a petal specific promoter from chrysanthemum (refer section 3).

The genetically modified phenotype is stable. The initial transgenic plants have now been vegetatively propagated for more than 4 years and the flower color phenotype and delphinidin levels measured in petals are stable (Noda et al., 2013).

3. Description of construct and genetic elements

3.1 Name of construct

The construct (binary transformation vector) used to generate the transgenic chrysanthemum is pB423 (Noda et.al., 2017).

3.2 Description of vector used to induce genetic change

The transgenic organisms were generated after co-cultivation with disarmed *Agrobacterium tumefaciens* strain EHA105 carrying the construct (Noda et al., 2017).

3.3 Description of construct

Figure 4 provides a diagram of the construct and table 1 (page 29) lists the genetic elements of the construct in the order in which they occur in the construct (from right border to left border).

All transgenic events generated with construct pB423 contain a neomycin phosphotransferase gene from *Escherichia coli*. This gene confers antibiotic resistance (Goldstein et al., 2005). Paromomycin was used as a selective agent during plant transformation.

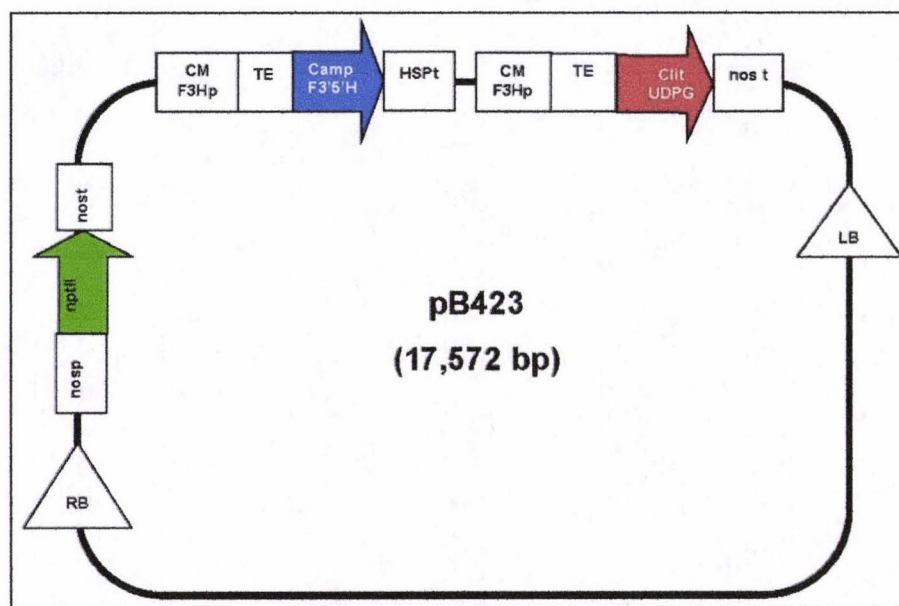


Figure 4. Diagram of construct pB423

3.4 Description of function of genetic elements

A brief description of the function of each genetic element is provided in table 2 (page 30).

3.5 Donor organisms

Donor organisms for all DNA sequences in the construct are listed in table 3 (page 31).

4. Taxonomy and biology of recipient organism

The recipient organism is chrysanthemum (commonly known as greenhouse chrysanthemum, cut mum, chrysanthemum, mum, florist's daisy, florists chrysanthemum). The term chrysanthemum encompasses varieties of chrysanthemum which have been bred specifically for cut flower production as well as varieties of chrysanthemum which are used for outdoor pot plant, bedding or garden use (such varieties are commonly known as garden mum, pot mum or outdoor mum). Many thousands of varieties of chrysanthemum have been developed by flower breeders, over hundreds of years. All varieties of chrysanthemum, whatever the intended use, are from the same species, *Chrysanthemum x morifolium* (Anderson, 2006). Other species of the *Chrysanthemum* genus are also cultivated and are sometimes referred to as chrysanthemum in common vernacular (table 4, page 32).

In this submission the recipient organism is **cut-flower varieties of the chrysanthemum only**. The intention is import of harvested cut-flowers.

4.1 Chrysanthemum domestication and cultivation

Chrysanthemum has a very long history of safe use by humans. Chinese and Japanese records indicate cultivation for many centuries (Ackerson, 1967a; Anderson, 2006) and chrysanthemum flowers have a central role in the culture of China, Japan and Korea. In these countries chrysanthemum flowers are not only used for ornamental and ceremonial purposes but also as part of traditional medicine (Hu, 2015; Huang, 1999). Edible cultivars have been grown in China for centuries (Li et al., 2011). From the 17th Century chrysanthemum was introduced to Europe and from there was cultivated through the rest of the world as European migration flowed to countries such as the USA, Canada, New Zealand and Australia. The chrysanthemum was imported to the USA in 1847 (Ameson, 1927) and has become established as a popular plant in the USA for landscaping, home use and commercial production (Anderson, 2006). Today cut flower chrysanthemum varieties are grown in virtually all temperate and subtropical areas and flowers of the species are one of the top ten most widely produced cut flowers in the world. Commercial cut flower varieties of chrysanthemum have been bred for the color and form of the flowers and further selection has been imposed for characters such as productivity, disease resistance and vase-life.

4.1.1 Cut flower varieties

Under natural light conditions, chrysanthemum flowers in the fall. Year-round production of cut flowers is possible through manipulation of day length under cultivation. Cut flower chrysanthemum varieties are defined by the way plants are managed and the way the ray and

disk florets are arranged (refer to 4.4.2 for details of flower structure). The most common flower forms in the US cut flower industry are shown on the next page in table 5 and figure 5. Other classifications are available, based on flower shape and plant form (Boase et al., 1997). In the US cut flower industry commonly used terms for flowers are standard, disbuds and sprays. Standards are large flowered cultivars, presenting a one single flower per stem. This is achieved by removal of lateral buds and shoots during growth, promoting one large floral meristem to develop. Smaller diameter standard type flowers are called disbuds. Spray type chrysanthemum flowers, such as typically found in bouquets, have a spray of smaller-flowers on a single stem. In these types the central meristem is removed to promote lateral growth. "Pom-poms" are common spray type flowers. The "santinis" and "madibas" are spray cut chrysanthemum with smaller size flower heads. The varieties described in this application are of the decorative, anemone or pom-pom form.

Table 5: Chrysanthemum flower forms

Flower form	Description
Single or Daisy	Composed of one or two rows of outer ray flowers surrounding a tight group of short disc florets.
Decorative	Like reflex blooms without the mop like appearance. Disk florets are completely concealed, ray florets usually don't radiate at more than a 90-degree angle to the stem.
Pompon	The blooms are fully double, of small size, and almost completely globular in form
Anemone	The disk florets are prominently featured, quite often raised and overshadowing the ray florets
Spider	The disk florets are completely concealed, and the ray florets are tube liked with hooked or barbed ends, hanging loosely around the stem

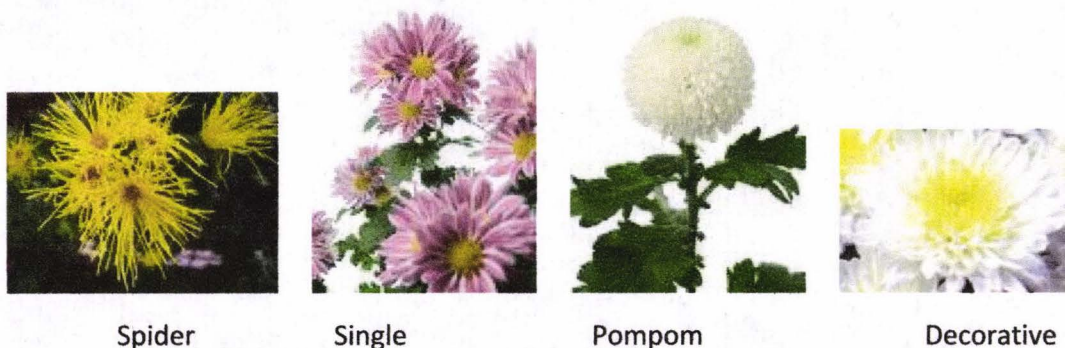


Figure 5. Examples of some of the different forms of chrysanthemum flowers. Further examples are provided by the US national chrysanthemum society (US national chrysanthemum society, 2013)

4.1.2 Chrysanthemum production and import in US

According to the most recent data from the USDA National Agriculture Statistics Service (USDA, 2016) chrysanthemum is produced in the USA as both pot plants and cut flowers;

- In 2015 there were 821 producers of pot chrysanthemum, across 15 states. Most growers were in the states of New York, Pennsylvania and Michigan. 47 million pots were sold, worth 124 million United States dollars (USD) at wholesale.
- There were 152 producers of potted flowering chrysanthemum for indoor use, across 26 states. 4 million pots were sold, worth 16 million USD at wholesale.
- There were 28 producers of cut flower chrysanthemum, the majority of whom are based in California. 70 million flowers were sold, worth 11 million USD at wholesale.

The number of imported cut flowers of chrysanthemum exceeds domestic production. Weekly import data is published by the USDA-Agricultural Marketing service (USDA-AMS, 2017) and collation of that data indicates 140 million stems were imported in 2016, of which 94% were imported from Colombia and 97% imported through Miami.

4.1.3 Cultivation methods for cut flower chrysanthemum

Figure 6 shows illustrative photographs of the production of cut flowers of chrysanthemum.



Figure 6. Photographs of production of cut flowers of chrysanthemum. From top left, then clockwise; mother plants used for production and harvest of cuttings, rooted cutting, rooted cuttings planted for flower production, flowering plants ready for flower harvest. Photographs were taken in Colombia

For flower production, chrysanthemum is vegetatively propagated and to produce plants cuttings are taken from 'mother plants'. These are continually pruned to produce a high number of vegetative cuttings from axillary buds. Cuttings are rooted in conditions of high humidity, after treatment with rooting powder. Rooted plants may be planted in soil or grown hydroponically, and are grown to flowering, after which the flowers are harvested, and plants destroyed to be replaced by new rooted cuttings. Garden chrysanthemum varieties are also largely vegetatively propagated (Anderson, 2005).

4.2 Taxonomy of recipient organism

4.2.1 *Chrysanthemum* genus

The *Chrysanthemum* genus belongs to the Asteraceae family. The Asteraceae or Compositae, is known as the aster, daisy or sunflower family, and is the second largest family of flowering plants with more than 20,000 species. As well as ornamental plants, this family contains commercially important food plants such as *Lactuca sativa* (lettuce), *Cichorium* (chicory), *Cynara scolymus* (globe artichoke), *Helianthus annuus* (sunflower), and *Helianthus tuberosus* (Jerusalem artichoke). At times, most *Chrysanthemum* species have been re-classified to the closely related genera *Dendranthema*, *Argyranthemum*, *Leucanthemum* and *Tanacetum*. However, at the end of the last century some species were returned to the *Chrysanthemum* genus (Brummitt, 1997, 1998; Nicolson, 1999) and for the purposes of this document we observe the taxonomic treatment of Oberprieler et al. (2007);

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliopsida

Order - Asterales

Family - Asteraceae (Compositae)

Sub-family Asteroideae)

Tribe -Anthemideae

Sub-tribe – Artemisiinae

Genus – ***Chrysanthemum***

Further information on the tribe Anthemideae is provided by Bremer and Humphries (1997). Oberprieler et al. (2007) and Lin et al. (2011) state the *Chrysanthemum* genus has 37 species. The genus *Chrysanthemum* has species with $n=9, 18, 27, 36$ and 45 chromosomes. The multiple ploidy levels in the *Chrysanthemum* genus is likely to be the result of interspecific hybridisation between two or three diploid species (Boase et al., 1997) with higher ploidy levels more common in species native to Asia (Dowrick, 1953). Cultivated and garden forms of chrysanthemum are of hybrid origin and are largely hexaploid ($2n=54=6x$) but because of sporting and subsequent selection, variation around this number is common (Dowrick and El-Bayoumi, 1966; Nazeer and Khoshoo, 1983). Chrysanthemum is therefore considered a polyan euploid complex (Boase et al., 1997). Guo et al. (2012) determined that the majority of 405 large flowered Chinese chrysanthemum cultivars were aneuploid or tetraploid. The

chromosome number of the transgenic varieties described in this document is not known but it is assumed they are hexaploid based on parentage.

4.2.2 Cultivated chrysanthemum

The most widely used scientific name of chrysanthemum is *Chrysanthemum x morifolium* Ramat. Other names in use in the older scientific literature are *Chrysanthemum x grandiflorum* C. sinense, *C. hortorium* and *C. hortum*. As chrysanthemum was one of the *Chrysanthemum* species placed in the *Dendranthema* genus for several decades the plant is also referred to in some scientific literature as *Dendranthema x grandiflora* Tzvelev (Anderson, 1987). In 1999 chrysanthemum was reclassified by the International Code of Botanical Nomenclature, from the genus *Dendranthema* to the genus *Chrysanthemum* (Brummitt, 1998; Nicolson, 1999).

4.2.3 Origin of cultivated chrysanthemum

Thousands of current and past cut-flower varieties of cultivated chrysanthemum have been developed for horticultural purposes. This development began in China thousands of years ago (Chen, 1985; Zhao et al., 2009) continued in Japan centuries ago and has continued worldwide since the mid-1800s. Because of the long history of cultivation, the taxonomic origin of the cultivated chrysanthemum is uncertain, though is assumed to have arisen from hybridisation (Ackerson, 1967b). The origin species most likely includes *Chrysanthemum indicum* (Brummitt, 1998; Dowrick, 1953; Ma et al., 2016; Lin et al., 2011), probably in addition to species such as *C. japonense*, *C. zawadskii*, *C. nankingense* and *C. vestitum* (Dowrick, 1953; Ma et al., 2016; Ohashi and Yonekura, 2004; Zhao et al., 2012). Natural hybrids between *C. indicum* and *C. vestitum* have been discovered in China (Nakata et al., 1992). The USDA plant database indicates *Chrysanthemum x morifolium* as “Pro.sp” – i.e. treated as a hybrid (USDA, NRCS, 2017).

4.3 Distribution

4.3.1 *Chrysanthemum* genus

The 37 *Chrysanthemum* species are all perennial flowering plants native to Asia and Europe (Dowrick, 1953). East Asia is the center of biodiversity with 22 species in China (Lin et al., 2011) and 18 native *Chrysanthemum* species in Japan (Ohwi, 1965). The species from which chrysanthemum is assumed to be derived, *Chrysanthemum indicum*, is found in Japan and China (Dowrick, 1953). The genus *Chrysanthemum* can be found in other parts of the world because of plant introduction.

Distribution in US flora

The taxonomic re-classification of the *Chrysanthemum* genus (refer 4.2.1) means that many species previously assigned to the genus are now assigned to other genera. There are now no *Chrysanthemum* species in the flora of North America (Barkley et al., 2017). Table 6 on page 33 lists the *Chrysanthemum* species and species previously classified as *Chrysanthemum*, according to the e-flora of North America (Barkley et al., 2017) and the USDA plant database (USDA, NRCS, 2017). Scientific names are cross referenced in the USDA plant database (USDA, NRCS, 2017). Most species listed in table 6 are introduced species, occurring as abandoned plantings and in disturbed sites (Barkley et al., 2017). Two species, *Leucanthemum vulgare*

(syn. *Chrysanthemum leucanthemum*) and *Tanacetum vulgare* (syn. *Chrysanthemum vulgare*) are very widely spread in the USA. Table 7 describes the distribution of the only species listed in table 6 which are native to the USA. There is no record in North America of inter-specific hybridization between the species listed in table 6.

Table 7: Species previously classified as *Chrysanthemum*, according to the e-flora of North America (Barkley et al., 2017), which are native to the US flora.

Scientific name	Synonym	Distribution in North America#
<i>Arctanthemum arcticum</i>	<i>Chrysanthemum arcticum</i>	Coastal Alaska and low-arctic Canada.
<i>Tanacetum bipinnatum</i> ssp. <i>bipinnatum</i>	<i>Chrysanthemum bipinnatum</i> (spp <i>bipinnatum</i>)	Dunes, other sandy sites, calcareous soils, coastal scrub
<i>Tanacetum bipinnatum</i> ssp. <i>huronense</i>	<i>Chrysanthemum bipinnatum</i> (spp <i>huronense</i>)	Dunes, other sandy sites, calcareous soils, coastal scrub
<i>Boltonia caroliniana</i>	<i>Chrysanthemum caroliniana</i>	Alluvial woods, swales, sandy wooded bottomlands; 10–300 m; N.C., S.C., Va
<i>Hulteniella integrifolia</i>	<i>Chrysanthemum integrifolium</i>	Yukon; Alaska

From Barkley et al., 2017

4.3.2 *Chrysanthemum morifolium*

Chrysanthemum x morifolium is only found as an escape from cultivation. Even in Asia, a centre of biodiversity for the *Chrysanthemum* genus, *C. morifolium* is a cultivated plant only (Ohashi and Yonekura, 2004; Zhao et al., 2009). In cultivation, the species is ubiquitous, as a cut flower or garden product in virtually all countries. It is most likely that the rare escapes that have been recorded have occurred from garden chrysanthemum varieties. *Chrysanthemum x morifolium* is not listed in the flora of North America (Barkley et al., 2017) but the USDA database (USDA, NRCS, 2017) contains records of escape from cultivation in Ohio, California, Pennsylvania and Massachusetts. These records indicate that escapes did not persist in Massachusetts (Cullina et al., 2011) and in California was a 1964 record of extensive growth in a yard (Hrusa et al., 2002). In Pennsylvania distribution is indicated in two counties only as an “occasional escape in fields, woods, railroad tracks and waste ground” (Rhoads and Klein, 1993).

4.4. Reproductive biology

4.4.1 Asexual reproduction

The chrysanthemum displays some features of a hardy perennial plant in that the plants are upright and are quick to branch. During or shortly before blooming stolons may grow at the base of the plant in some garden cultivars (Cathey, 1969). These stolons take the form of runners from lateral offshoots which under suitable environmental conditions may form roots on contact with the ground. Where they form, the stolons terminate in rosettes of small leaves which are a pre-requisite for over-winter survival when plants are grown outdoors. The rosettes must remain above ground (Cathey, 1969). Some garden chrysanthemum varieties produce

underground shoots or stolons which enable them to survive from year to year. Cultivars which do not produce stolons will not survive outdoors, though may survive a few degrees of frost without developing stolons. Florist chrysanthemums are easily winter-killed and in outdoor gardens, the flowering buds on florist chrysanthemums are often destroyed by frost before they can bloom. (Iowa State University, 2013). Commercial cut flower varieties cannot form stolons as plants are destroyed after flowers are harvested.

4.4.2 Flower structure

Figure 7 shows the primary structures of the flower of a cultivated cut-flower chrysanthemum variety.

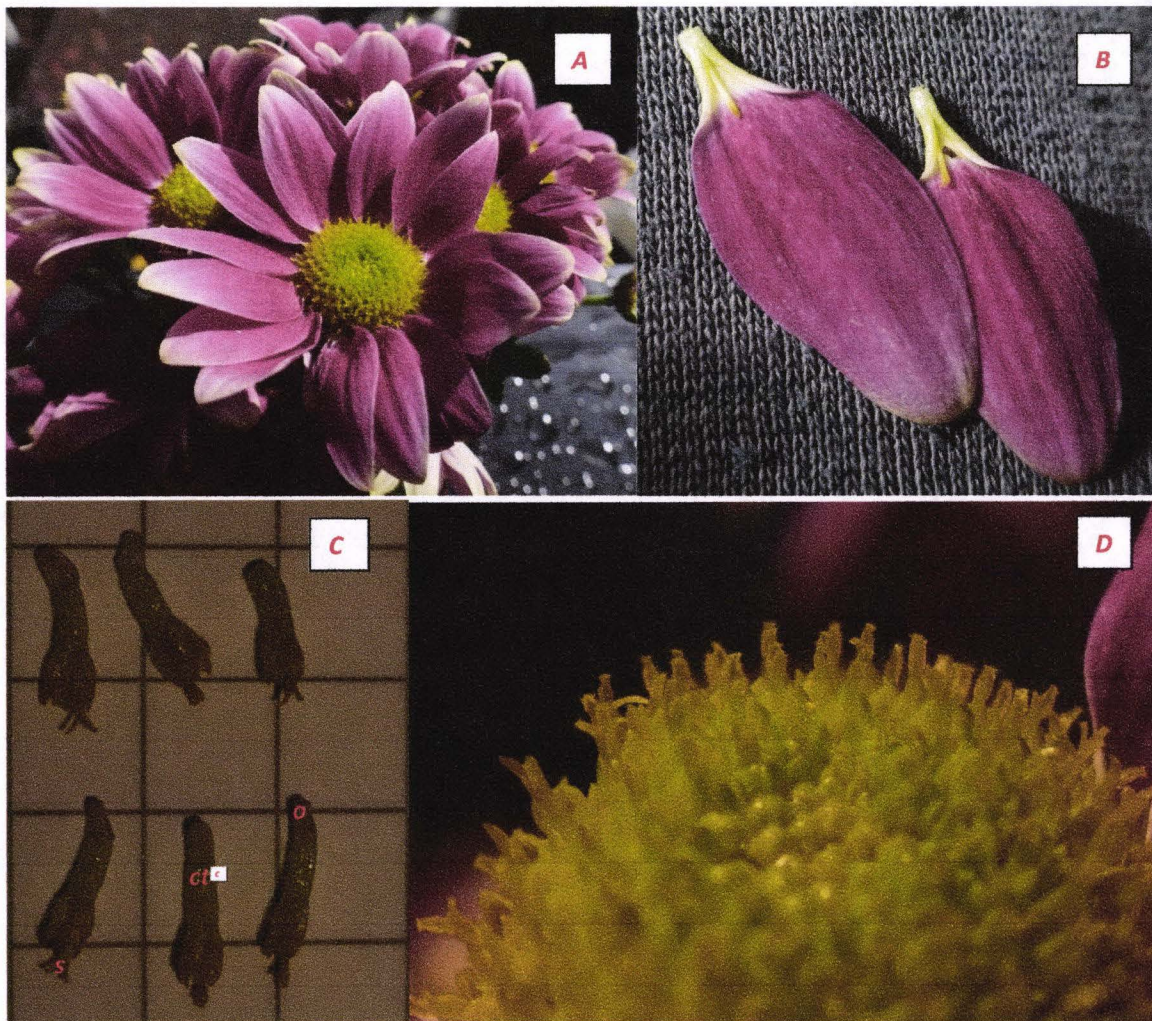


Figure 7. Illustrative photographs of a chrysanthemum flower. A. A complete flower head showing the yellow disc in the centre and the lilac (in this particular variety) ray flowers. B. Two ray flowers showing the corolla tube at the base of the flower. C. Isolated disc flowers showing stigma (s), corolla tube (ct) and location of the ovary (o). D. Close up of disc showing the multiple disc flowers.

The chrysanthemum flower head (capitula) is a compound structure comprising a disc (or “eye”) at the centre, and surrounding “ray”. The disc is comprised of many small individual flowers and the ray is comprised of much larger ray flowers. The pigmented petals in the ray flowers are the distinguishing feature of most cultivated cut-flower chrysanthemum varieties. Disc flowers have both male and female organs while ray flowers only have female organs (Kubitzki, 2007). In the disc flowers petals are fused into a corolla tube while in ray flowers the petals take the form of a long, extended lamina (figure 7).

4.4.3 Pollination biology

Chrysanthemum species are insect pollinated. When insects visit the capitulum they move from the margin to the centre to collect pollen and nectar. Pollinators are mainly bees, bumble bees, flies and other Hymenoptera (Frankel and Galun, 1977). Watanabe et al. (2006) also showed thrips and mites could also be pollen vectors. The most important pollinator of the Asteraceae family are solitary bees and pollination by flies is a characteristic of Asteraceae in montane areas (Kubitzki, 2007). Chrysanthemum flowers are adapted for insect pollination; pollen is heavy and sticky (facilitating a pollen mass that can be carried by insects) and the stigma has a low surface area. Despite production of up to 800,00 pollen grains per flower in some cultivars (Wang et al., 2014) wind-assisted pollen dispersal from cultivated chrysanthemum is unlikely and Shinoyama et al. (2008) demonstrated dispersal of pollen did not occur in artificial wind pollination experiments.

Flowers from cultivars which produce excess pollen have a “dusty” appearance and shorter vase life (Broertjes and De Jong, 1984; Fei et al., 2016; Wang et al., 2014) and selection for low pollen production or male sterility is an objective in breeding programs. Male sterility does occur in some chrysanthemum varieties and may arise after mutation breeding (Broertjes and De Jong, 1984). Anther indehiscence, or failure of anthers to crack and release viable pollen is one mechanism of male sterility (Fei et al., 2016; Wang et al., 2014). Some chrysanthemum cultivars do not produce any pollen (Wang et al., 2014).

Chrysanthemum pollen viability depends on genotype and environmental factors such as temperature and humidity. Chen et al. (2009) screened anemone type chrysanthemum varieties and found pollen viability was no more than 25% and less than 10% in 68% of varieties tested. Fei et al. (2016) measured pollen viability at 7% and 16% and counted 800 pollen grains per anther. Anderson and Ascher (2000) measured an average pollen viability of 70% in cultivated chrysanthemum.

The viability of chrysanthemum pollen drops quickly after anthesis. Kattera et al. (2014) showed pollen viability was lost within 2 days while Yang and Endo (2005) reported storage was only possible for up to 10 days at 4°C. Shinoyama et al. (2008) showed pollen viability was no greater than 4 days at 10 – 30°C.

4.4.4 Seed production

Compatibility

Chrysanthemum has a strong self-incompatibility system, as do all members of the Asteraceae (Kubitzki, 2007). Garden and cut-flower chrysanthemum varieties are normally highly self-incompatible (Drewlow et al., 1973; Ronald and Ascher, 1975c) and cultivated chrysanthemum has differing levels of SI or pseudo self-incompatibility, depending on variety (Anderson and

Ascher, 1996). Selection for reduced self-incompatibility is possible (Anderson and Ascher, 2000). Ronald and Ascher (1975b) showed that high temperature treatments partially overcame the effect of self-incompatibility in some chrysanthemum clones. De Jong and Kho (1982) determined that in incompatible combinations pollen tubes do not enter the style. Incompatibility in out crossing is in part due to the wide range of chromosome numbers found in commercial cultivars of chrysanthemum (Dowrick and El-Bayoumi, 1966). Crosses within the chrysanthemum species and with related species is possible as part of breeding programs (see 4.4.5) and as such are an important part of breeding for variety improvement, along with mutation breeding. Hexaploid species can be crossed more successfully with tetraploid species and hexaploid cultivars. However, it is very difficult to cross hexaploids with diploids without embryo rescue as embryos collapse during development (Boase et al., 1997; Watanabe, 1977; Zhang et al., 2016).

Seed set

Despite the high frequency of self-incompatibility, resulting in low or no seed set after selfing (Jain and Gupta, 1959; Anderson and Ascher, 1996) seed set is possible in chrysanthemum. The fruit of the chrysanthemum is a type of achene called a cypsela. One seed is formed per fruit in a successful fertilisation with little or no endosperm. seeds may be harvested 8 weeks after fertilisation (Boase et al, 1997; de Jong and Kho, 1982).

Mulford (1937) established there was no correlation between pollen viability and ultimate seed set. Anderson and Ascher (1996, 2004) provided data on seed set rates in chrysanthemum for selfed and outcrossed varieties. In those experiments selfing resulted in 0 – 8% seed set in two thirds of crosses while out crossing resulted in seed set rates of 16 – 92%.

In controlled breeding situations, seed set depends on optimization of temperature and light conditions. Under ideal conditions (the cooler temperatures of early Spring and fall) a good cross may yield 100 – 150 seeds per flower head (Ronald and Ascher, 1975c). Chrysanthemum does not normally set seed at high greenhouse temperatures (de Jong and Rademaker, 1989). A one day 40°C treatment reduced seed yield in both self- and crossed chrysanthemum (Ronald and Ascher, 1975b).

Seed viability and germination

Anderson et al. (1992) described 0 – 90% germination in seed from a chrysanthemum breeding program, noting post germination inbreeding depression after selfing in several varieties.

Shinoyama et al. (2008) showed hybridisation was possible in artificial crosses between self-incompatible chrysanthemum varieties and *C. japonense* and *C. pacificum* with seed germination rates of 8 – 25%.

4.4.5 Inter-generic hybridisation

There is a long history of interspecific hybridisation by breeders to incorporate wild *Chrysanthemum* genetics into cultivated chrysanthemum (Zhao et al., 2009). Shinoyama et al. (2008) showed hybridisation was possible in artificial crosses between self-incompatible chrysanthemum varieties and *Chrysanthemum japonense* or *Chrysanthemum pacificum*. Fukai et al. (2000a, 2000b) successfully carried out reciprocal crosses between chrysanthemum and ten related *Dendranthema* species native to Japan. Success was dependent on chrysanthemum cultivar but use of chrysanthemum as either female or pollen parent was possible without the need for embryo rescue. Chrysanthemum is compatible with the silver chrysanthemum, *Ajanía*

pacifica, a decaploid species and hand-pollination is sufficient to obtain hybrids between these species (De Jong and Rademaker 1989; Zhao et al., 2012). Another example where successful inter-specific hybridisation has been achieved between chrysanthemum and related species is *Dendranthema shiwogiku* (Aoyama et al., 1997; Fukai et al., 2000a, 2000b). Using delayed pollination and chemical treatments, Sun et al. (2010, 2011) achieved interspecific hybridisation between chrysanthemum and *Chrysanthemum nankingense*, *Chrysanthemum indicum* and *Chrysanthemum zawadskii* with success dependent on chrysanthemum variety. Zhang et al. (2016) showed that chrysanthemum could be crossed to tetraploid, but not diploid, *Chrysanthemum nankingense*.

Cheng et al. (2011) used embryo rescue to obtain a hybrid between chrysanthemum and *Chrysanthemum makinoi*.

Using ovule rescue, Zhu et al. (2014) obtained hybrids between chrysanthemum and *Artemisia vulgaris* (mugwort). The "Korean hybrids" of chrysanthemum are cultivars of the artificial hybrid *Dendranthema zawadskii* X *Dendranthema x grandiflorum*.

Intergeneric hybridisation in nature

Spontaneous hybridisation between cultivated chrysanthemum and other species is very rare. The flora of Japan (Ohwi, 1965) describes the occurrence of *Chrysanthemum x miyatojimense*, a hybrid between *Chrysanthemum morifolium* and *Chrysanthemum yezoense*. More recently, Nakata (1989) described the occurrence of small populations of possible natural hybrids between chrysanthemum and *Chrysanthemum wakasaense* in Kugami, Japan. Also in Japan, natural hybrids have been described between *C. morifolium* and *Chrysanthemum pacificum*, *Chrysanthemum kinokuniense* or *Chrysanthemum shiwogiku* (Ohashi and Yonekura, 2004).

4.5 Weediness

While the Compositae family contains many crops with economic value, this family also contains many weeds, some of which have spread throughout temperate areas of the world and are found in the USA.

4.5.1 *Chrysanthemum* genus

Chrysanthemum coronarium and *Chrysanthemum segetum* have established outside of cultivation in the USA (Arriagada and Miller, 1997). In addition, two species of *Chrysanthemum* found in the US flora (table 6) are listed as noxious weeds in the *Introduced, invasive, and noxious plants list* of the USA (USDA, 2017). These are;

- *Leucanthemum vulgare* syn. *Chrysanthemum leucanthemum* (oxeye daisy).
- *Tanacetum vulgare* syn. *Chrysanthemum vulgare* (common tansy).

Oxeye daisy (figure 8) is native to Europe but is now a weed in 40 countries, from Africa to Australia and in North and South America (Clements et al., 2004). Cultivars of this species are used as a bedding plant (Boase et al., 1997). A summer flowering plant, Oxeye daisy is found in every state in the US (USDA, NRCS. 2017) and is a declared weed in Colorado, Montana, Ohio, Washington and Wyoming (USDA, 2017). Through widespread distribution of seed in pastures, rangelands and some broad acre crops (Arriagada and Miller, 1997) oxeye daisy becomes a serious contaminant in seedlots. Pasture productivity is reduced as animals avoid grazing infested areas (Clements et al., 2004).

Common tansy is a native of Europe and after deliberate introduction as an ornamental is now widespread in the US where it grows well under varied climatic conditions. Found across the US except for Texas and the South-East corner states (USDA, NRCS, 2017) *Tanacetum vulgare* is a declared noxious weed in Colorado, Montana, Washington and Wyoming (USDA, 2017). Wolf et al. (2012) has shown the species to have high genetic heterogenicity in North American populations.



Figure 8. *Leucanthemum vulgare* syn. *Chrysanthemum leucanthemum* (oxeye daisy).

4.5.2 Cultivated chrysanthemum

Barkley et al. (2017) indicates that chrysanthemum is “not well established in the US flora” but may be “encountered as abandoned plantings”. In the USA there are very occasional reports of localised escape from cultivation (see 4.3.2) but these cannot be considered to represent the establishment of a weed. Though defined as an introduced plant, chrysanthemum is not listed as a noxious weed in any state weed list¹.

To our knowledge no wild chrysanthemum population has ever been found in the wild around production of chrysanthemum cut-flowers anywhere in the world. We are not aware of any reports naming *Chrysanthemum morifolium* as a weed, anywhere in the world.

4.6 Probability of gene dispersal

It is intended the flowers will be grown in Colombia and imported to the USA as cut flowers, where they will be distributed and consumed in the wholesale and retail environment. The probability of gene dispersal must therefore be considered in that context. Because the product

¹ <https://plants.usda.gov/java/noxiousDriver#federal>

is a cut flower insect mediated pollen flow to other chrysanthemum plants, or to related species, is extremely unlikely.

Seed set in imported chrysanthemum flowers

Theoretically, cultivated chrysanthemum is capable of sexual reproduction through seed set. However, seed dispersal from an imported cut chrysanthemum flower is not possible as seed set occurs 8 weeks after fertilisation (Boase et al., 1997; de Jong and Kho, 1982), which is longer than the longevity of a cut flower. Imported flowers will senesce and be discarded before any successful fertilisation could lead to seed set because a cut-flower will remain in a consumer's hand for a month at most before dying. An additional factor that minimizes any potential seed set is the fact self-incompatibility precludes selfing in chrysanthemum in most cases (Boase et al., 1997).

Pollen flow from imported chrysanthemum flowers

Though the possibility of pollination by wind is nil (Glosser, 1991) theoretically cultivated chrysanthemum is capable of hybridisation with other species through insect pollination. Intergeneric crosses have been used to transfer important genetic traits from wild species such as *Ajania Pacifica* to modern chrysanthemum cultivars (Zhao et al., 2009). The likelihood of such a hybridisation happening from imported cut flowers is very low. Pollen flow from the imported cut flowers would need to be mediated by insects, which would need to locate a flower with pollen and then reach a compatible recipient flower. The chance of these events successfully occurring is further minimised by the limited viability of the pollen after anthesis, which reduces the chance for pollen to germinate on styles of a recipient plant. Some chrysanthemum varieties, including anemone types, produce low amounts of viable pollen. In the floras of China and Japan, where chrysanthemum has been widely cultivated for centuries and there are compatible wild species, natural hybrids are very rare. None are identified in the flora of China (Lin et al., 2011) while the flora of Japan (Ohwi, 1965) describes the occurrence of *Chrysanthemum x miyatojimense*, a hybrid between *Chrysanthemum morifolium* and *Chrysanthemum yezoense*. The lack of hybrids could be in part due to the 1 to 2-month natural flowering period of most wild *Chrysanthemum* species (Lin et al., 2011; Zhao et al., 2009). Nakata (1989) described the occurrence of small populations of possible natural hybrids between chrysanthemum and *Chrysanthemum wakasaense* in Kugami, Japan but this was thought to be possible because of the very specific conditions in which pollen from cut flowers regularly offered at a shrine were transferred to a wild *Chrysanthemum* population within the shrine. That type of situation is not likely to occur in the US.

The likelihood of gene transfer via pollen to wild *Chrysanthemum* species in the US flora (such as oxeye daisy or common tansy) is extremely low, as fertile flowers of these species are not likely to be found in the environments in which the cut flowers will be used (e.g. homes, shops, restaurants etc.). There are no examples in the literature of inter specific hybridisation between chrysanthemum and either *Leucanthemum vulgare* or *Tanacetum vulgare*. Both these species occur in China and Japan, where there are native and cultivated *Chrysanthemum* species but there are no hybrids in the flora of either country (Lin et al., 2011; Ohwi, 1965). Furthermore, both these weeds are diploid (Clements et al., 2004; Keskitalo et al., 1998). It is very difficult to cross chrysanthemums with diploids as embryos collapse during development (Boase et al., 1997; Watanabe, 1977). *Chrysanthemum coronarium* and *Chrysanthemum segetum*, established outside of cultivation in the USA, are both diploid or tetraploid species

(Dowrick, 1953) and the species which have been successfully used in intergenic crosses in chrysanthemum (refer 4.4.5) do not occur in the US flora.

The evidence from the US flora is that wild populations of chrysanthemum are rare escapes from cultivation in gardens, limiting chrysanthemum as a possible recipient population. In commercial chrysanthemum production, flowers are removed. The high concentrations of chrysanthemum at production sites in the US are therefore not available as potential recipients.

Probability of inadvertent dispersal

Nearly all cultivated cut flower chrysanthemum varieties have lost the ability to grow stolons under normal growing conditions because of extensive breeding. It is therefore unlikely that plant establishment could occur because of inadvertent dispersal (through disposal of waste flowers for example). This is because the cut flowers do not have the physiological capacity to initiate stolon growth or establish plants through adventitious root formation from leaves, for example. It is also realistic to expect that in most situations in which the flower would be discarded this would be through usual waste management systems in the urban environment. Suntory Flowers works closely with growers from Colombia who have experience of large scale production of chrysanthemum. Chrysanthemum has never been found growing wild, even in the immediate vicinity of chrysanthemum growing areas where waste material has been discarded or has been left for composting. It would be expected to find wild populations of chrysanthemum if they had an inherent capacity for dispersal.

Conclusion

For reasons outlined above, our assessment is that there is no reasonable possibility of gene dispersal from the imported cut flowers and therefore no potential for the imported cut flowers to become weedy. Glosser (1991) stated there were no reports of feral seed producing chrysanthemum populations in the US, that chrysanthemum is not weedy and no sexually compatible plants occur in the USA.

The probability may be higher in situations such as Japan, where there are several indigenous species of *Chrysanthemum* and extensive chrysanthemum production (ca. 4,500 ha). In such situations, which are not comparable to a case where cut flowers are imported, a remote possibility of gene transfer between cultivated chrysanthemum and wild *Chrysanthemum* species exists. In the USA, despite decades of chrysanthemum production and common and widespread use of chrysanthemum in landscaping (including winter hardy garden chrysanthemum varieties) there are only very occasional examples of establishment outside of cultivation and these are most likely due to abandoned plantings of garden varieties rather than gene flow from imported cut flowers. In garden grown chrysanthemum there is a low possible of seed formation after self-fertilization or fertilization between adjacent plants. This would not apply to cut flower import.

There is no evidence in the US flora of hybrids between cultivated chrysanthemum and related species. We are also unaware of any such hybrids elsewhere in the world.

Though ornamental plants have sometimes escaped cultivation to become serious weeds (Dehnen-Schmutz *et. al.*, 2007; Hierro *et. al.*, 2005) to our knowledge this is not the case for cultivated varieties of chrysanthemum anywhere in the world.

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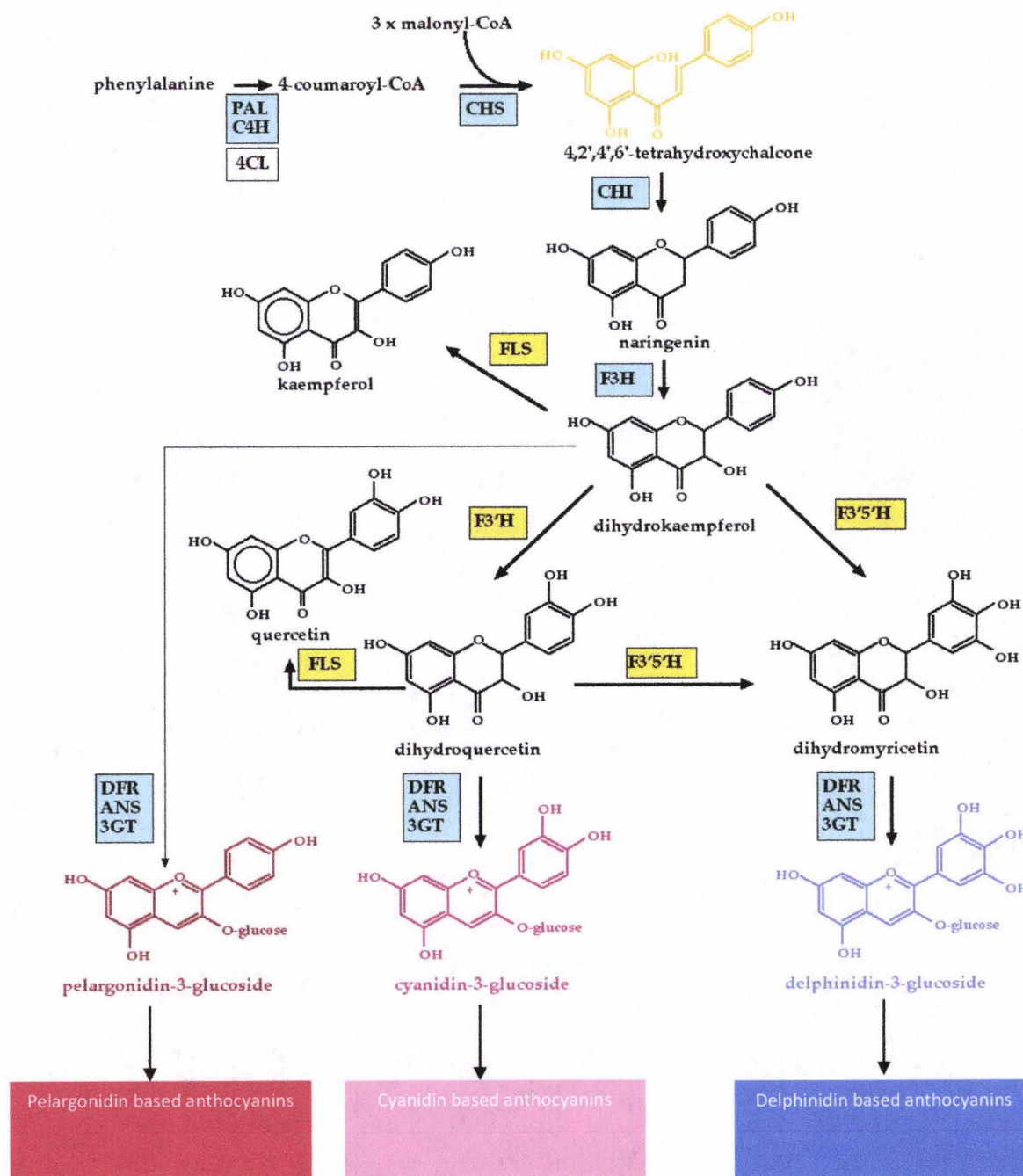


Figure 1. The anthocyanin biosynthesis pathway. Abbreviations include; CHS – chalcone synthase; CHI – chalcone isomerase; F3H – flavanone 3 β -hydroxylase; F3'H – flavonoid 3'-hydroxylase; F3'5'H – flavonoid 3', 5'-hydroxylase; DFR – dihydroflavonol 4-reductase; ANS – anthocyanidin synthase; FLS – flavonol synthase; 3GT – UDP-glucose: anthocyanidin 3-O-glucosyltransferase; PAL - phenylalanine ammonia-lyase; 4CL - 4-coumarate: CoA ligase; C4H - cinnamate 4-hydroxylase

Table 1. Genetic elements of pB423

Designation on Figure 4	Genetic Element Type	Genes and/or Regulatory Element Name	Source Organism Species Name [Common Name]
RB	T-DNA border repeat sequence	Right border	<i>Agrobacterium tumefaciens</i>
nosp	Regulatory element- promoter	Nopaline synthase gene promoter	<i>Agrobacterium tumefaciens</i>
nptII	Selectable marker gene	Coding sequence of nptII(neo) gene	<i>Escherichia coli</i>
nost	Regulatory element- terminator	Nopaline synthase gene terminator	<i>Agrobacterium tumefaciens</i>
CMF3Hp	Regulatory element- promoter	Flavanone 3-hydroxylase promoter	<i>Chrysanthemum morifolium</i> [chrysanthemum]
TE	Translation enhancer	5'-untranslated region of alcohol dehydrogenase gene	<i>Nicotiana tabacum</i> [tobacco]
Camp F3'5"H	Gene	Flavonoid 3',5'- hydroxylase coding sequence	<i>Campanula medium</i> [campanula]
HSpt	Regulatory element- terminator	Heat shock protein 18.2 gene terminator	<i>Arabidopsis thaliana</i> [arabidopsis]
CMF3Hp	Regulatory element- promoter	Flavanone 3-hydroxylase promoter	<i>Chrysanthemum morifolium</i> [chrysanthemum]
TE	Translation enhancer	5'-untranslated region of alcohol dehydrogenase gene	<i>Nicotiana tabacum</i> [tobacco]
Clit UDPG	Gene	UDP- glucose:anthocyanin 3',5'-O- glucosyltransferase coding sequence	<i>Clitoria ternatea</i> [butterfly pea]
nost	Regulatory element- terminator	Nopaline synthase gene terminator	<i>Agrobacterium tumefaciens</i>
LB	T-DNA border repeat sequence	Left border	<i>Agrobacterium tumefaciens</i>

Table 2. Function of genetic elements of pB423

Genetic element	Brief description of function
RB	Defines junction between T-DNA and plant genomic or vector DNA. Utilized in transfer of insert to plant cell and integration into the plant genome.
Nopaline synthase gene promoter	Constitutive promoter.
Coding sequence of nptII(neo) gene	Selectable marker conferring resistance to antibiotics such as paromomycin, and kanamycin, which are used for selection of transgenic plants <i>in vitro</i> .
Nopaline synthase gene terminator	Regulation of gene expression.
Flavanone 3-hydroxylase promoter	A petal-specific chrysanthemum promoter which has maximum expression efficiency in chrysanthemum (Noda et al., 2013).
5'-untranslated region of alcohol dehydrogenase gene	The inclusion of a translational enhancer derived from the tobacco alcohol dehydrogenase gene enhances the efficiency of translation of the introduced genes in chrysanthemum (Aida et al., 2008; Noda et al., 2013).
Flavonoid 3',5'-hydroxylase coding sequence	A campanula cDNA clone encoding the enzyme flavonoid 3',5'-hydroxylase (F3'5'H). Expression of this gene is required for the biosynthesis of delphinidin-related anthocyanins, which confer blue flower color (Noda et al., 2017). Campanula has been identified as the best source organism for optimal expression of the F3'5'H gene in chrysanthemum (Noda et al., 2013)
Heat shock protein 18.2 gene terminator	Arabidopsis terminator sequence. Regulation of gene expression.
UDP-glucose:anthocyanin 3',5'-O-glucosyltransferase coding sequence	A UDP-glucose: anthocyanin 3',5'-O-glucosyltransferase gene from butterfly pea. Expression functions to add two glucosyl residues to the delphinidin anthocyanin (Kowaga et al., 2007). The addition of these side groups confers a bluer hue to the anthocyanin (Noda et al., 2017).
LB	Defines junction between T-DNA and plant genomic or vector DNA. Utilized in transfer of insert to plant cell and integration into the plant genome.

Table 3. Description of donor organisms

Donor organism	Description
<i>Agrobacterium tumefaciens</i>	<i>Agrobacterium tumefaciens</i> is a plant bacterial pathogen. In the armed form, the bacterium causes crown gall disease in many dicotyledonous plant species (Mansfield et al., 2012). <i>Agrobacterium tumefaciens</i> is ubiquitous in nature and has a worldwide distribution in soil and in association with plants.
<i>Escherichia coli</i>	<i>Escherichia coli</i> has no plant pathogenicity, though certain strains may cause disease in humans. <i>E.coli</i> is an enterobacterium, and some bacterium in the group, such as <i>Erwinia</i> do exploit plants as hosts. <i>E.coli</i> is found in the gastrointestinal tract of animals (Todar, 2004). Certain strains of <i>Escherichia coli</i> may cause disease in humans, with toxic effects in some cases, associated with infection. The species is ubiquitous in nature with a worldwide distribution in numerous aquatic habitats and in association with animals and humans.
<i>Chrysanthemum morifolium</i> [chrysanthemum]	The donor organism is the same species as the recipient organism (refer section 4 for details of recipient organism)
<i>Nicotiana tabacum</i> [tobacco]	Tobacco (<i>Nicotiana tabacum</i>) is a non-pathogenic plant species. The species is an annual found in cultivation for the tobacco industry and is a non-epiphyte. The species is not listed as a weed by USDA-NRCS (USDA, 2017).
<i>Campanula medium</i> [campanula, Canterbury bells]	Campanula originates in southern Europe but has become naturalised in other parts of Europe. Typical habitats are drier, rocky environments. Campanula is an annual and is not listed as a weed by USDA-NRCS (USDA, 2017).
<i>Clitoria ternatea</i> [butterfly pea]	Butterfly pea is a short-lived, fast-growing climbing perennial herb. The plant is a climber but is non-epiphytic. The origin of the species is probably South America (Lim, 2014) though it is now distributed in tropical regions of all countries. <i>Clitoria ternatea</i> has a history of safe use as a medicinal plant (Mukherjee et al., 2008) and as an edible plant in South East Asia (Lim, 2014). The species is not listed as a weed by USDA-NRCS (USDA, 2017).
<i>Arabidopsis thaliana</i> [Arabidopsis, mouse-ear cress]	Thale or mouse-ear cress (<i>Arabidopsis thaliana</i>) is an annual plant found across much of Eurasia and is an introduced species in the USA (USDA, 2017). This species is not cultivated but is commonly grown for experimental research in plants. The species is listed as a weed in Kentucky (USDA, 2017).

Table 4. Chrysanthemum species commonly cultivated for ornamental and industrial purposes (Anderson, 2006; Boase et al., 1997; Dowrick, 1953; Soreng and Cope, 1991)

Species	Common name	Use#
<i>Chrysanthemum morifolium</i> syn. <i>Dendranthema grandiflora</i>	Chrysanthemum, mum, florist's chrysanthemum	F, G, P
<i>Chrysanthemum pacificum</i> syn. <i>Ajania pacificum</i>	Ajania, gold and silver chrysanthemum	F, G
<i>Chrysanthemum cinerariifolium</i>	Pyrethrum	I
<i>Chrysanthemum leucanthemum</i> syn. <i>Leucanthemum vulgare</i>	Oxeye daisy, field daisy	B, G
<i>Chrysanthemum lacustre</i> syn. <i>Leucanthemum lacustre</i>	Portuguese daisy	G
<i>Chrysanthemum mawii</i> syn. <i>Rhodanthemum gayanum</i>	Moroccan daisy	G
<i>Chrysanthemum coccineum</i> syn. <i>Tanacetum coccineum</i>	Pyrethrum daisy, painted daisy	G
<i>Chrysanthemum maximum</i> syn. <i>Leucanthemum maximum</i>	Shasta daisy, King Edward daisy	F, G
<i>Chrysanthemum indicum</i> syn. <i>Dendranthema indicum</i> (syn <i>C. japonicum</i>)	Indian chrysanthemum, chrysanthemum	G
<i>Chrysanthemum coronarium</i> syn. <i>Glebionis coronarium</i>	Crown daisy, rainbow daisy	G
<i>Chrysanthemum segetum</i> syn. <i>Glebionis segetum</i>	Corn daisy, corn marigold	G
<i>Chrysanthemum frutescens</i> syn. <i>Argyranthemum frutescens</i>	Marguerite, marguerite daisy, Paris daisy	B, F, G
<i>Chrysanthemum x rubellum</i> syn. <i>Dendranthema zawadskii</i>	Old fashioned mum	G
<i>Chrysanthemum wakasaense</i>	Seashore daisy	G
<i>Chrysanthemum balsamia</i> syn. <i>Tanacetum balsamia</i>	Costmary	G, H
<i>Chrysanthemum carinatum</i>	Tricolor chrysanthemum, garland chrysanthemum	G
<i>Chrysanthemum vulgare</i> syn. <i>Tanacetum vulgare</i>	Tansy, common tansy	G, H
<i>Chrysanthemum parthenium</i> syn. <i>Tanacetum parthenium</i>	Feverfew	G, H

B – bedding plant; F- cut flower; G – garden ornamental; H – herb; I – Insecticide; P – potted plant

Table 6: Chrysanthemum species and species previously classified as Chrysanthemum, according to the e-flora of North America (Barkley et al., 2017) and the USDA plant database (USDA, NRCS, 2017). “-” indicates the species is missing from the database or e-flora.

Species	Scientific name in USDA plant database	Scientific name in Flora of N. America	Common name
<i>Chrysanthemum</i> L.	<i>Chrysanthemum</i> L.	-	Daisy
<i>Chrysanthemum anethifolium</i> Willdenow; <i>C. foeniculaceum</i>	<i>Argyranthemum foeniculaceum</i>	<i>Argyranthemum foeniculaceum</i>	marguerite
<i>Chrysanthemum arcticum</i>	<i>Chrysanthemum arcticum</i>	<i>Arctanthemum arcticum</i>	Arctic daisy
<i>Chrysanthemum balsamita</i>	<i>Tanacetum balsamita</i>	<i>Tanacetum balsamita</i>	Costmary
<i>Chrysanthemum bipinnatum</i> (spp <i>bipinnatum</i>)	<i>Tanacetum bipinnatum</i> ssp. <i>bipinnatum</i>	<i>Tanacetum bipinnatum</i> ssp. <i>bipinnatum</i>	Lake Huron tansy
<i>Chrysanthemum bipinnatum</i> (spp <i>huronense</i>)	<i>Tanacetum bipinnatum</i> ssp. <i>huronense</i>	<i>Tanacetum bipinnatum</i> ssp. <i>huronense</i>	Lake Huron tansy
<i>Chrysanthemum carinatum</i>	<i>Glebionis carinatum</i> (Schousb.) Tzvelev	<i>Ismelia carinata</i>	tricolor daisy
<i>Chrysanthemum caroliniana</i>	-	<i>Boltonia caroliniana</i>	Carolina doll's-daisy
<i>Chrysanthemum cinerariifolium</i> (Tr evir.) Vis.	<i>Chrysanthemum cinerariifolium</i> (Tr evir.) Vis.	-	pyrethrum
<i>Chrysanthemum coccineum</i>	<i>Chrysanthemum roseum</i> , <i>Tanacetum coccineum</i>	-	pyrethrum daisy
<i>Chrysanthemum coronarium</i>	<i>Chrysanthemum spatiosum</i> , <i>Glebionis coronarium</i>	<i>Glebionis coronarium</i>	Crown daisy
<i>Chrysanthemum corymbosum</i>	<i>Tanacetum corymbosum</i>	-	corymbflower tansy
<i>Chrysanthemum frutescens</i>	<i>Argyranthemum frutescens</i>	-	marguerite
<i>Chrysanthemum incanum</i>	-	<i>Pentzia incana</i>	African sheep bush
<i>Chrysanthemum indicum</i> L.	<i>Dendranthema indicum</i> (L.) Des Moulins [excluded]	-	Indian chrysanthemum
<i>Chrysanthemum integrifolium</i>	<i>Hulteniella integrifolia</i>	<i>Hulteniella integrifolia</i>	Entire leaf daisy
<i>Chrysanthemum lacustre</i>	<i>Leucanthemum lacustre</i>	<i>Leucanthemum lacustre</i>	Portuguese daisy
<i>Chrysanthemum leucanthemum</i>	<i>Leucanthemum vulgare</i>	<i>Leucanthemum vulgare</i>	oxeye daisy
<i>Chrysanthemum majus</i> (Desf.) Asch.	<i>Chrysanthemum majus</i> (Desf.) Asch.	-	Daisy
<i>Chrysanthemum maximum</i>	<i>Leucanthemum maximum</i>	<i>Leucanthemum maximum</i>	max chrysanthemum (Shasta daisy)
<i>Chrysanthemum x morifolium</i>	<i>Chrysanthemum x morifolium</i>	-	florist's daisy
<i>Chrysanthemum nanum</i>	-	<i>Blennosperma nanum</i>	Glue-seed
<i>Chrysanthemum nipponicum</i>	<i>Nipponanthemum nipponicum</i>	<i>Nipponanthemum nipponicum</i>	Nippon daisy
<i>Chrysanthemum paludosum</i>	-	<i>Mauranthemum paludosum</i>	Creeping daisy
<i>Chrysanthemum parthenium</i>	<i>Tanacetum parthenium</i>	<i>Tanacetum parthenium</i>	Feverfew
<i>Chrysanthemum segetum</i>	<i>Glebionis segetum</i>	<i>Glebionis segetum</i>	Corn daisy
<i>Chrysanthemum serotina</i>	<i>Chrysanthemum uliginosum</i> , <i>Leucanthemella serotina</i>	<i>Leucanthemella serotina</i>	Giant daisy
<i>Chrysanthemum x superbum</i>	<i>Leucanthemum xsuperbum</i>	-	Shasta daisy
<i>Chrysanthemum vulgare</i>	<i>Tanacetum vulgare</i>	-	common tansy