# **MONITORING REPORT FOR GMO USES OTHER THAN CULTIVATION**

# CNL040201 FLO-40644-2 Florigene®Moonlite<sup>TM</sup>

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# 1. General information

## 1.1 Crop/traits

Carnation (Dianthus caryophyllus) with modified flower colour, variety Florigene®Moonlite<sup>TM</sup>.

# 1.2 Decision authorisation number pursuant to Directive 2001/18/EC and number and date of consent pursuant to Directive 2001/18/EC

### Florigene®Moonlite<sup>TM</sup>

Original decision authorisation number; C/NL/04/02

Number of original consent; C/NL/04/02.abb1

Date of original consent; July 11, 2007

Renewal decision authorisation number; C/NL/04/02/001

Number of renewal consent; C/NL/04/02 001.bes.1

Date of renewal consent; Feb 28, 2017

Consent holder; Suntory Flowers Limited, 4-17-5 Shiba, Minato-ku, Tokyo 108-0014 Japan

# 1.3 Decision authorisation number and date of authorisation pursuant to Regulation (EC) No. 1829/2003

Not applicable.

# 1.4 Unique identifier

FLO-40644-6

# 1.5 Report period from

July 1, 2020 to June 30, 2021

## 1.6 Other monitoring reports have been submitted in respect of cultivation

YES □ NO ■

## 2. Executive summary

Approximately 34 tonnes (1.9 million flowers) of Florigene® Moonlite™ were imported into the EU from July 1, 2019 to June 30, 2020, through a single importer in the Netherlands. Flowers were imported from Colombia (10%) and Ecuador (90%).

Results of general monitoring for the occurrence of genetically modified carnation in the EU were;

- The importer reported that they were not aware of any illegal growing and that neither they nor consumers have reported any adverse effects of handling the flowers.
- No reports were provided via the Florigene website. Florigene/Suntory received two questions from EU-based public, distributors or retailers in the period. One was from Italy, enquiring whether it was possible to buy flowers and one was a request for photographs to use in a publication.
- Information on survey work was provided by two botanical experts, from work in Croatia, Slovakia and the Republic of North Macedonia. There was no evidence of the establishment of any carnation populations in the wild, or of hybridisation with wild *Dianthus* species.
- 99 responses were received from 209 letters and emails sent to European botanical and plant conservation groups, botanical gardens, museums, amateur botanists, database managers and University departments. None of the respondents reported collecting or observing carnation populations established outside of cultivation. Wild type *Dianthus caryophyllus* was recorded in France and Italy.
- A review of literature related to *Dianthus* was carried out. None of the literature reviewed identified escape populations of cultivated carnation or hybrids with other *Dianthus* species in wild populations. Wild *Dianthus caryophyllus* was listed in two comprehensive studies of alien plants in Europe. A Europe wide vegetation review indicated 113 *Dianthus* species across more than 800,000 vegetation plots. *Dianthus caryophyllus* occurred in 0.74% of the total number of plots where a *Dianthus* species was recorded and was the 25<sup>th</sup> most abundant of the *Dianthus* species.
- Botanical and floral databases were searched for records of carnation and *Dianthus caryophyllus* made since the last monitoring report. Six new records of *Dianthus caryophyllus* L. were identified from Belgium, two from Germany, one from Sweden, and 13 from France. Photographs of the observations (where available) and follow up

communication with database managers indicated 21 of the 22 observations to be of 5-petal wild *Dianthus caryophyllus*, not carnation. The other instance was of a planted, pink flower, carnation plant.

The overall results are consistent with previous monitoring reports and indicate carnation is not present in nature in Europe. The monitoring this year supports previous observations that wild type *Dianthus caryophyllus* is rare and most common in France.

#### 3. Uses of GMOs other than cultivation

# 3.1 Commodity imports into the community

# 3.1.1 Commodity crop (GM and non-GM) imports into the community by country of origin

## GM product

GM product was imported from Colombia and Ecuador. Table 1 provides information on the imports of all transgenic carnation varieties imported into the EU during the reporting period. Information on the specific variety covered by this report is highlighted in red font.

Table 1. Tonnes of GM carnation imported into the EU from July 2020 to June 2021.

GM carnation variety	Quantity (tonnes)				
·	Imported from Ecuador	Imported from Colombia			
Florigene®Moonaqua™	36	8			
Florigene®Moonlite <sup>TM</sup>	30	4			
Florigene®Moontea <sup>TM</sup>	0	11			
Florigene®Moonberry <sup>TM</sup>	0	4			
Florigene®Moonvelvet <sup>TM</sup>	0	3			
Florigene®Moonvista <sup>TM</sup>	12	6			
All GM carnation varieties	78	36			

#### GM and non-GM product

At the time of accessing the EUROSTAT database to assess import of carnation flowers into the EU information was only available till the end of April 2021 for imports from Colombia and Ecuador and to the end of March 2021 for imports from most other countries. In order to estimate the percentage of imports which are GM we have therefore chosen to use the import data for the 12-month period from April 2020 to March 2021. Table 2 shows the data for imports of the GM carnation varieties over this period. Table 3 shows the combined total of GM and non-GM carnation flower imports<sup>1</sup>.

Table 2. Tonnes of GM carnation imported into the EU from April 2020 to March 2021.

GM carnation variety	Quantity (tonnes)		
	Imported from Ecuador	Imported from Colombia	
Florigene®Moonaqua <sup>TM</sup>	35	9	
Florigene®Moonlite <sup>TM</sup>	28	3	
Florigene®Moontea <sup>TM</sup>	0	9	
Florigene®Moonberry <sup>TM</sup>	0	4	
Florigene®Moonvelvet <sup>TM</sup>	0	3	
Florigene®Moonvista <sup>TM</sup>	10	6	
All GM carnation varieties	73	34	

<sup>&</sup>lt;sup>1</sup> http://epp.eurostat.ec.europa.eu/newxtweb/setupdimselection.do

Table 3. Estimated import of carnation (total of GM plus non-GMO) into the EU, April 2020 – March 2021.

Country of origin	Quantity (tonnes)*		
	NL imports	EU27 total imports	
Ecuador	232	254	
Colombia	9,654	11,540	
Other countries	13,702	16,147	
Total <sup>2</sup>	23,588	27.941	

<sup>\*</sup> From EUROSTAT (code 06031200; fresh cut carnations, DS-016890 trade since 1988 by CN8).

# Percentage of import which is GM

Table 4 shows the percentage of carnation flower import into the EU which is GM.

Table 4. Percentage of carnation flower import into the EU which were GM flowers. Data is calculated from tables 2 and 3.

GM carnation variety	Percentage of carnation imports			
	From Ecuador#	From Colombia##	From all extra- EU countries*	
Florigene®Moonaqua <sup>TM</sup>	13.77%	0.08%	0.16%	
Florigene®Moonlite <sup>TM</sup>	11.13%	0.02%	0.11%	
Florigene®Moontea <sup>TM</sup>	0.00%	0.08%	0.03%	
Florigene®Moonberry <sup>TM</sup>	0.00%	0.03%	0.01%	
Florigene®Moonvelvet <sup>TM</sup>	0.00%	0.03%	0.01%	
Florigene®Moonvista <sup>TM</sup>	3.84%	0.05%	0.06%	
All varieties	24.90%	0.24%	0.33%	

<sup>#</sup> GM imports into the E27 from Ecuador as a percentage of total GM plus non-GM product imported from Ecuador

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<sup>##</sup> GM imports into the EU27 from Colombia as a percentage of total GM plus non-GM product imported from Colombia

 $<sup>^</sup>st GM$  imports into the EU27 from all extra-EU countries (including Ecuador plus Colombia) as a percentage of total GM plus non-GM product

<sup>&</sup>lt;sup>2</sup> Reporter; EU27 2020 EXTRA

# 3.1.2 Commodity crop (GM and non-GM) imports into the community by country of destination

All imports of the GM product were into the Netherlands. Table 5 shows the percentage of carnation flower imports into the Netherlands which were GM.

Table 5. Percentage of carnation flower import into the Netherlands which were GM flowers. Data calculated from tables 2 and 3.

GM carnation variety	Percentage of carnation imports		
	From	From	From all extra-
	Ecuador#	Colombia##	EU countries*
Florigene®Moonaqua <sup>TM</sup>	15.07%	0.09%	0.04%
Florigene®Moonlite <sup>TM</sup>	12.19%	0.03%	0.01%
Florigene®Moontea <sup>TM</sup>	0.00%	0.09%	0.04%
Florigene®Moonberry <sup>TM</sup>	0.00%	0.05%	0.02%
Florigene®Moonvelvet <sup>TM</sup>	0.00%	0.04%	0.01%
Florigene®Moonvista <sup>TM</sup>	4.21%	0.06%	0.03%
All varieties	27.26%	0.30%	0.12%

<sup>#</sup> GM imports into NL from Ecuador as a percentage of total GM plus non-GM product imported from Ecuador

# 3.1.3 Analysis of data provided in 3.1.1 and 3.1.2

Approximately 34 tonnes of the GM event Florigene®Moonlite™ were imported in the monitoring period (July 2020 to June 2021). Imports were predominantly (90%) from Ecuador (table 2). The transgenic carnation event represents approximately 0.02% of total imports of carnation into the EU from Colombia and 11.1% of total imports of carnation into the EU from Ecuador (table 4). As the Netherlands dominates the import of extra-EU27 imports of carnation, similar percentages were recorded for import into the Netherlands alone; the transgenic carnation event represents approximately 0.03% of total imports of carnation into the Netherlands from Colombia and 12.2% of total imports of carnation into the Netherlands from Ecuador (table 5).

#### 3.2 General surveillance

# 3.2.1 Description of general surveillance

The general surveillance plan consisted of;

- 1. Importer questionnaire.
- 2. Survey reports. Florigene contacted a breeder and engaged the services of botanists to alert us to any wild carnation populations or unusual *Dianthus* hybrids. This year we have received information from two experts.
- 3. Mail out. A letter and email survey were carried out, in multiple languages. 209 contacts were made in 2021, compared to 213 in 2020 and 245 in 2019. Where communication was established by email last year this was continued as the means of communication. The breakdown of responses in 2021 is described in attachment 5.
- 4. Literature review (attachment 6) and database review (attachment 7).

<sup>##</sup> GM imports into NL from Colombia as a percentage of total GM plus non-GM product imported from Colombia

<sup>\*</sup>GM imports into NL from all extra-EU countries (including Ecuador and Colombia) as a percentage of total GM plus non-GM product

The same general monitoring plan was applied to all the transgenic carnation varieties which are imported into the EU. Accordingly, the information provided in attachments 1 to 7 is similar in the monitoring reports for each transgenic carnation event imported into the EU.

# 3.2.2 Details of industry, environmental, food and/or feed related surveillance networks used during general surveillance

Attachment 1. Breeders and experts contacted in 2021.

Attachment 2. Institutions contacted in 2021.

Attachment 7. Floral databases reviewed in 2021.

# 3.2.3 Details of information and/or training provided to importers, handlers, processors etc.

No training was provided.

# 3.2.4 Results of general surveillance

### Importer questionnaire

See attachment 3. The importer reported that they were not aware of any illegal growing and that neither they nor their consumers have reported any adverse effects of handling the flowers.

## Website feedback

Two queries were made to the Florigene website during the year. One was from Italy enquiring about possible purchases of flowers. The second was a request for photographs. Survey reports

Florigene received survey reports from two expert botanists. The results, summarised in attachment 4, reported no evidence of escape populations of transgenic carnation and no evidence of putative hybrids. Wild *Dianthus caryophyllus* populations were not found. Mail out

Overall response rate was 47.4%, which was significantly higher than response rates in 2020 (28.7%), 2019 (26.5%) and 2018 (29.2%) (Figure 1). The response rate to emails was higher than to postal enquiries.

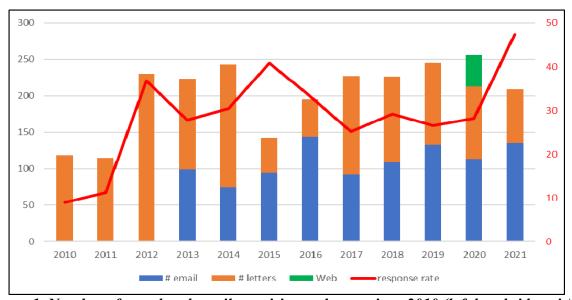


Figure 1. Number of postal and email enquiries each year since 2010 (left hand side axis) and overall response rate expressed as a percentage (right hand side axis). Response rate is calculated from the response to emails and letters only.

Details of the information obtained from the mail out is provided in attachment 5. No respondents reported populations of carnation.

## Literature review

Attachment 6 summarises the output from a literature review. A summation is provided in section to 3.2.6.

#### Database review

Attachment 7 lists the databases examined. No databases identified transgenic carnation, or hybrids between transgenic carnation and wild Dianthus populations. The databases provided 22 records of *Dianthus caryophyllus* in Europe made since the last monitoring report. Where photographs were available, all reports but one were of 5 petal wild type *Dianthus* species. A single carnation record from Belgium was confirmed to be a plant in cultivation.

## On-line floras

Last year, the broadcast to the website TelaBotanica (France) was well received and provided useful information from the general public. We approached two other similar on-line organisations to try and expand this approach. Neither were able to accommodate our request.

#### 3.2.5 Additional information

No adverse or unanticipated effects associated with production or sale of flowers of the transgenic event have been observed. Additional information relevant to the transgenic event is summarised below.

# **Production sites**

In May 2021 the transgenic carnation production area in Colombia was surveyed for the possible presence of escaped populations of cultivated transgenic carnation. The Ecuador site was surveyed in July 2021 (figure 2). No carnation plants were found outside of cultivation at either site.



Figure 2. Appearance of transgenic carnation composting area, Ecuador.

# Phenotypic stability

Off-type percentage in the event was measured in Colombia in April 2021. The flower colour modification phenotype remains stable with an observed off-type (to pink flowers) of 0.05%. The off-type flowers are not exported to the EU from either Ecuador or Colombia. Moonivory

In the last monitoring report, we described three flower colour variants obtained from Moonlite. Anthocyaninidin, dihydroflavonols and flavonol analysis was carried out for one of these varieties, (Moonivory) a flower of which is shown in figure 3. The lighter coloured parts of the Moonivory petal were analyzed and not the darker colour streaks.



Figure 3. Moonivory, a flower colour variant of Moonlite.

The results of the analysis are shown in table 6. It was found the lighter parts of the petal contained delphinidin and that the concentration of kaempferol and dihydrokaempferol was comparable to Moonlite. These two measurements confirmed there was expression of the introduced flavonoid 3', 5'-hydroxylase gene. However, concentrations of all anthocyanidins measured were approximately 15X lower than in Moonlite. Most probably, the phenotypic change in Moonivory is due to reduced expression or possibly inactivation of one or both<sup>3</sup> of the introduced DFR genes.

Flowers of the variety Moonivory are not exported to the EU.

Table 6. Concentration of the anthocyanidins delphinidin (Del), cyanidin (Cya) and pelargonidin (Pel), the dihydroflavonols dihydrokaempferol (DHK) dihydroquercetin(DHQ) and dihydromyricetin (DHM) and the flavonols kaempferol (K), quercetin (Q) and myricetin (M).

Variety			Con	centratio	n in pet	al (μg/g	FW)		
Variety	Del	Cya	Pel	DHM	DHQ	DHK	M	Q	K
Moonlite	66	6	22	0	0	886	29	14	3173
Moonivory	4	0.4	3	0	0	624	0.75	5	2591

#### 3.2.6 Review of peer-reviewed publications – Attachment 6

Attachment 6 provides details of the methodology and outcome of the literature review. Papers with relevance to carnation or *Dianthus caryophyllus* distribution, potential weediness, possible biosafety implications and/or genetic modification are briefly summarized and listed numerically in the reference list contained in attachment 6. Selected papers from that list are cited by their number in the list in the summation below. Evidence for escape of carnation from cultivation

None of the literature reviewed identified cultivated carnation, escape populations of cultivated carnation or hybrids with other Dianthus species in wild populations. Dianthus

<sup>&</sup>lt;sup>3</sup> Nakamura, N., Suzuki, T., Shinbo, Y., Chandler, S., & Tanaka, Y. (2020). Development of violet transgenic carnations and analysis of inserted transgenes. In The Carnation Genome (pp. 135-146): Springer.

caryophyllus was listed in two comprehensive studies of alien plants in Europe [3,4]. Vegetation survey data

A primary relevant literature source used was vegetation surveys. Wild populations of Dianthus longicaulis Ten. (syn. Dianthus caryophyllus) were noted by Biondi et al. [12] in Italy and in Corscia by Médail et al. [53]. A catalogue of the vegetation of the department of Bouches-du-Rhône, Southern France included two synonym species of Dianthus carvophyllus; Dianthus sciulus and Dianthus godronianus Jord. [64]. Dianthus siculus C. Presl,(syn. Dianthus caryophyllus) was nominated as a species which grows with Centaurea heywoodiana in the Nebrodi Mountains, Sicily, Italy [74]. In a review of more than 800,000 vegetation plots across Europe, Večeřa et al. identified 113 Dianthus species including Dianthus caryophyllus [89]. Dianthus caryophyllus was the 25th most abundant of the Dianthus species and occurred in 0.74% of the total number of plots where a Dianthus species was recorded.

The literature review identified several papers that used vegetation survey techniques in situations in which we might expect cultivated carnation to have an opportunity to establish were it to have a weediness propensity. These study sites, in which no Dianthus species were found, included canal and river banks [2,19], restricted areas [22] urban parks [7], rock walls [35, 57], abandoned orchards [77], garden waste [80] and historic road systems [32]. Casanelles-Abella et al. catalogued plants in managed public green spaces in Antwerp, Greater Paris, Poznan, Tartu and Zurich and found nineteen Dianthus species. Dianthus caryophyllus and Dianthus sp. (presumably pinks and/or carnation) were found in Zurich and Paris [18].

Information from vegetation surveys added to baseline information on distribution of Dianthus species other than Dianthus caryophyllus [1,5,8, 11,13, 21, 24, 42, 45, 66, 69, 71, 73, 76, 79, 81, 86].

## Published literature on the transgenic event

A detailed description of pigment chemistry in carnation, including discussion of pigments in transgenic carnation was made by Nakayama [60] and Morimoto et al. [55] included the variety in a study of the flavonols and anthocyanins present in over 100 carnation varieties. In addition to the expected delphinidin-related anthocyanins, cyanidin-based anthocyanins were detected.

A description of the binary vector used to create the transgenic carnation event and details of molecular characterisation of the inserts within the event was provided by Nakamura et al.

The Norwegian authority published a summary of their evaluation of the safety of the transgenic event in the European Journal of Nutrition & Food Safety<sup>4</sup>.

#### Related genetic modification research

The literature review identified protocols for carnation transformation [39] and *Dianthus* chinensis transformation [96].

One of the key genes introduced in Moonlite (flavonoid 3' 5'-hydroxylase gene) was overexpressed in transgenic lines of the plant Aconitum carmichaelii (chinese aconite) with a resulting enhancement of flavonoid levels [61]. By transferring the same gene into Curcuma alismatifolia (siam tulip) transgenic plants were obtained which accumulated anthocyanin in bracts [84].

Other information

<sup>&</sup>lt;sup>4</sup> Andreassen, Å. K., Asare, N. Y. O., Bakke, A. M., Jevnaker, A. M., Junttila, O., Sipinen, V. E., . . . Dalen, K. T. (2021). Final health and environmental risk assessment of genetically modified carnation Moonlite 123.2. 38. European Journal of Nutrition & Food Safety, 40-42.

Matveeva and Otten showed non-transgenic carnation has an intact copy of the Agrobacterium opine synthase gene cucumopine synthase (cus) [52]. Ozeki et al. provided a detailed description of genes involved in anthocyanin biosynthesis in carnation [67].

# 3.3 Case-specific monitoring

# 3.3.1 Description and results of case-specific monitoring (if applicable) Not applicable.

# **Processing (if applicable)**

EU member state	Point of entry/point of cultivation	Point of processing	Distance from point of entry/site of cultivation	Transport used
		Not applicable		

# 3.3.2 Monitoring and reporting of adverse effects resulting from accidental spillage (if applicable)

Not applicable.

# 3.4 Concluding remarks

There was no evidence of the establishment of the transgenic carnation event, or of any transgenic carnation event in the wild, or of introgression with wild Dianthus species. There has been no evidence of unexpected adverse effects on human health or the environment.

## 4. Summary of results and conclusions

#### Results

- 1. The importer reported that they were not aware of any illegal growing and that neither their staff nor consumers have reported any adverse effects of handling the flowers.
- 2. Reports from surveys carried out by two experts failed to identify Florigene® Moonlite<sup>TM</sup> in the wild and no evidence of hybridisation to this variety.
- 3. A mail out was carried out. None of the responses received identified any plants which could have been Florigene® Moonlite<sup>TM</sup>.
- 4. A review of recent peer-reviewed literature failed to identify any variety of cultivated carnation outside of cultivation in Europe.
- 5. A review of floral databases identified 22 collections made in the last 12 months of Dianthus caryophyllus. Photographs and follow up contact with collectors indicated 21 were wild type *Dianthus caryophyllus* (or synonyms) and one was a cultivated carnation. Conclusions

There was no evidence of the establishment of carnation of any variety in the wild, or of introgression. The data collected was consistent with the occurrence in nature in Europe of wild-type unimproved *Dianthus caryophyllus* i.e., the species is rare and is typically found in France.

# 5. Adaptation of the monitoring plan and associated methodology for future years

The literature and database review will be continued. Publicly available flora databases and research vegetation databases have been found to be the most relevant source of observation information and efforts will continue to be made to ensure all relevant European databases have been identified. Larger "citizen scientist" on-line depositories of observations are a new, useful, valuable resource.

The database review will be made in early June to allow time to contact the recorders of any new observations. We will continue to include taxonomic synonyms for *Dianthus caryophyllus* as search terms.

The literature review will be carried out in early June to allow time to contact authors of new literature. We will continue to include taxonomic synonyms for *Dianthus caryophyllus* as search terms.

We will continue to work with experts in the Balkans and continue to try and find botanical experts based in Italy and France.

We have prepared separate documentation which contains a proposal to remove the email/letter mail out component from the general monitoring. This document has been provided to the Netherlands CA.

Dated..... August 20, 2021

# Attachment 1. Breeders and experts contacted

Breeders	
Selecta Klemm GmbH and Co.	Hanfäcker 10
	70378 Stuttgart, Germany
Botanists	
Ss. Cyril and Methodius	Department of Botany and Dendrology
University in Skopje	Faculty of Forestry in Skopje
	MK-1000 Skopje
	Republic of North Macedonia
Slovak University of Agriculture	Department of Botany
in Nitra	Tr. A. Hlinku 2, 949 76 Nitra
	Slovakia

# Attachment 2. Mail out summary 2021

A list of institutions contacted in 2021 is shown below.

Organisation	City	Country
Botanical Garden, Universiteti I Tiranes	Tirana	Albania
Federal Ministry Of Agriculture, Forestry, Environment and Water Management	Vienna	Austria
Upper Austrian State Museum	Linz	Austria
Federal Public Service Of Public Health, Food Chain Security And Environment	Bruxelles	Belgium
Royal Belgian Institute of Natural Sciences	Bruxelles	Belgium
Institute Of Biodiversity and Ecosystem Research	Sofia	Bulgaria
National Museum of Natural History	Sofia	Bulgaria
University of Forestry - Sofia	Sofia	Bulgaria
Velebit Botanic Garden, Northern Velebit National Park	Krasno	Croatia
Plant Health and Quality Control Sector	Nicosia	Cyprus
Institute Of Botany, The Czech Academy of Sciences	Průhonice	Czech Republic
Charles University in Prague	Prague	Czech Republic
Vegetation Science Group, Department of Botany, Masaryk University	Brno	Czech Republic
National Museum	Prague	Czech Republic
Natural History Museum of Denmark	Copenhagen	Denmark
Estonian Museum of Natural History	Tallinn	Estonia
Plant Health Department, Estonian Plant Production Inspectorate	Saku	Estonia
Estonian University of Life Sciences	Tartu	Estonia
Natural History Museum and Botanical Garden, University of Tartu	Tartu	Estonia
Association Jardin Botanique du Val D'yser	Bambecqu	France
CBNSA (National Botanical Conservatory South Atlantic)	Audenge	France
Conservatoire Botanique National de Brest	Nantes	France
Conservatoire Botanique National du Massif-Central	Chavaniac-Lafayette	France
Église de Saint-Xist et Jardin Botanique	Le Clapier	France

Jardin Botanique de La Villa	Chemin	France
Jardin Botanique de l'Ecole Veterinaire d'Alfort	Maisons-Alfort	France
Jardin Botanique de Marnay Sur Seine	Aube	France
Jardin Botanique des Olfacties	Coëx	France
Jardin Botanique Pyrénéen	Melles	France
Jardin des Paradis	Cordes Sur Ciel	France
Jardin des Plantes	Rouen	France
Jardin Exotique and Botanique de Roscoff	Roscoff	France
l'Association des Parcs Botaniques de France	Paris	France
Le Jardin Botanique aÀ Durban-Corbières	Durban-Corbières	France
Le Parc Botanique de Neuvic	Neuvic-Sur-L'isle	France
Les Jardiniers De Maubeuge Et de la Vallée de la Sambre	Maubeuge	France
Les Jardiniers du Dimanche	Genay	France
Les Jardins D'eau	Carsac - Aillac	France
Museum D'histoire Naturelle d'Aix-En-Provence	Aix-En-Provence	France
Muséum d'Histoire Naturelle de Grenoble	Grenoble	France
Parc Botanique de Cornouaille	Combrit	France
Parc Botanique du Château d'Ouge	Ouge	France
Parc de La Teyssonnière	Buellas	France
Parc Et Roseraie du Château De Rambures	Rambures	France
Parc Zoologique Et Botanique de La Ville De Mulhouse	Mulhouse	France
Univ. Grenoble Alpes	Grenoble	France
Institut De Botanique, Université de Montpellier II	Montpellier	France
University of Rostock	Rostock	Germany
Bavarian Natural History Collections	Munich	Germany
Botanischer Garten der Universität Kiel	Kiel	Germany
Botanischer Garten der Universität Leipzig	Leipzig	Germany
Botanischer Garten für Arznei-Und-Gewurpflanzen	Oberholz	Germany
Centre of Natural History Hamburg	Hamburg	Germany
Abteilung Vegetationsanalyse und Phytodiversität, Georg-August-Universität Göttingen	Goettingen	Germany

Müritzeum - Nature Discovery Center	Waren	Germany
Natural History Museum Namu Bielefeld	Bielefeld	Germany
Neuer Botanischer Garten der Universität Göttingen	Göttingen	Germany
Senckenberg Society for Nature Research	Frankfurt	Germany
State Natural History Museum Braunschweig	Braunschweig	Germany
Stuttgart State Museum of Natural History	Stuttgart	Germany
Ubersee-Museum Bremen	Bremen	Germany
Natural History Museum of Crete	Heraklion	Greece
University of Patras	Panepistimiopolis	Greece
Institute of Ecology and Botany, Hungarian Academy of Sciences	Veratot	Hungary
Hungarian Natural History Museum	Budapest	Hungary
University of Pecs	Pecs	Hungary
National Biodiversity Data Centre	Carriganoc	Ireland
Chanousia Alpine Botanical Garden	La Thuile	Italy
La Sapienza University	Roma	Italy
Agricoltura e Foreste	Catanzaro	Italy
Alpine Botanical Garden Saussurea	Courmayeur	Italy
Civic Orto Botanico "L. Rota"	Bergamo	Italy
Dipartimento Fitosanitario	Torino	Italy
Dipartimento de Scienze Botaniche, Herbarium Mediterraneum Panormitanum	Palermo	Italy
Ufficio Servizi Fitosanitari	Saint-Christophe	Italy
Direzione Servizio Fitosanitario Regionale	Siena	Italy
Il Servizio Fitosanitario dell' Umbria	Perugia	Italy
Giardino Alpino "Paradisia"	Cogne	Italy
Giardino Aplino "Antonio Segni"	Veneto	Italy
Giardino Botanico Alpino "Castel Savoia"	Gressoney-Saint-Jean	Italy
Giardino Botanico Alpino "Giangio Lorenzoni"	Puos D'Alpago	Italy
Giardino Botanico Alpino "Giangio Lorenzoni" al Pian di Cansiglio	Tambre D'alpago	Italy
Giardino Botanico Alpino "San Marco"	Vicenza	Italy
Giardino Botanico Della Majella	Lama Dei Peligni	Italy

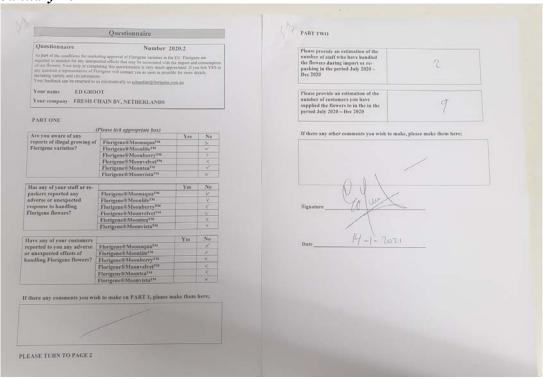
Giardino Botanico dell 'Isola Madre	Stresa	Italy
Giardino Botanico di Saussurea	Courmayeur	Italy
Giardino Botanico di Valbonella Via Della Foresta	Corniolo	Italy
Giardino Botanico Gole del Sagittario	Anversa Degli Abruzzi	Italy
Giardino Dei Semplici Facoltà di Farmacia Dipartimento di Scienze del Farmaco	Scalo	Italy
Giardino Esperia Club Alpino Italiano Localita' Passo del Lupo	Sestola	Italy
Ispezioni E Controlli Fitosanitari di Venezia	Venezia	Italy
Museo Civico Erbario	Bassano Del Grappa	Italy
Museo di Storia Naturale Erbario	Milan	Italy
Museo di Storia Naturale del Mediterraneo	Livorno	Italy
Institute of Biosciences and Bioresources, National Research Council of Italy	Bari	italy
Alto Adige Erbario, Naturmuseum Südtirol/Museo	Bolzano	Italy
Orto Botanico dell' università degli Studi di Siena	Siena	Italy
Orto Botanicodell 'università di Genova	Genova	Italy
Giardino Botanico "Loreto Grande", Parco Nazionale D'Abruzzo	Villavallelonga	Italy
Regional Institute for Floriculture	San Remo	Italy
Servizio Fitosanitario Regionale	Milan	Italy
Servizio Fitosanitario Regionale Liguria	La Spezia	Italy
Servizio Fitosanitario Regionale Liguria	Savona	Italy
The Reiza Alpine Botanical Gardens Bormio	Bormio	Italy
Università de Ferrara	Ferrara	Italy
Università Degli Studi di Genova	Genova	Italy
Università Degli Studi di Lecce	Lecce	Italy
Università Degli Studi di Roma "La Sapienza"	Rome	Italy
Università di Pisa	Pisa	Italy
University of Bologna	Bologna	Italy
University of Catania	Catania	Italy
University of Sassari	Sassari	Italy
Ministry of Agriculture	Riga	Latvia
Ministry Of Environmental Protection and Regional Development of The Republic of Latvia	Riga	Latvia

University of Montenegro	Podgorica	Montenegro
Natural History Museum, University of Oslo	Oslo	Norway
European Weed Research Society		Poland
University of Warsaw	Warsaw	Poland
Botanic Garden, University of Coimbra	Coimbra	Portugal
University of Lisbon	Lisbon	Portugal
Institute of Botany, Slovak Academy of Sciences	Banská Bystrica	Slovakia
Institute of Biology ZRC SAZU,	Ljubljana	Slovenia
Univ. of Barcelona	Barcelona	Spain
Complutense University of Madrid	Madrid	Spain
Institut Botànic de Barcelona Herbario	Barcelona	Spain
National Museum of Natural Sciences	Madrid	Spain
Royal Botanic Garden of Madrid	Madrid	Spain
University of Oviedo	Oviedo	Spain
University of the Basque Country	Leioa	Spain
Gothenburg University	Göteborg	Sweden
Swedish Museum of Natural History	Stockholm	Sweden
Basel Botanic Garden	Basel	Switzerland
Centre Alpien de Phytogéographie, Fondation Jean-Marcel Aubert	Champex-Lac	Switzerland
Musée Jurassien des Sciences Naturelles	Porrentruy	Switzerland
Natural History Museum of Geneva	Geneva	Switzerland
Schynige Platte Botanical Alpine Garden Association	Bern	Switzerland
Central Government Real Estate Agency, Ministry of The Interior And Kingdom Relation	Wageningen	The Netherlands
Naturalis Biodiversity Center	Leiden	The Netherlands
Utrecht University Botanic Gardens	Utrecht	The Netherlands
Wageningen University	Wageningen	The Netherlands
M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine	Kyiv	Ukraine
Yuriy Fedkovych Chernivtsi National University	Chernivtsi	Ukraine
Botanical Society of Britain And Ireland (BSBI)		United Kingdom
Glasgow Botanic Gardens	Glasgow	United Kingdom

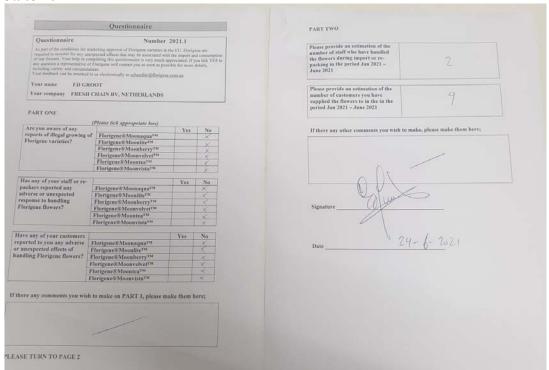
London Natural History Museum	London	United Kingdom
National Museum Wales	Cardiff	United Kingdom
National Museums Liverpool	Liverpool	United Kingdom
National Museums Ni	Holywood	United Kingdom
National Museums Scotland	Edinburgh	United Kingdom
Open University		United Kingdom
The James Hutton Institute	Aberdeen	United Kingdom

# Attachment 3. Importer questionnaire response

January 2021



## June 2021



# Attachment 4. Summary of survey data provided by experts

Florigene received reports from two experts, covering work in Croatia, Slovakia and Republic of North Macedonia. Neither of the experts found any indication of hybrids with transgenic carnations, populations of carnation or populations of wild *Dianthus caryophyllus*. Restrictions to movement imposed by the Covid-19 pandemic restricted the amount of field work possible, as was the case last year.

## Slovakia

Dates and locations of sampling are listed in table 1, with *Dianthus* species identified. Six *Dianthus* species were recorded, but not *Dianthus caryophyllus*.

Table 1. Details of Dianthus species identified in field work in Slovakia.

Month Location Species  Species			
July 2020	Štiavnické vrchy Mts., Hronská Breznica	Dianthus carthusianorum L.	
July 2020	village, meadows S from the village, 48°33'37.2"N 18°59'51.4"E	Dianthus deltoides L.	
July 2020	Štiavnické vrchy Mts., Ilija village, Tatárska lúka site near Sitno hill, 48°24'20.5"N 18°52'21.6"E	Dianthus carthusianorum L.	
August 2020	Pohronský Inovec Mts., Veľký Inovec hill, small meadow on top, 48°24'37.7"N 18°32'37.2"E	Dianthus carthusianorum L. Dianthus deltoides L.	
June 2021	Podunajská nížina lowland, Mužla, Čenkov farmstead, sandy dyke of the Dunaj River, 47°46'22.9"N 18°33'00.9"E	Dianthus pontederae A. Kern	
June 2021	Podunajská nížina lowland, Mužla, Čenkov farmstead, sandy site Čenkovský les forest, 47°46'41.0"N 18°31'08.0"E	Dianthus pontederae A. Kern	
June 2021	Podunajská nížina lowland, Modrany, abandoned vineyards N from the village, 47°50'07.7"N 18°21'03.2"E	Dianthus pontederae A. Kern	
June 2021	Podunajská nížina lowland, Modrany, abandoned Soviet tank training ground, 47°50'29.7"N 18°20'07.9"E	Dianthus armeria L. Dianthus pontederae A. Kern	
June 2021	Podunajská nížina lowland, Nána, Dank hill, dry grasslands, 47°49'07.8"N 18°38'35.7"E	Dianthus pontederae A. Kern	
June 2021	Považský Inovec Mts., Lúka village, dry slope on the eastern edge of the village, 48°39'44.3"N 17°53'36.4"E	<i>Dianthus praecox</i> subsp. <i>lumnitzeri</i> (Wiesb.) Kmeťová	
June 2021	Považský Inovec Mts., Lúka village, Bôrovište hill, 48°40'00.8"N 17°54'38.9"E	Dianthus praecox subsp. lumnitzeri (Wiesb.) Kmeťová	
July 2021	Podunajská nížina lowland, Mužla, Čenkov Farmstead, Čenkovská step Nature Reserve, 47°46'07.5"N 18°31'15.5"E	Dianthus serotinus W. & K.	
July 2021	Súdovce, Žarnový vrch hill, dry slopes, 48°14'14.6"N 18°49'20.0"E	Dianthus armeria L.	

# Croatia

One site of Dianthus ciliatus was identified, near Ražanac.

# Republic of North Macedonia

Dates and locations of surveys are listed in table 2.

Table 2. Details of field work in Republic of North Macedonia

Month	Location	Species
July and	Kale Fortress (Francuski grobishta	No Dianthus species identified
August 2020	[French graveyard]), Skopje	
April 2021	Moklishte (near Kavadarci),	Despite favourable conditions (such as
	Tikvesh region.	proximity to neighbouring cottages and
		weekend settlements) no Dianthus
		species identified
June 2021	Kozle (Vodno Mt. foothill). Vodno	No Dianthus species identified
	Mt. (Skopje region).	
T 0 1	***	N. D. J
Informal	Urban and peri-urban sites in the	No Dianthus species identified
	city of Skopje (municipalities and	
	bordering mountains)	

# Attachment 5. Summary of response to mail out

#### Mode of communication

Communication was by letter and email.

Emails were personalised to individuals and were written in English unless they were directed to new correspondents or old contacts who preferred to communicate in their own language. Communication to new contacts was made in the language of the destination country using on-line translation services. Letters were written in the language of the destination country.

In some communications, a photograph of a standard-type carnation flower was provided for comparison to a photograph of a flower from unimproved Dianthus caryophyllus (figure 1). This was provided as an information aid.

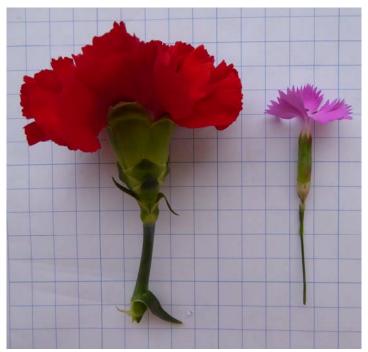


Figure 1. Copy of photograph provided with some communications.

# Breakdown of contacts

209 contacts were made to 7 different types of entity in 28 countries.

- Botanic gardens; 52 contacts
- Government (plant protection agencies, ministries); 16 contacts
- Museums and herbaria; 38 contacts
- Universities and research institutions: 28 contacts
- Botanical societies; 6 contacts
- Vegetation databases and web-based flora administrators; 18 contacts
- Unaffiliated individuals (citizen scientists, amateur naturalists, retired professionals etc.); 51 contacts

## Frequency of response to enquiry letters and emails

Table 1 provides a summary of response numbers, collated approximately 11 weeks after the letters and emails were sent out. As table 1 shows, overall response rate was 47.4%. This is the best response rate ever achieved to the annual mail out and is significantly higher than the overall mean of 28.5% for years 2016 – 2020.

The response rate to emails was, consistent with all previous surveys, significantly higher than that to postal enquiries. All responses to postal enquiries were delivered via email. Highest response rates were from botanical societies, vegetation databases and unaffiliated individuals (62% response rate from this group overall) and the lowest response rate was from government and botanical gardens (31% response rate from this group overall).

Table 1. Summary of 2021 response to mail out

<b>Communication mode</b>	Sent	Responses	%
Post	74	23	31.1
Email	135	76	56.3
Total (post and email)	209	99	47.4

The higher rate of response in 2021 is explained by the extension of time to allow a response (11 weeks compared to 6-7 weeks), a higher-than-average percentage of requests made by email (65% in 2021 compared to an average of 54% for the period 2016 – 2020) and a 57% response rate from unaffiliated individuals, who were, in 2021, approximately a quarter of all contacts made.

## Nature of response to enquiry letters and emails

The responses obtained may be summarised as;

- To provide details of observations of populations of the unimproved, wild Dianthus caryophyllus. These observations were all from France and included Dianthus godronianus and D.caryophyllus ssp. longicaulis with photographic evidence in some cases.
- To state that no observations of *Dianthus*, carnation, or transgenic carnation had been made in the past 12 months. Four records of carnation in Belgium were confrmed to be cultivated specimens.
- To provide links and/or information to old herbarium records of *Dianthus* caryophyllus (pre-2000).
- To provide new herbarium records of *Dianthus* species other than Dianthus caryophyllus.
- To provide information on the presence of Dianthus species other than Dianthus caryophyllus in the local floras and/or herbarium collections.
- To provide statements on absence of *Dianthus caryophyllus* and carnation in floras of local regions over the past year and historically.
- To provide opinions on the lack of invasiveness of *Dianthus caryophyllus*.
- To provide new sources of website data. Website leads were incorporated within the search outlined in attachment 7.
- To state they were unable to provide assistance.
- Four emails were returned as undeliverable. These are not included in the responses summarised in table 1.

None of the responses provided evidence of "escape" or naturalised population of carnation. This includes the information provided from 12 European-based vegetation databases. Two respondents indicated that the unimproved Dianthus caryophyllus population they observed in France could be an escape, based on location and proximity to urbanisation. A similar comment was made on historical records of unimproved Dianthus caryophyllus in Spain.

One respondent provided information from a French language flora which added useful baseline information on the taxonomy of *Dianthus caryophyllus* and relationshp between its synonyms<sup>5</sup>.

<sup>5</sup> Tison, J. M., Jauzein, P., Michaud, H., & Michaud, H. (2014). Flore de la France Méditerranéenne Continentale (Vol. 2080, p. 265). Turriers: Naturalia publications.

# Attachment 6. Literature review methodology

#### Source databases and journals

Literature searches were carried out using the following databases.

- AGRICOLA Article citation (NAL)
- Proquest -biological sciences
- Science Direct (Elsevier)
- Google Scholar

All papers published since 2020 in these three journals were also reviewed;

- Preslia
- Journal of vegetation science
- Vegetation classification and survey

#### Search terms

Search terms used were carnation, carnation biology, Dianthus, Dianthus biology, Dianthus fertilization, Dianthus gene, Dianthus genome, Dianthus medicinal, Europe flora, Europe plant survey, Europe plant checklist, Europe botany survey, Dianthus caryophyllus, vegetation survey, Europe vegetation, Dianthus arrosti, Dianthus caryophyllus var. coronarius, Dianthus gasparrinii Guss., Dianthus godronianus Jord, Dianthus longicaulis Ten., Dianthus saxicola, Dianthus siculus, Dianthus sylvestris subsp. longibracteatus, Dianthus sylvestris subsp. boissieri, Dianthus tarentinus, Dianthus virgatus.

Search terms were each used exactly as listed in normal font, with use of the filter of "since 2020". The primary focus of the literature review was seeking information on carnation and *Dianthus* populations outside of cultivation.

#### Citation search

43 key citations from literature reviews from previous monitoring reports were searched in google scholar for citing literature, which was then screened for content.

#### Literature review short list

The initial review identified hundreds of abstracts. Papers not considered for further review covered the chemistry of secondary products, essential oil preparation and analysis, non-European studies, horticultural studies relating to carnation production and breeding, edible flowers, physiological and biochemical studies relating to postharvest care in carnation, herbicide resistance and plant pathology studies. Abstracts concerning the coral species *Dianthus* or clove oil use were ignored.

Following this initial cull, 266 papers were short listed and read in full, including any supplementary information files provided with the paper. Papers with relevance to carnation or Dianthus caryophyllus distribution, potential weediness, possible biosafety implications and/or genetic modification are listed and briefly summarized in the reference list below. Included in this list are papers which provided vegetation surveys in European urban areas where escape of plants from cultivation might be expected, but in which no *Dianthus* species were identified.

No escape populations of cultivated carnation were identified in any of the 266 papers.

# Reference list

1. Alexandrova, A., Dimitrov, M., Vassilev, K., Sopotlieva, D., Pedashenko, H., & Tashev, A. (2020). Forest vegetation diversity of the Slivenska mountain

# (Eastern Stara planina, Bulgaria). Hacquetia, 19, 233-258. doi:10.2478/hacq-2020-0009

Dianthus giganteus and Dianthus moesiacus are included as diagnostic species in a vegetation survey in Eastern Bulgaria.

- 2. Anđelković, A., Marisavljević, D., & Pavlović, D. (2020). Analysis of the weed flora of the anthropogenically modified shorelines of the Danube-Tisa-Danube canal system. Acta herbologica, 29, 97-110. No Dianthus species were listed.
- 3. Arianoutsou, M., Bazos, I., Christopoulou, A., Kokkoris, Y., Zikos, A., Zervou, S., ... Tsiamis, K. (2021). Alien plants of Europe: introduction pathways, gateways and time trends. PeerJ, 9. doi:10.7717/peerj.11270 Dianthus caryophyllus is listed as one of 19 Dianthus species categorised as alien in Europe. The species was sub-categorised as "escape from confinement".
- 4. Axmanová, I., Kalusová, V., Danihelka, J., Dengler, J., Pergl, J., Pyšek, P., ... Boch, S. (2021). Neophyte invasions in European grasslands. Journal of Vegetation Science, 32, e12994.
  - 8,212 plant species were listed from approximately 100,000 grassland vegetation plots from the European Vegetation Archive. Dianthus caryophyllus was the only Dianthus species within a group of 536 neophyte (alien species introduced after 1500). D. caryophyllus was found in 0.3% of the sub-plots, in France only, in rocky habitats. The species is considered to be of European origin.
- 5. Bartolucci, F., Domina, G., Bagella, S., Barberis, G., Briozzo, I., Calbi, M., ... & Nepi, C. (2020). Notulae to the Italian native vascular flora: 10. Italian Botanist, 10, 47.

Seven Dianthus species are noted in the amendment to the Italian flora. None are Dianthus caryophyllus or synonyms.

6. Bauer, M., & Albrecht, H. (2020). Vegetation monitoring in a 100-year-old calcareous grassland reserve in Germany. Basic and Applied Ecology, 42, 15-

No Dianthus species were listed.

- 7. Bayón, Á., Godoy, O., Maurel, N., van Kleunen, M., & Vilà, M. (2021). Proportion of non-native plants in urban parks correlates with climate, socioeconomic factors and plant traits. Urban Forestry & Urban Greening, 63. doi:10.1016/j.ufug.2021.127215 No Dianthus species were listed.
- 8. Bazan, G., Baiamonte, G., Marino, P., & Schicchi, R. (2021). Mapping floristic diversity: a case study in Sicily. *Ecocycles*, 7, 38-46.

Dianthus arrostii ,Dianthus gasparrinii ,Dianthus minae ,Dianthus rupicola Biv. subsp. rupicola and Dianthus siculus C. Presl were the only Dianthus species identified in the population of 1,700 plant species found in the survey.

9. Benavent, J. E. O., & Lumbreras, E. L. (2020). Sobre l'enquadrament sintaxonòmic dels brugars valencians. Nemus: revista de l'Ateneu de Natura 10, 146-154.

Dianthus multiaffinis was identified in Valencian heathlands, Spain.

10. Berisha, N., Ćušterevska, R., Lluga-Rizani, K., Millaku, F., & Matevski, V. (2020). Relation between boundaries of protected areas and the distribution of vulnerable natural habitats - a case study from Sharri National Park, SE Europe. Ecological Questions, 32. doi:10.12775/eq.2021.008

Three Dianthus species were identified in the study; Dianthus integer, D. scardicus and D. sylvestris.

- 11. Berisha, N., Ćušterevska, R., Millaku, F., Kostadinovski, M., & Matevski, V. (2020). Contribution to the knowledge on the flora of Mt. Luboten, Sharri Mts., Kosovo. Thaiszia - Journal of Botany, 30. doi:10.33542/tjb2020-2-01 Ten Dianthus species were identified in the checklist of 853 species compiled for the area over a 4 year period. None of these were Dianthus caryophyllus or its synonyms.
- 12. Biondi, E., & Allegrezza, M. (2020). Syntaxonomy of *Pinus nigra* s.l. communities in the Erico-Pinetea class and their distribution in the central Apennines and Balkan province. Plant Biosystems 154, 248-258. Dianthus longicaulis Ten. Was the only Dianthus species identified during a vegetation survey carried out in the south-western sector of the Campo Imperatore plateau of Gran Sasso, Italy.
- 13. Bonari, G., Fernández-González, F., Coban, S., Monteiro-Henriques, T., Bergmeier, E., Didukh, Y. P., ... Acosta, A. T. (2021). Classification of the Mediterranean lowland to submontane pine forest vegetation. Applied Vegetation Science, 24, e12544. Dianthus caryophyllus is listed as a diagnostic species for a Pinus halepensis forest alliance located in Northern Africa. Data was drawn from vegetation subplots from Algeria, Morocco and Tunisia.
- 14. Bruschi, T., & Polverelli, L.(2020) La flora dei Tausani (San Leo, RN). Quad. Studi Nat. Romagna, 51, 41-94.
  - Three Dianthus species (Dianthus balbisii, Dianthus longicaulis and Dianthus monspessulanus) are included in a checklist of the flora of the Marecchia valley, Italy.
- 15. Bubel, K., Reczyńska, K., Pech, P., & Świerkosz, K. (2021). Secondary serpentine forests of Poland as a refuge for vascular flora. Diversity, 13, 201. Dianthus carthusianorum was the only Dianthus species identified in forest vegetation plots in South West Poland.
- 16. Calabrese, V., Carranza, M., Evangelista, A., Marchetti, M., Stinca, A., & Stanisci, A. (2018). Long-term changes in the composition, ecology, and structure of *Pinus mugo* scrubs in the Apennines (Italy). *Diversity*, 10. doi:10.3390/d10030070
  - Dianthus longicaulis was the only Dianthus species identified in a 2016 2017 survey of Abruzzo, Lazio, Molise and Majella National Parks, Italy.
- 17. Čarni, A., Ćuk, M., Zelnik, I., Franjić, J., Igić, R., Ilić, M., . . . Škvorc, Ž. (2021). Wet meadow plant communities of the alliance Trifolion pallidi on the Southeastern margin of the Pannonian plain. Water, 13. doi:10.3390/w13030381
  - Dianthus armeria was the only species of Dianthus identified in a vegetation survey in a region of the Pannonian Basin covering the border region of Eastern Croatia and Northern Serbia.
- 18. Casanelles-Abella, J., Frey, D., Müller, S., Aleixo, C., Alós Ortí, M., Deguines, N., ... Moretti, M. (2021). A dataset of the flowering plants (Angiospermae) in urban green areas in five European cities. Data in Brief, 37. doi:10.1016/j.dib.2021.107243
  - 2,146 plant species were catalogued across 80 common public green spaces (including botanical gardens) in Antwerp, Greater Paris, Poznan, Tartu and Zurich. Nineteen Dianthus species were found including Dianthus caryophyllus and Dianthus sp. (presumably pinks and/or carnation). Dianthus caryophyllus and Dianthus sp. were found in 11 sites in Zurich and one site in Paris.
- 19. Ceschin, S., & Salerno, G. (2021). Exploring plant species richness along the Tiber River within the city of Rome. Phytotaxa, 482, 143-158.

No Dianthus species were listed.

No *Dianthus* species were listed.

20. Chasapis, M., Samaras, D., Theodoropoulos, K., & Eleftheriadou, E. (2020). The vascular flora of Mt Tzena (northern Greece). *Flora Mediterranea*, 30, 55-63.

Dianthus corymbosus is the only Dianthus species found in Mt. Tzena.

- 21. Clua, A.B., (2020). The endemic flora of the Iberian peninsula: species richness, spatial phylogenetics and ecological differentiation. Doctoral thesis, Universidad Rey Juan Carlos, Spain.
  - The annotated checklist lists 15 *Dianthus* species, none of which are *Dianthus* carophyllus or its synonyms.
- 22. de Ronde, I., Haveman, R., van der Berg, A., & van Heusden, T. (2020). DUMIRA a management related vegetation plot database of Dutch military ranges. *Vegetation Classification and Survey, 1*, 155-161. doi:10.3897/vcs/2020/59869
- 23. Delbosc, P., Le Dez, M., Bouzillé, J.-B., Cianfaglione, K., & Bioret, F. (2021). Numerical classification of French vegetation of Carici caryophylleae Genistetea lobelii J.-C. Klein 1972. *Mediterranean Botany*, 42. doi:10.5209/mbot.68062

*Dianthus sylvestris* subsp. *Longicaulis* is the only *Dianthus* species named in the vegetative associations.

24. Di Cecco, V., Di Santo, M., Di Musciano, M., Manzi, A., Di Cecco, M., Ciaschetti, G., . . . Di Martino, L. (2020). The Majella National Park: a case study for the conservation of plant biodiversity in the Italian Apennines. *Italian Botanist*, 10, 1.

Dianthus carthusianorum L. subsp. tenorei and Dianthus guliae are included in a checklist of the Majella National Park, Italy.

25. Diviaková, A., Stašiov, S., Pondelík, R., Pätoprstý, V., & Novikmec, M. (2021). Environmental and management control over the submontane grassland plant communities in central Slovakia. *Diversity*, 13. doi:10.3390/d13010030

*Dianthus deltoides* and *D. carthusianorum* were the two *Dianthus* species identified in central Slovakian grasslands.

- 26. Enri, S. R., Nucera, E., Lonati, M., Alberto, P. F., & Probo, M. (2020). The Biodiversity Promotion Areas: effectiveness of agricultural direct payments on plant diversity conservation in the semi-natural grasslands of the Southern Swiss Alps. *Biodiversity and Conservation*, 29, 4155-4172.
  - *Dianthus carthusianorum* was the only *Dianthus* species in 242 vegetation surveys carried out in Ticino Canton, Switzerland.
- 27. Fanfarillo, E., Latini, M., Iberite, M., & Abbate, G. (2020). The segetal flora of Italy: an occurrence dataset from relevés in winter cereals and allied crop types. *PhytoKeys*, 161, 107.
  - *Dianthus nudiflorus* is the only *Dianthus* species in an Italy wide database containing 859 plant species growing within Italian winter cereal crops and allied crop types.
- 28. Ferrer-Gallego, P. P.(2021). Tipificación de seis nombres en el género *Dianthus* L.(Caryophyllaceae). *Flora Montiberica 79*, 9-27.

  Lectotypification of six *Dianthus* species, none of which are synonyms of *Dianthus caryophyllus*.
- 29. Piñar Fuentes, J. C., Raposo, M., Pinto Gomes, C. J., del Río González, S., Spampinato, G., & Cano, E. (2021). New contributions to the *Ericion umbellatae* alliance in the central Iberian Peninsula. *Sustainability*, 13, 5639.

Dianthus lusitanus is listed as a threatened species.

- 30. Gargano, M., Di Gristina, E., Domina, G., & Venturella, G. (2021). Trees and shrubs in the city of Bari (Italy). Fl. Medit, 31, 23-30. No Dianthus species was identified.
- 31. Gesti Perich, Josep, 2020. Catàleg de la flora vascular de Santa Coloma de Farners (la Selva, nord-est de Catalunya). Miconia, 4, 69-105.

A checklist of plants in the community of Santa Coloma de Farners, Caralonia, Spain includes 3 Dianthus species; D.armeria, D. pyrenaicus and D. seguieri.

- 32. Gianguzzi, L., & Bazan, G. (2020). The vegetation of a historic road system in the suburban area of Monte Pellegrino (Palermo, Sicily). Plant Sociology, *57*, 71.
  - No Dianthus species were listed.
- 33. Giulio, S., Acosta, A. T. R., Carboni, M., Campos, J. A., Chytrý, M., Loidi, J., . . . Janssen, J. A. (2020). Alien flora across European coastal dunes. Applied Vegetation Science, 23, 317-327. No Dianthus species were listed.
- 34. Giupponi, L., & Leoni, V. (2020). VegeT: An easy tool to classify and facilitate the management of seminatural grasslands and dynamically connected vegetation of the Alps. Land, 9, 473.

Dianthus monspessolanus was the only Dianthus species in the dataset, based on vegetation of the Taleggio Valley (Italian Alps). The species was present in 3 of 29 relevés.

- 35. Gjeta, E., Titus, J., & Titus, P. (2021). Plant species occupy different habitats on the fortress walls in Elbasan, Albania. Hacquetia, 20, 81-90. doi:10.2478/hacq-2020-0016 No Dianthus species were listed.
- 36. Gnatiuk, A. M., & Honchar, G. Y. (2020). Urban ornamental plants for sustenance of wild bees (Hymenoptera, Apoidea). Plant Introduction, 85-86. doi:10.46341/pi2020014

No *Dianthus* species were listed.

37. Guarino, R., Chytrý, M., Attorre, F., Landucci, F., & Marcenò, C. (2021). Alien plant invasions in Mediterranean habitats: an assessment for Sicily. Biological Invasions. doi:10.1007/s10530-021-02561-0

Dianthus arrosti (a synonym for Dianthus caryophyllus) was listed as a "diagnostic, constant and dominant species". This is the only *Dianthus* species identified in the study.

- 38. Hegedüšová, K., Korzeniak, J., Májeková, J., Stoica, A., Coldea, G., Kuzemko, A., ... Škodová, I. (2020). Syntaxonomical revision of the Triseto flavescentis-Polygonion bistortae alliance in the Carpathians. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 155, 16-41.
  - Dianthus barbatus subsp. Compactus is the only Dianthus species in the alliance, determined over a geographical region of the Carpathian Mountains covering Slovak, Polish, Romanian and Ukrainian Carpathians.
- 39. Jeong, H. Y., Naing, A. H., & Kim, C. K. (2021). Establishment of protocol for genetic transformation of carnation with 1-aminocyclopropanecarboxylate deaminase (acdS) gene. Journal of Plant Biotechnology, 48, 93-99.
  - Description of an Agrobacterium co-cultivation-based transformation protocol for carnation, using phosphinothricin selection.
- 40. Kalusová, V., Čeplová, N., Chytrý, M., Danihelka, J., Dřevojan, P., Fajmon, K., ... Řehořek, V. (2019). Similar responses of native and alien floras in

- European cities to climate. Journal of Biogeography, 46, 1406-1418. No Dianthus species were listed.
- 41. Kamil, S. S., Hussein, H. J., & Al-Marzoqi, A. H. (2020). Evolution of Antibacterial efficacy of Dianthus caryophyllus L. extracts against some hospitals pathogenic bacteria. International Journal of Pharmaceutical Research, 12, 1274-1279.

Antibacterial effect of solvent extracts from carnation flower buds was determined. Carnation variety was not identified in materials and methods.

- 42. Kaplan, Z., Danihelka, J., Chrtek Jr, J., Zázvorka, J., Koutecký, P., Ekrt, L., ... Grulich, V. (2019). Distributions of vascular plants in the Czech Republic. Part 8. Preslia, 91, 257-368. No *Dianthus* species were listed.
- 43. Khatib, C., Nattouf, A., & Agha, M. I. H. (2021). Ethnobotanical Survey of Medicinal Herbs in the Western Region in Syria (Latakia and Tartus). Research square, https://doi.org/10.21203/rs.3.rs-355008/v1

Carnation flower bud extracts are used traditionally in Syria to treat gum infections, gastrointestinal disorders, wounds and throat ailments.

44. Kundan, K., Deb, R., Dhama, K., & Krishna, D. The extracts of 16 plant seeds for their antiviral activity against herpes simples virus-1 (HSV-1, a DNA virus) and hepatitis A virus (HAV, a RNA virus). Advances Life Sci. Biotech. 3, 32 - 38.

Antiviral activity was confirmed in two viruses using an extract of carnation seed. No details of variety used was given.

45. Kunev, G., Tzonev, R., Tsiripidis, I., & Pachedjieva, K. (2020). Phytosociological study of submontane genistoid scrub communities from the Southeastern Balkans. Acta Botanica Croatica, 79, 170-184. doi:10.37427/botcro-2020-017

Dianthus pinifolius ssp. pinifolius, Dianthus corymbosus and Dianthus giganteus ssp. giganteus were identified in 156 vegetation plots in Bulgaria.

46. Küzmič, F., Šilc, U., Lososová, Z., Mucina, L., Chytrý, M., Knollová, I., . . . Tereshenko, S. (2020). European Weed Vegetation Database – a gapfocused vegetation-plot database. Phytocoenologia, 50, 93-100. doi:10.1127/phyto/2019/0337

No Dianthus species were listed.

47. Kwiatkowski, P. (2021). Basaltic Outcrops as Centers of Diversity for Xerothermic Plants in the Sudetes Mountains (Central Europe). Diversity, 13. doi:10.3390/d13040164

Dianthus carthusianorum L. is the only Dianthus species identified in 35 sampled areas of the Sudetes Mountains, Czech republic.

48. Lattanzi, E., Del Vico, E., Tranquilli, R., Farris, E., Marignani, M., & Rosati, L. (2021). An unknown hotspot of plant diversity in the heart of the Central Apennine: flora and vegetation outline of Mt. Pozzoni-St. Rufo valley (Cittareale, Rieti). PhytoKeys, 178, 111.

Four Dianthus species were identified in a mountainous area of Cittareale, Lazio, Italy. The four species were *Dianthus carthusianorum* L. subsp. Carthusianorum Dianthus deltoides L. subsp. Deltoides, Dianthus hyssopyfolius L and Dianthus longicaulis Ten.

49. Lisova, N., Syvyj, M., & Volik, O. (2021). Ecological analysis of the flora of the Kremenets Mountains National Nature Park (on the example of the mountains Divochi Skeli, Strakhova, Masliatyn, Chercha, Zamkova, Bozha). E3S Web of Conferences. 280, 11008 https://doi.org/10.1051/e3sconf/202128011008.

- Dianthus carthusianorum L. and Dianthus andrzejowskianus were identified in the study area, located in Ukraine.
- 50. Martinović, M., Valjak, N., Šegota, V., Bartolić, P., Jakovac, E., Stić, P., & Husnjak Malovec, K. (2020). Vascular flora of the educational trail "Okićnica "in the Žumberak-Samoborsko Gorje Nature Park (NW Croatia). Natura Croatica: Periodicum Musei Historiae Naturalis Croatici, 29, 109-121. Dianthus giganteus D' Urv ssp. croaticus is the only Dianthus species of 287 species on a checklist for a nature trail.
- 51. Mata, L., Andersen, A. N., Morán-Ordóñez, A., Hahs, A. K., Backstrom, A., Ives, C. D., ... Thomas, F. (2021). Indigenous plants promote insect biodiversity in urban greenspaces. Ecological Applications, e02309. No *Dianthus* species were listed.
- 52. Matveeva, T., & Otten, L. (2021). Opine biosynthesis in naturally transgenic plants: Genes and products. Phytochemistry, 189, 112813. doi:10.1016/j.phytochem.2021.112813
  - Non-transgenic carnation has an intact copy of the opine synthase gene cucumopine synthase (cus) from Agrobacterium.
- 53. Médail, F., & Pavon, D. (2021). Complément à la connaissance de la flore vasculaire de l'île Gargalu (Réserve naturelle de Scàndula, Corse occidentale). Le Journal de Botanique, 94, 11-27.
  - Dianthus longicaulis Ten. ( a synonym for D. caryophyllus) is included as the only Dianthus species on a checklist for Gargalu Island, Scàndula Nature Reserve, Corsica.
- 54. Milović, M., Jasprica, N., Tafra, D., Pandža, M., & Krpina, V. (2020). Prirodna obilježja Svilaje s pregledom istraživanja flore i vegetacije. Glasnik Hrvatskog botaničkog društva, 8(1), 29-50. doi:10.46232/glashbod.8.1.5
  - Dianthus sylvestris ssp. tergestinus was the only Dianthus species identified in a survey of Svilaja Mountain, Croatia.
- 55. Morimoto, H., Ando, Y., Sugihara, H., Narumi-Kawasaki, T., Takamura, T., & Fukai, S. (2021). Information on flower coloration and pigmentation in current carnation cultivars for use in future flower-color breeding Horticulture Journal doi: 10.2503/hortj.UTD-271
  - An analysis of the flower colour characteristics, epidermal cell characteristics and pigments of 110 carnation varieties, including four transgenic carnation varieties. The study provides an independent assessment of flower colour code and pelargonidin and cyanidin levels.
- 56. Mosyakin, S., & Mosyakin, A. (2021). Lockdown botany 2020: some noteworthy records of alien plants in Kyiv City and Kyiv Region. Ukrainian Botanical Journal, 78, 96-111.
  - No Dianthus species were listed.
- 57. Motti, R., Bonanomi, G., & Stinca, A. (2021). Biodeteriogens at a southern Italian heritage site: Analysis and management of vascular flora on the walls of Villa Rufolo. International Biodeterioration & Biodegradation, 162, 105252.
  - No Dianthus species were listed.
- 58. Musarella, C., Stinca, A., Cano-Ortíz, A., Laface, V., Petrilli, R., Esposito, A., & Spampinato, G. (2020). New data on the alien vascular flora of Calabria (Southern Italy). Annali di Botanica, 10, 55-66. https://doi.org/10.13133/2239-3129/14838
  - Nine Dianthus and sub-species were included in a checklist compiled from 660 samples taken from mountain top areas from mainland Greece and some

- Greek islands. None of the *Dianthus* species were synonyms with *Dianthus* carvophyllus.
- 59. Nakamura, N., Suzuki, T., Shinbo, Y., Chandler, S., & Tanaka, Y. (2020). Development of violet transgenic carnations and analysis of inserted transgenes. In The Carnation Genome (pp. 135-146): Springer.

A description of the binary vector and insertion sequences in all six of the transgenic carnation varieties marketed in the European Union.

- 60. Nakayama, M. (2020). Flower pigments responsible for cyanic, yellow, and cream-white coloration in carnation. In *The Carnation Genome* (pp. 61-79): Springer.
  - Detailed description of pigment chemistry in carnation, including discussion of pigments in transgenic carnation.
- 61. Nguyen, T. N. L., Hoang, T. T. H., Nguyen, H. Q., Tu, Q. T., Tran, T. H., Lo, T. M. T., ... Chu, H. M. (2021). Agrobacterium tumefaciens-mediated genetic transformation and overexpression of the flavonoid 3' 5'hydroxylase gene increases the flavonoid content of the transgenic Aconitum carmichaelii Debx. plant. In Vitro Cellular & Developmental Biology-Plant, https://doi.org/10.1007/s11627-021-10190-4 Over-expression of endogenous flavonoid 3' 5'-hydroxylase gene (under s control of 35S promoter) resulted in transgenic plants with 60% higher flavonoid concentration.
- 62. Nimura, M. (2020). Breeding of interspecific hybridization among carnation (Dianthus caryophyllus L.), Dianthus japonicus Thunb., and Dianthus× isensis Hirahata et Kitam. In The Carnation Genome (pp. 159-176): Springer.

Review of interspecific hybridisation techniques used by breeders within the Dianthus genus, including the species D. caryophyllus.

- 63. Nitarska, D., Boehm, R., Debener, T., Lucaciu, R. C., & Halbwirth, H. (2021). First genome edited poinsettias: targeted mutagenesis of flavonoid 3'-hydroxylase using CRISPR/Cas9 results in a colour shift. Plant Cell, Tissue and Organ Culture (PCTOC). doi:10.1007/s11240-021-02103-5 Genetic modification of the anthocyanin biosynthesis pathway using gene editing. The target gene was flavonoid 3-hydroxylase (F3'H) the partial suppression of which in transgenic plants lead to reduction in cyanidin accumulation and a change in bract colour from red to orange hues.
- 64. Noble, V., & Baret, J. (2020). Catalogue des végétations du département des Bouches-du-Rhône. In: CBN méditerranéen de Porquerolles, à paraître. A catalogue of the vegetation of the department of Bouches-du-Rhône, Southern France includes Dianthus balbisii and two synonym species of Dianthus caryophyllus; Dianthus sciulus and Dianthus godronianus Jord.
- 65. Nota, G., Ravetto Enri, S., Pittarello, M., Gorlier, A., Lombardi, G., & Lonati, M. (2021). Sheep grazing and wildfire: disturbance effects on dry grassland vegetation in the Western Italian Alps. Agronomy, 11, 6. Dianthus carthusianorum and Dianthus sylvestris were the two Dianthus species identified in the study area, the Susa Valley.
- 66. Novac, G. (2020). Produsele forestiere nelemnoase cu valoare furajeră din Republica Moldova. Studia Universitatis Moldaviae (Seria Științe Reale și ale Naturii), 136, 91-100.
  - Dianthus armeria is the only Dianthus species in a list of 416 plant species inventoried from forest flora in Moldova.
- 67. Ozeki, Y., Iijima, L., Higuchi, K., Miyahara, T., Sasaki, N., Tsujimoto, T., ... Suzuki-Wagner, A. (2020). Molecular mechanisms of carnation

flower colors via anthocyanin and flavonoid biosynthetic pathways. In The Carnation Genome (pp. 99-117): Springer.

Detailed description of genes involved in anthocyanin biosynthesis in carnation.

68. Perrin, S., Bick, F., & Simon, M. (2020). Contribution à l'inventaire botanique (Tracheophyta; Bryophyta; Marchantiophyta) du Sommerberg, commune de Niedermorschwihr (Haut-Rhin, France). Bulletin de la Société d'Histoire naturelle et d'Ethnographie de Colmar, 76, 27-34.

Dianthus carthusianorum L. was the only Dianthus species found in vegetation surveys of the Niedermorschwihr vineyard (Haut-Rhin, France).

69. Peruzzi, L., Viciani, D., Angiolini, C., Apruzzese, M., Banfi, E., Bonini, I., ... & Bedini, G. (2020). Contributi per una flora vascolare di Toscana. XII (739–812). Atti della Società Toscana di Scienze Naturali, Memorie, Serie B, 127, 101-111.

Dianthus armeria is the only Dianthus species listed in an inventory of the flora of Tuscany, Italy.

70. Pînzaru, P. (2020). Contribuții la studiul florei pădurii "Frunzești" din preajma orașului Cornești, Republica Moldova. Acta et commentationes (Ştiințe Exacte și ale Naturii), 9, 84-94.

Dianthus armeria is the only Dianthus species found in the flora of the "Frunzești" forest, Moldava.

71. Prodanović, D., Krivošej, Z., Amidžić, L., ĆIrić, S., Biberdžić, M., & Krstić, Z. (2020). Diversity and ecological analysis of serpentine flora in the Kosovo section of the Ibar river valley - comparison with the flora of nearby regions. Applied Ecology and Environmental Research, 18, 7289-7322. doi:10.15666/aeer/1805 72897322

Five Dianthus species were listed in a catalogue of the vascular plants of the Kosovo section of the Ibar river valley. None of the species were D.caryophyllus or synonyms.

72. Puig-Gironès R. (2020). Catàleg de flora vascular, fauna invertebrada i fauna vertebrada del parc natural de Sant Llorenç del Munt i L'obac. diputació de Barcelona, Barcelona. 96 pàgines.

The checklist of the national park lists Dianthus multiceps, Dianthus pungens and Dianthus seguieri subsp. Requienii out of a total of 1,186 plant species.

73. Răduțoiu, D. (2020). Ornamental plants species from spontaneous flora in Oltenia region, Romania. Scientific Papers-Series B, Horticulture, 64, 602-607.

Dianthus armeria was the only Dianthus species in a list of 189 "spontaneous decorative" plant species in a survey of urban and peri-urban areas within Oltenia province, Romania.

- 74. Raimondo, F., Spadaro, V., & Di Gristina, E. (2020). Centaurea heywoodiana (Asteraceae), a new species from the Nebrodi Mountains (NE-Sicily). Flora Mediterranea, 30, 369-376.
  - Dianthus siculus C. Presl, (syn. Dianthus caryophyllus) is nominated as a species which grows with Centaurea heywoodiana in the Nebrodi Mountains, Sicily, Italy.
- 75. Ravetto Enri, S., Nucera, E., Lonati, M., Alberto, P. F., & Probo, M. (2020). The Biodiversity Promotion Areas: effectiveness of agricultural direct payments on plant diversity conservation in the semi-natural grasslands of the Southern Swiss Alps. Biodiversity and Conservation, 29, 4155-4172. doi:10.1007/s10531-020-02069-4

- Dianthus carthusianorum is the only Dianthus species identified in 242 vegetation surveys from semi-natural grasslands in Ticino Canton, Switzerland. It is considered a significant indicator species.
- 76. Rodríguez-Rojo, M. P., Font, X., García-Mijangos, I., Crespo, G., & Fernández-González, F. (2020). An expert system as an applied tool for the conservation of semi-natural grasslands on the Iberian Peninsula. Biodiversity & Conservation, 29, 1977 – 1992.
  - A review of datasets from 12,204 vegetation plots from grassland habitats in the Iberian Peninsula and Macaronesian Islands (Azores, Canary Islands, Madiera, Cape Verde) identified five *Dianthus* species none of which were *D*. caryophyllus or synonyms.
- 77. Šantrůčková, M., Dostálek, J., & Frantík, T. (2020). Vegetation succession in extensive abandoned tall-trunk cherry orchards: a case study on Kaňk Mountain near Kutná Hora (Czech Republic). Hacquetia, 19, 127-136. doi:10.2478/hacq-2019-0017
  - Dianthus carthusianorum was the only Dianthus species identified in the study area. The authors indicate a habitat for the species in areas of medieval ore
- 78. Schellenberg, J., & Bergmeier, E. (2020). Heathland plant species composition and vegetation structures reflect soil-related paths of development and site history. Applied Vegetation Science, 23, 386-405. Dianthus deltoides was the only Dianthus species identified in the study of heathlands in northern Germany.
- 79. Sciandrello, S., Minissale, P., & Del Galdo, G. G. (2020). Vascular plant species diversity of Mt. Etna (Sicily): endemicity, insularity and spatial patterns along the altitudinal gradient of the highest active volcano in Europe. *PeerJ*, 8, e9875.
  - Dianthus rupicola and Dianthus nuiflorus are present in the inventory of 1,055 species in the flora of Mount Etna, Italy.
- 80. Šipek, M., & Šajna, N. (2020). Public opinions and perceptions of periurban plant invasion: the role of garden waste disposal in forest fragments. Management of Biological Invasions, 11, 733. No Dianthus species were listed.
- 81. Stoyanov, K. H., & Raycheva, T. G. (2020). Plant Composition in Grasslands and Field Margins in Agroecosystems of South Bulgaria. Ecologia Balkanica, 12, 1-12.
  - Dianthus corymbosus is the only Dianthus species found amongst 245 plant species found in 13 vegetation surveys across the Thracian Lowlands, Bulgaria.
- 82. Suffert, F., & Suffert, M. 'Phytopathological strolls' in the dual context of COVID-19 lockdown and IYPH2020: transforming constraints into an opportunity for public education about plant pathogens. bioRxiv preprint doi.org/10.1101/2021.02.03.429201;
  - A plant pathogen study which includes a photograph of a Dianthus longicaulis (syn. D. caryophyllus) flower.
- 83. Teofilovski, A. (2020). Some new and confirmed taxa in the flora of the Republic of North Macedonia. Acta Musei Macedonici Scientiarum *Naturalium*, 23, 31-37.
  - Dianthus superbus subsp. Superbus is noted for the first time in the flora of the Republic of North Macedonia.
- 84. Thamaragsa, N., Chundet, R., & Warut, U. (2020). Transformation of butterfly pea (Clitoria ternatea) F3'5'H (Flavonoid-3', 5'-Hydroxylase) gene into patumma (Curcuma alismatifolia) Blue Moon'. Thai Journal of Science

# and Technology, 9, 876-885.

- Expression of the F3'5'H gene under a ubiquitin promoter resulted in accumulation of anthocyanin pigments in bracts of transgenic plants. Nontransgenic regenerants were not pigmented.
- 85. Totsuka, A., Okamoto, E., Miyahara, T., Kouno, T., Cano, E. A., Sasaki, N., ... Ozeki, Y. (2018). Repressed expression of a gene for a basic helixloop-helix protein causes a white flower phenotype in carnation. Breed Sci, 68(1), 139-143. doi:10.1270/jsbbs.17072 Description of a possible mechanism for formation of white flowers and speckled flowers in normally pigmented carnation flowers. It was found expression of a transcriptional regulatory factor (bHLH) was necessary to induce expression of dihydroflavonol 4-reductase and other genes on the anthocyanin biosynthesis pathway.
- 86. Türke, I. J., Lontay, L., Serfőző, J., Zsólyomi, T., & Drozd, A. (2020). Pótlások Magyarország edényes növényfajainak elterjedési atlaszához XI. Contributions to the Atlas Florae Hungariae XI. Data from the Tokaj-Zemplén Mts and its surroundings Kitaibelia, 25, 239-241.

Dianthus deltoides was the only Dianthus species identified in the study.

- 87. U.S. Department of Agriculture, FAS GAIN report (2001). Agricultural Biotechnology Annual Colombia Report number CO2020-0037. Available at: https://apps.fas.usda.gov/newgainapi Accessed: 20/7/2021. Includes a review of approvals of genetically modified organism approvals in Colombia, including the GM carnation varieties imported into the European Union.
- 88. Urziceanu, M., Camen-Comănescu, P., Nagodă, E., Raicu, M., Sîrbu, I.-M., & Anastasiu, P. (2020). Updated list of non-native ornamental plants in Romania. Contribuții Botanice 55, 59 – 82. No Dianthus species were listed.
- 89. Večeřa, M., Axmanová, I., Padullés Cubino, J., Lososová, Z., Divíšek, J., Knollová, I., . . . Bonari, G. (2021). Mapping species richness of plant families in European vegetation. Journal of Vegetation Science, 32, e13035. Supplementary data provided with the paper provides data derived from more than 800,000 vegetation plots, plotting species distribution and relative abundance against habitat. Though the analysis is made on a taxonomic family basis distribution by plot total and habitat is provided for 113 Dianthus species including Dianthus caryophyllus (DC). It was noted DC is of European origin but is considered a neophyte in some European countries. On the basis of number of plots present, DC occurred in 0.74% of the total number of plots where a Dianthus species was recorded. DC was the 25th most abundant of the Dianthus species. 18% of the plots containing DC were forest, 52% grassland, 30% scrub and 0% wetland.
- 90. Vega, K. A., & Küffer, C. (2021). Promoting wildflower biodiversity in dense and green cities: The important role of small vegetation patches. Urban Forestry & Urban Greening, 62. doi:10.1016/j.ufug.2021.127165 Dianthus carthusianorum was included in the list of species surveyed in the study.
- 91. Vladimirov, V., Bancheva, S., Delcheva, M., Vassilev, K., & Pedashenko, H. (2020). Contribution to the knowledge of habitat and vascular plant diversity of Alibotush Reserve, Slavyanka Mountain (Bulgaria). Flora *Mediterranea*, 30, 19-32.
  - Dianthus pinifolius was the only Dianthus species identified in the survey area.
- 92. Vojík, M., Sádlo, J., Petřík, P., Pyšek, P., Man, M., & Pergl, J. (2020). Two

- faces of parks: sources of invasion and habitat for threatened native plants. Preslia, 92, 353-373.
- No *Dianthus* species were listed.
- 93. Wirth, T., Kovács, D., Sebe, K., & Csiky, J. (2020). The vascular flora of Pécs and its immediate vicinity (South Hungary) I.: species richness and the distribution of native and alien plants. Biologia Futura, 71, 19-30. No Dianthus species were listed.
- 94. Wirth, T., Kovács, D., Sebe, K., Lengyel, A., & Csiky, J. (2020). Changes of 70 years in the non-native and native flora of a Hungarian county seat (Pécs, Central Europe). Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, DOI: 10.1080/11263504.2020.1829734 No Dianthus species were listed.
- 95. Zdulski, J., Chabuz, W., Sawicka-Zugaj, W., Krawiec, A., Szulc, K., & Stobiecka, M. (2020). Wypas pastwiskowy jako czynna forma ochrony przyrody (Pasture grazing as an active form of nature protection) Zagrożenia środowiska i bezpieczeństwo żywności. DOI: 10.24326/mon.2020.8 Dianthus superbus is the only Dianthus species found in grazed meadows in a survey area in Poland.
- 96. Zhang, X., Wu, Q., Lin, S., Zhang, Z., Wang, Z., Wang, Q., . . . Fu, X. (2021). Regeneration and Agrobacterium-mediated genetic transformation in Dianthus chinensis. Scientia Horticulturae, 287, 110279. An Agrobacterium co-cultivation method was developed using kanamycin as a selective agent.
- 97. Zukal, D., Novák, P., Duchoň, M., Blanár, D., & Chytrý, M. (2020). Calcicolous rock-outcrop lime forests of east-central Europe. Preslia, 92, 191-211. doi:10.23855/preslia.2020.191

Three Dianthus species (D. carthusianorum agg., Dianthus praecox and Dianthus spiculifolius) were identified in a survey of releves located in Hungary, Slovakia, Romania and the Czech Republic.

#### **Attachment 7. Database information**

#### 7.1. Search terms

Databases previously identified were re-examined for any new entries listing *Dianthus* caryophyllus or *Dianthus godronianus* since the last monitoring report. In the event neither of these species were found the databases were checked for the following species;

- Dianthus arrosti C.Presl
- Dianthus caryophyllus var. coronarius L.
- *Dianthus gasparrinii* Guss.
- Dianthus longicaulis Ten.
- Dianthus saxicola Jord
- Dianthus siculus C.Presl
- Dianthus sylvestris subsp. longibracteatus (Maire) Greuter and Burdet
- Dianthus sylvestris subsp. boissieri (Willk.) Dobignard
- Dianthus tarentinus Lacaita
- *Dianthus virgatus* Pasq.

# 7.2. Databases previously examined

### 7.2.1 Records of Dianthus caryophyllus

• www.gbif.org (accessed June 14 2021)

Global Biodiversity Information Network. There were 2 entries from Europe since July 2020 listing *Dianthus caryophyllus*. No photographs were provided. The two observations are included in two databases described immediately below; http://waarnemingen.be and https://www.artportalen.se.

• http://waarnemingen.be(accessed June 14 2021)

Database of plant observations in Belgium. There have been three observations of *Dianthus caryophyllus* since July 2020; one in May 2021 and two in June 2021. All three were in the Antwerp region. Available photographs (example below) show pink flowered, cultivated pot *Dianthus*. The administrator was contacted for more details.



https://waarnemingen.be/users/364467/photos

## https://www.artportalen.se (accessed June 14 2021)

Database of the Swedish Species Information Centre. There has been one observation of Dianthus caryophyllus since July 2020. This was in October 2020 in the municipality of Mölndal. The observers were contacted for more details and confirmed the plant was an unimproved Dianthus, not carnation.

# http://www.tela-botanica.org (accessed June 14 2021)

The French botany network. Five new records of *Dianthus caryophyllus* L. have been posted (on the carnet de ligne) since July 2020. The dates and locations of the new records were;

Date	Location
6 June 2020	Montpeyroux
22 June 2020	Monieux
30 June 2020	Sumène
1 July 2020	Eyguières
14 July 2020	Villeneuvette

Images were available for two collections and both showed wild type form (five petal) flowers.

# http://www.naturedugard.org/accessed June 14 2021)

The database has two new entries for records of *Dianthus caryophyllus* in France. The observer was contacted for more information of the records which were from Saint Nazairede-Gardies (22 Aug 2020) and Le Vigan (5 June 2021).

http://www.naturgucker.de (accessed June 15 2021)

The database has one new entry for *Dianthus caryophyllus* since July 2020 from Baden-Württemberg (observation was made June 1 2021). The plant was a cultivated pink.

http://cbnmc.fr/cartoweb3/Chloris/atlas auv/menu auv.php (accessed June 15) A database of the flora d'Auvergne, south-central France. The database covers the departments of Allier, Puy-de-Dôme, Cantal and Haute-Loire. Dianthus caryophyllus and 9 sub-species of *D. caryophyllus* are listed. There were two records from 2020 that were not found last year.

• http://www.cbnbrest.fr/eCalluna/index.php (accessed June 15 2021)

Floristic database of the vascular flora of the Nouvelle-Aquitaine région, Basse-Normandie, Bretagne and Pays de la Loir regions of France operated by Conservatoire Botanique National de Brest (CBN). There were four observations in 2020. The observations were in departments Finistere (commune Plouarzel), Manche (Bricquebec, Granville) and Orne (Domfront).

• http://inpn.mnhn.fr/espece (accessed June 15 2021)

Database of French government (natural heritage service). There are 47 datasets behind the database one of these (**Pl@ntNet observations** described in 7.2) had records of *Dianthus caryophyllus* from 2020. Review of the **Pl@ntNet** site indicated these were non-transgenic carnation varieties in cultivation or in vases.

# 7.2.2 Databases with no new records of *Dianthus caryophyllus* since July 2020

• http://www.artsdatabanken.no (accessed June 15 2021)

Norwegian Species Observation service/ Norwegian Biodiversity Information Centre. There have been no observations of *Dianthus caryophyllus*, or synonyms, since July 2020.

• http://www.plantcol.be/search-plants.php (accessed June 15 2021)

A database of the living plant collections in nine botanical institutions in Belgium. Accessions of *Dianthus caryophyllus* are listed but there are no records of this species or its synonyms since 2018.

- http://www.bsbimaps.org.uk/atlas (accessed June 15 2021)
- Botanical records for the British Isles. The atlas of records for *Dianthus caryophyllus* and synonyms indicates no new records since July 2020.
  - http://www.florealpes.com (accessed June 17 2021)

Vegetation database of Hautes-Alpes, Corsica, Pyrenees and Provence. The flora provides photographs of *Dianthus saxicola* and *Dianthus godronianus*, the most recent of which are 2019. All photographs shown five petal unimproved plants.

- http://www.botagora.fr (accessed June 17 2021)
- Site could not be reached.
  - http://www.floraiberica.es(accessed June 17 2021)

Flora Iberica (Spain) website. *Dianthus boissieri* is listed as synonym of *Dianthus caryophyllus*. Taxa records were last updated in 2017.

• http://www.anthos.es, Anthos. (accessed June 17 2021)

Spanish plants information system. *Dianthus boissieri* is listed as synonym of *Dianthus caryophyllus*. The six photographs of flowers show five petal unimproved flowers.

• http://waarneming.nl/(accessed June 15, 2021)

A website of the biodiversity of the Netherlands. The website has been updated and there are no new listing *Dianthus caryophyllus* or its synonyms.

• http://www.anarchive.it (accessed June 17 2021)

Database lists hundreds of sample of *Dianthus caryophyllus* as species and five sub-species. No sample is younger than 2013.

- http://herbarivirtual.uib.es/cas-med/genere/2523.html (accessed June 17 2021) A database of the vascular plants in the western Mediterranean basin. Eleven *Dianthus* species are listed in the database, but not *Dianthus caryophyllus* or its synonyms.
  - http://www.sivim.info/sivi (accessed June 17 2021)

A database of the vegetation of Spain and France. No new entries listing *Dianthus caryophyllus*. The most recent of the 53 observations for the species is from 1982.

• http://biodiver.bio.ub.es/biocat (accessed June 17 2021)

A flora and vegetation database of Catalonia. Three entries list *Dianthus caryophyllus*. No new records since 2017.

• https://kasviatlas.fi/ (replaces http://www.luomus.fi/kasviatlas/taxa.php )(accessed June 17 2021)

Database of the Finnish museum of natural history, updated to June 2020. Eight Dianthus species are listed (some with sub-species). The only relevant record is a single record of a putative hybrid between *D.barbatus* and *D.caryophyllus* from the 1970s.

• http://www.greekmountainflora.info (accessed June 17 2021)

Collection of photographs of the flora of the Greek mountain region. Search function not operating. This website will be removed from the collection of datasets.

• data.mnhn.lu (accessed June 17 2021)

Database of the natural history museum, Luxembourg. No new entries listing *Dianthus caryophyllus*. There is a single entry from 1942.

• http://filotis.itia.ntua.gr/home/ (accessed June 17 2021)

Database for the natural environment of Greece. The Filotis database lists 45 *Dianthus* species but not *Dianthus caryophyllus* or any of its synonyms.

• http://www.flora-of-cyprus.eu (accessed June 17 2021)

Flora of Cyprus. The website lists four *Dianthus* species but not *Dianthus caryophyllus* or synonyms.

• http://www.nobanis.org/search.asp (accessed June 17 2021)

European invasive plant database. No change in status for *Dianthus caryophyllus*, which is listed as non-invasive for Denmark and Norway and no status for other European countries.

- http://www.verspreidingsatlas.nl/planten (accessed June 17 2021) Database of flora of the Netherlands. Lists 11 Dianthus species, but not Dianthus caryophyllus or synonyms.
  - http://www.biolib.cz/en/main/(accessed J June 17 2021)

A Czech Republic based library of biological observations with a focus on the Czech Republic area but also multiple checklists consolidating other databases. There are *Dianthus* species in the database and observers for these were contacted for more information. The database has not been updated for *Dianthus caryophyllus* since last reviewed. The linked distribution map in **https://www.pladias.cz** shows three observations for *Dianthus caryophyllus* the most recent of which was 2004.

• http://www.wsl.ch/land/products/webflora/floramodul1-en.html (accessed June 17 2021

A web-based flora of Switzerland. Nine *Dianthus* species are listed but not *Dianthus* caryophyllus or synonyms.

• http://www.floramarittime.it (accessed June 17 20212020)

A botanical database of the plants of the Maritime Alps region of Italy. 14 *Dianthus* species are listed but not *Dianthus caryophyllus* or synonyms.

• http://www.floraweb.de/(accessed June 17 2021)

A database of vegetation of Germany. *Dianthus caryophyllus* is listed. Records are from 1950.

• http://www.maltawildplants.com/wildplants (accessed June 17 2021)

A database of the wild plants of Malta. No *Dianthus* species are listed. The website was last updated in 2018.

• http://herbarium.univie.ac.at/database/search.php (accessed June 17 2021)

A database of herbarium specimens held within Austria. The database has 10 Dianthus caryophyllus specimens from Europe, the most recent of which is 2006 (no change from last vear).

# http://alienplantsbelgium.be (accessed June 17 2021)

Manual of the alien plants of Belgium. Dianthus caryophyllus is listed as a casual introduction along with five other *Dianthus* species. Information was last updated for Dianthus caryophyllus in 2019.

# http://www.iop.krakow.pl/ias/en (accessed June 17 2021)

A data base of alien species in Poland. Three *Dianthus* species, *D. barbatus*, *D. campestris* and D. serbanii are listed.

# http://hirc.botanic.hr/fcd/Search.aspx (accessed June 17 2021)

Floral database of Croatia. 21 Dianthus species are listed including Dianthus caryophyllus. No details of collection dates are provided for this species and no photographs aside from an herbarium specimen of a red-flowered cultivated carnation. This database was last updated in 2018.

## • www.infoflora.ch (accessed June 17 2021)

The national database of the flora of Switzerland. 16 Dianthus species are listed, including Dianthus caryophyllus. The sites identified in the distribution map are all defined as naturalised and rare. Three observations have been made since 2020 – all are to be validated.

http://www.flora-mv.de/index (accessed June 17 2021)

A floristic database of the Mecklenburg-Vorpommern region of Germany. *Dianthus* caryophyllus is listed in the flora but there have been no observations after 2009.

http://www.florius.cz (accessed June 17 2021)

Catalogue of the Union of Botanical Gardens, Czech republic. No records of Dianthus caryophyllus. Four new Dianthus entries in 2021.

• http://azoresbioportal.uac.pt/azores-species/ (accessed June 17 2021)

Database of the flora of the Azores, Portugal. No *Dianthus* species are listed.

http://plants.jstor.org// (accessed June 17 2021)

A global database of herbarium specimens sortable by date and species. The most recent Dinathus caryophyllus species is from 2002 and is of a wild type, not carnation. No additions since last review.

http://flora-on.pt/(accessed June 17 2021

On -line flora of Portugal. Eight *Dianthus* species are listed (excluding sub species) but the list does not include Dianthus caryophyllus or synonyms.

https://www.cbnalpin.fr/Atlas/AtlasFlore/CartesEspeces/MenuAtlas.htm (accessed June 17 2021)

An atlas of the flora of the French alps and foothills contained within the Alpine National Botanical Conservatory (CBNA) website. The atlas has distribution maps of 15 Dianthus species, including D. caryophyllus. The distribution map on the website indicates all observations are before 1990 except one which is only defined as "after 1990" with no access to observation details.

http://dryades.units.it/trieste/ (accessed June 17 2021)

A database of the city of Trieste, Italy. Two *Dianthus* species, but not *D. caryophyllus* or synonyms.

http://dryades.units.it/casentinesi/ (accessed June 17 2021)

Portal to the flora of the National Park of the Casentinesi forests, Monte Falterona and Campigna. Six *Dianthus* species are listed, but not D. *caryophyllus* or synonyms.

• http://dryades.units.it/prealpigiulie/(accessed June 17 2021)

Portal to the flora of the Julian Pre-Alps Natural Park. Five *Dianthus* species are listed, but not D. caryophyllus or synonyms.

- http://dryades.units.it/dolomitifriulane/ (accessed June 17 2021) Portal on the flora of the Friulian Dolomites Natural Park. Four *Dianthus* species are listed, but not D. caryophyllus or synonyms..
- http://www.prirodoslovni.com/inventarna/en/.(accessed June 17 2021) The online collections database of Natural History Museum Rijeka, Croatia. Specimens from 17 Dianthus species are listed, but not D. caryophyllus. Samples of Dianthus sp. are herbarium specimens dated before 2007.
  - http://www.species.be/nl/home.php (accessed June 17 2021)

A Belgium species list. Five *Dianthus* species are listed, but not *D.caryophyllus* or synonyms.

- https://www.pifh.fr/donnees/recherche par plante (accessed *June 17 2021*) A floral database of the région Auvergne-Rhône Alpes, France. The site does not provide photographs or collection dates but geolocation only. There are 27 Dianthus species and hybrids in the inventory, including Dianthus caryophyllus.
- http://dryades.units.it/triglav ita/ (accessed June 15, 2021) Portal on the flora of the Triglav National Park (Slovenia). Five Dianthus species are listed, but not D. caryophyllus or synonyms.
- http://dryades.units.it/udine/ (accessed June 15, 2021) Portal on the flora of the City of Udine. Three *Dianthus* species are listed, but not *D*. caryophyllus or synonyms.
- http://dryades.units.it/euganei/(accessed June 15, 2021) Information system on the vascular flora of the Euganean Hills. Four *Dianthus* species are listed, but not D. caryophyllus or synonyms.
- http://dryades.units.it/valerio/(accessed June 15, 2021) Information system on the vascular flora of Monte Valerio (Trieste). Two *Dianthus* species are listed, but not D. caryophyllus or synonyms.
- http://siflore.fcbn.fr (accessed June 15, 2021) Database within the National Botanical Conservatories of France www.fcbn.fr). Dianthus caryophyllus observations are listed since 2000. most recent observation is from 2014.
- http://daten.bayernflora.de/de/info pflanzen (accessed June 15 2021) The Botanischer Informationsknoten Bayern (Flora of Bavaria, Germany). There are no new (post July 2020) records of *Dianthus caryophyllus* or its synonyms.

#### 7.3. New databases

Thirteen new sites were identified. These will be included in future monitoring reports.

http://dryades.units.it/floritaly/index.php (accessed August 7 2021)

A consolidated flora of Italy. Dianthus caryophyllus is not listed but a synonym list against all Dianthus species indicates four species have synonyms to Dianthus caryophyllus sub species;

Dianthus caryophyllus L. subsp. gasparrinii (Guss.) Arcang. = Dianthus gasparrinii Guss.

Dianthus caryophyllus L. subsp. longicaulis (Ten.) Arcang. = Dianthus longicaulis Ten.

Dianthus caryophyllus L. subsp. virgatus (Pasq.) Arcang. = Dianthus virgatus Pasq.

Dianthus caryophyllus L. var. longicaulis (Ten.) Fiori = Dianthus longicaulis Ten.

# • https://pladias.cz/en/ (accessed August 7 2021)

PLADIAS; Database of the Czech flora and vegetation.

*Dianthus caryophyllus* is listed in the checklist. The most recent of the three observations is from 2004.

# • https://maps.biodiversityireland.ie (accessed June 21 2021)

National Biodiversity Data Centre of Ireland. There is a record for *Dianthus caryophyllus* from 2013 and details of the observation were sought. The plant was a wild type *Dianthus*.

# • https://plantnet.org(accessed June 15)

The *Pl@ntNet* project is a worldwide citizen science-based application for registration of plant observations. At the URL https://identify.plantnet.org/ there are nearly 3,000 images classified as "*Dianthus caryopyllus*" many of which are misclassifications of different *Dianthus* species and many of which are stock images of carnation in cultivation or as cut flowers. There were no photographs of the transgenic carnation varieties. The website has limited utility compared to **i-naturalist** (see immediately below).

# • https://www.inaturalist.org/observations (accessed June 18 2021)

A global database for citizen scientist observations. A powerful tool with date, location, and photograph of all observations. None of the images (no date constraint) for the genus *Dianthus* were of transgenic carnation. Since 2020 there have been 16 observations for *Dianthus caryophyllus* (including five synonyms). 11 showed five petal wild type flowers and 5 cultivated, non-transgenic, carnation.

# • https://nature.silene.eu (accessed June 17 2021)

Flora and Fauna of the Provence-Alpes-Côte d'Azur. The dataset includes *Dianthus caryophyllus* and includes observations from 2020. There are no means to access the observers or photographs of the observations.

# • https://easin.jrc.ec.europa.eu (accessed June 18 2021)

European Alien Species Information Network. Provides search function with location. A single entry for *Dianthus caryophyllus* was identified for the period in 2020 – 2021. The photograph linked to the observation was of a five-petal wild type flower.

- http://dryades.units.it/Roma (accessed June 15, 2021)
- Portal to the flora of the City of Rome. Three *Dianthus* species are listed, but not *D. caryophyllus* or synonyms.
  - http://euroveg.org (accessed June 21 2021)

The website of the European vegetation survey. The checklist within the site at https://www.synbiosys.alterra.nl/evc/ provides a search function of diagnostic species that includes many *Dianthus* species but not *Dianthus caryophyllus*. *Dianthus arrostii*, a synonym, is listed as a diagnostic species in the vegetation type "Siculo-Calabrian oromediterranean and upper mesomediterranean pulvinate scrub and related grasslands on siliceous substrates"

- http://portal.cybertaxonomy.org/flora-greece/intro (accessed June 21 2021)
  The vascular Plants Checklist of Greece. The web version of the flora is regularly updated.
  There are over 100 Dianthus species described but not Dianthus caryophyllus or synonyms.
  - RJB colecciones www. csic.es (accessed June 27 2021)

Website of the herbarium of the royal botanic garden, Madrid. Multiple sub-species of *Dianthus caryophyllus* are in the database. The most recent accession is from 1997.

• https://elurikkus.ee (accessed August 7 2021)

Estonia biodiversity database. The four records for *Dianthus caryophyllus* are historical herbarium accessions of the wild species.

• https:// ottluuk.github.io (accessed August 7 2021)

Estonian atlas of vascular plants complied by the society for the protection of heritage communities, the institute of agriculture and environment and the Estonian university of life sciences. Eight *Dianthus* species are mapped but not *Dianthus caryophyllus* or synonyms.