Unauthorised GM garden petunia varieties with orange flowers

COGEM advice CGM/170522-04

Summary

The Finnish authorities recently discovered that several of the orange coloured garden petunia varieties present on the Finnish market are genetically modified (GM). No GM garden petunia varieties have been authorised for cultivation, import, distribution or retail in the European Union. The GM petunia varieties (cuttings and seeds) detected in Finland originated from companies in the Netherlands and Germany. The companies involved have terminated all sales of the GM orange petunia varieties and withdrawn the petunia plants and seeds from garden centers and nurseries.

Upon the alert of the Finnish Food Safety Authority (Evira), the Dutch Human Environment and Transport Inspectorate (Inspectie Leefomgeving en Transport, ILT) launched an investigation and submitted to COGEM a request for advice on the possible impact of the unauthorised release of the GM petunias on the environment. In addition, ILT asked COGEM to advise on suitable methods for destruction of unauthorised GM petunias.

*The garden petunia (*Petunia hybrida*) is one of the most popular bedding plants throughout the world. The garden petunia is a so-called tender perennial, which is grown as an annual in many climate zones. The garden petunia may fertilise other garden petunia varieties or close relatives present in Europa. Garden petunia seedlings are observed at locations were they have been planted, but the majority of these reseeded garden petunias will not survive winter time. Garden petunias have no invasive or weedy characteristics and appear to be unable to establish and form self-sustaining populations in Europe.*

There are no reasons to assume that the transgenic traits (altered flower colour and antibiotic resistance) introduced in the GM garden petunias alter the fitness of the garden petunia or its relatives. Even though over a million of GM orange garden petunias have been sold, COGEM is not aware of any reports on petunia populations with orange flowers in Europa. In view of the above, and based on the information currently available, COGEM is of the opinion that GM garden petunias with an altered flower colour pose a negligible risk to humans and the environment.

The present COGEM advice is based on the information which is currently available on the unauthorised GM petunias. At this moment the origin of the GM petunias is not known and detailed information on the inserted elements is not yet available. When more detailed information becomes available, COGEM will update its advice.

1. Introduction

The Finnish authorities recently discovered that several of the orange coloured garden petunia varieties present on the Finnish market are genetically modified (GM) (i.e. Pegasus Orange Morn, Pegasus Orange, Pegasus Table Orange, Potunia Plus Papaya, Go!Tunia Orange, Bonnie Orange, Sanguna Patio Salmon, Sanguna Salmon and African Sunset).[1] No GM garden petunia varieties have been authorised for cultivation, import, distribution or retail in the European Union. The GM petunia varieties (cuttings and seeds) detected in Finland originated from companies in the Netherlands and Germany.[1]

Upon the alert of the Finnish Food Safety Authority (Evira), the Dutch Human Environment and Transport Inspectorate (Inspectie Leefomgeving en Transport, ILT) launched an investigation and commissioned RIKILT to analyse petunia varieties with orange flowers. RIKILT analysed the suspected petunia varieties by PCR with primer combinations[2,3,4] that cover the same genomic regions as targeted by Evira (P-35S, P-nos, NptII), other primer combinations[3] (T-CaMV 35S, T-OCS), as well as positive and negative controls. RIKILT confirmed that several orange petunia varieties (Go!Tunia Orange, Sanguna Salmon, Viva Orange, Viva Fire, Viva Orange Vein and African Sunset) are genetically modified.[5] For two of the analysed orange petunia varieties (Cascadias Indian Summer and Sanguna Patio Salmon) no transgenic elements were detected using the above mentioned primer combinations.

The companies involved have terminated all sales of the GM orange petunia varieties and have set out to withdraw the petunia plants and seeds from garden centers and nurseries.[5]

The ILT submitted to COGEM a request for advice on the possible impact of the unauthorised release of the GM petunias on the environment. In addition, ILT asked COGEM to advise on suitable methods for destruction of unauthorised GM petunias.

The present COGEM advice is based on the information which is currently (May 22nd, 2017) available on the unauthorised GM petunias. When more detailed information becomes available, COGEM will update its advice.

2. The genus *Petunia* and *Petunia hybrida*

The genus *Petunia* is native to South America (Brazil, Argentina and Uruguay) and belongs to the family of the Solanaceae.[6] *Petunia* species (*2n*=14) have a base chromosome number of x=7, unlike most species belonging to the Solanaceae which have a typical x=12 base chromosome number.[7]

The taxonomy of the genus *Petunia* has been subject to changes. Phylogenetic reconstruction of the 20 taxa in the genus revealed that it consists of two groups. The first group includes species (a.o. *Petunia integrifolia* and *Petunia inflata)* with purple flowers with a short corolla tube (except for *Petunia occidentalis* which has long corolla tubes). The species in this group are self-incompatible

and most likely exclusively pollinated by bees. The species in the second group (a.o. *Petunia axillaris*) have long corolla tube flowers with different corolla shapes and colors. Most plants in this group are self-compatible (except for some *P. axillaris* lineages) and pollinated by either moths, birds or bees.[6]

*2.1. The garden petunia (***Petunia hybrida***)*

The garden petunia (*Petunia hybrida* or *Petunia x atkinsiana*) is an ornamental plant and not used as food or feed. It is one of the most popular bedding plants throughout the world. In 2013, the garden petunia made the top 5 of bedding plants (11 million plants) sold by one of the largest international flowers and plants marketplaces of the world, Royal FloraHolland.[8,9]. Commercial varieties are either propagated by seed or vegetatively propagated.[10]

The garden petunia presumably originated from interspecific crosses between species of the different groups within *Petunia*, i.e. between *P. axillaris* and species of the *P. integrifolia* clade (*P. integrifolia* ssp. *integrifolia*, *P. inflata* or *Petunia interior*).[7,11] The first hybrids were produced by European horticulturalists in the early nineteenth century (ca. 1830). Hybrids were probably produced multiple times from different accessions of the two parental clades.[7,11]

The garden petunia is a so-called tender perennial, which is grown as an annual in many climate zones.[12] The garden petunia grows at daytime temperatures of 15°C to 25°C.[13] It does not like wet conditions[19] and is sensitive to frost[14,15], but may survive mild winters. It has been reported that at -5°C 50% of petunia plants die due to freezing injury. Gradual cold acclimation from 15°C to 3°C can decrease the temperature at which 50% of the petunia plants die to -6°C.[16,17]

The garden petunia can be planted in gardens after the last spring frost[19] and blooms from spring to fall. The majority of petunia varieties are self-compatible, but self-incompatible petunia varieties exist as well.[18] Cross-pollination occurs via insect pollination.

Many garden petunia varieties easily set seeds. After the petunia flower falls to the ground, the calyx begins to swell and turns brown. After a couple of days, it splits open and the seeds fall out.[19] Seeds from garden petunias can be collected and used to rear petunias the next growing season.
Seeds germinate within 7 to 10 days (at 21°C to 29°C). Ten to twelve weeks after sowing, the plants can be planted in the garden.[19] Although it is feasible to rear garden petunias from self-collected seed, it is easier to grow them from transplants.[19]

Although garden petunias have been cultivated throughout Europe since the early nineteenth century, feral populations appear to be extremely rare. In some of the European flora the garden petunia is mentioned as a casual or neophyte.[20,21,22,23] The garden petunia (*P. hybrida*) is mentioned as a rare species on the list of vascular plants of the Netherlands.[24] The garden petunia may reseed itself and seedlings are observed at locations were they have been planted.[25] In addition, there are a few exceptional reports of garden petunias that seem to have survived the winter in the Netherlands.[25]

Although there are occasional reports of *P. hybrida* in the Netherlands and other countries in Northwestern Europe,[21,22,24] it is most likely that these are reseeded garden petunias of which the majority will not survive wintertime. The garden petunia is not able to establish and form self-sustaining populations in Northwestern Europe.

No self-sustaining feral populations have been reported in Southern Europe. Garden petunias have been observed outside gardens,[23] but it is unclear whether the observed plants are seedlings that die out or plants that have established.

2.2. Relatives of the garden petunia

Other *Petunia* species, such as *P. integrifolia* (syn. *Petunia violacea*) and *P. axillaris* (syn. *Petunia nyctaginiflora*), are occasionally planted in gardens.[26,27,28,29] *P. integrifolia* is mentioned as a neophyte in Germany[30] and it has been reported as locally naturalised in Southern Europe.[31] It is unclear whether these reports refer to recurrent introductions of spontaneous seedlings from *P. integrifolia* garden plants or to the establishment of *P. integrifolia*.

3. Hybridisation

3.1 Hybridisation within the genus Petunia

The species within the *Petunia* genus have preserved their intercrossing ability, at least under experimental conditions. *Petunia exserta* (at least when used as a seed parent) and *P. axillaris* subsp. *axillaris* are cross-compatible with all *Petunia* taxa that were investigated except *P. occidentalis.*[32,33]

Despite the lack of intrinsic barriers to crossing between most of the *Petunia* species, hybrids in nature are rare. Natural interspecific hybrids have only been described between *P. exserta* and *P. axillaris* ssp. *axillaris* and between two subspecies of *P. axillaris.*[6]

P. axillaris and *P. integrifolia* can be crossed artificially and produce fertile hybrids capable of setting abundant capsules filled with viable seeds.[34] Although there are at least four native populations in which *P. axillaris* and *P. integrifolia* occur together, no natural hybrids have been detected.[34]

3.2. Hybridisation with closely related genera

The genus most closely related to *Petunia* is *Calibrachoa*. The species within these two genera have different chromosome numbers: for *Petunia 2n*=14 and for *Calibrachoa 2n*=18. The species within *Calibrachoa* used to belong to *Petunia*, but were transferred to a separate genus. Over two hundred populations composed of *Petunia* and *Calibrachoa* species are known, but no intergeneric hybrids were observed in these populations even though the same insect species (*Leioproctus* subgen. *Hexantheda* sp.) acts as a pollinator for both *P. integrifolia* and *Calibrachoa heterophylla.*[34] Crossing experiments confirmed that the two genera cannot hybridise. *P. axillaris* and *P. integrifolia* have been crossed with *Calibrachoa parviflora* and *C. heterophylla*, but no capsules were formed.[34] *P. axillaris* nor *P. exserta* could be successfully crossed with any species of *Calibrachoa*.[32,33]

4. Unauthorised GM garden petunias

Although the exact make-up of the GM garden petunias is not known at the time of this advice, it can be assumed that a transgene was inserted in the GM petunias that allowed the production of the pelargonidin pigment. At this moment, it is assumed that the GM petunia varieties contain the *DFR* gene of maize (also known as the *A1* gene).[35] Besides the transgene(s) involved in flower pigmentation, the GM petunias contain the antibiotic resistance gene *nptII*.

4.1. Development of petunias with modified flower colours

Pelargonidin is one of the three anthocyanin pigments (pelargonidin, cyanidin and delphinidin) and gives rise to orange-red flowers. It is formed from its precursor dihydrokaempferol (DHK) by the dihydroflavonol-4-reductase (DFR) enzyme. There are slight differences between DFR enzymes from different plants species. The natural DFR enzyme of petunia, for example, does not utilize DHK as a precursor and is therefore unable to synthesize pelargonidin. This is why regular petunias do not produce orange-red flowers.[36,37]

In 1987, German scientists produced the first genetically modified petunias by inserting the *DFR* gene from maize (*Zea mays*). These GM petunias accumulated DHK (owing to the deficiency of the *F3'H*, *F3'5'H* and *FLS* genes)[38] and it appears that besides the presence of the maize *DFR* gene, a DHK-accumulating background is required to synthesize sufficient amounts of pelargonidin. Accumulation of DHK is observed in natural mutants, but may also be achieved by genetic modification.

The flowers of these GM petunias were pale brick-red, and showed a variable pigmentation.[39] When different transgenic petunia lines containing the maize *DFR* gene were crossed with elite breeding material this resulted in stable bright-orange petunia cultivars with commercial potential.[36]

Other research groups in Finland and Japan introduced *DFR* genes from ornamental plants, such as gerbera and rose, into petunia, which resulted in intense and stable brick-red/orange petunias.[40,41,42,43]

COGEM notes that the investigation in the EU on the unauthorised release of GM petunias focuses on petunia varieties with orange flowers. These petunia varieties have been sold worldwide. It is currently unknown what the origin of the GM petunias is, therefore it cannot be ruled out that the GM petunias were used as breeding material to produce garden petunia varieties with different flower colours.[44] Noteworthy is that the U.S. Department of Agriculture's Animal and Plant Health Inspection (APHIS) states on its website that the GM petunia varieties involved produce various hues of orange, red and purple coloured flowers.[45,46] This opens the possibility that more commercial petunia varieties are genetically modified as currently envisaged.

4.2 Field experiments/releases of GM petunias

In Germany, over 30,000 transgenic petunia plants carrying a single copy of the maize *DFR* gene were planted in a field trial carried out by the Max Planck institute.[39] In 1996 and 1997, this GM petunia line was authorised in Germany for deliberate release in demonstration gardens with a small number of plants (20).[47,48,49,50]

In the United States there have been several applications for introduction of GM petunias. Since 1989, 88 applications concerning GM petunias have been filed. At least 23 of them are GM petunias with (possible) altered flower colour, two of which involve GM petunias with a maize *DFR* gene.[51] Field experiments with orange GM petunias have been carried out in the early nineties.[36]

In China, a GM petunia variety is commercially available. This GM variety developed by Beijing University expresses the *CHS* gene resulting in white pigmentation in flowers.[52,53]

5. Environmental risk assessment

The garden petunia (*P. hybrida*) is a very popular bedding plant, which has been cultivated since the early nineteenth century. It is a so-called tender perennial, which is grown as an annual in many climate zones. The garden petunia may reseed itself and seedlings are observed at locations were garden petunias have been planted. Garden petunias are sensitive to frost and only rare individuals survive the winter. The garden petunia has no invasive or weedy characteristics. The garden petunia is not able to establish and form self-sustaining populations in Northwestern Europe.

No self-sustaining feral populations have been reported in Southern Europe. Although garden petunias have been observed outside gardens in Southern Europe, it is unclear whether the observed plants are seedlings that die out or plants that have established.

Besides the garden petunia there are also some other *Petunia* species which are planted in gardens. In addition, *P. integrifolia (*an ancestral species of *P. hybrida*) is listed as a neophyte in Germany and it has been reported as locally naturalised in Southern Europe.

There are different traits, such as the size, scent, shape and colour of flowers that affect the attractiveness of flowers to pollinators.[54] As different pollinator species have a preference for different flower colours,[54] the orange colour of the petunia flowers may influence their attractiveness to pollinators and consequently the chance that the GM petunia varieties fertilise other garden petunias. In this environmental risk assessment, it is assumed that the GM garden petunias are able to fertilise other garden petunia varieties or close relatives present in Europe independent of their flower colour. Garden petunias and its seedlings are sensitive to frost, but in an exceptional case, a rare GM garden petunia or a GM seedling may survive the winter.

There are many species of plants other than petunia which have orange flowers and produce the pelargonidin pigment present in the GM petunias by nature. There are no indications that flower colour influences the fitness or weediness potential of these plants. Since the beginning of this decade, over a million of GM petunias with orange flowers have been sold in Europe. Despite the

large numbers of plants sold, there are no reports on petunia populations with orange flowers in Europe.

The GM petunias contain the *nptII* gene which confers resistance to aminoglycoside antibiotics, such as kanamycin and neomycin. The *nptII* gene is widely present in naturally occurring microorganisms. The chance of so-called horizontal gene transfer (HGT) between plants and bacteria is difficult to estimate, but the likelihood is extremely low. HGT has not been detected under field conditions.[55,56] The use of kanamycin and neomycine in clinical practice has been largely reduced, and is now mostly limited to veterinary practice. In 1998 and 2000, COGEM concluded that the presence of *nptII* genes in transgenic plants poses a negligible risk to humans and the environment.[57,58]

In 2004, EFSA also concluded that use of the *nptII* antibiotic resistance gene as marker gene in GM plants does not pose a risk to the environment or to human and animal health.[59] EFSA reconfirmed its conclusion on the safe use of *nptII* genes in GM plants in a statement published in 2007.[60]

6. Conclusion

Garden petunias have no invasive or weedy characteristics and appear to be unable to establish and form self-sustaining populations in Europe. There are no reasons to assume that the transgenic traits (altered flower colour and antibiotic resistance) introduced in the GM garden petunias alter the fitness of the garden petunia or its relatives. Even though over a million of GM orange garden petunias have been sold, COGEM is not aware of any reports on petunia populations with orange flowers in Europe. In view of the above, and based on the information currently available, COGEM is of the opinion that GM garden petunias with an altered flower colour pose a negligible risk to humans and the environment.

COGEM points out that GM petunias with orange flowers have been developed by several groups in various countries using different approaches. At this moment the origin of the GM petunias is not known and detailed information on the inserted elements is not yet available.

ILT commissioned further molecular analyses of the GM petunias. As soon as more detailed information on the inserted transgenic elements becomes available, COGEM will update its advice and reconsider whether its conclusion that the GM petunias pose a negligible risk to the environment, remains valid.

7. Destruction of unauthorised GM garden petunias

ILT asked COGEM to provide advice on the destruction of GM garden petunias. Destruction of the GM garden petunias is necessary because they are illegal in Europe. A document providing guidance on the destruction of GM petunias was recently published by APHIS.[61]

There are several methods that can be used to destroy plants such as freezing, incineration, drying out, cutting of the plant crown, the application of herbicides etc. In this advice, COGEM assumes

that a large number of plants have to be destroyed in a short time. Genetically modified organisms are usually killed by autoclaving or incinerating. Autoclaving is, however, only feasible for limited amounts of plant material.

There are several other methods such as the application of herbicides or composting, that kill large numbers of plants efficiently. The presence of seeds in plant material is, however, a factor complicating the destruction of plant material. Herbicides do not affect seeds and composting only kills seeds if the temperatures are sufficiently high over a longer period. In the Netherlands, industrial producers of compost have drawn up criteria for certified compost (so-called Keurcompost). One of these criteria is that the temperature in the compost heap has to reach 60°C for a minimum of two periods lasting at least three continuous days.[62] This is sufficient to kill seeds of weeds.[63]

In view of this, COGEM advises to destruct the GM garden petunias by incineration or industrial composting following the procedures for certified compost. If the GM petunia plant material does not contain seeds, herbicide treatments can also be used to destroy the plant material.

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