Adv. Hort. Sci., 2020 34(1S): 13-20 DOI: 10.13128/ahsc-7444

AHS
Advances in Horticultural Science

Postharvest aptitude of *Begonia semperflorens* and *Viola cornuta* edible flowers

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Key words: anthocyanins, antioxidant activity, fresh cut flowers, polyphenols, postproduction, pot plants, shelf-life.

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Citation:
DEMASI S., FALLA N.M., CASER M., SCARIOT V., 2020 - *Postharvest aptitude of Begonia semperflorens and Viola cornuta edible flowers* - Adv. Hort. Sci., 34(1S): 13-20

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 13 November 2019
Accepted for publication 4 May 2020

Abstract: The edible flowers are sold as pot plants or fresh cut produce and are attracting interest recently thanks not only to their organoleptic characteristics but also to their content in bioactive molecules. However, there is little information about the variations that these characteristics undergo during postharvest. In this study, the productivity and longevity of *Begonia x semperflorens-cultorum* Hort. and *Viola cornuta* L. pot plants were evaluated in an interior environment simulating the house conditions. Besides, the effect of cold storage (4°C) was evaluated on the aesthetic quality and the bioactive compounds content (total polyphenols, total anthocyanins, antioxidant activity through FRAP assay) of *B. semperflorens* and *V. cornuta* fresh cut flowers, using two different packaging, modelling a plastic box or a flowpack. The results suggest that *V. cornuta* could be a better choice for retailers because of its longer shelf life and better maintenance of its content in bioactive compounds, especially in the flowpack packaging. Conversely, *B. semperflorens* could be more suitable as pot plant, showing more adaptability and flower production in a domestic environment.

1. Introduction

Numerous flowers have been used in culinary arts since ancient times both in Europe, including *Rosa* L. spp., *Calendula officinalis* L., *Viola* spp., and *Taraxacum officinale* F.H. Wigg (Mlcek and Rop, 2011; Grzeszczuk *et al*., 2016; Fernandes *et al.*, 2017; Scariot *et al*., 2018), and in Asia and South-America, such as *Begonia* spp. (Laferrière, 1992; Basurto-Peña *et al*., 2003; Zheng *et al*., 2018). Nowadays, edible flowers are horticultural niche products, sold as pot plant or as fresh cut flowers, with increasing appeal for the food industry due to their organoleptic and healthy properties (Kaisoon *et al.*, 2012; Grzeszczuk *et al*., 2016; Lu *et al.*, 2016).

Edible flowers improve the sensorial qualities of food by adding colour, fragrance, flavour and visual appeal to culinary preparations (Kelley *et al*., 2001a; Mlcek and Rop, 2011; Koike *et al.*, 2015).

In the third millennium, several studies revealed the chemical composition of many wild and cultivated flowers, highlighting the presence of

important bioactive compounds, such as carotenoids and phenolics (Lu *et al.*, 2016). These phytochemicals with antioxidant activity are very important for plants, since they inhibit their natural senescence process, mainly caused by the presence of reactive oxygen species (ROS) (Mlcek and Rop, 2011). During metabolism ROS and other free radicals are produced in human body too, normally inactivated by an endogenous antioxidant system (Mlcek and Rop, 2011; Loizzo *et al.*, 2016). However, under stress conditions, in high load situations, because of lifestyle or pathological situations, these free radicals can accumulate, generating oxidative stress (Loizzo *et al.*, 2016) by reacting and damaging all types of biomolecules such as lipids, proteins, carbohydrates, and DNA (Kaisoon *et al.*, 2012). If damaged DNA is left unrepaired, it may become cancerous (Mlcek and Rop, 2011; Kaisoon *et al.*, 2012; Li *et al.*, 2014). Thus, a diet rich in antioxidants, which can scavenge free radicals, can reduce the oxidative stress and may be a strategy to prevent some chronic conditions (Kaisoon *et al.*, 2012; Loizzo *et al.*, 2016; Lu *et al*., 2016). Epidemiological data showed that dietary patterns were significantly associated with the prevention of these chronic diseases, especially when rich in antioxidants (Kaisoon *et al.*, 2012), including carotenoids and phenolics (Koike *et al*., 2015; Grzeszczuk *et al*., 2016).

As a result of the increased knowledge of the edible flowers' properties, the consumers' demand of this kind of product is increasing worldwide (Fernandes *et al*., 2017; Pires *et al*., 2019; Falla *et al*., 2020), thanks to the increased attention to the quality of foodstuffs and to the content of individual compounds (Rop *et al.*, 2012; Lu *et al*., 2016).

However, edible flowers are highly perishable and have a short shelf life (petal abscission and discoloration, flower wilt, dehydration, and tissue browning start to appear 2-5 days after harvest), which limits their marketability (Koike *et al.*, 2015; Fernandes *et al.*, 2018). Fresh cut edible flowers are typically packaged in small, rigid, plastic boxes, in order to protect them from desiccation and to preserve their frail structure (Kelley *et al*., 2003; Kou *et al.*, 2012).

It is noteworthy that consumers eat with their eyes well before they taste with their mouths, thus it is important to maintain the visual appeal of a flower on market; quality is essential: consumers want more varieties of top quality plants with a longer shelf life (Kelley *et al.*, 2001 b). Nevertheless, edible flower's postproduction technology still receives less attention than that of other horticultural products, such as vegetables and fruits, because edible flowers' production is still low and it is a niche market (Fernandes *et al.*, 2018).

Temperature is usually the most important environmental factor limiting shelf life of horticultural products (Kelley *et al*., 2003): both respiration and transpiration processes are considered as the major causes of postharvest losses and poor quality in produce. Thereby, controlling temperature of storage is very important since these factors directly influence the two metabolic processes mentioned above, extending the product's shelf life (Flores-López *et al.*, 2016).

A flower's short shelf life may cause not only a rapid decrease in visual quality, but also a rapid loss of its nutraceutical compounds, however very few articles reported the effects of storage on quality of edible flowers, and even less investigated these effects on their nutraceutical compounds (Landi *et al.*, 2015). Therefore, it would be interesting to deepen the knowledge on whether the loss of nutraceutical compounds in edible flowers during storage occurs more or less quickly than the loss of visual quality. This information could promote the consumption of edible flowers at visual quality levels less than perfect, with minor flaws (Kelley *et al.*, 2001 b).

Thus, the aim of this work was to evaluate two common edible flowers' species (*Begonia x semperflorens-cultorum* Hort., commonly referred to as *Begonia semperflorens*, and *Viola cornuta* L.) as 1) pot plants, by evaluating the productivity and longevity in an interior environment simulating the domestic conditions; and 2) fresh cut flowers by evaluating the shelf life and the content in biologically-active compounds (total polyphenols, anthocyanins) and antioxidant activity, when stored at 4°C, testing two types of packaging (a plastic box closed with its own lid or closed with a plastic film in a flowpack).

2. Materials and Methods

Pot plant postproduction

The potted flowering plants of *Begonia semperflorens* (10 plants) and *Viola cornuta* (36 plants) were obtained from the nursery Fratelli Gramaglia (Collegno, Italy; 45°05'22.4'' N, 7°34'26.4'' E, 302 m a.s.l.).

Plants were kept at room temperature (about 20°C), in a peat-based substrate, hand-watered when needed, throughout the harvest period: 30 days for begonias and 11 days for violas. Every two days

opened flowers were harvested and weighed to evaluate the flowering longevity and productivity. For each plant, the number of flowers produced was counted and the weight of flowers suitable to be consumed (opened flowers in good visual conditions) was calculated: namely each plant's productivity.

Fresh cut flower postharvest

Fresh flowers harvested when fully open and in good visual conditions were put into plastic packages (Ondipack 250 cc, 123x114x50 mm, polypropylene, Plemet, France), 5 g of flowers for each box (Fig. 1).

Two packaging methods were assessed, for both species:

- Plastic box closed with its own plastic lid (abbr. PP; 8.96 g);
- Plastic box without its lid, inserted into a bi-oriented polypropylene plastic film (abbr. BOPP; 6.04 g).

The plastic films were folded and closed on three sides through heat-sealing (FR400, Ferplast, Cuneo, Italy), modelling a flowpack.

Fig. 1 - Flowers of *V. cornuta* (A) and *B. semperflorens* (B) freshly harvested and put in the plastic package.

Flowers were stored at 4°C, in refrigerators with a glass door [Fiocchetti fridge, Luzzara (RE), Italy], simulating markets' shelves conditions, with four repetitions per packaging type.

Every two days, each package was weighed, in order to obtain data about the flowers' weight variation.

The visual appeal of flowers was scored on a 9-point scale based on visual observation of the degree of decay (Aquino-Bolaños *et al.*, 2013; Landi *et al*., 2018), where 9 was assigned to flowers without imperfections, 5 was the limit of marketability of the product (the limit of acceptability for the consumer), while 1 was the value of a decomposing flower, at the end of its life cycle.

Ultrasound extraction

At the beginning of the trial, so that the day of harvest (t0) could be represented, about 5 g of flowers of both *B. semperflorens* and *V. cornuta* were collected from pot plants; the same was done after storage, when the flowers reached grade 5 of the visual scale. One of the four packages stored per method was taken and the 5 g of flowers contained in it were stored at -80°C until analysis. Flower samples were grinded with liquid nitrogen, then 0.5 g of grinded plant material were put into a glass tube, to which 25 ml of a 50% aqueous MeOH (methanol) solution were added. Three repetitions were carried out for each sample. The tubes were put into the ultrasound extractor (23 kHz, Reussarl, Drap, France) for 15 minutes at room temperature. The obtained phyto extract was filtered with paper filters (Whatman filter papers No. 1, Whatman, Maidstone, UK) and the obtained solution was stored at -20°C for further analysis.

Total polyphenols

The total phenolic content was determined following the Folin-Ciocalteu method (Singleton *et al*., 1999).

The analysis was performed as follows: 750 µl of diluted 1:10 Folin reagent were mixed with 150 µl of phytoextract and 600 µl of Na_2CO_3 (7.5%) in each plastic tube. Samples were left in the dark at room temperature for 30 minutes. Absorbance was measured at 765 nm by means of a spectrophotometer (Agilent Technologies, Cary 60 UV-Vis, Santa Clara, CA, United States), and the results were expressed in milligrams of gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g FW).

Total anthocyanins

The total anthocyanin content in the extracts was determined through the pH-differential method as indicated by Lee *et al*. (2005) and Giusti and Wrolstad (2005).

The analysis was performed as follows: 1 ml of phytoextract was put into a 10 ml flask, and then made up to volume with an aqueous buffer solution at pH 1 (KCl and HCl). The same was made in a second flask with an aqueous buffer solution at pH 4.5 ($C_2H_3NaO_2$and $C_2H_4O_2$). Samples were put in the dark at room temperature for 20 minutes. Absorbance of both flasks was measured at 515 nm and 700 nm by means of a spectrophotometer (Agilent Technologies, Cary 60 UV-Vis, Santa Clara, CA, United States), and the results were expressed in milligrams of cyanidin-3-O-glucoside per 100 grams of fresh weight (mg C3G/100 g FW).

Antioxidant activity - FRAP assay

The method used to evaluate the antioxidant activity is the FRAP (Ferric ion Reducing Antioxidant Power) assay as indicated by Benzie and Strain (1996).

The antioxidant activity was determined mixing 30 µl of phytoextract with 90 µl of deionised water and 900 µl of FRAP reagent. The samples were then placed at 37°C for 30 minutes. Absorbance was measured at 595 nm by means of a spectrophotometer (Agilent Technologies, Cary 60 UV-Vis, Santa Clara, CA, United States). Results were expressed as millimoles of ferrous iron equivalents per kilogram of fresh weight (mmol Fe^{2+}/kg FW).

Statistical analysis

All data were subjected to the statistical analysis for the homogeneity of variance (Levene test).

Weight variations were compared using a one-way ANOVA test.

Mean comparisons between data obtained from the two different packages during postharvest were performed using an independent samples t-test, by means of the SPSS 25 software (version 25.0; SPSS Inc., Chicago. Illinois).

3. Results

Pot plant productivity

Begonia semperflorens and *V. cornuta* pot plants showed differences in the number of flowers produced over time. On average, from the day of arrival at the laboratory, plants of *B. semperflorens* produced flowers for 30 days (with an initial production of about 38 flowers per plant, and a final production of 1-2 flowers per plant) (Fig. 2), with an average total productivity of 11 flowers per plant per day. Pot plants of *V. cornuta* bloomed for 11 days (starting with an average of 21 flowers per plant up to 3-4 flowers per plant) (Fig. 2), with an average total productivity of 9 flowers per plant per day.

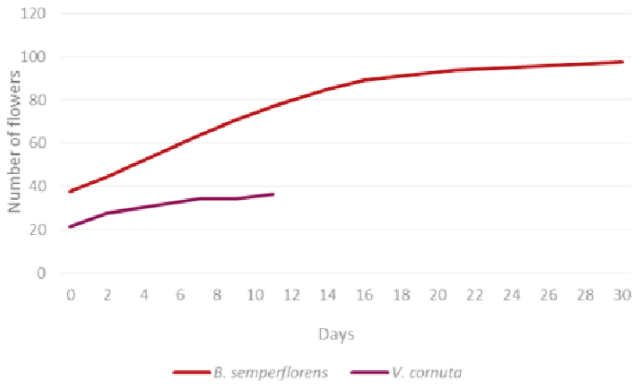

Fig. 2 - Flower production of *B. semperflorens* (red) and *V. cornuta* (purple). Data are shown in a cumulative curve.

Cut flower shelf life

As shown in figure 3, the shelf life of *B. semperflorens* and *V. cornuta* assessed at 4°C is quite different: the first species reached the limit of marketability (grade 5) after 9 days (both in PP and in BOPP) (Fig. 3, red lines), while the viola flowers remained acceptable for the consumer up to two weeks (both for PP and for BOPP) (Fig. 3, purple lines).

During storage up to grade 5, the flowers did not show significant weight variations in both packaging type (Table 1).

Bioactive compounds

The total polyphenol and anthocyanin content, and the antioxidant activity (FRAP) of *B. semperflorens* and *V. cornuta*'s flowers are reported in Table 2. Values are referred to flowers freshly picked, corresponding to grade 9 (t0), and after storage at 4°C, when they reached grade 5 of the visual scale (i.e. limit of marketability), that corresponded to 9 days for begonias and 16 days for violas (Fig. 3).

Comparisons aimed to highlight flower differences between the two species and along time within the same packaging and between the two types of packaging (Table 2). Variations are visualized in figure 4. Concerning the pot plants, *V. cornuta* flowers at t0 showed higher values of both polyphenols ($p<0.001$) and antioxidant activity ($p<0.001$) than *B. semperflorens*, while this latter showed a higher content in anthocyanins ($p<0.05$) than *V. cornuta*.

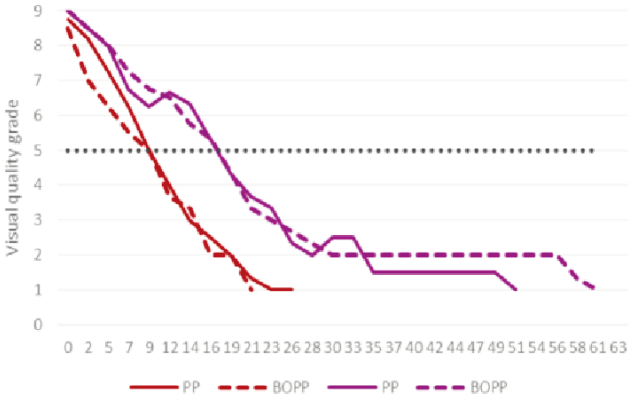

Fig. 3 - Trend of visual quality during storage for *B. semperflorens* (red) and *V. cornuta* (purple). Data are shown as mean values. The intersection of the curves with the dotted horizontal line corresponds to the marketability limit (grade 5). PP= plastic box + lid; BOPP= Plastic box without its lid, inserted into a bi-oriented polypropylene plastic film.

Regarding the bioactive compound's evaluation during the post-harvest, begonia flowers kept in PP encountered a decrease of all parameters (polyphenols: -45.78%; anthocyanins: -85.33%; antioxidant activity: -52.35%) while in BOPP only the anthocyanins decreased (-99.44%). Viola flowers kept in PP encountered an increase in anthocyanins (+202.5%) and a decrease of antioxidant activity (-34.21%) while total polyphenols were constant (Table 2). In BOPP all the parameters decreased (phenolic content -76.51%, antioxidant activity -88.05%, anthocyanins -32.52%).

Table 1 - Flower weight variation during cold storage (4°C) up to the grade of marketability

Flower species	Day	Weight (g) PP	Weight (g) BOPP
Begonia semperflorens	0	5.25	5.04
	2	5.25	5.04
	4	5.25	5.03
	7	5.24	5.18
	9	5.23	5.01
	p	NS	NS
Viola cornuta	0	5.18	5.43
	2	5.18	5.43
	4	5.14	5.44
	7	5.16	5.47
	9	5.12	5.41
	11	5.10	5.40
	14	5.17	5.38
	16	5.17	5.37
	p	NS	NS

PP= plastic box + lid; BOPP= Plastic box without its lid, inserted into a bi-oriented polypropylene plastic film). Data are shown as mean values. Comparisons between data were performed using a one-way ANOVA analysis.

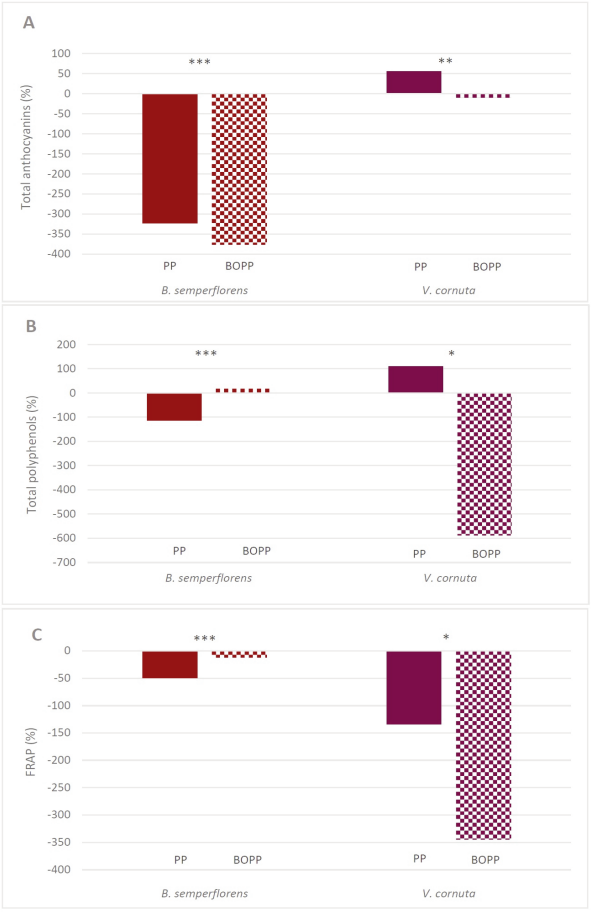

Fig. 4 - Percentage variation of the content of A) total anthocyanins, B) total polyphenols, C) antioxidant activity (FRAP) in *B. semperflorens* (red) and *V. cornuta* (purple), up to grade 5 of visual quality scale, depending on the type of packaging (stored at 4°C). The statistical analyses were made separately on *B. semperflorens* and *V. cornuta* values. Mean comparisons between data were performed using an independent samples T-test. * p≤0.05. ** p≤0.01. ***p≤0.001. Axis 0 uses the t0 value as a reference. PP= plastic box + lid; BOPP= Plastic box without its lid, inserted into a bi-oriented polypropylene plastic film.

Table 2 - Total polyphenols, total anthocyanins, and antioxidant activity (FRAP) at grade 9 (day of harvest) and grade 5 (i.e. limit of marketability, corresponding to 9 days for begonias and 14 days for violas) of visual quality scale of *B. semperflorens* and *V. cornuta* flowers stored at 4°C in two different packaging

Flower species	Packaging	Total polyphenols (mg GAE/100 g FW)			Total anthocyanins (mg C3G/100 g FW)			Antioxidant activity FRAP (mmol Fe^{2+}/kg FW)		
		Grade 9	Grade 5	*p*	Grade 9	Grade 5	*p*	Grade 9	Grade 5	*p*
Begonia semperflorens	PP	246.71	133.75	**	378.67	55.57	***	95.23	45.38	**
	BOPP	246.71	264.77	NS	378.67	2.12	***	95.23	83.49	NS
	p	-	***		-	***		-	***	
Viola cornuta	PP	767.26	877.01	NS	27.76	83.99	**	391.89	257.84	*
	BOPP	767.26	180.26	***	27.76	18.74	*	391.89	46.83	*
	p	-	*		-	**		-	*	

PP= plastic box with its own lid; BOPP= flowpack. Data are shown as mean values. * p≤0.05. ** p≤0.01. *** p≤0.001. Mean comparisons between data were performed using an independent samples T-test.

4. Discussion and Conclusions

This study aimed to evaluate the aptitude of two common edible flower species, i.e. *B. semperflorens* and *V. cornuta*, to be sold as pot plants or fresh cut flowers.

The growing conditions adopted in this study (i.e. 18-20°C and low lighting) were useful to simulate the maintenance of pot plants in a domestic environment, so to give information on productivity to the final consumer. These conditions are unlikely to be fully appropriate, especially for violas. Indeed, *V. cornuta* plants are more productive at temperatures from 4 to 10°C (Ball, 1991; Nau, 1998). Cooper and Watson (1952) noticed that flowers sizes are also bigger when plants grow at night-time temperatures of 10°C. A frequent harvest could concur to cause a general reduction in productivity and in flowers size too. In this study, where flowers were picked every 2-3 days, a change in flowers weight was observed. Begonia flowers weighted 0.5-0.6 g at the beginning of the experiment and 0.3-0.4 g at the end, while viola flower varied from 0.2 g to 0.1 g. Begonias flowers, moreover, showed petals discoloration during the last days of harvest.

Few data are available in literature about *B. semperflorens* and *V. cornuta* so that comparisons with results of other studies are difficult. Some information could be found in other congener species. Low temperature (4°C) and natural lighting during storage mimed the retailer conditions. Data about *B. semperflorens* agreed with those assessed by Friedman *et al*. (2007) in flowers of *Begonia elatior* and *B. semperflorens* that were stored in plastic trays for about ten days at 2-5°C. *Viola cornuta* shelf life was in accordance with the data found by Kelley *et al.* (2003) in *Viola wittrockiana,* that was considered marketable after two weeks of storage at 5°C. Regarding the phytochemical content, results were partially discordant with those found by Benvenuti *et al.* (2016) in *V. wittrockiana* that showed a higher antioxidant activity but also a higher anthocyanins content than *B. semperflorens*.

Results of works that evaluated the content of edible flower phytochemicals during cold storage are sometimes conflicting. Aquino-Bolaños *et al*. (2013) observed a reduction in the nutraceutical values of squash (*Cucurbita pepo* L.) edible flowers, conversely Friedman *et al*. (2007) found no differences in anthocyanins content in *B. semperflorens* flowers. Landi *et al*. (2018) analysed *B. semperflorens* flowers too, finding a general constancy in the nutraceutical values during storage. Data obtained in this study showed that the phytochemical content of *B. semperflorens* decreased during storage in the plastic box closed with its own lid, while in the flow pack the total phenolic content, and the antioxidant activity, remained constant. The best way to store flowers of *B. semperflorens* could be therefore the flowpack, preferably a perforated one to prevent condensation of vapours on their inner surface (Mlcek and Rop, 2011). Conversely, *V. cornuta* flowers seemed to better preserve its characteristics in the plastic box closed with its own lid, showing a certain constancy in polyphenols and antioxidant activity, and a significantly increased level of anthocyanins, while in the flow-pack showed a significant reduction in all three parameters.

In conclusion, our data confirm that *B. semperflorens* and *V. cornuta* are suitable for edible flower production both in term of shelf life and phytochemical characteristics. *Begonia semperflorens* seems preferably marketable as pot plants, thanks to its better adaptability to grow in the domestic environment and longer flowering. Conversely, *V. cornuta* flowers resulted more suitable as fresh cut produce, showing a longer shelf life and preserving better the phytochemical characteristics during storage at 4°C.

New technological approaches (ethylene inhibitors, modified atmosphere packaging, edible film coatings, high hydrostatic pressure, irradiation, etc.) could further improve the distribution and marketing efficiency of edible flowers, contributing to their success in the market (Fernandes *et al.*, 2018).

Acknowledgements

The research was funded by the program Interreg Alcotra Francia Italia V-A, project n. 1139 “ANTEA - Attività innovative per lo sviluppo della filiera transfrontaliera del fiore edule”

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