



## CENTRO FLORA AUTOCTONA

Via Bertarelli, 11 – 23851 - Galbiate LC  
Tel. +39 0341 542266 – Fax. +39 0341 240216  
<http://centroflora.parcobarro.it> - [centroflora@parcobarro.it](mailto:centroflora@parcobarro.it)



## Orchid conservation using *in vitro* techniques a practical course for the germination, cultivation and transplantation of temperate-zone terrestrial orchids



Regione  
Lombardia

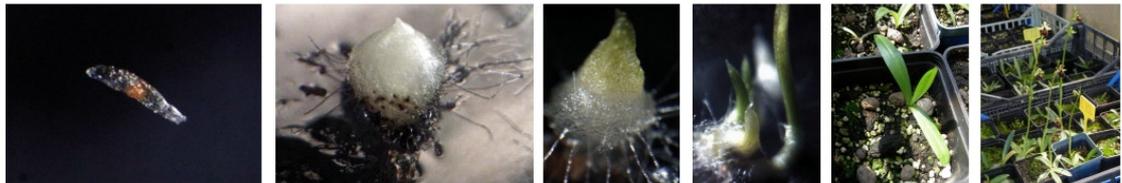


Fondazione Minoprio



### Dr. Simon Pierce

Department of Agricultural and Environmental Sciences  
University of Milan



A comprehensive course for professional conservationists who wish to learn how to apply *in vitro* techniques for orchid production and conservation. The course will include practical hands-on laboratory work and seminars on the subjects of:

- how to hand-pollinate orchids and collect and store seed,
- how to organise the laboratory for *in vitro* work,
- how to produce various agar media and use equipment such as the autoclave, laminar flow hood and pH meter,
- how to sow seed and cultivate seedlings *in vitro*,
- how to cultivate seedlings in soil,
- how and when to transplant plants into the wild.

**Date:** 18<sup>th</sup> – 22<sup>nd</sup> March 2013

**Location:** The Laboratory for the Conservation of Plant Biodiversity (Laboratorio per la conservazione della biodiversità), Centro Flora Autoctona della Regione Lombardia, Parco Monte Barro, Villa Bertarelli, via Bertarelli 11, Galbiate (LC).

### Coordinator/Secretary:

Roberta Ceriani

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**Requirements:** no previous *in vitro* experience is required. As only 8 places are available, preference shall be given to candidates that can demonstrate involvement in practical plant conservation work. Participants must bring a clean lab coat, and are invited to bring orchid seed they wish to sow (although seed will also be provided during demonstrations).

The course **inscription fee** will be € 1.000.00; discounts may be available for special circumstances (e.g. proof of current student status, proof of current apprenticeship, etc.). The fee includes full board accommodation at Hotel Le Torrette of Pescate, Lecco ([www.hotellettorrette.com](http://www.hotellettorrette.com)) from the midday meal of **Monday 18<sup>th</sup> March** until the midday meal of **Friday 22<sup>nd</sup> March 2013**. Included are: booking of a single room, buffet breakfast, lunch and dinner menus with three courses (first, second and dessert) and drinks (1/4 litre of wine and half a litre of mineral water per person); transport from the hotel to the site of the course during the hours of the course programme (the timetable will be finalized at the time of inscription). Also included in the inscription fee are Laboratory consumables (reagents, power, stationary, etc.) and insurance whilst working in the laboratory.

### **Further information about the course:**

The course is open to a maximum of **8 participants**. In the case of a greater number of participation requests than can be accommodated, the organizers reserve the right to propose future dates for further courses.

The course will be conducted in the **English language**, unless all participants are native speakers of Italian.

With the aim of facilitating the organisation of the course and optimising the course programme, participants are requested to fill in a brief **questionnaire**.

The completed application form should be sent to the secretary of the course, within the deadline of Friday the **8<sup>th</sup> February 2013**, at the following address:

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via Bertarelli, 11 - I-23581 Galbiate (LC)  
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Acceptance of the application request shall be communicated by mail only to admitted candidates, to the address indicated on the application form, within the **12<sup>th</sup> February 2013**. Payment of the inscription fee should be carried out within 15 days of the confirmation of application, or not later than Thursday the **28<sup>th</sup> February 2013**, via money transfer to our bank account (IBAN: IT96V0521651300 000000001600), indicating in the subject matter the surname of the participant and the statement "Corso Orchidee CFA".

For further information please contact Roberta Ceriani, on +39 (Italy) 0341 542266 from 9:00 to 14:00 local time from Monday to Friday, or by email: [centroflora@parcobarro.it](mailto:centroflora@parcobarro.it)

The programme, application form and questionnaire are available at the end of this document, and are downloadable from the following website: <http://centroflora.parcobarro.it>.



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### Further information on the Centro Flora Autoctona (CFA) and orchid conservation

The mission of the CFA is to promote the use and conservation of plant species native to the Lombardy region, mainly via the provision of certified native plants, seeds and seed mixtures, for environmental recuperation work and conservation projects within the territory. Since 2000 the CFA has produced 160,000 plants of 167 species, and has collected and seedbanked germplasm of over 800 species from 490 locations.

The main laboratory of the CFA, situated in the picturesque surroundings of Villa Bertarelli on the edge of Monte Barro park, was designed for the study of seed germination and in particular *in vitro* methods for the germination of species that are rare or difficult to germinate and cultivate *ex situ*. Between 2009 to 2011 one of the largest orchid propagation and conservation projects in the world, Project ORCHIS, was undertaken, during which more than 10,000 plants of 14 species were successfully germinated, cultivated and returned to the wild to reinforce natural populations in Parco Monte Barro and the nearby Parco delle Orobie Bergamasche. Whilst habitat conservation is essential for orchid conservation, *ex situ* conservation activities such as these have proved useful for species that are found in extremely reduced populations and are more prone to inbreeding depression. New methods and approaches to the propagation of small, inbred populations have been developed at the laboratory by Dr. Simon Pierce, and this has led to the successful propagation and repopulation of the rare Italian endemic *Ophrys benacensis*, for the first time in the world, and other particularly rare species (*Dactylorhiza traunsteineri*, *Goodyera repens*, *Orchis provincialis*) and problematic species such as *Pseudorchis albida*.

Facilities include: a laminar flow hood for *in vitro* work, two professional growth chambers with photoperiod and day/night temperature control, a seedbank with a humidity-controlled atmosphere and freezers, all equipment necessary for the production of agar media, a bench top autoclave, stereomicroscope with digital camera, a greenhouse with an automatic watering system.



The 14 species of native orchid reproduced from seed at the CFA and used to restock wild populations during project ORCHIS: *Anacamptis morio*, *A. pyramidalis*, *Coeloglossum viride*, *Dactylorhiza traunsteineri*, *Goodyera repens*, *Gymnadenia conopsea*, *G. odoratissima*, *Nigritella nigra* ssp. *rhellicani*, *Orchis provincialis*, *Ophrys apifera*, *O. benacensis*, *O. sphegodes*, *Pseudorchis albida*, *Serapias vomeracea*.

Photos © Simon Pierce.



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Work on orchids by Dr. Pierce, conducted at the lab and at Parco Monte Barro, has resulted in a number of peer-reviewed scientific articles and books:

**Pierce S., Ceriani R.M., Villa M. & Cerabolini B. 2006.** Quantifying relative extinction risks and targeting intervention for the orchid flora of a natural park in the European pre-alps. *Conservation Biology* 20(6): 1804-1810.

**Pierce S., Ferrario A. & Cerabolini B. 2010.** Outbreeding and asymbiotic germination in the conservation of the endangered Italian endemic orchid *Ophrys benacensis*. *Plant Biosystems* 144(1): 121-127.

**Pierce S. 2011.** *The Conservation of Terrestrial Orchids*. Kindle eBook edition (English language edition translated from the Italian print edition, *La Conservazione delle Orchidee Terrestri: dalle Alpi alla Pianura Padana Lombarda*). The Native Flora Centre of the Lombardy Region (CFA), Galbiate, Italy. (Available from Amazon.com, .co.uk and .de).

**Pierce, S. 2011.** *The Conservation of the Native Orchids of Lombardy*. Parco delle Orobie Bergamasche. Available in .pdf format from:  
[http://www.parcobarro.lombardia.it/cfa/index.php?option=com\\_content&task=view&id=78&Itemid=1](http://www.parcobarro.lombardia.it/cfa/index.php?option=com_content&task=view&id=78&Itemid=1)

**Pierce S. & Cerabolini B.E.L. 2011.** Asymbiotic germination of the White Mountain Orchid (*Pseudorchis albida*) from immature seed on media enriched with complex organics or phytohormones. *Seed Science and Technology* 39(1): 199-203.



75 plants of *Ophrys benacensis* produced *in vitro* during project ORCHIS were transplanted at the Costa Perla site, Monte Barro, from an original population of just 10 plants. The first of the plants to flower after transplantation is pictured here. Photo © Simon Pierce.



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# **Orchid conservation using *in vitro* techniques**

## **Programme of the course**

### **Day 1 (18<sup>th</sup> March): Introduction to the *in vitro* lab, orchid hand pollination and seed collection**

**09:00 – 12:30**

Welcome to Monte Barro

- 1). Short welcome speech from the President of Parco Monte Barro,
- 2). Brief presentation of the structure of the course,
- 3). Introductory seminar on *in situ* conservation and when additional *ex situ* conservation is necessary: "Why use *ex situ* conservation for orchids?"

Tour of the lab and growth facilities. Familiarisation with the *in vitro* lab and equipment, discussion and planning of the *in vitro* procedure to be used on Day 2.

**12:30 – 14:00** Lunch break at the hotel

**14:00 – 17:30**

Practical demonstration of hand pollination of *Ophrys sphegodes* in the field, in the Monte Barro Park (note that we cannot guarantee the timing of the flowering of the species, and this activity may be moved to Friday 22nd March depending on the season/weather/behaviour of the plants).

Techniques for seed collection and storage.

**17:30** Participants will be taken to their accommodation.

### **Day 2 (19<sup>th</sup> March): Preparation of agar media**

**09:00 – 17:30** (with 1 hour break for lunch)

Practical demonstration and practice session for:

- 1). the preparation of media, choosing agar media and additives,
- 2). the use of the pH meter,
- 3). the use of the autoclave,
- 4). the addition of heat-sensitive hormones to the medium,
- 5). the production and short-term storage of agar plates.

On this first day of *in vitro* work 4 of the 8 course participants will be guided as they prepare agar plates with growth media tailored to species that they wish to sow, which will also be used as a demonstration for all participants.

### **Day 3 (20<sup>th</sup> March): Sowing orchid seeds *in vitro***

**09:00 – 17:30** (with 1 hour break for lunch)

On morning of this second day of *in vitro* work the remaining 4 participants will have the opportunity to prepare agar plates with growth media tailored to species that they wish to sow.

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Demonstration and practical session for the asymbiotic sowing of orchid seeds (participants will have the opportunity to sow seeds they have brought with them).

- 1). Surface sterilisation and washing of seeds and whole fruits,
- 2). Plating out seeds,
- 3). Storage and incubation of seeds sown on Petri dishes, with an introduction to the use of growth chambers and temperature and light requirements of orchids.

The first 4 participants will be guided as they sow seed (seed will be provided, or participants can sow their own).

### **Day 4 (21<sup>st</sup> March): Sowing orchid seeds *in vitro* (continued) and cultivating orchid seedlings *in vitro***

**09:00 – 17:30** (with 1 hour break for lunch)

The second 4 participants will be guided as they sow seed (seed will be provided, or participants can sow their own).

*In vitro* cultivation of protocorms and seedlings, including re-plating and the transfer of seedlings between *in vitro* containers.

How to organise a typical day of *in vitro* work – from agar powder to seeds in the growth chamber in a single day.

In the afternoon extra time will be available for participants to practice their *in vitro* technique and discuss specific problems encountered during the practical sessions.

### **Day 5 (22<sup>nd</sup> March): The transplantation of plants into the wild**

**09:00 – 12:30**

In the historic greenhouse of Villa Bertarelli:  
Cultivation in pots and the requirements of species from different habitats:

- 1). Which type of growth medium or soil to use,
- 2). Temperature and light requirements,
- 3). Watering regimes.

Transplantation into the wild:

- 1). When and how to transplant,
- 2). Visit to Costa Perla (within Parco Monte Barro) to see some of the orchids transplanted during project ORCHIS.

Discussion of interaction with the public/presentation of results during conservation projects.

**12:30**

Lunch at the hotel. End of course.



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### Application form for the course “Orchid conservation using *in vitro* techniques”

Surname and name: \_\_\_\_\_

Institution: \_\_\_\_\_

Address: \_\_\_\_\_

Telephone: \_\_\_\_\_

e-mail address: \_\_\_\_\_

#### Information regarding your stay:

Is a vegetarian menu required?  yes  no

Single room:  Double room:

#### Information regarding your activities and expectations of the course:

	Yes	No
Are you, or your institution, currently involved in the germplasm banking of orchids?		
Are you, or your institution, currently involved in the propagation of native orchids from seed?		
Would knowledge of orchid propagation techniques contribute to future conservation activities carried out by your institution?		
You are interested in the conservation of which species/genera?	-	-
Would you be interested in sowing your own orchid seed during the course?		



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Information regarding your level of experience with *in vitro* techniques  
(note that none is required for attendance of the course, but this information will help us to tailor the course to your needs):

	<b>Yes</b>	<b>No</b>
Do you have a basic working knowledge of <i>in vitro</i> technique		
Are you familiar with the operation of laboratory equipment used during <i>in vitro</i> procedures? (laminar flow hood, autoclave, pH meter)		

Date: \_\_\_\_\_

Signature and stamp:

\_\_\_\_\_



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### **How to arrive at the location of the course, at the town of Galbiate (Via Bertarelli 11)**

#### **By car:**

##### **From the direction of MILAN:**

SS 36 (Milano – Lecco); exit Lecco Oggiono-Civate then follow signposts for PARCO MONTE BARRO SEDE in the town of Galbiate.

##### **From the direction of COMO (and CANTON TICINO - CH):**

SS639 (Como Lecco); merges with SS 36 (Milano – Lecco); exit Lecco Oggiono-Civate then follow signposts PARCO MONTE BARRO SEDE in the town of Galbiate.

##### **From the direction of BERGAMO (and Aeroporto Orio al Serio):**

SS639: after the village of Calolziocorte, cross the Adda river; follow the SP72; in the village of Pescate follow signposts for Galbiate taking the SP60.

#### **By Train and Coach/bus:**

##### **Arrival at the station of LECCO:**

Coaches of the company LECCO TRASPORTI – SAL; coach D55, on the Lecco – Oggiono – Annone route; exit from the stop “LECCO F.S.” to the stop “Galbiate (Autostazione)”

##### **Hours and prices:**

trains: <http://orario.trenitalia.com>

coaches: <http://www.leccotrasporti.it/oraristampabili.asp>

#### **By air:**

Orio al Serio International Airport: bus navetta “Airpot Bus- Linea 1” from the airport to Bergamo station (<http://www.atb.bergamo.it>); then trains for LECCO (see above).



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### BY TAXI

#### From the city of Lecco

Lecco taxi services: tel. 0341 364490

#### From Orio al Serio international airport:

Radiotaxi: tel. 035 4519090

On the map are indicated: the centre of Galbiate (in the vicinity of the course location); Hotel Le Torrette in the town of Pescate; the railway station of Lecco.



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