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The World Bamboo Congress in Antwerp: a dream come true

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Welcome to the IXth World Bamboo Congress, in Antwerp.

On behalf of IKEBANA and the University of Antwerp, on behalf of Geert Potters, Frances Schutte and myself, I wish all of you a warm welcome to the IXth World Bamboo Congress in Belgium, for the first part at the University of Antwerp, and for the second part in Merksplas.

The history

Almost exactly three years ago we had a meeting at Oprins Plant with Jan Oprins and Corneel Dewindt and they asked me to explore the possibility of EFRO-funding. EFRO stands for European Fund for Regional Development, funding. Deadline was exactly six days later. My answer was yes, on one condition: Geert Potters' time schedule. Fortunately, this was no problem and after one week of hard but enjoyable work, the project was submitted and granted. The project is called IKEBANA, International Knowledge Center for Bamboo, Northern Campinas-Antwerp. Antwerp because of the University, and Northern Campinas because the main co-financing came from Oprins Plant, engaged already a long time in bamboo research.

The goal of IKEBANA is to promote the use of bamboo in agriculture, in a broad sense. Frances Schutte was appointed as program manager. Our two-year project involved two major meetings on biomass, inviting competitors from *Miscanthus* and poplar/willow/SRC sides to create biomass platform, focusing on real data for farmers. Energy from biomass is a hot topic, but in great need of real data, for all energy crops. The step from research institute to farmer's adoption is a difficult one.

The final or ultimate goal of the EFRO-IKEBANA project was to organize a World Bamboo Congress, focusing on research. The organization of a World Bamboo Congress required the approval of the World Bamboo Organization WBO, and to this goal a bid book was prepared. Being at a University and having funding of EFRO, we had all pieces together to achieve our goal. So in fact, the basic planning was already done two years ago. And we got permission to organize WBC, at least the part on Bioscience and Bioengineering. Architecture and Design was going to be organized in Toulouse in September 2012.

Belgium, a hothouse for bamboo research

In some sense, bamboo research is not cutting edge research. The genome is more complicated than *Arabidopsis*, controlled hybridization and genetic transformation is not yet possible, and so on, so there is not much glory to be gained with bamboo research. On the other hand, bamboo is a REAL plant, and a wild, natural resource, with many possible uses. Both from a biological point of view (Bamboo as centerpiece of the grass family, a giant grass with gargantuan inflorescences..) and from a technological point of view, bamboo is interesting and has in the past led to some nice or even great advances. Square bamboo even entered into mathematics as the archetype of Lamé curves, or anisotropic metrics.

There is much to be studied. And we find research in almost every university and every university college today in Flanders and Belgium. Much of it started more than 15 years ago when we applied for research funding with IWT and Europe. One year later we could organize a workshop in Meise to explore the potential of using bamboo in Western Europe. Later this resulted in the Bamboo for Europe project, coordinated by Joris Devos of Cobelgal, combining 9 partners from Belgium, France, Germany, Spain and Portugal. This successful project then was carried over in the Bamboo Thematic Network. In one decade bamboo research was carried out at all universities and the level of research has steadily been increasing. This can be witnessed in the program today with the talks of Laura Van Hoyweghen (University of Gent), Suzanne Van den Akker, Davina Van Goethem and Litsa Bogaerts (research at University of Antwerp) and the crew from Aart Willem Van Vuure at the University of Louvain.

The congress Part A: Bamboo Bioscience, Bioengineering and Agroforestry Potentials

Belgium has become a real hothouse of bamboo research, and it seemed very logical to us to have the next bamboo congress at the University, symbolizing the research focus. This could increase the level of research in the future with a platform that is dedicated to the communication of scientific results, and at the same time, show to other fields that bamboo, either as a plant or as a material is really worth trying. Previous WBC's have been held at private estates (Linda Garland, Bali & Prafrance for example) or in hotels or resorts. It is the first time that it is really organised at a university, with the explicit aim and hope that future WBC's will follow the same path.

The National Organizing Committee was chaired by Geert Potters, and the scientific committee by Johan Gielis. Frances Schutte was appointed for organizational & financial work, with a lot of help from Susanne Lucas on the promotional side. We built a strong scientific committee:

Walter Liese, Germany
Lynn Clark, USA
Azmy Mohammed, Malaysia
Rajani Nadgauda, India
Amita Pal, India
Tesfaye Hunde, Ethiopia
David Midmore, UK/Australia
Shozo Shibata, Japan
Chris Stapleton, UK
Jinhe Fu, China
Ximena Londono, Colombia
Pablo Vanderlugt, The Netherlands
Geert Potters, Belgium
Johan Gielis, Belgium

All scientific papers have been reviewed by two scientists. More than 2/3 of the reviewers is from within the field of bamboo, and about 20% had no prior research in this field. 45% of the reviewers were Belgian, 55% from outside Belgium. Here we could use our own extensive academic network for specific papers or topics including special statistics or molecular biology. The list:

Amita Pal, Rajani Nadgauda, Azmy Mohamed, Walter Liese, Pablo Van der Lugt, Tesfaye Hunde, Jinhe Fu, Geert Potters, Shozo Shibata, Chris Stapleton, David Midmore, Roeland Samson, Laura Van Hoyweghen, Aart Willem Van Vuure, Joris Van Acker, Raf Dewil, Mike Temmerman, Marcel Jansen, Roland Valcke, Victor Brias, Evelyn Rottke, Jules Janssen, Andry Widyowijatnoko, Frances Schutte, Sarah Lebeer, Roland Caubergs, Davina Van Goethem, Richard Murphy, Khosrow Ghavami, Elizabeth Magel, L.B. Singha, Anil Sood,

Contents of the Proceedings

Session 2. Biology, Physiology and Ecology

Table of Contents

Freezing avoidance in tropical Andean bamboos

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Abstract

Frost resistance was compared in five species of different life forms of the neotropical woody bamboo genus *Chusquea* growing along a cloud forest-paramo gradient, between 2250-4010 m asl in the Venezuelan Andes. *C. purdieana* and *C. serrulata* are viny bamboos of the upper montane cloud forest-treeline ecotone (2200-2700 m and 2450-2900, respectively); whereas *C. angustifolia C. spencei* and *C. guirigayensis* are shrublike bamboos associated to swampy low elevation paramos (2525 m) in the first case, a broad range of paramo ecosystems (2670-3600 m) in the second, and dry high elevation paramos in the third (3800-4010 m). Intercellular ice nucleation and 50 % tissue injury temperatures were estimated in these five species under laboratory conditions in order to determine frost resistance mechanisms. No significant differences were observed among ice nucleation and tissue injury temperatures in any of these species, indicating that all avoid intercellular ice nucleation through supercooling (-12.3 to -10.1 °C). We conclude that variations in supercooling capacity and freezing injury are not related to life form, habitat or elevation and that freezing temperatures are not a determining factor in the altitudinal distribution of tropical Andean bamboos.

Keywords

supercooling capacity, tropical Andes, *Chusquea*, Bambusoideae, altitudinal gradients.

Introduction

Temperature is one of the main environmental factors that affect plant distribution. In the tropical Andes, life-form distribution usually responds to changing environmental conditions along altitudinal gradients, and those that reach the highest altitudes have adapted successfully to resist low temperature environments (Larcher 1995, Körner 1998).

In the Venezuelan Andes, low temperature resistance mechanisms have been described for giant rosettes (Goldstein et al. 1985), trees (Rada et al. 1985, Cavieres et al. 2000, García-Núñez et al. 2004; Azócar et al. 2007, Rada et al. 2009), dwarf shrubs (Squeo et al. 1991, Azócar 2006), herbs (Azócar et al. 1988; Squeo et al. 1991, Azócar 2006, Azócar and Rada 2006) and a wide variety of grasses (Márquez et al. 2006). Above treeline limits, freezing temperatures may occur any night of the year and surviving below zero temperatures is achieved either by frost tolerance (withstanding ice formation in intercellular spaces) or avoidance (withstanding temperatures well below zero without ice formation in any of the tissues) mainly through deep supercooling (Levitt 1972; Beck *et al.* 1982, 1984, Sakai and Larcher 1987, Pearce 2001).The former has been described as a mechanism utilized in harsher environments with prolonged below zero temperatures, whilst the latter, in less severe environments (Larcher 1995, Körner 1998, Sakai and Larcher 1987). In general, freezing avoidance represents the most common mechanism exhibited by woody species of the tropical high Andes (Squeo *et al.* 1991, Azócar 2006, Azócar and Rada 2006, Rada et al. 1985, 1987, 2009), given that periods below 0 °C may occur any night of the year, but remain close to zero lasting only a few hours (Monasterio and Reyes 1980, Azócar and Rada 2006). Avoidance through supercooling in leaves is achieved in tropical Andean giant rosettes and dwarf shrubs thanks to compact mesophylls with low apoplastic water contents (Rada et al. 1987, Azócar 2006, Azócar and Rada 2006). In other paramo woody species like *Polylepis sericea*, avoidance is achieved by the accumulation of osmotically active solutes and carbohydrates (Rada et al. 1985).

In contrast with woody life forms, herbaceous plants including grasses tolerate intercellular ice formation (Squeo et al. 1991, Márquez et al. 2006). Bamboos regarded as "woody grasses", are associated to a diversity of high altitude tropical ecosystems, from cloud forests to high elevation paramos (Clark 1995, Monasterio and Molinillo 2003, Clark and Ely 2011). However, how this group responds to freezing temperatures remains unknown. Temperate Japanese bamboos of the genera *Sasa* and *Sasamorpha* avoid frost formation in foliage tissues through deep supercooling, reaching freezing temperatures between -22 and -15 ºC (Ishikawa 1984, Sakai 1976, 1995, Tanaka 2002). In the tropical Andes, all of the high elevation bamboos belong to the genus *Chusquea* (Clark 1989, Clark 1995, Bussman 2004, Niño et al. 2006, Clark and Ely 2011), and the greatest species diversity may be found in Andean cloud forests, between 2400-2800 m a.s.l (Clark 1995). At higher altitudes (3000-4000 m asl), species diversity decreases markedly, and the viny life-form is replaced by a shrublike one. In the Venezuelan Andes the *Chusquea* species that grow along the upper cloud forest-paramo ecotone are: *C. purdieana* (2200-2700 m asl), *C. serrulata* (2450-2900 m asl), *C. angustifolia* (2525-2800 m asl), *C. spencei* (2670-3600 m asl) and *C. guirigayensis* (3800-4010 m asl). The first two species are upper cloud forest viny bamboos, whereas the remaining three species are shrublike and differ in their altitudinal distribution: *C. angustifolia* grows in swampy low paramos, *C. spencei* along the treeline-paramo ecotone, and *C. guirigayensis* exclusively in high elevation paramos.

At present no studies on the effect of freezing temperatures on high altitude bamboos have been conducted. Two main questions are addressed in this study: Firstly, how do Andean bamboos respond to freezing temperatures? One would expect the species of lower altitudes to be frost avoiders and those of higher elevations frost tolerant. Secondly, does freezing resistance increase in woody bamboos along the altitudinal gradient?

Materials and methods

Field sample collection and thermal analysis were carried out from 2008 through 2010. Fresh leaf samples were collected at different intervals, during both dry and rainy seasons, along an altitude gradient ranging from 2450 to 4010 m asl, comprising upper cloud forests, treeline ecotones, low and high paramos. Samples of *C. purdieana* and *C. serrulata* were collected at 2450 m asl in Monte Zerpa cloud forest (08° 38 92' N and 71° 24 63' W). *C. angustifolia* was collected in Las Piñuelas at 2525 m (N 8º 37'35 and W 71º24'), which constitutes the lowest paramo of the Venezuelan Andes. *C. spencei*, due to its broad altitudinal range of 930 m (Ely 2009), was collected at three different altitudes: paramos Las Coloradas (N 8°28′ and W 71°57′) at 2670 m, La Culata (N 08° 44 99' and W 71° 04.17') at 3025 m asl, and La Aguada (N 8° 35' and W 71° 09') at 3320 m asl. *C. guirigayensis*, was collected in the páramo Piedras Blancas (N 8º53' and W 70º 57), at 4010 m asl. Mean annual rainfall at these five sites are: 2286 mm in Las Piñuelas, 2520 mm in Monte Zerpa, 1877 mm in Las Coloradas, 1780 mm in La Culata, 1573 mm, in La Aguada (Ely 2009) and 800-900 mm in Piedras Blancas (Kiyota 2011).

Species description

Extensive descriptions of the vegetative characters for these species may be reviewed in Clark (1989), Niño *et al*. (2004), Ely (2009) and Kiyota (2011). Vouchers of the five species collected are deposited in MERC, Instituto Jardín Botánico de Mérida, Faculty of Sciences, University of The Andes, Mérida, Venezuela.

Chusquea angustifolia (Soderstr. and C. Calderón) L.G. Clark. (*F. Ely et al. 2009*, v.s. 44 *MERC*). Shrub of variable height, 0.2-1.7 m high, that grows between 2520-2800 m asl Foliage leaves pubescent, somewhat sticky, lanceolate, coriaceous, blades 6-8 cm long x 0.2-5 mm wide.

Chusquea guirigayensis Niño, L.G. Clark and Dorr. (*F. Ely et al. 2009*, v.s. 43 *MERC*). Miniature shrub, typically 20-50 cm high (exceptionally 120 cm). In Mérida, this species grows between 3800-4010 m asl Foliage leaves glabrous, triangular to lanceolate, consistency markedly coriaceous, blades 1-2.5 cm long x 0.3-0.5 cm wide.

Chusquea purdieana Munro. (*F. Ely & Borregales 2006*, v.s. 15 *MERC*). Woody climber, usually 2-6 m high that grows between 2200-2700 m asl Foliage leaves glabrous, membranous, blades 6.5-8.2 cm long x 3.8-4.2 cm wide.

Chusquea serrulata Pilger. (*F. Ely et al*. 2006 v.s. 18 *MERC*). Woody climber, usually 3-6 m high, that grows between 2450-2900 m asl. Foliage leaves glabrous, linear, papery blades 18-30 cm long x 0.4-0.6 cm wide.

Chusquea spencei Ernst. (*F. Ely et al*. 2006 v.s. 1 *MERC*). Shrub, ranging from 0.8-3 m high, growing between 2670-3650 m asl Foliage leaves glabrous, linear-lanceolate, coriaceous, blades 5-14 cm long x 0.2-0.6 cm wide.

Climate measurements

Air temperature (°C) and relative humidity (RH) data from the last 10 years were considered, as well as measurements performed at these six localities; from February to October 2008 in Monte Zerpa, La Culata and La Aguada, and from May to November 2010 in Las Piñuelas, El Molino and Piedras Blancas. Data were registered every 15 minutes with portable data loggers at each site (*HOBO*, *Pro Series*. Onset, Massachusetts, USA). In all of the study sites, sensors were placed at 1.5 m above ground level, protected from direct full sunlight. These were also placed at ground level in the paramos Las Piñuelas, El Molino and Piedras Blancas.

Thermal analysis

The relationship between tissue ice nucleation and injury temperatures determine frost resistance mechanisms. If these two temperatures coincide well below zero, we refer to freezing avoidance. In frost tolerant plants, ice nucleation temperatures occur close to 0°C while injury at significantly lower temperatures.

Intercellular ice nucleation temperatures were measured in fresh leaves collected at the six sites. A minimum of four trials were carried out in n=18-20 samples per species. Leaf samples of all species were collected at different intervals of the dry (January through March) and the rainy (May through September) seasons. Sampling consisted of five young culms per species of different genets collected from culms separated at a minimum distance of 10 m. Culms were cut at ground level in the field, placed in water and the ends cut again under water to avoid formation of air bubbles in xylem vessels (Lei and Koike 1998). In the lab, culms were covered with black plastic bags and allowed to rehydrate overnight. A total of five leaf samples were placed in small glass test tubes, tightly sealed with rubber stoppers in order to avoid tissue moisture changes. Copper-constantan thermocouples were inserted in the tissue samples and temperature was continuously monitored with a 5-channel data logger connected to a PC. Test tubes were immersed in a refrigerated alcohol bath (*NESLAB*, mod. RTE-111). Temperature was then lowered progressively from 5 ºC to -25 ºC at a rate of approximately 7.5 ºC/h and monitored continuously with specially designed software (*Planta-ICAE*). Intercellular ice nucleation was registered through the formation of exotherms, the result of an abrupt increase in temperature generated by heat released during the freezing process.

Determination of injury temperatures

50 % Injury temperatures were determined in these five species through the electrolyte leakage method used previously by Ishikawa (1984) and later modified by Lindén (2002). Electrical conductivity (µS) was measured in leaf tissues previously submitted to decreasing low temperatures, from 5 ºC to -25 ºC, and submerged in deionized water (with an initial electrical conductivity of 0 µS). Increases in electrical conductivity resulted from electrolyte leakage due to release of potassium ions as a consequence of cell wall rupture. Tissue samples were submitted to the same frost-inducing procedure described previously. This procedure was performed in n=18-20 samples per species. At 5 ºC intervals, three tubes were withdrawn from the refrigerated bath, leaf samples removed from the tubes and placed in clean plastic containers with 15 mm^3 of deionized water. The containers were then refrigerated at 6 ºC during 48 h, and electrical conductivity was measured with an ExStik digital conductimeter, mod. EC500 (*Extech Instruments*, U.S.A). After measurements were performed, complete tissue rupture was induced by submerging samples briefly in liquid nitrogen and placing them again in their respective containers. These were refrigerated again for 48 h and electrical conductivity was measured afterwards. This last measurement corresponded to the electrical conductivity of samples after 100 % leakage had occurred. Tissue injury was estimated as the temperature at which 50 % leakage occurred through the following equation:

$$T_{50\%} = \frac{\text{Initial Electrical Conductivity}}{\text{Final Electrical Conductivity}} * 100$$

Initial Electrical Conductivity corresponded to the temperature to which the tissue was exposed before withdrawing the tube from the refrigerating bath, whereas Final Electrical Conductivity represented the electrical conductivity of the sample after inducing complete tissue rupture.

Data analysis

Ice nucleation temperatures were represented graphically in box plots (Sigma Plot, ver. 10.0), and average temperatures and standard deviation errors were estimated. U Mann-Whitney non-parametric tests were carried out to determine whether or not differences between intercellular ice nucleation and injury temperatures were statistically significant for each species. Kruskall-Wallis tests (*SPSS Statistics*, ver. 17.0) were performed to determine statistically significant differences regarding ice nucleation and injury temperatures among these five species.

Results

Climate characteristics

Below zero temperatures occurred only above 3000 m, and were relatively infrequent at treeline limits (3025-3320 m) where they remained very close to zero, being more frequent in the upper open paramo limits (3800-4010). The lowest night temperatures were registered in Páramo de Piedras Blancas followed by Páramo La Aguada (Table 1). The frequency of nights with freezing temperatures increased during the dry season (end of December through the end of March 2008) above 3000 m, representing a 10 % of the total of days registered at 3000, 18 % at 3320 m, and 41 % at 4010 m.

Table 1. Average air temperatures registered at the six study sites. Maximum and minimum temperatures registered are indicated in parentheses.

Study site	Average Temperature (°C)		Average Min. temperature (°C)		Average Max. temperature (°C)	
	Soil	Air	Soil	Air	Soil	Air
Monte Zerpa (2450 m)	ID	13.02	ID	8.03 ± 0.01 (6.53)	ID	17.06 (20)
Las Piñuelas (2525 m)	13 ± 0.03	14± 0.03	10 ± 0.2 (6.6)	8.5± 0.1 (4.6)	25 ± 0.6 (32)	20± 0.2 (25)
Las Coloradas (2670 m)	11 ± 0.02	12 ± 0.02	7.5 ± 0.2 (4.6)	9.0 ± 0.1 (7.0)	15 ± 0.2 (19)	17 ± 0.2 (21)
La Culata (3025 m)	ID	9.25±0.07	ID	4.3 ± 0.65 (-0.16)	ID	8.8±0.08 (19)
La Aguada (3320 m)	ID	7.6±0.1	ID	2.3 ± 1.0 (-0.68)	ID	20±0.06 (24)
Piedras Blancas (4010 m)	8.6 ± 0.1	6.2± 0.04	1.5 ± 0.1 (-12)	2.0 ± 0.1 (-4)	27 ± 1.0 (46)	15 ± 0.4 (21)

ID: insufficient data due to technical difficulties with the loggers

Intercellular ice nucleation and injury temperature

Both ice nucleation and 50 % injury temperatures were consistent in all of the trials. In these five species, average intercellular freezing temperatures varied between -12.1 and -10.1 °C, whereas average 50 % injury values ranged between -12.3 and -10.3 °C (Table 2). No statistically significant differences were observed between intercellular ice nucleation temperatures (exotherm formation temperature) and injury temperatures in any of the species along the altitudinal gradient, indicating that in these five *Chusquea* species, intercellular ice nucleation was avoided through a moderate supercooling capacity.

Intercellular ice nucleation temperatures did not decrease linearly along the altitude gradient in this genus (Figure 1); nor did they vary significantly between the two viny cloud forest species (*C. purdieana* and *C. serrulata*); nor amongst the latter and the paramo shrublike species growing at the lower and upper limits of this gradient (*C. angustifolia* at 2520 m, the genets of *C. spencei* at 2670 m, and *C. guirigayensis* at 3800-4010 m). However, significant differences were observed between the genets of *C. spencei* growing at lower and upper limits of its distribution range (2670 m vs 3025-3320 m), and among genets of *C. spencei* above 3000 m and the remaining four species (Figure 1, Table 2).

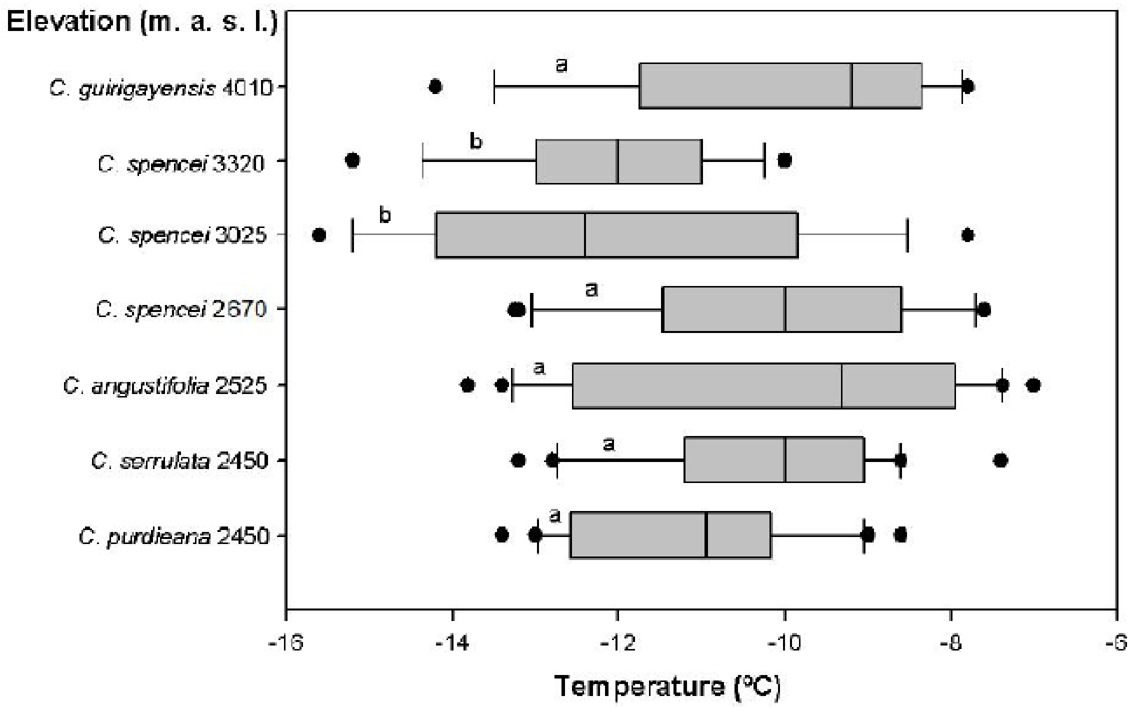

Figure 1. Relationship between ice nucleation temperatures and altitude for five *Chusquea* species along the 1560 m altitudinal gradient. Black circles represent extreme (high and low) values of ice nucleation temperatures in each case. Different letters depict significant differences (p<0.05).

Table 2. Average intercellular ice nucleation and 50% injury temperatures measured in these five *Chusquea* species. Maximum and minimum temperatures are indicated in parenthesis.

Species/elevation (m.asl)	Intercellular ice nucleation temperature (°C)	50 % Injury temperature (°C)
C. purdieana (2450)	(-9.0) -11.0 ± 0.3[a] (-13.4)	(-10.8) -11.5 ± 0.3[a] (-12.4)
C. serrulata (2450)	(-9.0) -10.5 ± 0.3[a] (-13.0)	(-9.6) -10.1 ± 0.4[a] (-11.9)
C. angustifolia (2525)	(-7.4) -10.8± 0.2 [a] (-13.8)	(-11.4) -12.3 ±0.3[a] (-13.1)
C. spencei (2670)	(-7.6) -10.6± 0.4[a] (-13.3)	(-8.4) -10.2 ± 0.4[a] (-13.7)
C. spencei (3025)	(-7.9) -12.1 ± 0.5[b] (-15.6)	(-9.8) -12.0 ± 0.2[b] (-13.5)
C. spencei (3320 m)	(-10.4) -12.0 ± 0.3[b] (-15.0)	(-9.8) -12.2 ± 0.3[b] (-15.9)
C. guirigayensis (4010 m)	(-8.0) -10.3± 0.4[a] (-14.2)	(-9.0) -10.6 ±0.3[a] (-12.0)

Different letters depict significant differences ($p<0.05$).

Discussion

Chusquea is distinguished as the genus with the greatest diversity regarding species, habitats, life forms and altitudinal distribution in tropical Andean ecosystems (Clark 2001), yet above 3000 m, species diversity decreases markedly in this genus. These variations in species abundance suggest that freezing temperatures could be a determining factor in woody bamboo distribution in tropical mountain ecosystems. We had initially assumed that the bamboos growing below treeline limits (3000 m), which are not exposed to freezing temperatures, such as the viny species (*C. purdieana* and *C. serrulata*) and the genets of *C. angustifolia* and *C. spencei* of low paramo ecosystems (2520 and 2670, respectively) were altogether devoid of freezing resistance mechanisms in which case, ice nucleation and injury temperatures should have occurred very close to 0 °C, as described for other woody species of the upper cloud forest-paramo ecotone (Cavieres *et al*. 2000). Nevertheless, all five species avoided intercellular ice formation through a moderate supercooling, regardless of their life-form, habitat plant height, foliage leaf size or consistency. Supercooling capacity values were higher in these *Chusquea* species than those reported for other paramo woody species (Rada et al. 1985, 2009; Cavieres et al. 2000), and comparable to those reported for giant rosettes of the genus *Espeletia* (Goldstein *et al*. 1985, Rada *et al*. 1987). Another unexpected result was that neither intercellular ice nucleation nor 50 % injury temperatures varied significantly between the species situated at the upper and lower limits of the cloud forest-paramo gradient (2450 and 4010 m). Only *C. spencei* presented a slight increase in its supercooling capacity with increasing altitude, as the differences between the genets growing below and above 3000 m suggest. A possible explanation for the uniform supercooling capacity observed in these five species is that after the last glaciation, cloud forest and paramo boundaries suffered repeated displacements, with paramo ecosystems descending as low as 2000 m (Van der Hammen 1974, 1988, 2000, Salgado-Labouriau et al. 1977, 1992). In addition, minimum air temperatures have also increased considerably during the last decades, reducing the frequency of nocturnal frosts, as recent microclimate studies indicate (Monasterio and Reyes 1980, Azócar and Rada 2006, Azócar 2006, Ely 2009, Kiyota 2011).

Our results suggest that neither plant height nor elevation necessarily condition freezing resistance mechanisms in this group. *C guirigayensis* with the smallest height and growing at the highest altitudes responds to freezing temperatures in the same manner as the other species studied. Freezing resistance mechanisms differ among herbaceous tussock grasses and strongly lignified grasses such as bamboos, regardless of whether they grow in temperate (Ishikawa 1984, Tanaka, 2002, Ashworth and Pearce 2001, Liu and Osborne 2008) or tropical climates (Márquez et al. 2006). Tussock grasses of the Venezuelan paramos tolerate extracellular freezing, with intercellular ice nucleation temperatures between -6.3 and -3 °C, and 50 % injury of foliage tissues occurring between -18 and -9.8 °C (Márquez et al. 2006). Tussock grasses are subjected to lower temperatures and for longer intervals; due to their proximity to the ground where the temperatures are lowest in the air-soil gradient, therefore foliage tissues are exposed to freezing temperatures in their early development, in contrast with woody bamboos, in which developing organs are protected from extreme temperatures by thick culm leaves until they have reached maturity (Ely 2009). In the Venezuelan paramos, where freezing air temperatures typically remain close to 0 °C and last for only a few hours , a moderate supercooling capacity combined with the protection of developing organs should be sufficient to impede the formation of intercellular frost in foliage tissues.

These results support the studies conducted in the Japanese species of *Sasa* and *Sasamorpha*, differing only in their greater supercooling capacity (-22 to -15 °C, Sakai 1976, 1995, Ishikawa 1984), which have likely evolved as an adaptation to the seasonal winters (Tanaka 2002). Based on these results we also conclude that the altitudinal distribution of *Chusquea purdieana*, *C. serrulata*, *C. angustifolia* and

C. spencei in the Venezuelan Andes is not conditioned by freezing temperatures, but more likely by other environmental factors not taken into account in the present study.

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The authors would like to thank Jhony Márquez and Williams Dugarte for their assistance in the field. This research was partly funded by a Misión Ciencias-Ministerio de Ciencias y Tecnología grant, LOCTI funds granted by the Clínica Albarregas, by the Interamerican Institute for Global Change (IAI-CRNII-005) and the Consejo de Desarrollo Científico Humanístico Tecnológico y de Arte through the grant C-710-10-01-F.

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