Article

Weak Apical Control of Swiss Stone Pine (*Pinus cembra* L.) May Serve as a Protection against Environmental Stress above Treeline in the Central European Alps

Walter Oberhuber [1], Theresa Andrea Geisler [1], Fabio Bernich [1] and Gerhard Wieser [2,*]

[1] Department of Botany, Leopold-Franzens-Universität Innsbruck, Sternwartestraße15,
     A-6020 Innsbruck, Austria
[2] Department of Alpine Timberline Ecophysiology, Federal Research and Training Centre for Forests,
     Natural Hazards and Landscape (BFW), Rennweg 1, A-6020 Innsbruck, Austria
*   Correspondence: gerhard.wieser@uibk.ac.at; Tel.: +43-512-573-933-5120

Abstract: At the treeline in the Central European Alps, adverse climate conditions impair tree growth and cause krummholz formation of Swiss stone pine (*Pinus cembra* L.). Multi-stemmed trees (tree clusters) are frequently found in the treeline ecotone and are generally thought to originate from seed caches (multiple genets) of the European nutcracker (*N. caryocatactes*) or due to repeated damage of the leader shoot by browsing or mechanical stress (single genet). Additionally, lack of apical control can lead to upward bending of lateral branches, which may obscure single-genet origin if the lower branching points are overgrown by vegetation and the humus layer. The multi-stemmed growth form may serve as a means of protection against extreme environmental stress during winter, especially at wind-exposed sites, because leeward shoots are protected from, e.g., ice particle abrasion and winter desiccation. The aims of this study therefore were to analyze in an extensive field survey: (i) whether weak apical control may serve as a protection against winter stress; and (ii) to what extent the multi-stemmed growth form of *P. cembra* in the krummholz zone is originating from a single genet or multiple genets. To accomplish this, the growth habit of *P. cembra* saplings was determined in areas showing extensive needle damage caused by winter stress. Multi-stemmed saplings were assigned to single and multiple genets based on determination of existing branching points below the soil surface. The findings revealed that upward bending of lateral branches could protect saplings against winter stress factors, and, although multi-stemmed *P. cembra* trees were primarily found to originate from multiple genets (most likely seed caches), about 38% of tree clusters originated from upward bending of (partially) buried branches. The results suggest that weak apical control of *P. cembra* in the sapling stage might be an adaptation to increase survival rate under severe climate conditions prevailing above treeline during winter.

Keywords: apical control; multi-stemmed growth form; *Pinus cembra*; treeline

1. Introduction

The alpine treeline is a conspicuous climate-driven ecological boundary, which designates the upper elevational limit of tree growth [1–3]. There is extensive evidence that at high altitude cold temperatures during the growing season, which directly limit cell division and differentiation in meristematic tissues ("carbon-sink-limitation hypothesis"), are a major cause of treeline formation [4–7]. Above the treeline, adverse climatic conditions (e.g., frost drought, wind abrasion, and late frosts)

frequently occur such that height growth of trees is strongly suppressed and tree stature is dominated by stunted multi-stemmed architecture (krummholz).

Swiss stone pine (*Pinus cembra* L.) is a key species forming the alpine treeline in the Central European Alps [8], which is reached at about 2400 m–2500 m above sea level (a.s.l.) under natural conditions, i.e., without human interference [3]. The heavy wingless seeds of *P. cembra* are almost exclusively dispersed by the European nutcracker (*Nucifraga caryocatactes* L.). At sheltered sites, krummholz forms of this species may be found up to ca. 2800 m a.s.l. (see [9]). Three growth forms of *P. cembra* can be distinguished: (1) single-trunk; (2) single genet multi-trunk caused by repeated damage of the leader shoot; and (3) multi-genet tree cluster attributable to multiple germination of seed caches [10]. The latter two forms are morphologically similar and generally thought to be distinguishable only by genetic analysis [11]. Although several coniferous tree species, e.g., spruces, firs, and larches, are able to form clonal tree groups by layering, i.e., formation of adventitious roots on buried lateral branches [12–14], cluster formation by vegetative sprouting has not been reported to occur in *P. cembra* [2,3]. Most pine species have a dominant main stem and distinct lateral branches which grow shorter and more horizontal than the vertical leader shoot [15], i.e., strong apical control is exerted by the leader shoot. Lack of apical (hormonal) control, e.g., due to mechanical or biotic damage of the leader, leads to upward bending of branches [16]. Shoot architecture of *P. cembra* is generally characterized by a vertical main trunk and upward bending of lateral branches.

This study aimed to increase our understanding of tree adaptation to extreme environmental conditions prevailing in the treeline ecotone. There are no reports on the influence of apical control on resistance against environmental stress in the treeline ecotone. Therefore, in an extensive field survey, we evaluated whether weak apical control may protect *P. cembra* against winter stress (frost desiccation, and snow mold). Furthermore, determining a morphological feature that consistently distinguishes single genet multi-trunk trees from multi-genet tree clusters originating from seed caches was a second aim of this study.

2. Materials and Methods

The study area is situated in the treeline ecotone on Mt. Patscherkofel (2246 m a.s.l.; 47°12′ 33″ N; 11°27′40″ E), which is located in the Central European Alps in western Austria. Mean annual temperature and precipitation (1967–2015) at the top of Mt. Patscherkofel are 0.2 °C and 883 mm with minimum in winter (December–February: 139 mm), respectively. The geology is dominated by gneisses and shist. Three sites (based on information gathered in Table 1) were selected on a ridge facing W to WNW (slope 15°–20°) above the current treeline at ca. 2100 m–2150 m a.s.l. Selected sites are frequently exposed to extreme winds (foehn), frequently reaching >100 km/h. Exposed mineral soil is common and dominating plants (*Loiseleuria procumbens* (L.) Desv., *Calluna vulgaris* (L.) Hull., *Juncus trifidus* L. and lichens, e.g., *Thamnolia vermicularis* (Sw.) Ach.ex Schaer and *Alectoria ochroleuca* (Hoffm.) A. Massal) are known to be resistant against wind, frost and winter desiccation [8].

Table 1. Number of *P. cembra* individuals at a wind-exposed ridge above treeline belonging to single- or multi-stemmed growth form. Multi-stemmed growth form is divided into multi-genet individuals developing from seed caches, and single-genet individuals, which developed upright growing lower branches. Percentages are given in parenthesis. For multi-stemmed growth form the mean number of shoots ± standard deviation per site are given. Significant differences (*p* < 0.05) in the mean values of the three plots (± SD) between single- and multi-stemmed and between multi-genet and single genet, respectively, are marked in bold and italics.

		Single Stemmed		Multi-Stemmed		
site	n			*n* shoots (mean ± SD)	Multi-genet	Single genet
1	31	11 (35)	20 (65)	2.9 ± 1.3	9 (45)	11 (55)
2	30	6 (20)	24 (80)	3.5 ± 1.6	20 (83)	4 (17)
3	31	9 (29)	22 (71)	2.6 ± 0.6	13 (59)	9 (41)
Mean		*9 ± 3 (28 ± 8)*	*22 ± 2 (72 ± 8)*	3.0 ± 0.5	14 ± 6 (62 ± 19)	8 ± 5 (*38 ± 19*)

The study was conducted on *P. cembra*, which is the dominant and widespread conifer along the treeline ecotone. European larch (*Larix decidua* Mill.) is scattered at some locations. The importance of apical control in *P. cembra* saplings as a mean to protect against winter stress was studied at wind exposed ridges and depressions with a long duration of snow cover showing pronounced needle damage after winter. In each of the three sites, one 100 m^2 plot was established where all *P. cembra* saplings (single stemmed and clusters, i.e., multi-stemmed trunks) without any visible damage to the leader shoot were counted. Multi-stemmed trunks were assigned to single and two or more genets by digging out all leaders until able to be traced back to the main stem and determining whether below the soil surface upward bending of lateral branches (i.e., single genet) or vertical leader shoots originating from seed caches (i.e., multi-genets) occurred.

Differences in the overall mean number between single stemmed and multi-stemmed *P. cembra* individuals were analyzed by one-way analysis of variance (ANOVA). One-way ANOVA was also used to test for differences between multi-genet and single genet saplings. A probability level of *p* < 0.05 was considered as statistically significant. Statistical analyses were made with the SPSS 16 software package for windows (SPSS, Inc., Chicago, IL, USA).

3. Results

Stem height and diameter of selected saplings (*n* = 92) were 39.2 ± 22.4 cm and 1.9 ± 1.2 cm, respectively, and annual shoot growth amounted to 4.4 ± 1.4 cm (mean values ± standard deviation). These growth variables were not significantly different among single-stemmed and multi-stemmed saplings. A potentially protective function of weak apical control in *P. cembra* against severe winter drought is depicted in Figures 1 and 2. Several examples of upward bending lower branches with connection to the main stem below the soil surface, whose vertical growing lateral branches are obscured by the humus layer and intensively growing dwarf-shrubs (*L. procumbens*, *C. vulgaris*), are depicted in Figure 3.

Figure 1. *Pinus cembra* sapling with upward-bending lateral branches (**left**) and *P. cembra* showing strong needle damage on the wind-exposed side caused by ice particle abrasion and winter desiccation, while sheltered leeward side remained largely undamaged (**right**).

Figure 2. Multi-stemmed appearance of *P. cembra* individuals (single genet) showing strong needle damage on the wind-exposed side caused by ice particle abrasion and winter desiccation, while sheltered leeward side remained largely undamaged (**left**). Upright growing lower branch after vegetation and raw humus layer was removed (**right**).

Figure 3. Examples of weak apical control of excavated basal branches of *P. cembra* saplings showing no damage to the leader shoot.

Above the treeline, the number of single stemmed *P. cembra* individuals (9 ± 3) was significantly lower ($p = 0.02$) when compared to the number (22 ± 2) of multi-stemmed *P. cembra* saplings, which corresponds to 28 ± 8% and 72 ± 8% single- and multi-stemmed *P. cembra* saplings, respectively (Table 1). The multi-stemmed growth form originates from 14 ± 6 (62 ± 19%) seed caches from multiple genet individuals and 8 ± 5 (38 ± 19%) seed caches from upright growing branches, i.e., single genet individuals (Table 1), which however was not statistically significant ($p = 0.19$). Upward-bending branches of a *P. cembra* sapling growing in a depression with long-lasting snow cover and frequent occurrence of snow mold is shown in Figure 4.

Figure 4. Branches of *P. cembra* in a hollow with long-lasting snow cover, where *Rhododendron ferrugineum* L. is the dominating shrub (**left**). Predominantly dead lower branches caused by snow mold. Note shoot tips with undamaged green needles (**right**).

4. Discussion

4.1. Multi-Stemmed Growth Form

Multi-stemmed trunks of *P. cembra* are frequently occurring in the alpine treeline ecotone [11,17,18]. This growth form is frequently reported to result from seed caches [11,19] or damage of the leader shoot caused by browsing or mechanical disturbance (e.g., winter desiccation, wind abrasion, frost, and avalanche [2,20]). In the case of destroyed apical meristems, apical control is lost and lateral branches bend upwards and form vertical shoots [16]. Here, we report that, in about 38% of multi-stemmed *P. cembra* saplings, lower branches at the base of the stem tend to grow upright without any visible damage to the leader. Hence, weak apical control may exist in *P. cembra* at the sapling stage, which can be explained by the competitive-sink hypothesis developed in [21]. This hypothesis states that branches compete with the subjacent stem for branch-produced carbohydrates, i.e., when the subjacent stem sink for carbohydrates is small, as is to be expected at the sapling stage, the branch is largely released from apical control and develops vertical growth.

At wind-exposed ridges, tree cluster formation favors growth of dwarf-shrubs (especially *L. procumbens* and *C. vulgaris*) by locally trapped litter and wind-blown organic and soil particles, which may increase nutrient content and water storage capacity of the soil [2]. Because the branching point close to the soil surface may be overgrown by vegetation and development of a humus layer, the origin from a single genet can be obscured. By carefully removing vegetation and the humus layer, multi-stemmed trunks of saplings and small trees can unequivocally be allocated to lateral branches of a main stem (single genet) or genetic different individuals (multi genet) originating from seed caches. Multi-stemmed trunks merge with time, which survive and grow better than single-stemmed trees, most likely due to better structural stability against wind [11,22,23]. Upright branches will ultimately merge with increasing tree age and radial growth, forming multi-stemmed trees, which—without genetic analysis—can mistakenly be assigned to originate from multi genet seed caches.

Analyzing the genetic diversity in multi-stemmed *P. cembra*, the authors of [11,23] found that 7 out of 22 and 1 out of 4 *P. cembra* tree clusters, i.e., 30% and 25%, respectively, were single genet multi-stemmed trees. Their findings are quite similar to our morphological approach, which was based on determination of the branching pattern below the soil surface. Hence, the assumption of a genetically controlled delay in dormancy release from varying stem diameter in multi-stemmed clusters may be false, because multi-genet shoots can be hidden by upright growing branches belonging to the same single genet tree individual.

4.2. Protective Function of Cluster Formation and Weak Apical Control During Tree Establishment

In the alpine treeline ecotone, natural regeneration of *P. cembra* is concentrated on locally higher terrain, such as ridges, shoulders and rock buttresses where wind exposure is high, snow depth is low, snow cover duration is short and there is sparse vegetation. Tree seedling establishment is controlled by micro-relief and/or shelter from low stature alpine vegetation, e.g., dwarf shrubs [24,25]. Once seedlings emerge from the ground vegetation and are exposed to the convective conditions of the atmosphere, wind effects on exposed ridges are important for tree survival during winter [3,12,17]. Damage of needles and buds is common at wind-exposed sites with little snow cover due to injuries from abrasive blowing snow, ice and mineral particles (for review see [26]), which reduces cuticle resistance leading to winter desiccation. Winter desiccation results from gradual water loss through transpiration, which cannot be compensated due to deep and long-lasting soil frost at snow-free ridges [27–29]. Saplings growing in clusters or single genet trees with multi-stemmed trunks caused by weak apical control create a wind-barrier effect that reduces mechanical damage at the sheltered leeward side especially during winter. This is corroborated by the finding that afforestation of *P. cembra* in groups in the subalpine zone yielded greater success than planting solitary trees ([30]; for a review, see [31]). Tree groups produce a snowdrift at their leeward side, which prevents deep soil frost and provides soil moisture at the beginning of the growing season [19]. On the other hand, weak apical control is also favorable for tree establishment in snow-rich concave topography and leeward slopes. Here, lower branches are less likely to be at risk for occurrence of snow mold under a long-lasting snow cover, if lower branches grow upright in the early sapling stage. Age- and/or size-related changes in apical control are to be expected, however, as a tree faces different challenges to survival with increasing tree age and/or size (cf. [32]). Hence, findings of this study suggest that upward bending of basal branches, i.e., a weak apical control in the sapling stage, might be an adaptation to extreme environmental conditions prevailing in the alpine treeline ecotone in cool temperate and boreal mountains, especially during winter. Our interpretation is corroborated by strong apical control found in co-occurring European larch (*L. decidua* [33]), which, due to its deciduous behavior, is less prone to winter drought and snow mold.

5. Conclusions

The results of this field survey suggest that cluster formation due to weak apical control of lateral branches at the stem base might be an adaptation to extreme environmental conditions prevailing above treeline within the study area. About one third of multi-stemmed *P. cembra* clusters in the krummholz zone belonged to a single genet. Multi-stemmed trees serve as protection against winter stress factors (snow and ice particle abrasion, and winter desiccation), have a better structural stability against wind and possibly improve resource acquisition due to trapping of litter and wind-blown soil. However, as this study was carried out within the inner Alpine dry zone of the Central European Alps, where the local climate is strongly influenced by southern "Foehn" type winds [34], further research needs to clarify the above demonstrated growth patterns of *P. cembra* saplings at sites differing in environmental conditions, especially winter stress.

References

1. Wieser, G.; Tausz, M. *Trees at Their Upper Limit: Treelife Limitations at the Alpine Timberline*; Springer: Dordrecht, The Netherlands, 2007.
2. Holtmeier, F.K. Mountain Timberlines: Ecology, Patchiness, and Dynamics. In *Advances in Global Change Research*; Springer: Berlin, Germany, 2009; Volume 36, p. 437.
3. Körner, C. *Alpine Treelines: Functional Ecology of the Global High Elevation Tree Limits*; Springer: Basel, Switzerland, 2012; p. 220.
4. Körner, C. A re-assessment of high elevation treeline positions and their explanation. *Oecologia* **1998**, *115*, 445–459. [CrossRef] [PubMed]
5. Grace, J.; Berninger, F.; Nagy, L. Impacts of climate change on the tree line. *Ann. Bot.* **2002**, *90*, 537–544. [CrossRef] [PubMed]
6. Oberhuber, W. Influence of climate on radial growth of *Pinus cembra* within the alpine timberline ecotone. *Tree Physiol.* **2004**, *24*, 291–301. [CrossRef] [PubMed]
7. Körner, C.; Paulsen, J. A world-wide study of high altitude treeline temperatures. *J. Biogeogr.* **2004**, *31*, 713–732. [CrossRef]
8. Ellenberg, H.; Leuschner, C. *Vegetation Mitteleuropas mit den Alpen: In Ökologischer, Dynamischer und Historischer Sicht*; Verlag Eugen Ulmer: Stuttgart, Germany, 2010.
9. Available online: https://www.lfi.ch/resultate/meldungen/logbuch.php (accessed on 12 July 2019).
10. Linhart, Y.B.; Tomback, D.F. Seed dispersal by nutcrackers causes multi-trunk growth form in pines. *Oecologia* **1985**, *67*, 107–110. [CrossRef] [PubMed]
11. Tomback, D.F.; Holtmeier, F.K.; Mattes, H.; Carsey, K.S.; Powell, M.L. Tree clusters and growth form distribution in *Pinus cembra*, a bird-dispersed pine. *Arct. Alp. Res.* **1993**, *25*, 374–381. [CrossRef]
12. Jeník, J. Clonal growth in woody plants: A review. *Folia Geobot.* **1994**, *29*, 291–306. [CrossRef]
13. Bellingham, P.J.; Sparrow, A.D. Resprouting as a life history strategy in woody plant communities. *Oikos* **2000**, *89*, 409–416. [CrossRef]
14. Del Tredici, P. Sprouting in temperate trees: A morphological and ecological review. *Bot. Rev.* **2001**, *67*, 121–140. [CrossRef]
15. Turnbull, C.G. Shoot architecture II: Control of branching. In *Plant Architecture and Its Manipulation*; Turnbull, C.G., Ed.; Blackwell Publishing: Oxford, UK, 2005; Volume 17, pp. 92–120.
16. Wilson, B.F. Apical control of branch growth and angle in woody plants. *Am. J. Bot.* **2000**, *87*, 601–607. [CrossRef]
17. Tranquillini, W. *Physiological Ecology of the Alpine Timberline: Tree Existence at High Altitudes with Special Reference to the European Alps*; Springer: Berlin, Germany, 1979.
18. Kratochwil, A.; Schwabe, A. Wuchsformen der Arve (*Pinus cembra* L.) in Abhängigkeit von der ornithochoren Ausbreitung-im Vergleich mit Weidbuchen (*Fagus sylvatica* L.). *Diss. Bot.* **1993**, *196*, 107–134.
19. Holtmeier, F.K.; Broll, G. Broll Feedback effects of clonal groups and tree clusters on site conditions at the treeline: Implications for treeline dynamics. *Clim. Res.* **2017**, *73*, 85–96. [CrossRef]
20. Schuster, W.S.; Mitton, J.B. Relatedness within clusters of a bird-dispersed pine and the potential for kin interactions. *Heredity* **1991**, *67*, 41–48. [CrossRef]
21. Wilson, B.F.; Gartner, B.L. Effects of phloem girdling in conifers on apical control of branches, growth allocation and air in wood. *Tree Physiol.* **2002**, *22*, 347–353. [CrossRef] [PubMed]
22. Till-Bottraud, I.; Fajardo, A.; Rioux, D. Multi-stemmed trees of *Nothofagus pumilio* second-growth forest in Patagonia are formed by highly related individuals. *Ann. Bot.* **2012**, *110*, 905–913. [CrossRef] [PubMed]
23. Höhn, M.; Ábrán, P.; Vendramin, G.G. Genetic analysis of Swiss stone pine populations (*Pinus cembra* L. subsp. *cembra*) from the Carpathians using chloroplast microsatellites. . *Acta Silv. Lign. Hung* **2005**, *1*, 39–47.
24. Smith, W.K.; Germino, M.J.; Hancock, T.E.; Johnson, D.M. Another perspective on altitudinal limits of alpine timberlines. *Tree Physiol.* **2003**, *23*, 1101–1112. [CrossRef]
25. Renard, S.M.; McIntire, E.J.; Fajardo, A. Winter conditions—Not summer temperature—Influence establishment of seedlings at white spruce alpine treeline in Eastern Quebec. *J. Veg. Sci.* **2016**, *27*, 29–39. [CrossRef]
26. Holtmeier, F.K.; Broll, G. Wind as an ecological agent at treelines in North America, the Alps, and the European Subarctic. *Phys. Geogr.* **2010**, *31*, 203–233. [CrossRef]

27. Larcher, W. Frosttrocknis an der Waldgrenze und in der alpinen Zwergstrauchheide auf dem Patscherkofel bei Innsbruck. *Veröffentlichungen Mus. Ferdinandeum Innsbr.* **1957**, *37*, 49–81.

28. Holzer, K. Winterliche Schäden an Zirben nahe der alpinen Baumgrenze. *Cent. Gesamte Forstwes.* **1959**, *76*, 232–244.

29. Hadley, J.L.; Smith, W.K. Influence of krummholz mat microclimate on needle physiology and survival. *Oecologia* **1987**, *73*, 82–90. [CrossRef] [PubMed]

30. Aulitzky, H.; Turner, H.; Mayer, H. Bioklimatische Grundlagen einer standortsgemäßen Bewirtschaftung des subalpinen Lärchen-Arvenwaldes. *Mitt. Eidg. Anst. Forstl. Vers.* **1982**, *58*, 327–577.

31. Schönenberger, W. Cluster afforestation for creating diverse mountain forest structes—A review. *For. Ecol. Manag.* **2001**, *145*, 121–128. [CrossRef]

32. Day, M.E.; Greenwood, M.S.; Diaz-Sala, C. Age- and size-related trends in woody plant shoot development: Regulatory pathways and evidence for genetic control. *Tree Physiol.* **2002**, *22*, 507–513. [CrossRef] [PubMed]

33. Prendin, A.L.; Petit, G.; Fonti, P.; Rixen, C.; Dawes, M.A.; von Arx, G. Axial xylem architecture of Larix decidua exposed to CO_2 enrichment and soil warming at the tree line. *Funct. Ecol.* **2018**, *32*, 273–287. [CrossRef]

34. Fliri, F. *Das Klima der Alpen im Raume von Tirol*; Universitätsverlag Wagner: Innsbruck, Austria, 1975.