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Research Report

Cryocampsis: a biophysical freeze-bending response of shrubs and trees under snow loads

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Abstract

We report a biophysical mechanism, termed cryocampsis (Greek *cryo-*, cold, + *campsis*, bending), that helps northern shrubs bend downward under a snow load. Subfreezing temperatures substantially increase the downward bending of cantilever-loaded branches of these shrubs, while allowing them to recover their summer elevation after thawing and becoming unloaded. This is counterintuitive, because biological materials (including branches that show cryocampsis) generally become stiffer when frozen, so should flex less, rather than more, under a given bending load. Cryocampsis involves straining of the cell walls of a branch's xylem (wood), and depends upon the branch being hydrated. Among woody species tested, cryocampsis occurs in almost all Arctic, some boreal, only a few temperate and Mediterranean, and no tropical woody species that we have tested. It helps cold-winter climate shrubs reversibly get, and stay, below the snow surface, sheltering them from winter weather and predation hazards. This should be advantageous, because Arctic shrub bud winter mortality significantly increases if their shoots are forcibly kept above the snow surface. Our observations reveal a physically surprising behavior of biological materials at subfreezing temperatures, and a previously unrecognized mechanism of woody plant adaptation to cold-winter climates. We suggest that cryocampsis' mechanism involves the movement of water between cell wall matrix polymers and cell lumens during freezing, analogous to that of frost-heave in soils or rocks.

Keywords: stem bending biophysics, response to freezing, cold adaptation, woody plants, tundra albedo

Significance Statement:

This paper describes a recently discovered biophysical bending response (*cryocampsis*) of woody stems to subfreezing temperatures, wherein they bend more under the same load below than above freezing, causing shrubs to lie down under the snow, protecting them from winter hazards. Cryocampsis is conspicuous in Arctic and boreal zones with cold-winter climates, but also occurs (with lesser magnitudes) in a number of species of more southerly (except tropical) latitudes.
Cryocamptic bending intriguingly occurs despite the normal stiffening of frozen biological materials. It requires that stems be well-hydrated. Conversion of water to ice in the presence of plant cell wall polymers, thus has important mechanical and ecologically adaptive consequences, heretofore unrecognized, besides increasing tundra winter/spring albedo, significantly reducing global warming.

Introduction

Unlike most animals, land plants cannot move (except by seed, spore, or rhizome dispersal) to avoid environmental hazards. Perennial plants that successfully inhabit cold-winter climates have typically evolved one or more strategies such as frost hardiness, winter-deciduous leaf abscission, or dying down to subterranean overwintering structures such as rhizomes or bulbs. This last is not an option for trees or shrubs, and the other two, although general, may be insufficient for enduring severely cold winters such as those of the Arctic. Many tundra shrubs lie down, often nearly to the ground surface, under winter snow (Fig. 1B). This shelters them from winter environmental hazards, and also affects ecosystem energy balance: Sturm et al. (1) described how coverage of tundra shrubs by snow from winter into spring increases the tundra surface's albedo, thereby reflecting much of the spring sunlight. Absorption of this by shrub branches would not only warm the surrounding air and contribute to global atmospheric warming, but also reduce the duration of the reflective snow cover through spring, thus causing more sunlight absorption and conversion into heat.

After being freed of snow in late spring, Arctic shrubs normally return to their more erect, summer habit (Fig. 1A). Pomeroy et al. (2), and several references cited by Liston and Hiemstra (3), collected data on shrub/snow behavior needed for modeling tundra albedo; Liston and Hiemstra (3) and Ménard et al. (4, 5) modeled it, based (3) on snow burial of standing shrubs, or (4, 5) on including shrub bending under snow loads, assuming an instantaneous-elastic (Hooke's Law) bending response. We also

Competing Interest: The authors declare no competing interest.
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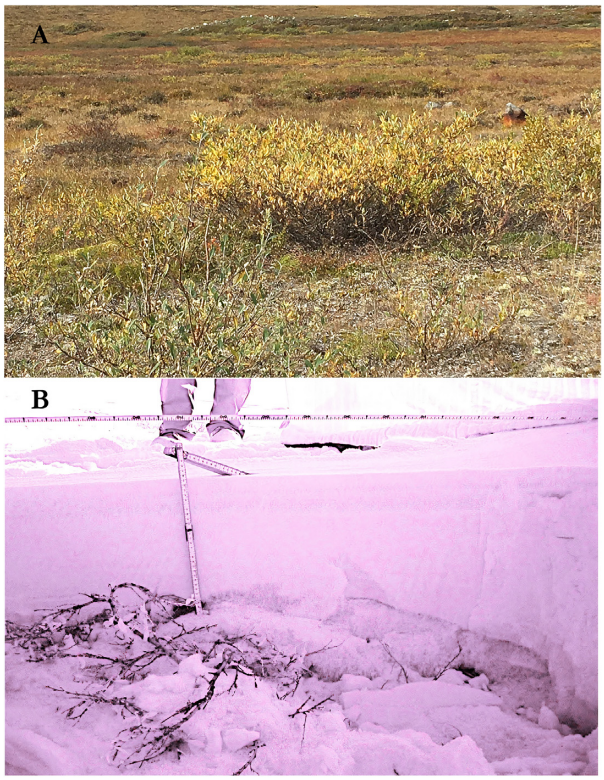

Fig. 1. Arctic tussock–tundra dwarf shrubs at different seasons. (A, above) in late summer (August) near Toolik Lake, Alaska, when leaves of the deciduous shrubs were senescing, and (B, below) in early spring (same general area, taken with spring sunshine) after substantial winter snowfall, excavated to reveal the bent-down, now leafless shrub branches. Compare these with the relatively vertical disposition of several shrub stems that can be seen in (A): center (large yellow leaves), *Salix glauca*; far left and far right (small leaves), *Betula nana*; and nearest foreground (left and center, few leaves), *S. alaxensis*. Although many of the shrub stems in (A) are hidden by foliage, it is clear that they are not bent down like those in (B). These latter, despite having been freed, by excavation, from their previous snow loads, have not straightened upward like those in (A). Thus, their downbending was not simply elastic; we now know it must have involved cryocampsis. For scale, the *S. glauca* shrub in (A) is ~1 m high, while the snow depth in (B) was about 40 cm; the (frozen) moss/soil substrate surface was just below the level to which the snow was excavated for this photograph.

investigated tundra shrub bending, finding that it involves a combination of instantaneous (Hookean) and time-dependent (retarded) elasticity (6).

While measuring this, with an apparatus outdoors (for typical springtime Arctic temperatures) at the Toolik Field Station in northern (68.4°N) Alaska, we serendipitously discovered (thanks to having started a repeat bending test on a tundra shrub branch just before a late-June snowstorm dropped the temperature to −6°C) that the branch segment that we had previously bending-tested above 0°C surprisingly bent more, under the same load, when it was exposed to −6°C. We soon ascertained that for Arctic tundra shrubs, extra bending was a genuine effect of subfreezing temperature. We named this freeze-bending response *cryocampsis* (Greek *cryo-*, cold, and *campsis*, bending).

Results

The cryocampsis response

We discovered and investigated cryocampsis using an apparatus [Figure S1 (Supplementary Material); ref. (6)'s Fig. 2] for measuring cantilever bending of isolated branch segments (firmly held, horizontally, at the basal end and caused to bend by a load hung from the free, apical end). Figure 2 gives a representative example of bending time courses for an Arctic tundra shrub (*Salix glauca*) branch segment when it was first cantilever-loaded, and then later unloaded, under different temperature (hereafter, "*T*") combinations, showing some of the characteristics of cryocampsis. Above freezing (Fig. 2A), upon either loading or unloading, the branch's initial, instantaneous elastic deflection was followed by a time-dependent bending creep, due mainly to retarded elasticity (6, 7).

Below freezing (Fig. 2B), the branch's instantaneous deflections upon either loading (hereafter "LI": see Materials and Methods for acronyms used here) or unloading the same weight as in Fig. 2(A) were about half of those when it was not frozen. This stiffening agrees with ordinary intuition, and with previous measurements on frozen vs. unfrozen wood (8–12). However, after loading the branch at −12°C (later moved to −15°C), substantial creep continued much longer than it did above freezing, causing considerably greater total downward bending (Fig. 2A and B).

To separate, from retarded elasticity and time-dependent irreversible bending, the effect of freezing upon bending, we first impose a bending load on the stem segment at an above-freezing *T* (Fig. 2C). After it completes its bending creep (usually within 18 to 24 h) we move it, while still loaded, to about −15°C. At this *T*, a cryocampsis-capable branch undergoes additional bending [hereafter, "FB" (freeze-bending)], faster than it bent during the latter part of its preceding creep (Fig. 2C and D). FB can occur at a *T* as high as −6°C, as noted in the Introduction. FB is largely completed within about 2 h. We presume that the slower, but ultimately greater, bending of a branch when first loaded at < 0°C (Fig. 2B) as compared with > 0°C (Fig. 2A), consists of cryocamptic FB combined with a retarded-elastic deflection that is slowed below 0°C, compared with its progression above freezing.

Recovery from cryocamptic freeze-bending

If a branch segment that has undergone cryocamptic FB is unloaded while still frozen (Fig. 2B and C), it recovers by an instantaneous elastic unbending that is only about half as great as its LI under the same load above 0°C (e.g. Fig. 2A and D). This is followed by a limited retarded-elastic recovery, which leaves the segment tip below its original, preloading elevation. If the branch is then brought above 0°C (far right in Fig. 2B and C), it unbends rapidly at first, and eventually returns nearly or completely to its original elevation by a retarded-elastic recovery similar to that in the right-hand part of Fig. 2(A).

If, after undergoing cryocamptic FB, a branch segment is instead warmed above 0°C while still carrying its load (hereafter, "LW"), it raises this load substantially (Fig. 2D). This is surprising because, as shown below, upon thawing the branch becomes much more elastically flexible than it was while frozen, so it might instead have been expected to bend further under its load. However, we have repeatedly observed that loaded, frozen, cryocampsis-capable branches raise their load upon thawing.

When a previously frozen and then thawed (while still loaded) Arctic shrub branch segment is then unloaded (far right in Fig. 2D), it undergoes an immediate elastic recovery similar to its previously measured LI, having recovered its instantaneous elastic compliance. It subsequently returns to, or almost to, its original elevation after completing a retarded elastic recovery (Fig. 2D) similar to, or somewhat greater than, that which a never-frozen segment undergoes after unloading (Fig. 2A). Thus, the large effect

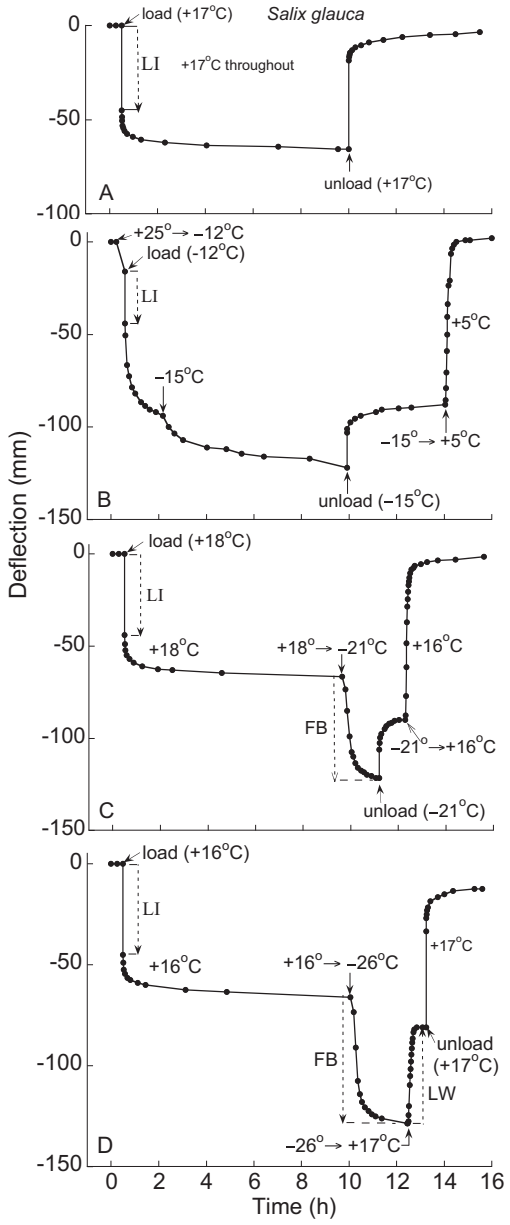

Fig. 2. Bending of a cantilever-loaded branch segment from the tundra shrub *S. glauca* from the Alaskan North Slope at different temperatures. Deflection of the segment's tip was recorded after loading a 177-g weight just behind its tip, and after subsequently unloading it, after various temperature ("*T*") changes. Negative values represent downward deflection from the tip's initial elevation. The gap preceding the first data point beyond each loading or unloading gives the "instantaneous" (<1 s) elastic deflection ("LI"). (A) Segment was loaded, and later unloaded, at +17°C throughout. (B) Segment was first moved from +17°C to −12°C, and 20 min later was loaded; at 2.2 h, when the surrounding (outdoor) *T* had risen to −11°C, the segment was moved into a −15°C chamber, in which it was subsequently unloaded; 4 h later it was moved to +5°C. In (C) and (D), the segment was loaded at >0°C, then after completing its retarded-elastic bending it was moved to <−20°C, inducing cryocamptic freeze-bending ("FB"). (C) After completing this, the segment was unloaded at −21°C; 1 h later it was returned to +16°C. (D) After undergoing FB at −26°C, the still-loaded segment was returned to +16°C; after raising its load ("LW") as far as it would, it was unloaded (followed by retarded-elastic recovery). Dotted-line vertical arrows denote magnitudes of deflections assigned to LI, FB, and LW (acronyms used in the text and listed in the Appendix). Branch segment was 300 mm long, 8.6/6.5 mm in basal (clamped)/apical (free) diameter, and was collected on 2008 August 26 from a *S. glauca* shrub near the University of Alaska's Toolik Field Station (68°37.7' N, 149°35.7' W).

of freezing to induce additional bending is almost completely reversible, but only after a branch is *both* relieved of its load *and* is brought above 0°C. Hysteresis is clearly involved in the response.

The cycles illustrated in Fig. 2(C) or (D) can be obtained repeatedly with the same branch segment, so they involve no irreversible alterations. Therefore, cryocampsis involves no irreversible decrease in either instantaneous or retarded elastic moduli like that reported by Mishiro (13) after freezing woods in liquid N_2 (−196°C).

Historical aspects

As far as we know, cryocampsis has not been reported from previous mechanical measurements on wood or woody branches at subfreezing *T*s (e.g. 8–12). A few accounts in the 19th century botanical literature, cited by Pfeffer (14), reported winter downbending, below freezing, of leafless (deciduous) tree branches, which reversed upon subsequent thawing, so was probably cryocampsis. Possible reasons why this was subsequently ignored include (a) Arctic and certain boreal hardwoods, which respond most conspicuously below freezing, have rarely if ever been previously tested mechanically; (b) softwoods (conifers), although often tested mechanically, respond less conspicuously below freezing than do hardwoods; and (c) previous measurements lacked the postloading temperature downshift, which reveals cryocamptic FB most strikingly (Figs. 2C and D, and 3A and B).

Quantifying cryocampsis

Previously loaded and frozen, cryocampsis-capable hardwood branches usually raise their load, when warmed above 0°C (LW), by only about half to three-fourths of the distance that they had descended during the preceding exposure to subfreezing temperature [e.g. Figs. 2D, and 3A and B; mean fractional recovery (LW/FB) of 0.58 ± .018 ($n = 125$) in our tests on the species of Arctic shrubs cited below]. Since these branches' LW is an important part of their recovering their initial elevation after subsequent unloading, we consider that a load-lifting response to thawing is an essential feature of cryocampsis. It distinguishes cryocampsis from mechanical effects due to freezing injuries, and from other bending effects of freezing that are not substantially reversible by thawing while still loaded. We, therefore, in what follows, adopted the temperature-shift procedure in Figs. 2(D) and 3(A) and (B) (normally using a test *T* of ∼ −15°C, that of the freezer room available to us) as the standard test for comparing cryocamptic responses among different species.

We normalize (divide values of) FB or LW by, respectively, the initial instantaneous elastic deflection (LI), or the FB that precedes the warming that produces LW, so the standard assay's results are two numbers, FB/LI and LW/FB. Both are obtained from a single bending time course like that in Fig. 3(B) (except for recovery *T* normally being +21° rather than +2.5°C, as there). So that the two assay numbers can easily be distinguished, we normally express FB/LI as a % of LI, but LW/FB as a simple fraction; these normalizations are justified in Methods. With a view toward what is probably ecologically significant, for hardwoods (dicots) we set a lower limit of 30% for FB/LI and 0.3 for LW/FB, that an assay normally must satisfy to indicate definite cryocampsis (for softwoods, see below).

We found, by this test, substantial cryocampsis in 11 out of 12 erect (as against prostrate) Arctic shrub species available for testing. The one available Arctic tree species, *Populus balsamifera,* gave tests in the lower part of the cryocampsis range defined above. However, the occurrence, and magnitude, of cryocampsis was very different in woody plants from lower-latitude biomes.

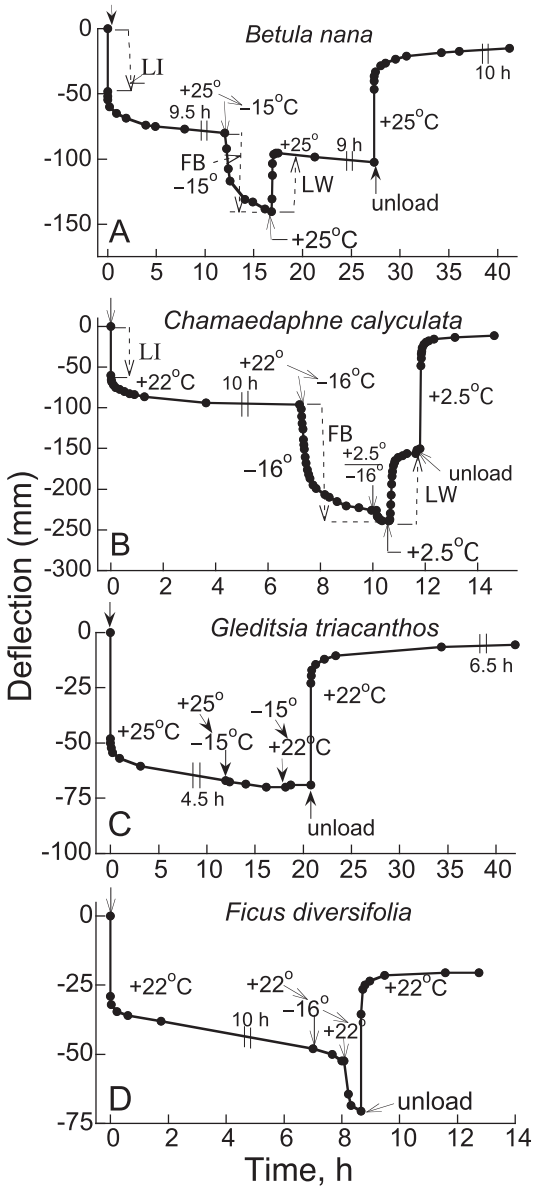

Fig. 3. Tests for cryocampsis, similar to that in Fig. 1(D), performed on branch segments from several woody species. (A) the Alaskan Arctic tundra shrub *Betula nana* (dwarf birch), (B) the boreal shrub *Chamaedaphne calyculata* ("leatherleaf"), (C) the temperate-zone deciduous tree *Gleditsia triacanthos* (honey locust), and (D) a tropical shrub, *Ficus diversifolia* (a species of fig). Unlabeled solid, downward arrow at each time course's starting point indicates initial loading. Vertical pairs of small lines across a plot line show where blocks of time were deleted from the plot to better display periods of rapid flexure. Dotted-line vertical arrows indicate LI, FB, and LW, as in Fig. 2. In (A), the gradual decline that follows the sharp rise that occurred upon transfer back to +25°C is irreversible bending (6) that resumed at that *T* under the load, and later became visible by the tip's failure to return fully to its initial elevation. In (B), at "+2.5°/−16°" the stem received 2 min at +2.5°C and was then returned to −16°C, a treatment that causes a small additional cryocamptic downbending, probably as a result of relief from bending restriction by internal ice (9). Stem segment lengths, midpoint diameters, and applied loads were (A), 260 and 6.0 mm, 177 gm; (B), 250 and 3.2 mm, 26.5 gm; (C), 275 and 3.75 mm, 66.2 gm; and (D), 187 and 5.9 mm, 234 gm. Collections were from (A) near Toolik Lake on the Alaskan North Slope, (B) the boreal forest near Fairbanks, Alaska, (C) ornamental broadleaf stand near Cliff, New Mexico, and (D) the tropical biology greenhouse at the University of Alaska Fairbanks. With many tropical plants other than (D), significant downbending occurred upon exposure to −16°C, followed by similar or greater downbending upon thawing, instead of by a rise as in (A) and (B).

Cryocamptic responses of species from different biomes

Table 1 gives biome mean values of FB/LI and LW/FB for a total of more than 800 branches from a total of nearly 200 woody species from five biomes (one-way ANOVA, $F_{5,191} = 22.7609$, $P < 0.0001$ for FB/LI). The tropical sample's FB/LI mean was close to zero, and the three mid-latitude samples' means only slightly greater, the differences between most of them being statistically insignificant in Tukey's post hoc tests. The boreal species' mean FB/LI was intermediate between any of the four lower-latitude biomes' and the Arctic's ones, these differences being highly significant, except for those involving the desert sample, whose *n* was small.

Although at least some biome LW/FB means differ across biomes ($F_{5,191} = 3.1659$, $P = 0.0038$; Table 1), what is instead important here is the difference between these numbers and the cryocampsis LW/FB threshold of 0.3. The Arctic and boreal LW/FB biome means differ from 0.3 by some five to six times their SEs, whereas the lower-latitude LW/FB means differ from 0.3 less than, to only slightly more than, their SEs. Thus, the former means are derived from multiple cryocampsis-positive species, whilst the latter means likely are derived mostly from species that do not show cryocampsis.

The largest cryocamptic response that we encountered is actually from a primarily boreal shrub, *Chamaedaphne calyculata* (Ericaceae). About one-third of the stems of this species that we tested from interior Alaska freeze-bent by more than 200% of their LI, and recovered a fraction 0.6 to 0.7 of that bending when thawed under load (LW/FB), a truly spectacular performance (Fig. 3B; *maximum response* columns in Table 2). Its most weakly responding stems freeze-bent by more than 100% of LI, leading to a biome-mean FB/LI of 158% (Table 2).

Table 2 lists all 36 species (from all sampled biomes) that gave species-mean FB/LI values greater than 30% in our tests. These data and those in Table S1 (Supplementary Material) provide the following observations:

(a) The 10 species giving the strongest response (FB/LI > 65%) are all shrubs, #s 1 and 4 being evergreen and the rest, deciduous. All are from the botanical families (cladistic groups) Betulaceae, Salicaceae, or Ericaceae (Table S1, Supplementary Material).

(b) All the species in Table 2 are hardwoods (dicotyledons). Almost half (12) are trees; only six are evergreen. All but four (#s 13, 23, 32, and 36) satisfy both of the above-stated criteria for genuine cryocampsis (mean FB/LI ≥ 30%; mean LW/FB ≥ 0.3).

(c) Although 23 species are from the Arctic or boreal biomes, nine species [excluding the four noted in (b)] are native to either temperate, desert, or Mediterranean habitats; therefore, cryocampsis is not restricted to habitats with heavy winter snowfall or severely low winter *T*s.

(d) Although a slight majority (19) of the 36 species with strong cryocampsis (Table 2) are from the botanical families in (a), among those with mean FB/LI < 65% 12 other botanical families are represented. Thus, cryocampsis probably originated independently in multiple families.

(e) Many of the species with weaker cryocampsis, especially those with a large SE (indicating a wide spread between maximum and mean responses), included one or more branches that did not satisfy the 0.30 LW/FB criterion, even though other branches of the same species did. This suggests that the requirements for cryocampsis to occur might be rather subtle.

Table 1. Mean freeze-bending parameters of species from different biomes. Means preceded only by different letters are significantly different, those with the same letter are not.[1]

Biome/wood type	*n*	Mean			
		FB/LI % of LI	± S.E.	LW/FB[2] Fraction of FB	± S.E.
Arctic	12	A 64.9	± 7.6	AB 0.52	± 0.027
Boreal[3]	40	B 29.8	± 4.7	A 0.64	± 0.055
Temperate	60	C 14.9	± 1.8	AB 0.43	± 0.054
Desert	6	BCD 15.4	± 9.5	AB 0.30	± 0.107
Mediterranean	52	CD 13.9	± 2.1	B 0.27	± 0.031
Tropical	27	D 1.8	± 0.6	B 0.22	± 0.196
Mid-latitude[4]					
Hardwoods	129	A 20.7	± 2.0	A 0.33	± 0.024
Softwoods	29	B 8.4	± 1.1	B 0.84	± 0.087

[1]In Tukey's HSD post hoc tests following one-way ANOVAs, for differences between biomes, or in Kruskal–Wallis tests for differences between hardwoods and softwoods.
[2]This column's tropical, med., and desert LW/FB means would be negative if, in calculating species means, negative LW values (= branch tip declined upon thawing) had not been assigned the value 0 (see Methods). The last four FB/LI means in the upper part of the table would be 0.5% to 1% lower if the same adjustment had not been made there. It did not affect the uppermost two means in either column.
[3]No softwoods had a mean FB/LI > 22%, so they depress the boreal mean FB/LI, which would be 38.9 ± 6.3% if only hardwoods were included, still substantially lower than the Arctic (all hardwoods) mean, due to the low FB/LI values of many boreal hardwoods.
[4]Includes species from boreal, temperate, and Mediterranean biomes only. Species from Arctic, desert, and tropical biomes are omitted from the comparison because our samples from them contained only hardwood species, no softwoods (gymnosperms).

(f) The wide distribution of FB/LI values among branches within species and the continuous spread of mean values across different species suggest that cryocampsis is probably inherited quantitatively, rather than in Mendelian fashion.
(g) Cryocampsis is probably constitutive in species that exhibit it, rather than being induced solely by prior exposure to low temperatures, since several species that occur in cold-winter climates but which we sampled from mild-winter, above-freezing habitats (#s 12, 16, 27, 29, and 33) showed definite cryocampsis.

Below 30% FB/LI, the occurrence pattern is similar to that in the lower part of Table 2. Table S2 (Supplementary Material) lists 19 species that gave mean FB/LI values between 30% and 20%. About half of these species had mean LW/FB values > 0.3, suggesting marginal cryocampsis. A large majority of species tested from nontropical, lower-latitude biomes, and even almost half of our 40 tested boreal species (Species Survey section, Supplementary Material), gave mean FB/LIs below 20% and mean LW/FBs mostly < 0.3, so did not have significant cryocampsis. Many of these gave a test like that in Fig. 2(C), where neither FB nor LW occurred, whilst tests of certain others resembled that of many tropical species (Fig. 2D).

Tropical species

Most of those tested bent downward at least slightly upon freezing, but when then thawed, rather than lifting their load, they bent down further, irreversibly, and often much more than in Fig. 2(D) (a relatively modest example). This suggests that their FB was due not to cryocampsis, but to freezing injury causing loss of living-cell turgor pressure involved in these stems' support, and the mechanical consequences of this loss continue to develop when the stem is later thawed. The resulting (often very large) negative LW/FB values, while useful in indicating that the preceding FB was not cryocamptic, are not meaningful for calculating numerical values for comparing the strength of cryocampsis among species, so for use in Table 1 both were adjusted to 0. None of the tested tropical species, even those few that did not exhibit a freezing-injury mechanical effect, showed genuine cryocampsis, since they consistently failed to raise their loads after thawing (LW ≤ 0).

Softwoods

Conifers, from the three intermediate-latitude biomes from which we sampled both them and hardwoods (dicotyledons), gave a mean FB/LI that was significantly lower than that of the hardwoods (χ^2 = 10.6003, df = 1, P = 0.0011; Table 1). In contrast, the softwoods' mean LW/FB was significantly higher than hardwoods' (χ^2 = 31.926, df = 1, P < 0.0001), because 45% of the 29 tested softwoods had LW/FB means close to, or in four cases even above, 1.0 (Table S3, Supplementary Material). Thus when thawed, they raised their loads on average at least as much as, or sometimes even more than, they had declined when subjected to −15°C. In contrast, hardwoods, as noted above, almost never gave a mean LW/FB > 0.8. The ≥1 mean LW/FB of many softwoods indicates that their FB is genuine cryocampsis (see Supplementary Material).

Scaling up cryocampsis measurements to field conditions

In larger-scale tests comparable to those in Figs. 2(D) and 3, but on longer, thicker, older branches under much larger imposed loads, *S. glauca* and *Betula nana* branch segments up to 30 mm in diameter and ~1 m long gave values of FB/LI similar to those obtained from the younger, smaller branch segments (mostly ~6 to 8 mm in diameter, ~300 mm long) of the same species in the tests shown in Figs. 2 and 3 and Tables 1 and 2. This suggests that observations from our standard bending assay would apply at least approximately to entire branches of Arctic shrubs, such as those in Fig. 1(B), the downward curvature of whose thicker, older stem portions in spring at T < 0°C is clearly visible.

Cryocampsis's effect to decrease branch elevation outdoors in winter should exceed the effect that might be expected from our FB/LI assay data. This is because the FB/LI values are normalized relative to a branch's instantaneous elastic bending (LI) under the given load at +21°C, but LI under subfreezing conditions in the field will be lower than LI at +21°C, so FB will be a larger fraction of it. LI under subfreezing conditions is smaller because the T coefficients of ordinary (Hookean) elastic compliance are positive

Table 2. Species giving mean freeze-bending responses of > 30%. Listed are (a) the ratio of freezing-induced bending to instantaneous elastic deflection (FB/LI) expressed as %, and (b) upon subsequent thawing, the ratio of rise in tip elevation to the preceding downbending (LW/FB) expressed as a fraction, for both the maximum and the mean (± SE for *n* tests) for each species. Species are listed in decreasing order of mean FB/LI.

Sp.	Species[1]	Native habitat[2]	Maximum[3]		Mean				
No.			FB/LI % of LI	LW/FB	FB/LI % of LI	± SE	LW/FB	± SE	*n*
1	*Chamaedaphne calyculata*	bor	242	0.61	157.9	± 12.1	0.69	± .055	17
2	*Salix hastata*	arc	147	0.69	100.2	± 11.9	0.53	± .035	10
3	*Alnus viridis ssp. fruticosa*	arc	169	0.55	97.4	± 8.3	0.55	± .030	16
4	*Ledum groenlandicum*	bor	127	0.63	91.2	± 7.9	0.60	± .040	10
5	*Betula nana*	arc	150	0.89	89.2	± 5.0	0.66	± .033	30
6	*Salix glauca*	arc	151	0.77	88.5	± 5.7	0.65	± .030	33
7	*Salix arbusculoides*	arc	110	0.53	75.8	± 18.5	0.37	± .088	4
8	*Betula neoalaskana*	bor	109	0.63	75.5	± 9.6	0.59	± .056	6
9	*Alnus viridis ssp. fruticosa*	bor	115	0.45	71.0	± 8.5	0.45	± .039	12
10	*Salix richardsonii*	arc	121	0.73	66.0	± 6.9	0.53	± .044	19
11	*Chilopsis linearis*	des	97	0.43	61.6	± 16.9	0.43	± .053	4
12	*Baccharis pilularis*	med	93	0.66	60.8	± 4.9	0.43	± .046	12
13	*Platanus racemosa*	med	104	0.65	60.5	± 20.6	0.27	± .136	5
14	*Viburnum edule*	bor	87	0.68	58.5	± 13.5	0.72	± .034	5
15	*Salix alaxensis*	arc	102	0.44	57.6	± 5.1	0.45	± .027	20
16	*Tilia platyphyllos*	tem	78	0.44	56.7	± 5.1	0.47	± .046	10
17	*Salix pulchra*	arc	92	0.85	53.4	± 3.2	0.52	± .044	29
18	*Arctostaphylos uva-ursi*	bor	101	0.71	51.5	± 15.3	0.62	± .086	5
19	*Betula glandulosa*	bor	64	0.94	51.2	± 3.2	0.82	± .026	6
20	*Rosa acicularis*	bor	80	0.57	49.4	± 7.7	0.50	± .057	8
21	*Platanus wrightii*	tem	57	0.44	46.5	± 3.8	0.41	± .020	4
22	*Alnus rubra*	tem	83	0.59	44.7	± 14	0.31	± .095	8
23	*Hedera helix*	tem	59	0.16	44.1	± 7.6	0.20	± .047	7
24	*Salix alaxensis longistylis*	arc	47	0.48	41.1	± 3.3	0.46	± .024	4
25	*Shepherdia canadensis*	arc	87	0.78	38.1	± 14	0.49	± .142	5
26	*Populus balsamifera*	arc	75	0.43	38.0	± 3.8	0.41	± .040	18
27	*Liquidambar styraciflua*	tem	69	0.78	35.0	± 13	0.69	± .024	5
28	*Sorbus sitchensis*	bor	43	0.24	33.5	± 4.4	0.33	± .052	4
29	*Juglans major*	tem	44	0.34	33.4	± 3.0	0.30	± .014	5
30	*Quercus lobata*	tem	52	0.27	33.4	± 4.5	0.36	± .066	10
31	*Betula kenaica*	bor	40	0.52	33.3	± 2.2	0.48	± .072	4
32	*Populus balsamifera*	bor	41	0.34	32.6	± 2.4	0.23	± .083	9
33	*Holodiscus discolor*	med	63	0.31	31.6	± 7.9	0.43	± .061	7
34	*Elaeagnus commutata*	bor	64	0.33	31.1	± 3.7	0.46	± .093	5
35	*Vitis monticola*	tem	54	0.57	31.0	± 5.9	0.53	± .084	6
36	*Nerium oleander*	med	100	0.47	30.2	± 14	0.21	± .086	7

[1]Species with mean FB/LI values < 30% are listed in Tables S2 and S3 (Supplementary Material) and its Species Survey text; growth forms, taxonomic (evolutionary) affinities, vernacular names, natural occurrence, and provenances (for bending tests) of all the species in this paper's tables are given in Table S1 (Supplementary Material).
[2]Species that occur in >1 biome were sampled only from the biome(s) listed for them in this column. Biome abbreviations: arc, Arctic; bor, boreal; des, desert; med, Mediterranean (climate); and tem, temperate. No tropical species qualified for inclusion here (none had FB/LI > 30%).
[3]Highest FB/LI of any of the *n* tests made on branches of the given species, and the LW/FB value that accompanied it (often, but not always, the highest LW/FB for this species).

(compliance decreases with decreasing *T*). The details of this are considered in the Supplementary Material. They lead to a formula for Φ, the fractional increase in field bending expected for a given lab test FB/LI value:

$$\Phi = \frac{(\mathrm{FB/LI})}{\xi_i + \xi_r * \varphi}, \quad (1)$$

in which the ratios of tensile (Young's) moduli between +21°C and the $T < 0$°C in both the field and the lab freezing test are ξ_i and ξ_r for instantaneous (i) and retarded (r) elasticity, respectively, and φ is the ratio of retarded to instantaneous bending at +21°C.

This formula indicates that for lab FB and field *T*s of −15°C, FB/LI values should be multiplied by 1.3 to give the field bending enhancement by cryocampsis, a not insignificant correction. However, all the factors in formula (1) vary with *T* and with species, so a completely general number cannot be given for Φ. Also, to get from Φ to actual field bending, atmospheric factors determining snowfall rate, and winter shoot morphologies (e.g. marcescent leaves) influencing snow capture by vegetation, would also have to be taken into account. The "Scaling-up…" section of the Supplementary Material presents a rough calculation to correct Φ for differences between field and cryocampsis assay *T*s < 0°.

Cryocampsis and winter survival of buds

That cryocampsis is ecologically significant in cold-winter climates is suggested by the abundance of strong cryocamptic responses in species native to high latitudes. Its ecological benefits likely derive from protecting shrub branches from winter injury

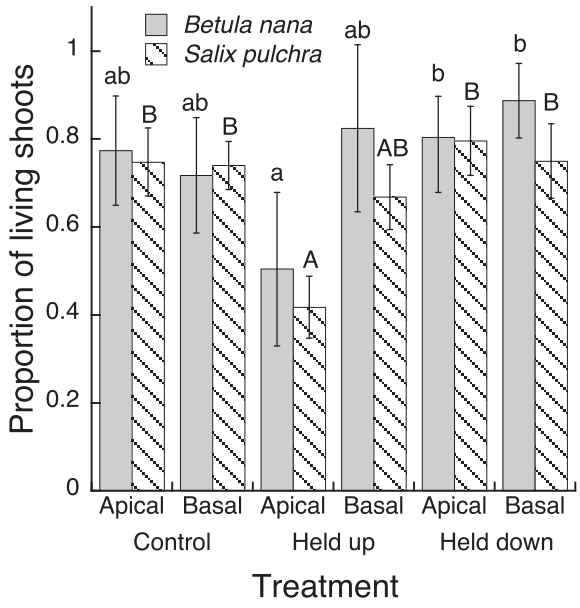

Fig. 4. Effect of snow cover on winter survival of Arctic tundra shrub buds. Bars show living shoots (including live buds and catkins) as a proportion of total bud positions in spring 2008, from buds produced in 2007, on tagged, apically or basally located, branchlets on branches of *B. nana* (gray bars) or *S. pulchra* (hatched bars) that were either held upward by tying to a stake, held down to the ground, or not manipulated (controls), over the 2007 to 2008 winter. All except the apical, held-up branches were below the winter snow surface (the untied, apical ones because of elastic plus cryocamptic downbending under their snow loads); the apical, tied-up ones were at least 30 cm above the snow surface. Treatments that do not share a common letter differ significantly in Tukey's post hoc tests: capital letters for *S. pulchra*, lower case for *B. nana*. For *S. pulchra*, error bars indicate 1 SE for untransformed data; for *B. nana*, error bars indicate 95% CI estimated from the statistically best, variance-structured model (see Table 3; Tables S5 and S6, Supplementary Material).

by helping to get, and keep, them below the winter snow surface. To test this, we ran an experiment (Fig. 4), over two successive winters, wherein the position of tundra shrub shoots was manipulated by tieing and holding arrangements, to be either above or below the snow surface. A significantly lower proportion of surviving shoots and buds was found in spring on tagged branchlets of *S. pulchra* that were located on branches that had been tied upward to keep them above the snow surface over the preceding winter, compared with buds that were kept below the snow surface (Fig. 4; Table 3). A similar pattern was seen for branches of *B. nana* (Fig. 4; Table 3), but not quite as strong, so significance of some of the *B. nana* treatment-by-position combinations was intermediate in Tukey's HSD tests (Fig. 4).

Mean shoot mortalities for winter-exposed (tied up) shoots of *S. pulchra* or *B. nana* were respectively 2.8 or 2.5 times the mortality of shoots below the snow surface for the winter of 2008 to 2009 and 4.8 or 3.0 times for the winter of 2007 to 2008. Mortality was about 20% for buds on basal branches that were either tied up or held down below the snow surface, and also for buds on untied basal shoots (similarly below the snow surface; Fig. 4). This implies that tieing itself was not responsible for the much greater mortality of tied-up, apical shoots.

Physical basis for cryocampsis

A freezing-induced deflection of 19 mm under an apical load of 68 g was obtained both before and after completely stripping the bark from an *S. pulchra* branch segment. Similar results were obtained with stems of *C. calyculata*, in Denmark, by Frederik Ridder-sholm. They indicate that the changes responsible for cryocampsis occur in the wood (xylem).

Since xylem is composed mainly of dead cells, it is perhaps not surprising that cryocampsis still occurs after all the living cells of a branch segment have been killed by treating the stem with steam (5 to 10 min at 100°C). Steam-killed *B. nana* gave an FB that was 80.7% of that prior to killing, with an LW/FB of 0.66, while *S. richardsonii* gave an FB that was 63.4% of that prior to killing, with an LW/FB of 0.51. Cryocampsis thus appears to be due to mechanical properties of the thick secondary cell walls of the xylem's mostly dead cells.

Steam-killing would eliminate the turgor pressure of any living cells. Therefore, failure of steam-killing to suppress cryocampsis shows that this bending cannot be based on any low-temperature effect on living cell turgor pressure, like that reported for shrinkage of alpine conifer bark during freezing (15).

That killed stems, lacking metabolism, can do the work required to lift a load during thawing, implies that the energy for this work comes from potential energy that was conserved elastically in their cell walls during cryocamptic FB. It might appear that the stem's elastic compliance has to increase, when exposed to subfreezing temperature, by 2- or 3-fold (for Arctic shrubs or for *C. calyculata*, respectively) in order for an existing load to double or triple its deflection during FB. However, data in Fig. 1(A)–(C) and previous work [e.g. (8, 9, 11)] indicate that a well-hydrated branch's elastic compliance is only about half as great at −15°C as it is at > 0°C, the opposite of what is needed to explain cryocamptic FB. Thus, a simple increase in bending compliance is not the basis for this FB.

A mechanical alternative to explain cryocamptic bending would be a low-*T*-induced downshift, under a load, in the null point for elastic bending (a temporary irreversible bending, that persists only until $T > 0$°C). This null point would be the elevation to which a branch segment, post FB, recovers when unloaded while still frozen (as in Fig. 2B and C). Loading is apparently required to shift the null point. This is shown by post-FB reclamping of a branch segment in different orientations (e.g. upside down), then reloading and refreezing it, because the renewed FB and null point shift always occur in the direction of the new load**.** A possible physical basis for such a downshift in null point and its reversal upon thawing involves interactions with water.

Role of water in cryocampsis

Cryocamptic FB disappears if a branch segment is allowed to air-dry to a moisture content less than about 20% of its dry weight (Fig. 5). The response can be restored if the moisture content is raised back toward its initial value after moderate dehydration, and can be at least partially restored by the partial rehydration following more complete dehydration (Fig. 5). Therefore, water (probably xylem cell wall water) appears to be involved in the FB process. This requirement is a further reason why cryocampsis has not been recognized, heretofore: most previous bending measurements have been performed on air- or kiln-dried wood with a moisture content of ≤ 12%, at which cryocampsis scarcely occurs (Fig. 5).

Discussion

Subfreezing *T*s in the range of −5° to −15°C cause many well-hydrated Arctic tundra shrubs, and some boreal forest shrubs and

Table 3. Analysis of deviance (type III) for data in Fig. 4. Data were the proportion, (surviving buds and shoots)/(total prewinter bud positions), on shrub branchlets. Fixed effects were treatment (held up, held down, and no manipulation), position (apical vs. basal), and treatment by position interaction (T*P). Ndf and Ddf = numerator and denominator degrees of freedom, respectively. Bold values indicate significance at $\alpha = 0.05$.

Species	*S. pulchra*				*B. nana*			
Factor	Ndf	Ddf	χ^2	P	Ndf	Ddf	χ^2	P
Treatment	2	104	18.4406	**<0.0001**	2	98	8.6739	**0.01308**
Position	1	104	0.1844	0.6677	1	98	4.5438	**0.03304**
T*P	2	104	6.5470	**0.0379**	2	98	6.1249	**0.0468**

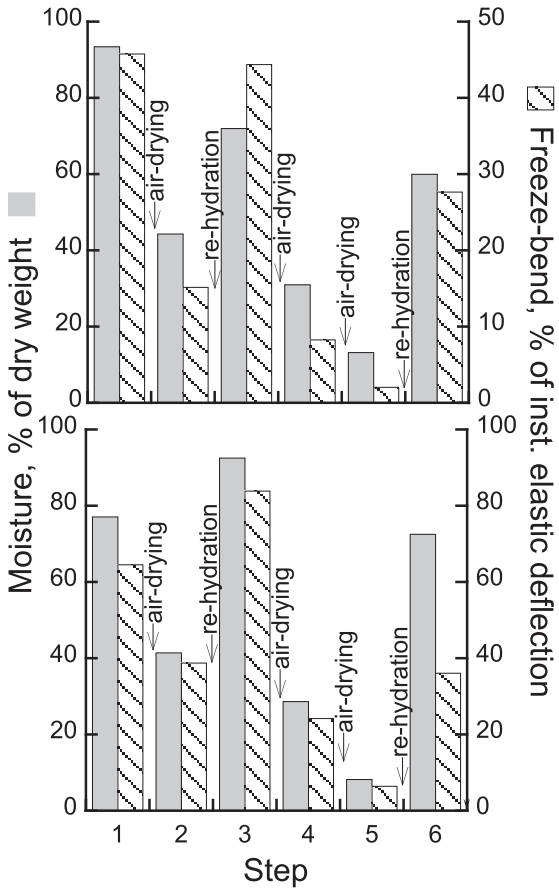

Fig. 5. Cryocamptic bending (cross-hatched bars) and moisture content (gray bars) after dehydration and subsequent rehydration of two stems of *S. glauca* (upper and lower panels, respectively). From their initial condition (column 1) they were retested after successive periods of air-drying (columns 2, 4, and 5) interspersed with rehydration by soaking in cold (3°C), deionized water (columns 3 and 6). FB [expressed as % of initial (undehydrated) LI] was fully restored by rehydration after moisture had been reduced to about 50% of its initial content, and was partially restored by rehydration after drying to the point that almost completely suppressed cryocampsis. Rehydration was for ~29 h for column 3, and for 5 days for column 6. Dehydration prior to column 2 was by standing 10 days in laboratory air at 21°C, and for columns 4 and 5 was in gently moving laboratory air warmed to 43°C for ~40 h. Comparable effects of dehydration on FB were obtained with several other Arctic shrubs (*S. pulchra*, *S. alaxensis*, and *S. richardsonii*; *B. nana*; and *Alnus viridis* ssp. *fruticosa*).

trees, to reversibly bend down more under applied bending loads such as snow, than they would be expected to do based on their above-freezing elastic compliance. Their high-latitude habitats suggest an ecological connection with a cold-winter climate. Hydrated biological tissues generally stiffen, rather than becoming more compliant, upon being frozen. That Arctic shrubs' Hookean stiffness rises sharply, rather than falling, as they undergo cryocamptic FB suggests that cryocamptic bending may be due instead to an observed freezing-induced downshift in a stem's bending elasticity null point.

Ecological significance of cryocampsis

Cryocampsis will tend to cause tundra shrub branches to bend lower during winter than they would have done from the weight of their snow load alone, so it should increase a shoot's chances of spending the winter and much of the subsequent spring below the snowline. Having branches well-below the winter snow surface may be especially important to shrubs in Arctic tundra, which typically receives only modest winter snowfall that is often less than would completely cover them if their branches retained their summer elevation. Subsurface positioning should physically shelter buds from abrasion by wind-driven snowflakes and other ice particles, as well as from winter desiccation (16), and from injury by low-temperature extremes, by snow's insulating action. It should also help shrubs avoid bud loss due to avian (17, 18) and mammalian (19) bud-eating herbivores that feed predominantly on buds above the snow surface.

A second beneficial effect of cryocampsis for shrubs should occur when winter snow gets windblown off branches at $T < 0$°C. The loading hysteresis involved in cryocampsis (Fig. 2B and C) would keep them from rising up as much as they do after the late-spring thaw, thus improving their chances of becoming promptly recovered by further snowfall within the given winter. Cryocampsis should, therefore, be ecologically adaptive for tundra shrubs, conferring additional protection against shrub winter mortality, as the results in Fig. 4 suggest, and thus might well have evolved by natural selection.

A third ecological benefit of cryocampsis, for boreal trees and tall shrubs, whose upper branches cannot descend below the snow line, is to tip the angle of lateral branches downward toward the vertical. Considering how easily freshly deposited snow can be made to fall from branches, even the ~26% increase in field bending that would result from the modest ~20% FB/LI of the best-responding boreal conifers would probably help them avoid snow loads that could break their branches or trunks (*cf.* 20). Thus, cryocampsis might well be naturally selected for, in boreal trees and tall shrubs.

A separate explanation is needed for how a snow-loaded Arctic shrub branch can bend down *far below* the snow surface (Fig. 1B). The snow load that at first drives ordinary elastic and cryocamptic bending will become relaxed as a branch bends enough to make mechanically significant contact with the snow surface below it, temporarily stopping its downbending. Over a period of days, snow recrystallization metamorphism (21) will weaken the snow below

the branch, allowing it to bend down further, elastically and by cryocampsis. Subsequent snowstorms will both bury it further below the snow surface, and increase the snow load on it, tending to depress it by elastic and cryocamptic bending even more. Cryocamptic bending will also increase if the *T* becomes increasingly negative. By this combination of effects a branch can approach the ground (Fig. 1B), keeping it beneath the snow, and thus maintaining the high albedo surface of tundra much longer through the Arctic spring than if it lacked cryocampsis. This is important to the reflective role of shrub snow covering in slowing global warming.

Possible mechanism for cryocampsis

Cryocamptic FB is probably due to migration, below 0°C, of water from within xylem cell walls, to the ice that forms by freezing of water that is inside the xylem cell lumens (cell chambers). Freezing-induced dehydration of xylem cell walls has long been inferred (22–24, 25, pp. 62–64). Hans Kübler (26, 27) used it to explain frost-crack formation in tree trunks (28). He ascribed this water migration ("Ausfrieren von Wasser") to the vapor pressure of water associated with cell walls exceeding that of the water ice within the xylem cell lumens (29), presumably because the chemical potential of water associated with cell walls is higher than that of crystalline water (ice) at $T < 0$°C.

How freezing-induced cell wall dehydration could lead to cryocamptic bending involves several possible mechanisms. Collapse of normally (>0°C) water-filled wall capillary channels through dehydration could allow additional compressive or extensile strain to occur on the branch's lower or upper side, respectively, in the latter case by allowing additional Poisson's Ratio transverse contraction (which is coupled to longitudinal extension) to occur. Capillary channel collapse would lead to juxtaposition of previously separated wall polymers, which could allow new, noncovalent bonding to develop between some of them, somewhat like the new bonds that presumably form when stems bend irreversibly under a bending load (6). Such juxtapositions can be seen in models of hydrated and dehydrated xylem secondary wall nanostructure drawn by Penttilä et al. [(30), their Fig. 9] reflecting results from their physical measurements. This bonding would downshift the bending null point, as our results indicate occurs. Refilling the capillary channels with liquid water released during thawing could break some of these new bonds, causing the null point to rise toward the originally prevailing one, and allow the segment tip to rise by this same amount.

The extent to which such changes occur and lead to FB would depend both upon how much wall dehydration actually occurs at Ts < 0°C, and upon how extensive the resulting wall structural changes are, such as new interpolymer bond formation, or the amounts of swelling and shrinking of cell walls caused by hydration or dehydration that occur through the freeze/thaw cycle. Any of these changes could depend on species-specific wall polymer structure, and/or on biochemical factor(s) that either restrict or promote exchange of water between cell walls and adjacent lumens [e.g. (31) and refs. there cited]. They could differ between cryocampsis-positive and -negative species.

Melting of ice under high pressure, like what is involved in glacier flow (32), might also be involved in FB. Frozen water within water-filled xylem cells must interact mechanically with the large cell wall stresses that develop in near-surface xylem under a cantilever load. If the pressure on the ice in lower-side cells reaches the melting threshold, liquid water would be released there and tend to move toward the upper side where the stress is tensile, and lower water potential prevails. Removing water volume from the lower side would cause wall contraction, and addition of this water to the upper side ice would cause wall extension, so their combined action would increase downward bending. It is not yet clear whether the pressure within the lower-side cells can become large enough to support this potential mechanism.

Pertinence of recovery hysteresis to possible mechanisms

That loaded, cryocampsis-positive hardwood branch segments typically do not return fully upon thawing to their prefreezing, loaded elevation (LW/FB < 1), suggests that some of the new intermolecular bonds, formed during freezing while loaded, persist within the xylem cell walls after thawing. That such segments return gradually, after unloading, to nearly their original elevation suggests that the new bonds are not collectively strong enough to escape eventual disruption by the wall's preexisting internal elastic bonding forces, when these latter are no longer opposed by a load. Variations in the ratio between the opposing actions of preexisting elastic and newly induced (by freezing) null-point-displacing forces might explain the rather high variation in FB/LI and LW/FB values often observed among different branches of the same species.

That many softwoods instead have LW/FB values ~1 could indicate that little similarly thawing-resistant bonding develops in their cell walls during cryocamptic FB. This might in turn explain why softwoods, even from severely cold-winter climates like that of Fairbanks, possess limited FB/LI values compared with the most responsive hardwoods (Table 2 vs. Tables S3, Supplementary Material).

The biophysical processes suggested above as involved in cryocampsis may be related to the phenomenon of "frost-heave" that is frequently destructive for roads and building foundations in cold-winter climates. Frost-heave is due to flow of water, at $T <$ 0°C, from the capillary store of water in a porous matrix (soil and rock) to ice lenses that have formed somewhere in the material (33). A connection between cell wall freezing-dehydration and frost-heave actually occurred to Kübler (34). A more recently developed thermodynamic basis for frost-heave water flow (35–39) might, thus, apply also to cryocampsis.

Conclusions

Cryocampsis causes woody stems to bend more under a load at subfreezing than above-freezing temperatures. It occurs predominantly in plants from high latitudes, and may be ecologically beneficial in snowy, cold-winter habitats. Its ecological importance extends to the level of global energy balance and climate change, through its role in positioning tundra shrubs well-below the winter and spring snow surface. This increases the tundra's springtime albedo enough that much of the incident spring solar energy is reflected by the tundra, rather than being absorbed by shrub branches, which would cause atmospheric warming (1, 3). Cryocampsis requires hydrated stems, and likely involves migration of water molecules from xylem cell walls to ice that forms in cell chambers during freezing. It is biophysically curious and counter-intuitive for many, including some physicists, whom we asked what results they would expect from the experiment that most strikingly detects it (Fig. 2C or D). Further investigations into its mechanism may illuminate some novel physical or biophysical principles.

Materials and methods

Methods for collecting and preparing stem segments and identifying them to species are given in the Supplementary Material and in ref. (6), along with description and illustration of our bending measurement apparatus [Figure S1 (Supplementary Material); ref. (6)'s Fig. 2].

Bending measurements

Our standard cryocampsis assay is almost exactly the protocol shown in Fig. 3(B). Briefly, the basal end of a stem segment ∼300 mm long and (usually) 6 to 8 mm in basal diameter was clamped rigidly and horizontally to a vertical rod by a bar-to-bar clamp. At normally about +21°C, a weight sufficient to cause 30 to 40 mm of instantaneous initial downward deflection of the free end was hung from that end, into which a projecting pin had been inserted before mounting the segment in the apparatus. This pin's tip enabled precise recording of the segment's free end's elevation by a sequence of pencil marks made on the adjacent recording sheet, showing the pin tip's location at successive times.

After its initial downward Hookean (LI) deflection, the stem segment (Fig. 3B) underwent time-dependent bending (creep) at +21°C, which was completed after 20 to 24 h, and was then shifted to about −15 ± 2°C by moving the apparatus into a freezer room. After completing (within ≤ 2 h) any downward bending (FB) that this $T < 0$°C induced, the stem received a brief (2 min) exposure to a $T > 0$°C (usually +22°C, but instead +2.5°C in Fig. 3B), followed by return to −15°C for several minutes. For stems that gave substantial FB this regularly gives a few mm of further downbending, which we added to the preceding −15°C deflection to get the total FB. A final return was made to $T > 0$°C (usually +21°C), yielding an upward deflection (LW), followed after about 20 min by unloading, yielding instantaneous and retarded upward recoveries. For additional details, see the Supplementary Material and ref. (6).

Unless stated otherwise, numbers for FB or LW are normalized to (i.e. divided by) the specimen's LI or FB, respectively, obtained from other steps in the same cryocampsis assay; thus FB/LI or LW/FB. FB has a load dependence similar to that of LI, so normalization to FB/LI corrects for differences in both elastic modulus and applied loads between tested branches. LW/FB expresses the fractional recovery, upon thawing, of the bending that had occurred during the preceding exposure to sub-freezing T. It is important for distinguishing cryocamptic FB from different, irreversible effects of freezing on stem bending in some, especially tropical, plants.

Species survey (Tables 1 and 2; Tables S2–S4, Supplementary Material)

A total of four or more (rarely three) FB determinations, all (if possible) on branches from different individuals, were made for each species. The FB/LI and LW/FB data for each species were separately averaged, yielding species means, and these means were in turn averaged for all the species that we sampled from each biome, to yield biome means (Table 1). However, tested hardwood species with mean FB/LIs < 20% are simply listed by name under the biome headings in the Supplementary Material's species survey section; their (low) species means contributed to the biome means (Table 1), but are not listed individually in Table 2 or Table S2 (Supplementary Material).

Stem segments from many tropical and some other (mostly Mediterranean) plants bent downward, irreversibly, in the assay protocol's post-freeze warming (LW) step, rather than upward as Arctic and boreal ones almost invariably do. Freezing of frost-sensitive, living cells eliminates their turgor, which evidently contributes to these stems' mechanical support. No recovery of elevation during thawing occurred, so LW/FB for such species was assigned a value of 0. Turgor loss likely also contributed to the downbending that such stems usually underwent during the freezing (FB) step that preceded the LW step. As this negative FB value represented mechanical failure, rather than cryocamptic FB, such FBs were also assigned a value of 0. The Supplementary Material considers related consequences of atypical bending.

Shrub branch-holding experiment (Fig. 4)

In autumn 2006 at Alaska's Toolik Field Station (Lat. 68.6° N), some of the larger, stouter, branch systems of two *B. nana* and two *S. pulchra* shrubs were lifted and tied upward onto vertical stakes driven into the ground, so that their apical branchlets were at an elevation (well-above 0.5 m above ground level) that would prevent them from descending below the ∼0.5-m winter snow surface even if they bent downward. Basal branchlets (located < 0.5 m above the ground surface), on the tied branches would be below the snow surface whatever their inclination. A total of two other, nearby, entire *B. nana* and *S. pulchra* shrubs were pinned down to the ground by U-shaped garden clamps pushed into the soil, to confine both apically and basally located branchlets below the snow surface. A total of two other, nearby similar individual shrubs of each species, that were not manipulated, were chosen as untreated controls. The following spring, we randomly selected and tagged 10 apical and 10 basal branchlets, on each shrub, that had been produced by primary growth during the previous year. Survival of shoots from buds that had occurred on each tagged branchlet during the past winter was determined by enumerating the living buds and shoots (including catkins) on it, as a fraction of its total number of bud positions. After the second winter (i.e. in 2008) shoot survival was similarly determined for bud positions on the shoot system into which each of the initially tagged branchlets had developed during the intervening (2007) growing season. Recognition of bud positions was based on the known shoot growth morphology of these species [(40) for *B. nana*, and standard woody shoot morphology for *S. pulchra*]. Thus, there were two individual shrubs of each species in each treatment, with initially 20 tagged branchlets per shrub, although some tags were lost by the end of the second winter.

Statistical analyses

Wherever used here, ± denotes a standard error of the mean (SE).

Species survey

FB/LI and LW/FB species means (Table 1) were checked for normality and homogeneity of variance, and analyzed by one-way analysis of variance (ANOVA) with biome as the main effect, followed by Tukey's HSD post-hoc tests.

Overall means of FB/LI and LW/FB for hardwoods and softwoods in only the boreal, temperate, and Mediterranean biomes (because softwoods occurred only in our samples from those biomes), were compared using a non-parametric Kruskal–Wallis test with Chi-square approximation (Table 1). A two-way ANOVA with biome and wood type (softwood vs. hardwood) as main effects and a biome by wood type interaction was also run for FB/LI and LW/FB data from those biomes. Data were rank-transformed to achieve normality and homogeneity of variance. In these analyses, biome by wood type interaction terms were not significant, and the effects of biome on FB/LI and LW/FB for hardwoods

and softwoods separately were qualitatively similar to the overall effects shown in Table 1, so are not shown here. Analyses of FB/LI and LW/FB were run using JMP Pro software v.13.2 (SAS Institute, Inc.).

Shrub branch-holding experiment (Fig. 4)

Data were expressed, for each tagged branchlet, as the ratio of living shoots plus live buds and catkins in spring to total buds at the end of the previous growing season. Separately, for *B. nana* and *S. pulchra*, we tested how the ratio of living/total shoots was affected by position (apical vs. basal), treatment (tied up, held down, and control), and position by treatment interaction, as fixed effects (Table 3). For *S. pulchra*, the living/total bud ratio was rank-transformed to achieve normality and homogeneity of variance. For *B. nana* rank transformation was not helpful, due to non-independence and heteroscedasticity. Therefore, we performed model selection for both species' data using Akaike's Information Criterion (AIC) and visual inspection of the residuals, to identify the best random effect and variance structure (41, 42) (AIC values are given in Tables S5 and S6, Supplementary Material), using generalized least-squares and random-effect models. For *B. nana*, the best model included variance weighted by individual shrub, but no random effect (Table S5, Supplementary Material). For *S. pulchra,* no random effect or variance structure was needed; rank transformation was sufficient (Table S6, Supplementary Material). Significance of fixed effects was assessed using Type III Analysis of Deviance (ANOVA) and Tukey's post hoc HSD tests. Model fitting was by Restricted Maximum Likelihood (REML). These statistics were run using R version 3.5.2 (43) including the R library packages "car" v. 3.0-10 (44), "nlme" v. 3.1-137 (45), "emmeans" v. 1.6.0 (46), and "effectsize" v. 0.4.4-1 (47).

Abbreviations used in the Text

FB: Freeze-Bending: mm decline (negative) in tip elevation upon transfer to ∼−15°C of a previously (at +21°) cantilever-loaded stem segment.

LI: Initial, instantaneous, downward elastic deflection (mm, negative) upon cantilever-Loading of a segment, normally at +21°C, unless stated otherwise.

FB/LI (%): 100 x freeze-bending (FB), divided by the segment's previous initial, instantaneous downbending (LI) when it was loaded at +21°C. As both LI and FB are negative numbers, FB/LI is positive for branches that show cryocampsis.

LW: change in tip elevation (mm, positive if upward) of a previously Loaded, and then frozen, stem segment when, still loaded, it was Warmed above 0°C (usually to +21°C) and kept there (still loaded) until recovery ceased.

LW/FB: LW (positive, at least for cryocampsis-positive species) divided by −FB to give a positive number expressing the fractional recovery from the preceding FB upon thawing of a still-loaded segment, unless the segment bent downward upon thawing, the then-negative value being adjusted to 0 (see Methods).

T: temperature in °C.

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Supplementary material

Supplementary material is available at *PNAS Nexus* online.

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Authors' contributions

P.M.R. and M.S.B.H. designed and performed the research, analyzed the data, and wrote the paper.

Data availability

Quantitative data that we have gathered on levels of cryocamptic response of many Dicot and Gymnosperm species, primarily but not exclusively from Alaska, is contained in a master Excel file, which will be archived with the Arctic Data Center.

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