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# Ultrasound Acoustic Emissions from Bark Samples Differing in Anatomical Characteristics

By

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With 2 figures

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Summary

KIKUTA S. B. 2003. Ultrasound acoustic emissions from bark samples differing in anatomical characteristics. – Phyton (Horn, Austria) 43 (1): 161 – 178, with 2 figures. – English with German summary.

This study examines ultrasound acoustic emissions (UAE) from various bark types differing in anatomical characteristics. UAE were detected in dehydrating bark strips from twigs of two conifers (*Pinus nigra* ARNOLD, *Taxus baccata* L.), and four woody dicotyledons (*Hedera helix* L., *Malus sylvestris* MILL., *Sambucus nigra* L., and *Tilia platyphyllos* SCOP.) with varying contents of dead mechanical elements in the cortex and in the secondary phloem.

If filled with water, non-conducting bark elements (sclereids, fibre-sclereids, secondary phloem fibres and periderm cells) emitted ultrasound during dehydration. Signal production varied between the species and the bark layers studied. Pressure infiltration increased the number of UAE conspicuously. UAE were also registered from infiltrated sections of bottle cork (*Quercus suber* L.) and infiltrated walnut shells (*Juglans regia* L.) built of sclereids only and characterised by lignified secondary cell walls.

Stems of *Sphagnum* sp. emitted UAE, probably originating in hyalocysts, dead non-conducting water storage cells in the leaves.

Living cells (inner epidermes of bulb scales of *Allium cepa* L. and collenchyma strands from the herbaceous stem of *Lamium maculatum* L.) produced only very few UAE.

The results suggest that ultrasound acoustic emissions during dehydration do not only occur in cavitating conducting xylem elements but also in non-conducting, dead cells with thick walls.

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## Zusammenfassung

KIKUTA S. B. 2003. Ultraschallemissionen in anatomisch unterschiedlichen Rindentypen. – Phyton (Horn, Austria) 43 (1): 161 – 178, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

In der vorliegenden Arbeit werden Ultraschallemissionen (UAE) aus verschiedenen Rindentypen, die sich in anatomischer Hinsicht unterscheiden, beschrieben. UAE wurden aus austrocknenden Rindenstücken von Zweigen zweier Koniferen (*Pinus nigra* ARNOLD, *Taxus baccata* L.) und vier holziger Dikotylen (*Hedera helix* L., *Malus sylvestris* MILL., *Sambucus nigra* L., und *Tilia platyphyllos* SCOP.), die unterschiedliche Anteile von toten Festigungselementen in Rinde und Bast aufwiesen, aufgenommen.

Wenn sie mit Wasser gefüllt waren, emittierten die nicht leitenden Rindenelemente (Sklereiden, Fasersklereiden, Bastfasern, Peridermzellen) während der Austrocknung Ultraschallsignale. Die Zahl der Ultraschallsignale variierte in den untersuchten Arten und Rindenschichten. Druckinfiltration erhöhte die Zahl der UAE beträchtlich. Auch infiltrierte Schnitte von Flaschenkork (*Quercus suber* L.) und infiltrierte Stücke von Nuss-Schalen (*Juglans regia* L.), die ausschließlich aus Sklereiden mit verholzten Sekundärwänden bestehen, emittierten UAE.

Stämmchen von Torfmoos (*Sphagnum* sp.) produzierten UAE, die vermutlich in den toten, wasserspeichernden Hyalinzellen in den Blättchen entstanden.

Lebende Zellen, z.B. Innenepidermen aus Zwiebeln von *Allium cepa* L. und Kollenchymstränge aus krautigen Stängeln von *Lamium maculatum* L., sandten nur eine unbedeutende Zahl von UAE aus.

Die Ergebnisse weisen darauf hin, dass Ultraschallemissionen während Austrocknung nicht nur in kavitierenden wasserleitenden Elementen des Xylems auftreten sondern auch in nicht leitenden toten Zellen mit verdickten Wänden.


## Introduction

Ultrasound detection is a well-established method for recording cavitation, the breakage of the water column in xylem conduits of plants. Since the first application of this technique by TYREE & DIXON 1983, an impressive body of data on ultrasound acoustic emissions (UAE) from conducting xylem elements of

(1) stems or stem segments (TYREE & DIXON 1986, DIXON & al. 1988, TYREE & SPERRY 1989a, BORGHETTI & al. 1991, 1993, COCHARD 1992, SOBRADO & al. 1992, TOGNETTI & BORGHETTI 1994, VAN DOORN & JONES 1994, JACKSON & al. 1995, RASCHI & al. 1995, VAN DOORN & SUIRO 1996, NARDINI & SALLEO 2000)

(2) leaves (NEUFELD & al. 1992, KIKUTA & al. 1997, SALLEO & al. 2000, 2001, NARDINI & al. 2001)

(3) leaf petioles (TYREE & al. 1993, HACKE & SAUTER 1996) and

(4) roots (TOGNETTI & BORGHETTI 1994, HACKE & SAUTER 1996) has accumulated.

UAE were also detected from cavitating fibres in the xylem (SANDFORD & GRACE 1985, SPERRY & al. 1988).

Ultrasound acoustic emissions are vibrations recorded by a microphone sensor. TYREE & DIXON 1983 discussed possible sources of vibrations: oscillation of hydrogen bonds in water, elastic oscillations in tracheid walls, torus aspiration, and structural failure in the sapwood. There is strong experimental evidence that the ultrasound acoustic emissions are caused by elastic oscillations in the cell wall

of the conducting elements following a cavitation event. When conducting elements contain water under increasing tensions, air seeding (ZIMMERMANN 1983) leads to cavitation: Air is pulled through pores in the pit membrane into the lumen of the conduit. The air bubble formed expands in the lumen. The pressure rises rapidly as liquid water at negative pressure is converted to water vapour very near vacuum pressure (SPERRY & al. 1988). Due to the rapid pressure rise the conduit walls relax and oscillate radially in and out, thus causing vibrations which are detected as ultrasound acoustic emissions (TYREE & SPERRY 1989a). Since the rate of emissions increases with increasing water loss and decreasing xylem pressure potential, the sudden release of tension in the elastic cell wall seems a plausible explanation (see also RITMAN & MILBURN 1988) for the origin of UAE. Ultrasound emissions are stopped when the plant organs take up water, thus raising the water content (TYREE & DIXON 1983, KIKUTA & al. 1997).

For detailed information on the cavitation process, the acoustic method for detection of cavitation, its advantages and disadvantages, and the equipment used see reviews by PICKARD 1981, TYREE & SPERRY 1989b, MILBURN 1993.

Ultrasound acoustic emissions were also reported from non-vascular tissues. RITMAN & MILBURN 1988, 1990 used annular cells of fern sporangia as a test system for cavitation in small non-conducting cells. Cavitation of water was monitored simultaneously by acoustic detection and visual analysis. The number of UAE recorded from a saturated sporangium dehydrating in air closely approximated the average number of cavitable annular cells.

RITMAN & MILBURN 1988, 1991 compared audible acoustic emissions (AAE, in the low-frequency range of 0.2-2.0 kHz) and ultrasound acoustic emissions (UAE, in the high-frequency range of 100-300 kHz) in dehydrating plant tissues of differing anatomical characteristics from various species. Considerable differences in signal production and registration were found: *Ricinus* petioles and isolated vascular bundles of *Plantago* produced AAE only. Annular cells of fern sporangia and stem segments of *Acacia* and *Ricinus* cut shorter than the longest conducting elements produced UAE only. The largest conduits in stem segments emitted both AAE and UAE, cavitating fibres and tracheids emitted predominately UAE. In phloem fibres no acoustic activity was detected. The authors concluded that the production of AAE or UAE depends on the tissue type. They observed that high water content and high elasticity of the cell wall efficiently increased ultrasound absorption, that is, UAE were filtered out, whereas AAE were not. Tissues in which both AAE and UAE were registered had large conducting elements and stiff cell walls. No evidence was found that AAE or UAE were caused by tissue fracture (MILBURN & JOHNSON 1966, TYREE & al. 1984, RITMAN & MILBURN 1988).

So far, the ultrasound technique has not been applied to detect emissions from bark, a complex tissue composed of different cell types including mechanical elements with thick walls. The term bark is used to describe all tissues located outside the vascular cambium regardless of their specific structure (ESAU 1977, TROCKENBRODT 1990). The purpose of this study was to examine ultrasound acoustic emissions from various bark types differing in anatomical characteristics.

## Material and Methods

### Plant material

Between November and April, 2- to 4-yr-old twigs of two conifers, *Pinus nigra* ARNOLD (*Pinaceae*), *Taxus baccata* L. (*Taxaceae*), and four dicotyledonous woody species, *Hedera helix* L. (*Araliaceae*), *Malus sylvestris* MILL. (*Rosaceae*), *Sambucus nigra* L. (*Caprifoliaceae*), and *Tilia platyphyllos* SCOP. (*Tiliaceae*), were collected from adult specimens growing in the Botanical Garden of the University of Agricultural Sciences Vienna. After collection the twigs were brought to the laboratory within 5-10 min, recut under water and saturated by placing their cut ends in distilled water for about 24 h. To minimise transpiration during saturation, twigs were enclosed in black plastic bags.

Pieces of bark were removed from saturated twigs by setting two horizontal rings (at a distance of about 20 mm) and one connecting vertical cut with a fresh razor blade. Then the bark was carefully peeled off. Cross sections of the detached bark strip were made by hand and examined under a light microscope to make sure that all tissues outside the vascular cambium were included.

Measurements were done on strips of (i) complete bark (including periderm), (ii) bark without periderm, and (iii) isolated periderm. In experiments (ii) and (iii) the periderm was stripped off gently from the other tissues of the bark and the successful separation again checked on cross sections under the microscope. In preliminary experiments the six study species had been selected with regard to easy peeling of the bark and removal of the periderm.

In addition, UAE measurements were extended to infiltrated sections of commercial bottle cork from *Quercus suber* L. and walnut shell (*Juglans regia* L.).

### Anatomical characteristics of the bark specimens

The species studied varied in their content of mechanical elements in the cortex and in the secondary phloem. The tissues found in the bark types measured are characterised as follows:

Sclerenchyma is a supporting cortex tissue comprising fibres, sclereids, and fibre-sclereids as an intermediate cell type (ESAU 1977).

Fibres are usually long, slender cells with lignified or non-lignified secondary walls.

Sclereids develop from phloem parenchyma cells in either conducting or non-conducting phloem, vary in form and have usually thick, strongly lignified secondary walls with numerous, commonly simple and branched pits (EVERT 1963, ESAU 1977, FAHN 1990, TROCKENBRODT 1990). Sclereids may occur in layers or clusters or appear isolated among other types of cells. Stone cells, one of various categories of sclereids, are more or less isodiametric in form.

Fibre-sclereids are derived from parenchyma cells in non-conducting phloem. They grow intrusively and at maturity are difficult to distinguish from phloem fibres.

Phloem fibres develop directly from meristem derivatives, are long and slender, have thickened walls and show apical growth. The term primary phloem fibre applies to a fibre differentiated from protophloem elements in the vascular bundles. Primary fibres outside the vascular bundles are called perivascular fibres (ESAU 1969, 1977). Secondary phloem fibres are located in the secondary phloem and differentiate directly from fusiform cambial initials. They show intrusive growth and mature in the conducting phloem.

Cork (phellem) is part of the periderm, the compound secondary protective tissue that replaces the epidermis (ESAU 1977, MAUSETH 1988, FAHN 1990). Cork cells are approximately prismatic in shape and usually arranged in compact radial rows, the tissue lacking intercellular spaces. The walls are suberised.

(1) In the bark of *P. nigra* (black pine) no sclerenchyma cells or secondary phloem fibres are present (HOLDHEIDE 1951, ESAU 1977). The walls of the sieve cells are thick, but not lignified (MAUSETH 1988). Two to three layers of the phelloderm consist of rectangular cells with thick walls. Phlobaphene cork consists of 12 and more layers (HOLDHEIDE 1951).

(2) According to ESAU 1977 secondary phloem fibres are produced in *T. baccata* (common yew) in uniserate tangential bands, whereas HOLDHEIDE 1951 reported that fibre-sclereids, irregularly arranged in tangential bands, are found, but no fibres. For many years no thick-walled mechanical elements may be formed at all. In the bark strips used in this study, neither fibre-

sclereids nor secondary phloem fibres were found; only parenchyma cells with many pits in the radial walls and sieve cells could be distinguished. The periderm consists of three to four layers of cork cells with mostly thick and colourless walls, the meristematic phellogen, and the phelloderm forming two to three cell layers (HOLDHEIDE 1951).

(3) In the bark of *M. sylvestris* (wild crab) the supporting cells in the secondary phloem are fibres in small groups or tangential bands at the end of the growth increments. In some branches and twigs they may be missing (HOLDHEIDE 1951, EVERT 1963). The sclerenchyma cells are fibre-sclereids (Fig. 1a); they are commonly associated with crystal-containing cells. The periderm consists of six to ten layers of radially flattened cork cells with thick tangential walls.

(4) In *S. nigra* (common elder) fibre-sclereids are arranged in small, tangential to scattered groups in the secondary phloem (Fig. 1b); secondary phloem fibres are absent (HOLDHEIDE 1951). Very thick layers of cork cells are formed.

(5) In *T. platyphyllos* (large-leaved lime) fibres occur on the periphery of the phloem (primary phloem fibres) and also within the secondary phloem (secondary phloem fibres) where they appear in parallel tangential bands (Fig. 1c) (HOLDHEIDE 1951, ESAU 1977). In the periderm seven to twenty layers of cork cells are formed. Below the periderm four to five layers of cortex parenchyma cells with thick walls are found, inwardly followed by parenchyma with thin walls.

(6) Bark of *H. helix* (ivy) contains fibre-sclereids and stone cells (Fig. 1d). Fibre-sclereids are found either as single isolated elements or arranged in small groups in the secondary phloem. The lumen is wide, the walls are strongly lignified. Stone cells are mainly present in the outer cortex layers in loose groups of small to medium size. The walls of the stone cells are comparatively thin. The periderm is regular and contains cork cells characterised by extremely thickened tangential walls with branched pit canals. The lumen is hardly perceptible. All cell walls are strongly lignified. The phelloderm consists of seven to ten layers of rectangular cells. Below the periderm seven to ten layers of cortex parenchyma cells with thick walls and pit canals are found. Numerous oxalate crystals are present. Cortex parenchyma cells with thin walls follow in the radial direction.

(7) The shell of walnut (*J. regia*) is made up entirely of sclereids (ESAU 1977).

(8) Walls of bottle-cork cells (*Q. suber*) contain ultra-thin pores of 60 nm diameter, always arranged in groups, which are supposed to have developed from plasmodesmata (SITTE 1955, 1962). In native phellem these blocked pores contain dense and oxidizable material, probably aged pectins and lignins. Ordinarily, there are no pits in the thin walls of bottle cork cells, the lumina are filled with air. Bottle cork is highly impervious to water (ESAU 1977).

(9) Measurements of UAE were done on main stems (50 to 60 mm long) of *Sphagnum* sp. to find out whether hyalocysts, dead non-conducting water storage cells in the leaves of peat moss, emit ultrasound signals.

(10) As a comparison, UAE measurements were also done on living tissues:

(i) Isolated inner epidermes peeled off from bulb scales of *Allium cepa* L.

(ii) Collenchyma cell strands, visible as ridges in the peripheral regions of the herbaceous stem of *Lamium maculatum* L..

Preparation of cross sections

Samples of complete bark were taken from 3-yr-old twigs of the six species studied and cut into 40 to 50 µm cross sections with a sliding microtome (Reichert, Vienna, A). Sections were stained with astrablue / safranine, dehydrated, and mounted in Euparal (GERLACH 1984). Sections were observed under a light microscope (CHS Olympus Optical Company) to identify elements in the cortex and secondary phloem. Photographs were taken with a digital camera (C-4040ZOOM CAMEDIA, Olympus Optical Company). Digital images were enlarged and processed with Adobe Photoshop Version 6.0 (Adobe Systems Incorporated, San José, CA, USA). Since no mechanical structures were found in the cross sections of the two conifers, photographs of the dicotyledons only are presented.

Measurement of ultrasound acoustic emissions

Ultrasound acoustic emissions (UAE) were detected with an I15I UAE transducer (d = 18 mm) connected to a 4615 Drought Stress Monitor (DSM) (both instruments by Physical Acoustics

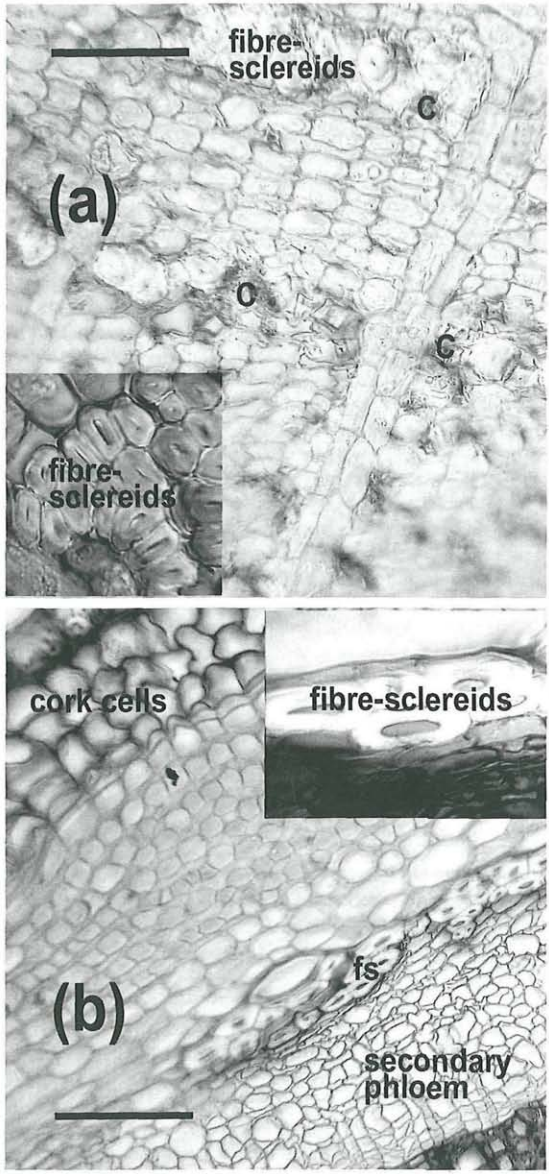
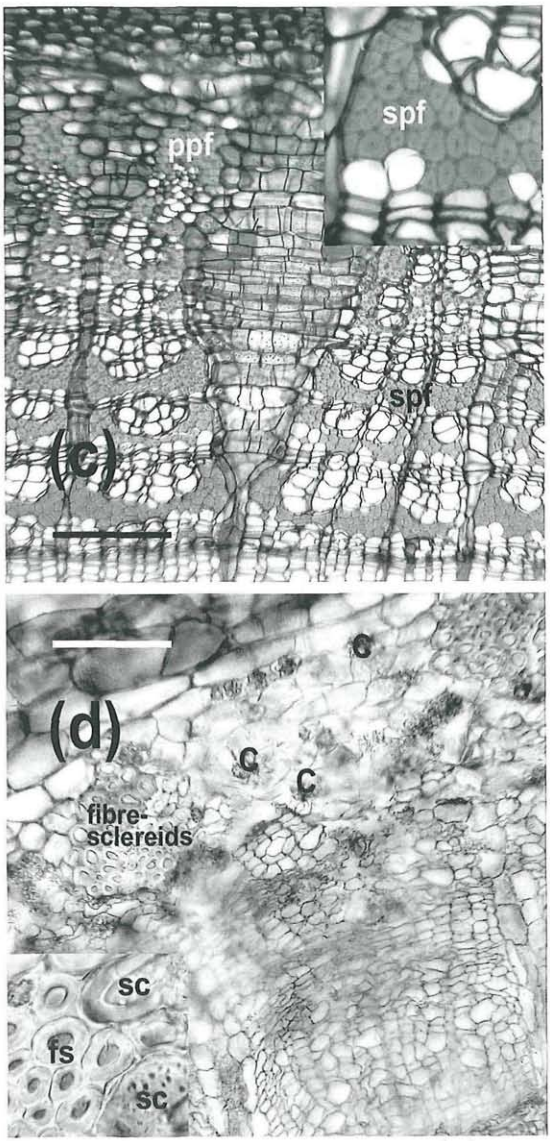



Fig. 1. Cross sections (40 to 50 µm) of complete bark. (a) *Malus sylvestris*; (b) *Sambucus nigra*; (c) *Tilia platyphyllos*; (d) *Hedera helix*. bar = 100 µm. c: crystal-containing cell; fs: fibre-sclereid; ppf: primary phloem fibre; spf: secondary phloem fibre; sc: stone cell.



Corporation, Princeton, NJ, USA). Total gain of the DSM was set at 70 dB (the monitor amplifier set at 50 dB, the head-stage amplifier fixed at 20 dB). The background registered by a sensor freely exposed in the laboratory air was less than ten signals per day. The UAE transducer was attached to the inner side of strips of (i) complete bark, (ii) bark without periderm, and (iii) isolated periderm. During dehydration of the bark strips on the laboratory bench the cumulative numbers of UAE were recorded with a notebook computer every two seconds until signal emission ceased completely.

After UAE detection all samples were scanned (Epson GT-7000) and the area determined by means of SigmaScan (Image Analysis, Version 2.0, Jandel Scientific 1995). The mean value of the areas of all bark strips measured was 368.1 ± 14.4 $mm^2$ and thus higher than the area of the circular end face of the I15I UAE transducer (254.3 $mm^2$) in contact with the samples. This means that the edges of the tissue samples were not positioned immediately below the sensor. However, they were certainly within the listening distance over which UAE are detected, that was found to range between 5 and 40 mm depending on the species as well as on tissue characteristics and water content (TYREE & al. 1984, TYREE & SPERRY 1989a, RITMAN & MILBURN 1991).

Before the beginning and at the end of the UAE measurements the fresh weight (FW) of the bark strips was taken. After the measurement the samples were dried to constant weight for 24 h at 100 °C. The values of dry weight (DW) and fresh weight were used to calculate the water content (WC) in percent of the dry weight:

$$WC \text{ in } \% \text{ of } DW = [(FW - DW) / DW] * 100$$

Cumulative numbers of ultrasound acoustic emissions (cUAE) recorded were related to the area as well as to the dry weight of the samples and are presented as cUAE/$mm^2$ and cUAE/mg, respectively, in the tables. Thus it was possible to compare and rank the species regarding their signal production.

Bark strips were either measured immediately after peeling or, prior to UAE measurement, infiltrated with distilled water at an overpressure of 1.0 MPa applied for 15 min to a beaker standing in a pressure chamber (model 3000, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA). Sections of bottle cork and walnut shell were infiltrated in the same way.

Statistical analysis

Data were analysed with the SPSS 9.0 for Windows® statistics package (SPSS Inc., Chicago, IL, USA). Values are given as means ± standard error. Mean values of two independent sample groups were tested for significance of differences with the Mann-Whitney U-test. Mean values of several samples were compared with the Kruskal-Wallis H-test. Differences were accepted as significant if P was ≤ 0.05.

## Results

The total number of UAE per unit bark area (cUAE/$mm^2$) was lowest in *P. nigra* (0.63 ± 0.24) and highest in *H. helix* (25.95 ± 4.01) (Table 1). There were two distinct subsets of samples, one consisting of *P. nigra, T. baccata, and M. sylvestris,* the other of *S. nigra, T. platyphyllos,* and *H. helix.* Within the groups, the differences were not statistically significant. The ratio of cUAE/$mm^2$ was significantly smaller ($P \leq 0.001$) in the first subset. The number of emissions was also related to the dry weight of the samples. The ratio cUAE/mg was lowest in *P. nigra* (0.65 ± 0.14) and highest in *T. platyphyllos* (127.72 ± 35.43) (Table 1). Again, there were the same two subsets of data differing significantly ($P \leq 0.001$).

Table 1. Ratio between cumulative number of ultrasound acoustic emissions and sample area (cUAE/mm²) or sample dry weight (cUAE/mg), detected from complete bark strips dehydrating in air. Values are means ± SE; *n* = number of replicates. Different letters indicate statistically significant differences ($P \leq 0.001$) between the species.

| Species | cUAE related to sample area (cUAE/mm²) | *n* | cUAE related to sample dry weight (cUAE/mg) | *n* |
|---|---|---|---|---|
| *Pinus nigra* | 0.634 ± 0.242 a | 11 | 0.653 ± 0.138 a | 11 |
| *Taxus baccata* | 1.542 ± 0.548 a | 13 | 6.159 ± 2.179 a | 13 |
| *Malus sylvestris* | 2.232 ± 0.334 a | 8 | 12.798 ± 2.204 a | 8 |
| *Sambucus nigra* | 15.177 ± 3.023 b | 8 | 91.184 ± 13.562 b | 8 |
| *Tilia platyphyllos* | 23.724 ± 5.887 b | 8 | 127.717 ± 35.435 b | 8 |
| *Hedera helix* | 25.947 ± 4.009 b | 11 | 81.568 ± 15.032 b | 11 |

In Fig. 2a, b typical time courses of cUAE from strips of complete bark dehydrating in air under laboratory conditions are presented. The two conifers showed low ratios of cUAE/mm²: 0.29 in *P. nigra* and 0.32 in *T. baccata*. In the dicotyledons conspicuously more emissions were registered, resulting in higher ratios of cUAE/mm²: 2.39 in *M. sylvestris*, 12.15 in *S. nigra*, 20.40 in *H. helix*, and 24.03 in *T. platyphyllos*. The shape of the curves differed considerably between the species, and was distinctly sigmoid only in *H. helix*. In Fig. 2c, d the time courses of the same samples are given as percentage of the total numbers of emissions. Obviously, there were conspicuous differences in time elapsed before 50% of total signals were reached (Table 2): *T. platyphyllos* < *P. nigra* < *M. sylvestris* < *T. baccata* < *H. helix* < *S. nigra*, the rate of signals per hour being highest in *T. platyphyllos* and lowest in *T. baccata*.

Table 2. Time elapsed until 50% and 100%, respectively, of the total numbers of ultrasound acoustic emissions (UAE) were reached in dehydrating strips of complete bark. Values are derived from representative time courses shown in Fig. 2c, d. 'Emission rate' denotes average signal production per hour (UAE/h) until reaching 50% of total emissions. Water content (WC) at the end of UAE detection is given for strips of complete bark as percentage of dry weight (DW). Values are means ± SE; *n* = number of replicates. Different letters indicate significant differences at the $P \leq 0.001$ level.

| Species | Time elapsed until 50% UAE (h) | Emission rate until 50% UAE (UAE/h) | Time elapsed until 100% UAE (h) | WC in % DW at end of UAE production | *n* |
|---|---|---|---|---|---|
| *Pinus nigra* | 7.7 | 7.1 | 21.9 | 32.74 ± 4.08 a | 11 |
| *Taxus baccata* | 19.5 | 3.2 | 39.0 | 17.64 ± 1.89 a | 13 |
| *Malus sylvestris* | 13.3 | 28.5 | 43.4 | 8.27 ± 0.20 b | 8 |
| *Sambucus nigra* | 23.7 | 128.1 | 89.8 | 11.06 ± 2.36 b | 8 |
| *Tilia platyphyllos* | 2.1 | 1288.8 | 21.3 | 8.43 ± 0.27 b | 8 |
| *Hedera helix* | 22.9 | 204.9 | 47.0 | 8.84 ± 0.51 b | 11 |

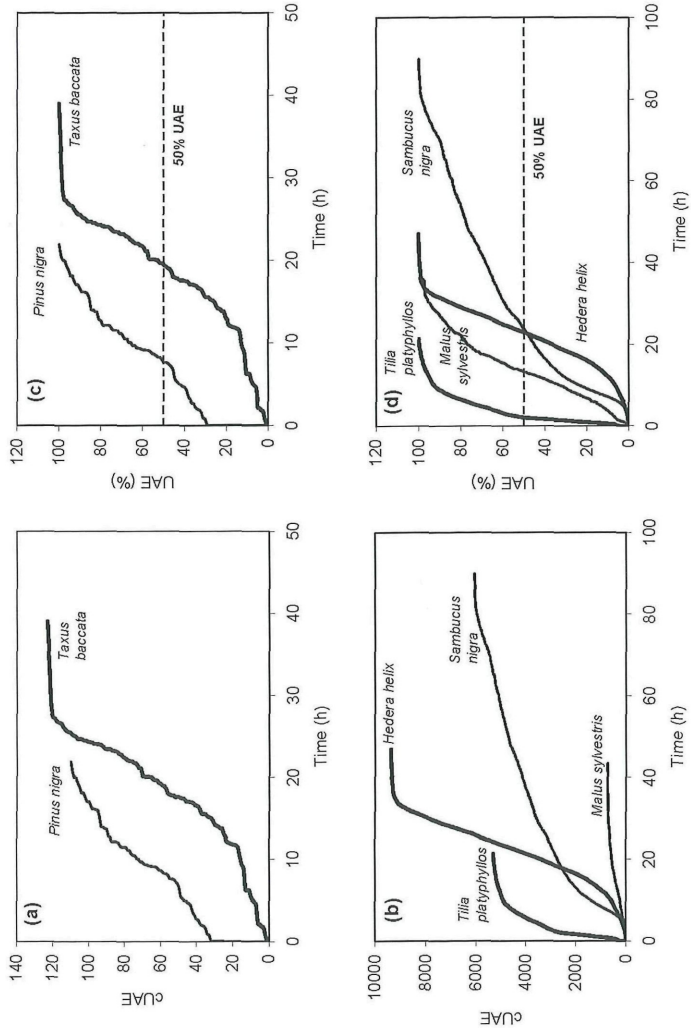



Fig. 2. (a), (b): Representative time courses of cumulative numbers of ultrasound acoustic emissions (cUAE) of dehydrating strips of complete bark. (c), (d): Time courses of the same samples given as percentage of the totals of ultrasound acoustic emissions, UAE (%).

Table 3. Cumulative number of ultrasound acoustic emissions per sample area (cUAE/mm$^2$) and per sample dry weight (cUAE/mg) from bark strips without periderm. In strips of isolated periderm cUAE were related to sample area only. Values are means ± SE; $n$ = number of replicates. Different letters indicate significant interspecific differences.

| Species | Bark without periderm | | | | | | Isolated periderm | | |
|---|---|---|---|---|---|---|---|---|---|
| | (cUAE/mm$^2$) | | $n$ | (cUAE/mg) | | $n$ | (cUAE/mm$^2$) | | $n$ |
| *Malus sylvestris* | 1.834 ± | 0.226 a | 8 | 14.773 ± | 2.096 a | 8 | 0.785 ± | 0.365 a | 6 |
| *Sambucus nigra* | 0.221 ± | 0.025 a | 8 | 4.269 ± | 1.262 a | 8 | 13.838 ± | 2.841 b | 6 |
| | | | | | | | | $P \leq 0.05$ | |
| *Tilia platyphyllos* | 18.891 ± | 1.917 b | 10 | 79.667 ± | 6.495 b | 10 | 4.587 ± | 0.741 a | 8 |
| | | $P \leq 0.01$ | | | $P \leq 0.05$ | | | | |
| *Hedera helix* | 9.071 ± | 1.670 c | 10 | 56.215 ± | 14.152 b | 10 | 20.830 ± | 3.043 b | 8 |
| | | $P \leq 0.01$ | | | | | | $P \leq 0.05$ | |

Time courses to reach 100% of total emissions showed just one change in the ranking of the species: *T. baccata* < *M. sylvestris*. When signals had stopped, the water content of complete bark strips calculated as percentage of the dry weight was significantly higher ($P \leq 0.001$) in the two conifers than in the four dicotyledons (Table 2).

Removal of the periderm had no significant influence on the water content at the end of UAE measurement. Signal production of bark strips without periderm differed considerably between the four dicotyledons (Table 3). The ratio cUAE/mm² was lowest in *S. nigra* (0.22 ± 0.02) and highest in *T. platyphyllos* (18.89 ± 1.92). There was no significant difference between *M. sylvestris* and *S. nigra*, whereas *T. platyphyllos* and *H. helix* were significantly different at the 1% level. The same ranking was found for the ratio cUAE/mg which was significantly different ($P \leq 0.05$) between the four species, with smaller mean values for *M. sylvestris* and *S. nigra* compared to *T. platyphyllos* and *H. helix* (Table 3). Isolated periderm emitted cUAE/mm² as low as 0.78 ± 0.36 in *M. sylvestris* and as high as 20.83 ± 3.04 in *H. helix* (Table 3), the values differing between the four dicotyledons at the 5% level.

The sum of cUAE/mm² for strips of bark without periderm and for isolated periderm was very close to the value of cUAE/mm² measured on complete bark. There were however distinct differences between species concerning the contribution of UAE from isolated periderm to the total number of signals (Table 4). Emissions from complete bark were set as 100%, signals from strips of bark without periderm and from isolated periderm were calculated as the aliquot percentage. While in *S. nigra* isolated periderm contributed 98.4% to the total UAE production of complete bark, in *T. platyphyllos* only 19.5% were attributable to the periderm. *Vice versa*, bark without periderm contributed the smallest percentage in *S. nigra* and the highest in *T. platyphyllos*. The small disparities in the results of cUAE/mm² from complete bark in Tables 1 and 4 are due to different data sets measured on samples taken for different experiments.

Table 4. Contribution of cUAE/mm² measured in strips of bark without periderm and strips of isolated periderm, calculated as percentage, to the totals of cUAE/mm² from complete bark. Values of cUAE/mm² from complete bark were set as 100%.

| Species | Complete bark cUAE related to sample area (cUAE/mm²) | *n* | Bark without periderm Percentage of total cUAE/mm² (%) | *n* | Isolated periderm of complete bark (%) | *n* |
|---|---|---|---|---|---|---|
| *Malus sylvestris* | 2.619 | 8 | 70.0 | 8 | 30.0 | 6 |
| *Sambucus nigra* | 14.059 | 8 | 1.6 | 8 | 98.4 | 6 |
| *Tilia platyphyllos* | 23.478 | 8 | 80.5 | 10 | 19.5 | 8 |
| *Hedera helix* | 29.902 | 12 | 30.3 | 10 | 69.7 | 8 |

Pressure infiltration of the bark strips resulted in higher cUAE/mm², the increase varying with species and tissue (Table 5). In the conifers, infiltration of complete bark induced the highest increase (11000% *in P. nigra* and 6600% in *T. baccata*). Pressure infiltration of bark strips without periderm caused a smaller increase of signal production than in complete bark (650% in *P. nigra* and 3700% in *T. baccata*). In *T. platyphyllos* and *H. helix* infiltration induced a similar rise in signal production both in complete bark (221%) and bark without periderm (200%). This suggests that structures other than periderm are the prime source of additional signals in these species.

Table 5. Emission rates per sample area (cUAE/mm²) from non-infiltrated and infiltrated samples of complete bark and bark without periderm. Values are means ± SE; $n = 5 - 6$; ns = non significant. Non-infiltrated vs. infiltrated variants were tested for significance of differences with the Mann-Whitney U-test.

| Complete bark | | |
|---|---|---|
| Species | non-infiltrated (cUAE/mm²) | infiltrated (cUAE/mm²) |
| *Pinus nigra* | 0.292 ± 0.150 | 32.337 ± 0.577 ns |
| *Taxus baccata* | 0.535 ± 0.213 | 35.337 ± 3.833 $P \leq 0.01$ |
| *Tilia platyphyllos* | 16.170 ± 1.928 | 38.512 ± 3.990 $P \leq 0.01$ |
| *Hedera helix* | 24.397 ± 4.134 | 50.028 ± 2.567 ns |

| Bark without periderm | | |
|---|---|---|
| Species | non-infiltrated (cUAE/mm²) | infiltrated (cUAE/mm²) |
| *Pinus nigra* | 0.820 ± 0.058 | 5.314 ± 0.577 ns |
| *Taxus baccata* | 0.137 ± 0.047 | 5.065 ± 1.779 ns |
| *Tilia platyphyllos* | 18.891 ± 1.917 | 38.228 ± 7.953 $P \leq 0.01$ |
| *Hedera helix* | 12.652 ± 0.845 | 25.058 ± 11.356 $P \leq 0.05$ |

Table 6 shows that pressure infiltration induced a more pronounced increase in cUAE/mm² in *M. sylvestris* than *S. nigra* both in complete bark and bark without periderm, whereas the increase in isolated periderm strips was higher in *S. nigra*.

From infiltrated sections (100 to 120 µm) of commercial bottle cork 17.32 cUAE/mm² or 73.94 cUAE/mg (n=3) were recorded, from infiltrated samples of walnut shells 63.78 cUAE/mm² or 50.01 cUAE/mg (n=3), whereas non-infiltrated sections, as could be expected, did not produce any signals.

Main stems of *Sphagnum* sp. emitted 186.67 ± 28.00 cumulative ultrasound acoustic emissions (n = 5).

Pieces of isolated inner epidermes of bulb scales from *A. cepa* emitted 5.40 ± 1.69 cUAE (n = 6), collenchyma strands of *L. maculatum* 21.50 ± 8.50 cUAE.

Table 6. Emission rates per sample area (cUAE/mm²) from non-infiltrated compared to pressure infiltrated specimens. Values are means ± SE; $n = 4 - 5$; ns = non significant. Non-infiltrated vs. infiltrated variants were tested with the Mann-Whitney U-test.

| Complete bark | | | |
|---|---|---|---|
| Species | non-infiltrated (cUAE/mm²) | infiltrated (cUAE/mm²) | |
| *Malus sylvestris* | 2.162 ± 0.709 | 25.612 ± | 0.582 $P \leq 0.05$ |
| *Sambucus nigra* | 15.177 ± 3.023 | 20.895 ± | 1.844 ns |
| **Bark without periderm** | | | |
| Species | non-infiltrated (cUAE/mm²) | infiltrated (cUAE/mm²) | |
| *Malus sylvestris* | 1.834 ± 0.226 | 26.088 ± | 3.823 $P \leq 0.05$ |
| *Sambucus nigra* | 0.221 ± 0.025 | 1.044 ± | 0.250 ns |
| **Isolated periderm** | | | |
| Species | non-infiltrated (cUAE/mm²) | infiltrated (cUAE/mm²) | |
| *Malus sylvestris* | 0.785 ± 0.365 | 3.056 ± | 0.427 $P \leq 0.05$ |
| *Sambucus nigra* | 9.920 ± 2.595 | 16.658 ± | 3.514 $P \leq 0.05$ |

## Discussion

Measurement of ultrasound acoustic emissions (UAE) on complete bark strips dehydrating in air showed that signal production varied between the species and the bark layers studied, obviously depending on the cell types present. For the two conifers (*P. nigra*, *T. baccata*) the lowest cumulative numbers of UAE per sample area and sample dry weight were calculated (Table 1). In these species neither sclerenchyma cells nor secondary phloem fibres were present. In *M. sylvestris*, the ratios cUAE/mm² and cUAE/mg were higher than in the conifers, but the difference was statistically not significant. Fibre-sclereids in the sclerenchyma were identified as mechanical elements (Fig. 1a). The other dicotyledons (*S. nigra*, *T. platyphyllos*, *H. helix*) emitted significantly higher ($P \leq 0.001$) cUAE per sample area and sample dry weight. In common elder small groups of fibre-sclereids were arranged in the secondary phloem (Fig. 1b). In large-leaved lime primary and secondary phloem fibres were present (Fig. 1c). Ivy was the only species studied where stone cells occurred in the outer cortex (Fig. 1d). In addition, fibre-sclereids with strongly lignified cell walls were present in the secondary phloem. In ivy, the highest ratio cUAE/mm² was measured.

These results suggest that certain dead cell elements in barks, such as sclereids (including stone cells), fibres, fibre-sclereids and secondary phloem fibres emit ultrasound during dehydration in air. A prerequisite is that the elements are filled with water.

Time courses of cUAE varied distinctly between the species (Fig. 2). The water content at the end of UAE registration was significantly higher in the conifers than in the dicotyledons (Table 2). Thus, it is supposed that in the conifers the periderm cells cavitate more easily than, for instance, in *S. nigra*.

Bark strips without periderm produced fewer signals per sample area and sample dry weight (Table 3) than complete bark, but the differences were statistically not significant.

From isolated periderms of *S. nigra* and *H. helix* considerable numbers of UAE were recorded, whereas in *T. platyphyllos* ultrasound emission from periderm was low (Table 4). This means that in *S. nigra,* where thick layers of cork cells are formed, most UAE were emitted from the periderm, in *T. platyphyllos* from elements other than the periderm, presumably from phloem fibres. Most probably the developmental stage of the periderm and especially the fraction of water-filled cells are the cause of differences between species.

Pressure infiltration of the bark strips prior to dehydration increased the number of UAE conspicuously in all species and bark layers (Tables 5 and 6). Due to infiltration, evacuated elements were filled with water and emitted ultrasound signals during the following dehydration. In the two conifers studied it was obviously the periderm that reacted to pressure infiltration with an enormous increase in cUAE/mm² (Table 5). Surprisingly, in bark without periderm, there was also an infiltration-induced increase in cUAE/mm² in the conifers, although statistically not significant. In *P. nigra*, this increase may be explained by infiltration of schizogenous resin ducts in the cortex. Resin ducts were identified in cross sections under the light microscope. In *T. baccata*, no resin ducts are present but large intercellular spaces between the cortex parenchyma cells were observed. Probably, the intercellular spaces are infiltrated due to overpressure. It may be supposed that the walls of cells adjacent to the intercellulars oscillate on dehydration of the tissue and thus produce ultrasound signals.

UAE originated in infiltrated sections of bottle cork, and in infiltrated walnut shells built of sclereids only and characterised by lignified secondary cell walls.

Ultrasound emissions were registered in *Sphagnum* stems, probably caused by air-seeding (ZIMMERMANN 1983) in the hyalocysts, dead non-conducting cells. On dehydration a bubble is pulled through the pores in the cell wall. Consequently, the lumen is filled with air. Water released flows to the adjacent cells, thus increasing their water content. This process of air-seeding in hyalocysts was also observed under the microscope (LEWIS 1988).

The small number of UAE recorded from living cells of onion epidermes and collenchyma strands confirms the observations of RITMAN & MILBURN 1991 that ultrasound signals originate in small thick-walled cells as a consequence of cavitation events within fibres. In living cells, snapping of plasmodesmata (J.A.

MILBURN, pers. communication to H. RICHTER) or collapse of cell walls by sacking or buckling (OERTLI 1989) may produce audible acoustic emissions but no ultrasound signals.

In conclusion, these results are proof that ultrasound signals during dehydration in air may be produced also in cells other than cavitating xylem elements, such as phloem fibres, sclerenchyma and periderm cells.

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