Birds Girdling Activity on Exotic Tree Species as a Form of Adaptive Behavior?¹

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Abstract—Four tree species in the Kostelec n. Č. l. arboretum (Czech Republic) have been repeatedly damaged by *Dendrocopos medius*. The unique aspect of this otherwise common behavior called girdling consists in regularly visiting the same trees every spring, although there are more than 1.200 tree species within the arboretum. We monitored transpiration, leaf phenology and the chemical composition of the xylem sap of girdled and non-girdled trees. Spectral analysis revealed slightly higher amounts of sugars, especially saccharose, in *Cladrastis* Raf. as the most regularly girdled tree among other conditions, comparing girdled to non-girdled trees. Higher transpiration rates were not confirmed in connection with girdling—quite the opposite—*Cladrastis* Raf. as the most highly favored tree for girdling showed the lowest transpiration rates (in average 6 kg water per day within spring months) compared to other non-girdled trees. We presume that the birds do not choose a particular tree on the basis of any visible or chemical traits but they examine many trees within their territory. Afterwards they probably remember the position of trees whose xylem sap starts to flow early in the spring compared to other trees, as their transpiration stream is enriched with sweet organic substances that represent an advantage for the forthcoming nesting period.

Keywords: Cladrastis kentukea (Dum. Cours.) Rudd, FTIR spectra, middle-spotted woodpecker *Dendocopos medius*, phenology, transpiration stream, xylem sap

DOI: 10.1134/S1995425517020135

INTRODUCTION

General Overview

Phloem sap is nutrient rich, full of sugar and without toxins or feeding deterrents, but it is consumed by a restricted range of animals (Douglas, 2006). Sap trees are used almost solely by birds (with the exception of a few mammals) using tree sap, such as the Yellow-bellied Glider (*Petaurus australis*) (Goldingay, 1987). Nevertheless, none of them is native to middle Europe. Consumption of phloem and xylem sap by birds in the middle European region was first reported by Turček (1952, 1961) and girdling was described by Kučera (1972) and Simon (1984). Even so, all published papers lack a physiological approach and rely on describing this phenomenon. The explanation for why some tree species are attacked while the others from the same genus are overlooked remains missing.

Eberhardt (2000) mentioned several factors that influence tree selection for girdling although most of them are microhabitat or tree health factors. Moreover

¹ The article is published in the original.

his suggestion concerning girdling depends upon sap throughout the year, which is not our observation.

In Europe girdling activity is connected with birds from the family *Picidae—Dendrocopos major*, *D. medius*, *D. leucotos and D. minor*, *Picoides tridactylus*, *Dryocopus martius* and *Picus viridis* (Turček, 1961). Sap-well design sometimes enables recognition of the bird species—e.g. one sided wells not clustered are specific to the white headed woodpecker (Kozma, 2010). Sap drippings are also utilized by other bird species as march tit (*Parus palustris*), great tit (*Parus major*) and finch (*Fringilla coelebs*).

The increment of girdled trees is significantly lower than non-girdled trees. According to Simon (1984), over the decade of heavy girdling the loss in current periodic diameter increments was equal to 0.97 cm within 10 years, representing 46% of total diameter increment. Moreover, girdling is supposed to change auxin levels, influencing the total increase, number of branches and shape of the crown, although these consequences are difficult to prove (Simon, 1984).

Nowadays more than 40 tree species are recognized as sap trees and most are of exotic origin (Turček, 1961). Among the trees susceptible to girdling in central Europe, these species are annotated: Norway Spruce—*Picea abies* (L.) Karst., Silver Fir—*Abies alba* Mill., pines—*Pinus sylvestris* L., *Pinus nigra* Arnold, *Pinus rigida* Mill., *Pinus cembra* L., oaks—*Quercus robur* L., *Quercus cerris* L., yew—*Taxus baccata* L. (Turček, 1949) and limes (*Tilia sp.*) (Simon, 1984). Sap resin and cambium consumption is also observed in coniferous trees, especially Norway Spruce—*Picea abies* (L.) Karst.

Morphological traits or health conditions of girdled trees are often mentioned regarding to girdling. Turček (1952) supposed that suppressed individuals, individuals impaired by wind, hoofed animals or individuals inappropriate from a forest-site point of view (e.g. pine in oakland biocenosis) are preferred for girdling. Girdling is then considered as a method of eliminating undesirable tree species due to their more frequent insect or fungi attack or vulnerability reduction.

On the opposing side, O'Donnell and Dilks (1994) claims two groups of girdled trees not common in the biocoenose (e.g. exotic species): (1) Healthy trees, solitary or trees with expected higher transpiration rates or phloem sap respectively. (2) Suppressed individual trees with forked tops or damaged roots and the expected changed physiology. What is not the reason for choice seems to be: thin bark, large crowns, tree height or pattern of microhabitat (Eberhardt, 2000).

We hypothesized that there are three physiological reasons why birds choose specific trees for attack; a) a higher transpiration rate in spring (higher sap-flow rate) and/or b) specific xylem sap composition in spring when solutes are transferring from roots to bursting buds and new twigs or c) greater difference in bud-burst timing, as earlier trees should be more prone to birds. Regarding these questions, targets of the study are:

Do bird species mostly attack exotic trees in the arboretum and are there any links between girdling and physiological traits, such as transpiration, spring leaf phenology or chemical composition of the sap?

MATERIALS AND METHODS

Experimental Site Characteristics

This investigation of tree girdling was done in arboretum in Kostelec about 50 km east from Prague (50°01' N, 14°51' E). Arboretum Kostelec is situated about 3 km from Kostelec nad Černými lesy, on a south exposed slope. The elevation above sea level is 300 to 345 m. The area is 12.40 ha. In the Arboretum Kostelec grew 1599 taxa of trees and shrubs, of them 959 species, 93 hybrids and 574 lower taxa.

Tree species description (girdled and selected non-girdled trees of the same genus).

The observation of sap-feeding birds was done on the *Tilia* × *moltkei* Späth ex Schneid., *Tilia americana* L. and *Cladrastis kentukea* (Dum. Cours.) Rudd – all these species are visited every spring and their bark is covered by sap wells forming round dots in regular circles from the bottom to the top of the tree, including thicker branches (Fig. 1).

On the other hand *Tilia cordata* Mill., *Tilia plathy-phyllos* Scop. and *Tilia neglecta* Spach are only visited irregularly by birds. There are several dots on their bark instead of rings evident in girdled trees. These three tree species were selected as representatives of non-girdled trees. American Linden is rarely cultivated in the Czech Republic, as well as *Cladrastis ken-tukea* (Dum. Cours.) Rudd, which is native to eastern North America.

Tilia × *moltkei* Späth ex Schneid. is a hybrid between *Tilia americana* L. × *Tilia petiolaris* DC. These individuals are the most attractive for birds, especially tree 25 years in age are most sought after by birds. The holes for sucking sap are deployed around the perimeter in rings, across the height of the trunk and older branches.

Tilia americana L. a medium-sized to large tree reaching a height of 15 to 25 (40) m. Strong sucking of sap was observed on the 25 year-old trees on the trunk and older branches.

Cladrastis kentukea (Dum. Cours.) Rudd is a small to medium-sized deciduous tree, reaching 10-15 (27) meters in height with typically low branching and smooth gray bark. Moderate sucking of the sap was observed in the previous 6 years. The holes are mostly on the sunlit side of the trunk at a height of about 2 m.

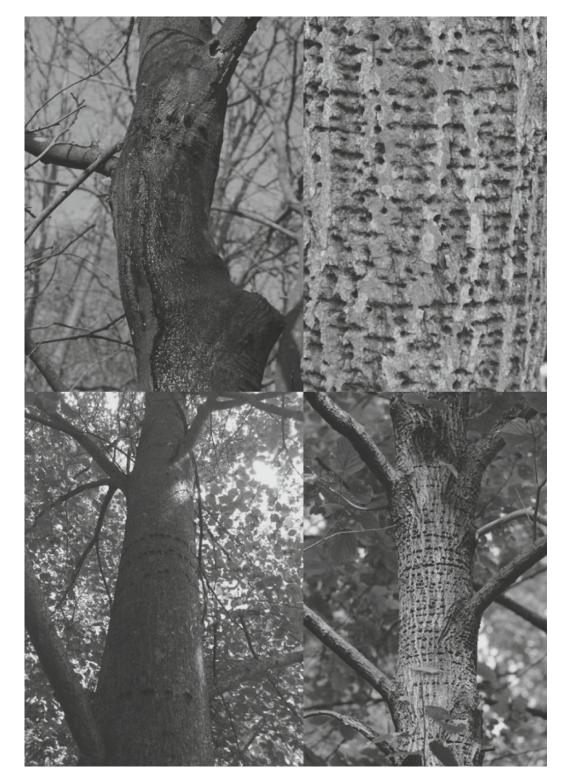


Fig. 1. Girdling on *Cladrastis kentukea* (Dum. Cours.) Rudd (top left) and *Tilia* × *moltkei* Späth ex Schneid. (top right), *Tilia euchlora* K. Koch (bottom left) and *Tilia americana* L. (bottom right) by *Dendrocopos medius* in arboretum Kostelec.

Birds Observation

Direct observation of birds visiting the trees was not successful during the spring 2014 and the moving sensor camera did not record any presence of the bird, although new sap holes occurred on the stem. For that reason 3 time-lapse cameras (Brinno TLC 200 (Tai-wan)) with 1280×1024 resolutions were installed in front of the investigated trees, shooting a photograph

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every minute over the next year. The set of photographs was recorded on a flash disk in .avi format.

Observation of three tree species—*Cladrastis kentukea* (Dum. Cours.) Rudd and both *Tilia* × *moltkei* Späth ex Schneid. was done from the end of February to the beginning of May. The camera was mounted from the south (*Cladrastis*) or east (*Tilia*) on the bark of other tree in the neighborhood of the one investigated. Although the pictures were taken every minute, one bird species—*Dendrocopos medius* (probably the same one) was recorded twice and for several minutes, although new holes on observed and non-observed trees occurred.

The middle spotted woodpecker is 20–22 cm long. There is a red crown on the head, the upperparts are predominantly black with white. The middle spotted woodpecker lives on a diet of insects and larvae, seeds and fruits and xylem sap also. It prefers broadleaves or mixed forest nevertheless forested area should be more than 10 ha (Gorman and Kokay, 2004). There are up to 6000 breeding pairs in the Czech Republic. *Dendrocopos medius* is an extra protected bird species as the sites of its natural occurrence are vanishing. From this point of view arboretum serves as a refuge for *D. medius* in heavy populated landscape of Central Bohemia Region.

Sampling Procedure

Methodology for spectral analysis and mass spectrometry of tree sap. At the end of September we collected whole branches, including the phloem, from all investigated trees. Branches were obtained from the branches from all azimuthal directions and stored in a freezer for several hours before the phloem was removed in the lab. FTIR ATR spectra (range 400- 4000 cm^{-1} , 64 scans, resolution 2 cm⁻¹) were recorded on FTIR a Nicolet 6700 spectrometer (Thermo Scientific, United States). All the spectra were smoothed and normalized using Omnic 8.0 software (Thermo Scientific), then exported in CSV format to Origin 6.0 software (Microcal Origin, United States) for further processing (PPT smoothing, ATR and/or baseline correction, normalization, etc.) and preparation of the graphs.

The spectroscopic data obtained were then statistically processed using basic describing statistics and principal component analysis (PCA) as the applications of Statistica 9.0 (Statsoft, United States) and TQ Analyst 8.5.21 (Thermo Scientific) software. The quantitative analysis of carbohydrates—glucose, fructose and saccharose in tree sap was done using LC/MS/MS. After sample preparation, carbohydrates were ionized by Cs⁺ after their separation by normal phase liquid chromatography on an amino based column. The analysis was done according to the methodology of Rogatsky et al. (2005).

Methodology for transpiration stream measurement. Sap flow was measured in one position in different azimutal directions only on *Tilia* species and *Cladrastis*. Trunk Sector Heat Balance method was used for transpiration stream measurement—for more detailed description of the method see Čermák and Nadezhdina (2011). In 2013, the measurement began on February 20th and ended May 8th and transpiration stream was estimated in kg/hour in different stems.

RESULTS

Bird Observation and Characterization of Girdled Trees

After approx. 3 weeks of minute-records of 3 trees. a record of the Dendrocopos medius that girdled the tree was found. It visited Cladrastis kentukea (Dum. Cours.) Rudd early in the morning in March, girdled in several heights of the tree but was not there longer than 7 minutes (electronic supplementary material). Sap wells originating from *Dendrocopos medium* are generally less common and it often visits sap wells originating from *D. major*—it is recognized as the main originator of girdling (Vačkář oral communication). In mountain regions Picoides tridactylus is often observed girdling and beech girdling was found in the nesting locality of Dendrocopos lecotos (Vačkář oral communication). Sap wells are rarely visited during the rest of the vegetation period. Presence of D. medius is a proof of the significance of small and diverse ecosystems with number of exotic tree species for various bird populations (Gray and van Heezik, 2015).

From the total of all angiosperm trees 5% are visited regularly. An overview of all linden in the arboretum and quantification of pricks on them is shown in the Table 1.

The character of the holes on our investigated trees was different due to varied cork cambium activity. On the linden well aligned holes in the rings always extend into the wood and badly overgrown. There is obviously a violation of cork cambium, so the holes are also visible for many years. *Cladrastis* holes are arranged in rings, initially small then get bigger and fill up the colored precipitate organic and inorganic substances in the sap. Birch holes are individual, mainly on the branches and due to the intensive cork cambium activity are hard to recognize after one year. The oft mentioned morphological traits as suppressed individuals or individuals not common in the biocoenose seem not to be important.

Physiological Properties of Girdled Trees

Transpiration stream. Our expectations that transpiration will be higher in girdled trees due to the higher pressure of water with organic substances compared to non-girdled were not proven. The transpiration stream in *Cladrastis* was very low during the entire measurement period (February 20–May 8, 2014)

Table 1. Survey of lime species in the arboretum with respect to the extent of girdling by *Dendrocopos medius*. Scale: no—no sign of bird's visit, low—up to 5% of the bark covered with pricks, medium—up to 25% and high—more than 50% of the bark

Species	Age	Extent of girdling
<i>Tilia</i> × <i>flavescens</i> "Glenleven"	19	Low
<i>Tilia</i> × <i>flavescens</i> "Glenleven"	19	Low
Tilia × moltkei	57	High
Tilia × moltkei	54	High
<i>Tilia</i> × <i>vulgaris</i> "Pallida"	19	Low
Tilia × vulgaris	54	Low
Tilia americana	54	High
Tilia cordata	58	Low
Tilia cordata	84	Low
Tilia euchlora	34	Medium
Tilia mongolica	31	Low
Tilia neglecta	54	High
Tilia petiolaris	34	No
Tilia petiolaris	34	No
Tilia platyphyllos "Fastigiata"	53	Low
Tilia platyphyllos "Laciniata"	57	Low
Tilia platyphyllos	54	Medium
Tilia platyphyllos	54	Low
Tilia tomentosa	56	Low

although it was the only tree this year with a massive attack of *D. medius* (Fig. 2).

The differences between girdled and non-girdled tree groups (*Tilia cordata* Mill., *Tilia plathyphyllos* Scop. and *Tilia neglecta* Spach are non-girdled) were not confirmed (Table 2) even in case of transpiration

calculation with respect to different diameters, which enables comparison of trees among them as the diameter is a covariate.

At the same time we did not confirmed coincidence of transpiration and bud burst as mentioned by several authors (Simon, 1984). Our sap from bird pricks was recorded on February 20th although bud bursting of *Cladrastis* started in the middle of May. Similarly there is weak connection between the sum of effective temperatures and bud bursting. The threshold of effective sum of temperatures (above 5°C) occurred more than one month (March 12th, the sum of effective temperatures was 6.8°C) before bud bursting of American and local linden on April 21th.

Spectral analysis of tree sap. The FTIR spectra of the phloem sap samples ($1800-900 \text{ cm}^{-1}$) obtained from four tree species—*Cladrastis kentukea* (Dum. Cours.) Rudd, *Tilia americana* L., *Tilia cordata* Mill., and *Tilia* × *moltkei* Späth ex Schneid. show a variability that can be explained by specificity in chemical composition (Fig. 3). Spectral differences in the median spectra (Fig. 4) reflect non-similarities in the phloem composition for the species studied. Strong absorption in the regions of $1800-1500 \text{ cm}^{-1}$ (C=O and C=C stretching) and $1200-950 \text{ cm}^{-1}$ (CO and CC stretching) are characteristic for the main constituents, i.e. phenolics and sugars, respectively.

In these two regions FTIR spectra demonstrate pronounced variability within each species. Especially *Tilia cordata* Mill. with no girdling and *Cladrastis kentukea* (Dum. Cours.) Rudd are markedly different from the rest of the investigated trees. It is evident from the spectra that the samples from *Tilia cordata* Mill. have specific composition of phenolics, whereas *Cladrastis kentukea* (Dum. Cours.) Rudd differs from the other species in sugar composition. The IR band near 1743–1739 cm⁻¹ (pronounced for *Cladrastis kentukea* (Dum. Cours.) Rudd and *Tilia cordata* Mill.) was

Table 2. Average transpiration \pm standard deviation per tree and transpiration per 1 cm of diameter from February 20th 2013 to the start of increasing transpiration. Identical letters indicate homogeneous groups with statistically insignificant differences (P > 0.05). Girdled group of trees in bold

Tree	Transpiration per tree	Transpiration per 1 cm of the diameter	Start of increasing transpiration
Tilia americana ^c	8.7 ± 3.9	0.10 ± 0.05	15.4
<i>Cladrastis^c</i>	5.7 ± 2.7	0.08 ± 0.04	Did not start till May 8th
Tilia cordata ^{ab}	6.1 ± 5.6	0.04 ± 0.03	19.4
Tilia × moltkei ^{ab}	1.8 ± 1.3	0.03 ± 0.02	18.4
Tilia × moltkei 2^{ab}	1.8 ± 2.1	0.02 ± 0.02	29.4
Tilia neglecta ^a	0.7 ± 1.7	0.01 ± 0.02	23.4
Tilia plathyphyllos ^c	10.6 ± 5.0	0.09 ± 0.04	18.4

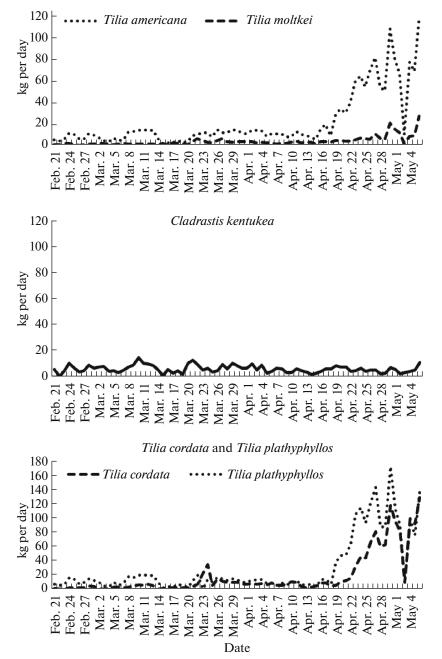


Fig. 2. Sum of transpired water during investigation period (21.2.-8.5.2014) in trees clustered according their origin. Transpiration is expressed per one tree (kg/day). First two figures are transpiration of girdled trees, the last of non-girdled trees.

assigned to C=O stretching vibration in esters (Wu et al. 2011). This band is typical for volatile oils. Stretching vibration of unconjugated C=O in xylans may also contribute in this region (Popescu et al., 2010). By contrast, band near 1732–1727 cm⁻¹ (pronounced for *Tilia americana* L. and *Tilia* × *moltkei* Späth ex Schneid.) could be assigned to the same vibration in organic acids or conjugated esters. Intense broad band near 1634–1644 cm⁻¹ was assigned mainly to C=C stretching vibration in phenolics with some

contribution of water bending. The weak bands or shoulders at 1520–1514 cm⁻¹ (pronounced for *Cladrastis kentukea* (Dum. Cours.) Rudd and *Tilia* × *moltkei* Späth ex Schneid.) are typical for aromatic acids (Popescu et al., 2013, 2010).

Weak band or shoulder near 1455 cm⁻¹ arose from scissoring vibration of CH₂, and the next bands neat 1373, 1330, and 1320 have contribution of the CH and OH bending vibrations in sugars and lignin. The band near 1250-1246 cm⁻¹ with the shoulder near 1270 cm⁻¹

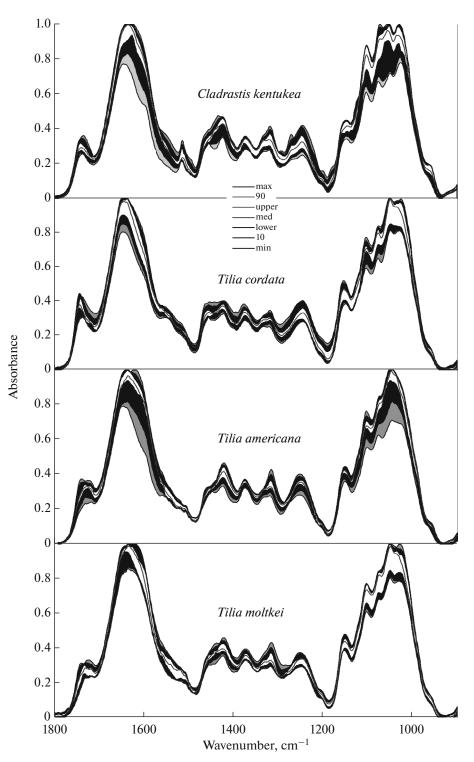


Fig. 3. Statistical distribution of the FTIR spectra (1800–900 cm⁻¹) for the phloem sap samples obtained from four tree species *Cladrastis kentukea* (Dum. Cours.) Rudd, *Tilia americana* L., *Tilia cordata* Mill. and *Tilia* × *moltkei* Späth ex Schneid. (n = 10 for each species).

arouse mainly from CO stretching vibration in acids and esters. The band centered near 1154 cm⁻¹ (all the *Tilia* species) or at 1146 m⁻¹ *Cladrastis kentukea* (Dum.

Cours.) Rudd was assigned to glycosidic bond COC stretching vibration in oligo- and polysaccharides. The envelope of highly overlapped bands at 1103-950 cm⁻¹

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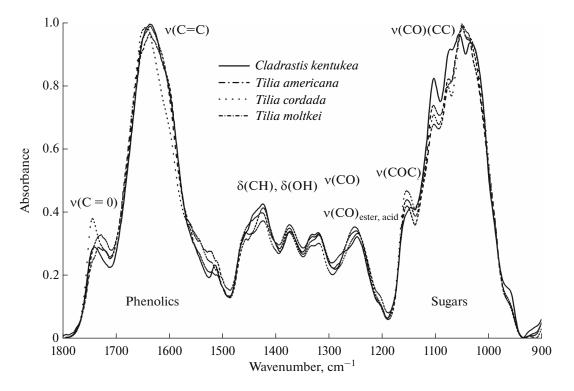


Fig. 4. Overlay of median FTIR spectra for the phloem sap samples obtained from four tree species *Cladrastis kentukea* (Dum. Cours.) Rudd, *Tilia americana* L., *Tilia cordata* Mill. and *Tilia* \times *moltkei* Späth ex Schneid. (n = 10 for each species).

arose from coupled CO and CC stretching vibrations with some contribution of COH bending originated from sugars (Wu et al., 2011; Popescu et al., 2013).

The spectral region of $1200-900 \text{ cm}^{-1}$ was found to be sensitive to origin of the phloem sap samples. This region of four median spectra was used for multivariate analyses (HCA, PCA) with the aim of discrimination of tree species. Both the dendrogram of similarity (Fig. 5a) and the 3D component score graph PC1 vs PC2 vs PC3 (Fig. 5b) showed the discrimination of genera *Cladrastis* and *Tilia*.

In addition, it is evident from these graphs that species *Tilia cordata* Mill. and *Tilia* \times *moltkei* Späth ex Schneid. are closer together than *Tilia americana* L. From the dendrological point of view it is the opposite from expected because *Tilia americana* L. is one of the parent trees of *Tilia* \times *moltkei* Späth ex Schneid.

DISCUSSION

Why Some Trees are Girdled and Others Not

The hypothesis concerning girdling practice by certain part of population or individuals only are supported by Klíma (1958) because of scattering of girdled tree. Girdling of rare exotic species by *D. medius* could then be seen as another kind of adaptive behavioral strategy to improve its diet variety. E.g. Acorn Woodpecker (*Melanerpes cactorum*) was observed feeding

their nestlings with insects dipped into sap as it is a welcome source of energy during the breeding cycle (Eberhardt, 2000). The utilization of sap may allow birds to avoid energy shortages while migrating (not the case of *D. medius*) and as a source of energy in time of low insect availability (Pejchar, 2004).

During the investigation period it was found that there are many trees in arboretum with at least with one prick on the stem (Acer saccharum Marsh., Carpinus betulus L., and several species of Betula L. genus). Some of them as Cladrastis kentukea (Dum. Cours.) Rudd or *Tilia* × *moltkei* Späth ex Schneid. are visited every year and the surface of their bark is girdled from base to the top of the crown. This observation lead us to the suspicion about the cognitive maps formation in *Picoidae* family as the girdling is welcome source of energy early in the spring. According to many papers concerning bird spatial memory (Mayer et al., 2013), birds are able to remember the location of previously hoarded food. They use local and spatial cues for orientation in the landscape and the preference of food hoarding birds to use spatial cues is probably part of their adaptation mechanism to the environment in which they live (Feenders and Smulders, 2011). The ability of birds to return home or find their colony was studied in shearwaters as they are forced to pinpoint their breeding colony in featureless open ocean. According to Reynolds (2015) shearwaters rely on olfactory cues for oceanic navigation because the dimethyl sulfide as a product of

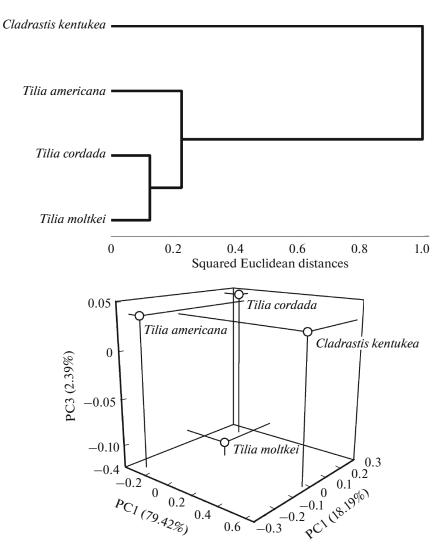


Fig. 5. Dendrogram of similarity (4a) and the 3D component score graph PC1 vs PC2 vs PC3 (4b) for the median FTIR spectra (1200–900 cm⁻¹) of the phloem sap samples originated from *Cladrastis kentukea* (Dum. Cours.) Rudd, *Tilia americana* L., *Tilia cordata* Mill. and *Tilia × moltkei* Späth ex Schneid. (n = 10 for each species).

coastal phytoplankton signalize the off-shore proximity. In pigeons (Columba livia) at least two strategies are used in homing-compass information and landmark guidance (e.g. Biro et al., 2006). Cognitive navigational map was proved in flight experiment by Blaser et al. (2013): pigeons knew their geographical position in relation to targets (food loft or home). On the basis of our physiological measurement we conclude that not the transpiration, phenology nor chemical composition of the xylem sap are the reason of the girdling. Because of the at least one prick on the stem of many trees in the arboretum we formulated one suspicion: D. medius and other birds involved in girdling probably do not sort out particular tree on the basis of physiological traits but they examine more trees in their territory and create "a map of confident trees" with reliable xylem sap streaming early in the spring.

ACKNOWLEDGMENTS

English language correction by Mr. Jim Freeman is gratefully acknowledged.

The authors declare that they have no conflict of interest.

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