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A NOTE ON THE OBSERVABLE BARK COLORATION OF QUAKING ASPEN (*POPULUS TREMULOIDES*)

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ABSTRACT.—The bark of quaking aspen (*Populus tremuloides*), a clonal hardwood that is dominant in many Rocky Mountain forests, varies from white to orange to green among both ramets and populations. The proximate causes of this color variation remain controversial. We collected 72 samples of aspen bark from 11 locations in Boulder County, Colorado, and used microscopy, dissection, and thin layer chromatography to assess the structural and biochemical basis for the color gradient. Our study shows that the cork cells closest to the cork cambium of the aspen were consistently orange and conglutinated. Outward from this layer, the cork cells transitioned from orange to white; they aged and simultaneously lost cohesion. Green bark was visible due to thinnest cork layers, which revealed subcortical chlorenchyma tissue, the only bark tissue in which photosynthetic pigments were detected. Tests for photosynthetic pigmentation in the cork were negative. Comparison to standards indicated that β-carotene is not the pigment responsible for the orange hue of the cambium. We conclude that the powdery substance found on the tree surface is composed of bark cells, and their color variation seen on the aspen bark is attributed to the cell's pigment content and thickness, although the molecule responsible for cork cell coloration remains unidentified.

RESUMEN.—La corteza de los álamos (*Populus tremuloides*), una madera clonal dominante en varios de los bosques de las Montañas Rocosas, varía de blanco a anaranjado y a verde entre rametos y poblaciones. Las causas inmediatas de esta variación en el color continúan siendo controversiales. Colectamos 72 muestras de corteza de álamo de 11 localidades en el condado de Boulder, Colorado, y utilizamos microscopía, disección y cromatografía en capas finas para evaluar la base estructural y bioquímica del degradado de color. Nuestro estudio muestra que las células de corcho que más se aproximaron al corcho del cámbium del álamo eran anaranjadas y estaban aglutinadas de manera constante. Hacia el exterior de esa capa, las células de corcho pasaban de anaranjado a blanco; envejeciendo y, simultáneamente, perdiendo cohesión. La corteza verde observable se hizo visible debido a las capas más delgadas del corcho que reveló tejido subcortical, el único tejido de la corteza en el que se detectaron los pigmentos fotosintéticos. Las pruebas de la pigmentación fotosintética en el corcho fueron negativas. La comparación con estándares indicó que el caroteno-β no es el pigmento responsable del tono anaranjado del cámbium. Llegamos a la conclusión de que la sustancia en polvo que se encuentra en la superficie del árbol se compone de células de la corteza, y la variación de color visto en la corteza del álamo se debe al contenido de pigmento de la célula y a su grosor, aunque la molécula responsable de la coloración de las células de corcho permanece sin identificar.

Populus tremuloides Michx. (quaking aspen) is one of the most emblematic species of Rocky Mountain ecosystems and has the widest geographical range of any tree in North America. Individuals occur in cool temperate regions from Canada to Mexico, from New England to the Pacific Northwest, and throughout the continental mountain ranges (Debyle and Winokur 1985, Perala 1990, Jelinski and Cheliak 1992, Argus et al. 2010). Many physiological adaptations to the varied climates encountered by this species across its range have been documented, including rapid postfire colonization, clonal reproduction, and bark photosynthesis (Clausen et al. 1989, Romme et al. 1995, Wu et al. 2000, DeWoody et al. 2008). Perhaps one of the most curious traits, which has long puzzled ecologists, botanists, and natural historians, is the variation in color of the powder-like substance found on the surface of aspen bark.

Clonal colonies of aspens often vary in powder color. The coloration ranges from light orange to stark white. In nature, the color and thickness of the powder found on aspen trees is responsible for the observable color of the bark. From our preliminary field investigations, we observed that all aspens have a uniform dark orange and green hue beneath the powder surface due to the presence of cork tissue and photosynthetic chlorenchyma, respectively (Pearson and Lawrence 1958, Schaedle

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et al. 1967, Aschan et al. 2001). Studies on aspen ecology and evolution have generated numerous working hypotheses to explain the color variation found in this powdery substance. Marr (1947) observed that aspens with whiter bark experienced greater frost damage than trees with orange bark. Cottam (1954) observed that aspens with orange bark occupied higher elevations than aspens with white bark. Mitton and Grant (1980) noted that young, orange-bark trees produced female flowers whereas white-bark trees produced male flowers, but these authors speculated that the result may have been spurious. Weber and Wittmann (2010) stated that white powder is sometimes associated with the presence of lichen thalli. Additionally, some popular culture references describe the powder itself as a sunblock, a natural source of yeast, and a medicine, without indicating if the substance itself is bark tissue or part of another organism (Van Horn 2014).

No study has addressed the structure and chemistry of aspen bark coloration. Recent studies of tree species with similar bark morphology (*Eucalyptus accedens* W. Fitzg., *Vachellia xanthophloea* Benth.) led us to hypothesize that the powdery substance on the surface of aspen trees is sloughed cork cells (Venter and Venter 1996, Majer et al. 2004). We further suggest that the coloration differences are caused by sunlight deteriorating residual photosynthetic pigments in initially orange cork cells, bleaching them white over time. To test our hypothesis, we conducted qualitative microscopy and chromatography studies of the different tissue layers that comprise the periderm across natural aspen stands. In this note, we report on the anatomy of the aspen periderm in relation to the powdery substance, document the presence of photosynthetic pigmentation in the powder responsible for coloration, and suggest possible functions of the aspen bark powder in nature.

METHODS

Field Sampling

Fieldwork was conducted by the first author in winter between January and March 2014. Seventy-two samples of periderm tissues of *Populus tremuloides* were collected in Boulder County, Colorado, at 2 river valleys, Boulder Canyon and Lefthand Canyon, across an elevation gradient of 1615–2560 m (5300–8400 ft.) Eleven sites were haphazardly selected based on ease of accessibility. Twelve trees were sampled at each site. Six samples were collected from different trees within 6 m (20 ft.) of one another. Six extra samples were taken from isolated trees not more than 60 m (200 ft.) from the core group of sampled trees. All sampled trees were >1.5 m in height. Incisions were made with a pocketknife on the south face of each tree and a thin 3 × 2-cm tissue sample was collected. Each sample comprised all peridermal tissue layers between and including the secondary phloem and cork. This was confirmed by a visual assessment of the color of the deepest sampled tissue; secondary phloem was tan with red sap, whereas chlorenchyma was green. The most superficial layer in our samples was composed of the powder substance, and the deepest layer was chlorenchyma.

Field samples were marked with an identification number, placed in cloth bags, and refrigerated within 4 hours of collection. Cloth bags were used so that samples would dry completely while refrigerated (Average relative humidity for Boulder from January to March from 2010 to 2013 ranges from 29.5% to 70%; FOEHN 2015). Samples were kept refrigerated for 2 weeks or until uniformly dry. Dryness was assessed by relative tendency of the material to break. Prior to laboratory study, samples were checked for microbial activity and water content. Wet or decayed samples were not used in the study. Aspens infected with the fungus *Cytospora* spp. Ehrenb. exhibit a localized neon orange hue, which is caused by a change in the cork tissue chemistry induced by the fungus. Another symptom of *Cytospora* infection is a smooth bark texture resulting from destruction and rot of the vascular cambium directly subtending the area of infection (Jacobi 1991). We visually inspected and field-verified infected trees by outer bark texture and vascular cambium health. We excluded infected trees from our field sampling.

Microscopy

Thin cross sections of the bark tissue were made by hand and viewed under an Olympus SZX10 stereo and an Olympus BX51 compound microscope. Micrographs were taken using a Qimaging Retiga 2000R camera and

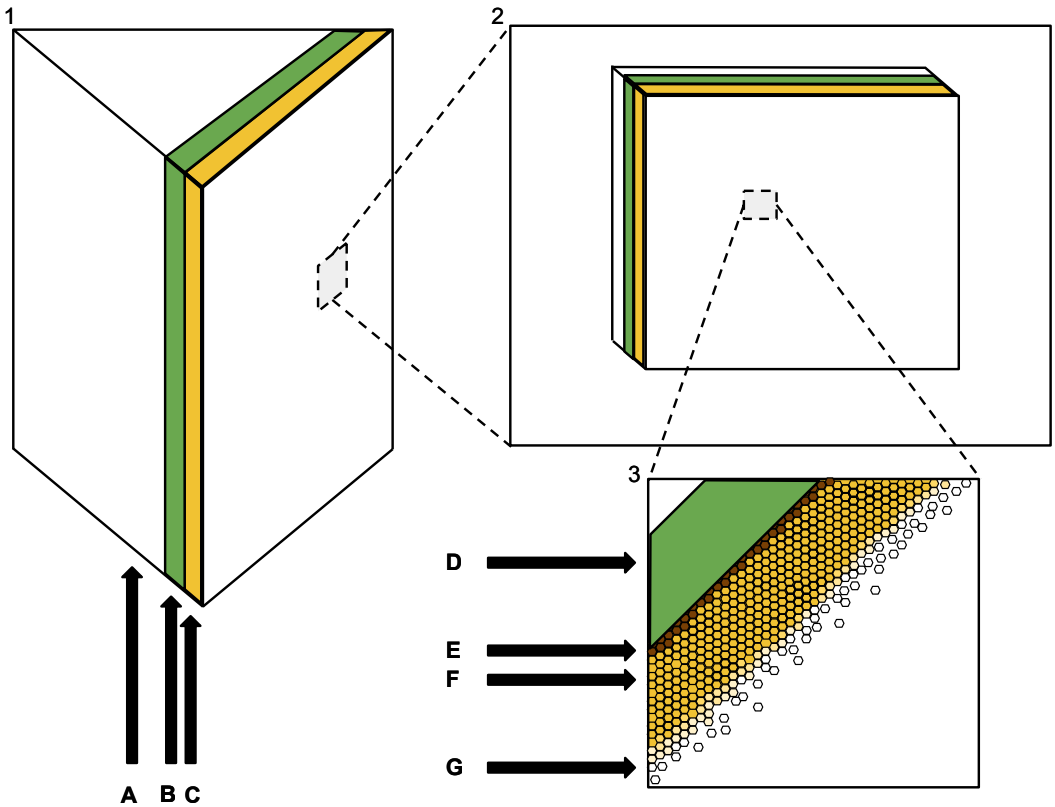

Fig. 1. Tissue layers visible upon primary inspection of tree surface. (**A + B**) Diagram 1 depicts the wood subtending the layer of photosynthetic chlorenchyma, depicted in green. (**C**) Bark layer is comprised of the cork and cambium. Diagram 2 exemplifies size and depth of bark samples collected. Diagram 3 represents results from cross-section micrographs of bark samples. (**D + E**) Chlorenchyma is seen subtending cork cambium, depicted in brown. (**F**) Arranged in a hexagonal lattice, orange cork cells, initiated by the cork cambium, are seen transitioning to white on the edge of bark layer. (**G**) White cork cells are shed readily and accumulate in a powder on the surface of the tree. In this study, we found that samples were variable in cork cell layer thickness and some samples lacked white cork cells, which impacted macroscopic color differences. Scale bar = 80 microns (μm).

Qcapture Pro 7 software (http://www.qimaging.com) on a Dell desktop computer.

Thin Layer Chromatography (TLC)

Thin layer chromatography was conducted to assess the presence of photosynthetic pigments in chlorenchyma, cambium, and cork tissues of varying colors detectable qualitatively by the human eye. Methods followed TLC protocols described in Wellburn (1994) and Thakare et al. (2010), with minor modifications. Bark tissues were separated from each other manually with a razor blade then cut into 1 × 1-cm squares. Tissue samples of chlorenchyma, cambium, loose orange cork, and loose white cork were placed into separate 1.5-mL plastic vials. The vials were frozen in liquid nitrogen and pulverized in a Spex Geno/Grinder (Metuchen, NJ) at 600 cycles per second.

Extracts from macerated tissues were made in ceramic wells with HPLC-grade acetone (100%). Dried carrot and spinach samples were processed in a similar manner and used as standards during chemical tests.

Extractions were spotted on Analytical Chromatography TLC Silica Gel 60 F_{254} aluminum plates and transferred to a homemade mobile phase chamber. Our mobile phase solution consisted of a 50:40:10 ratio of petroleum ether:chloroform:isopropyl alcohol. All reactions were completed in low-light settings to preserve pigment integrity. Solvent action was arrested 2 cm from the top of the

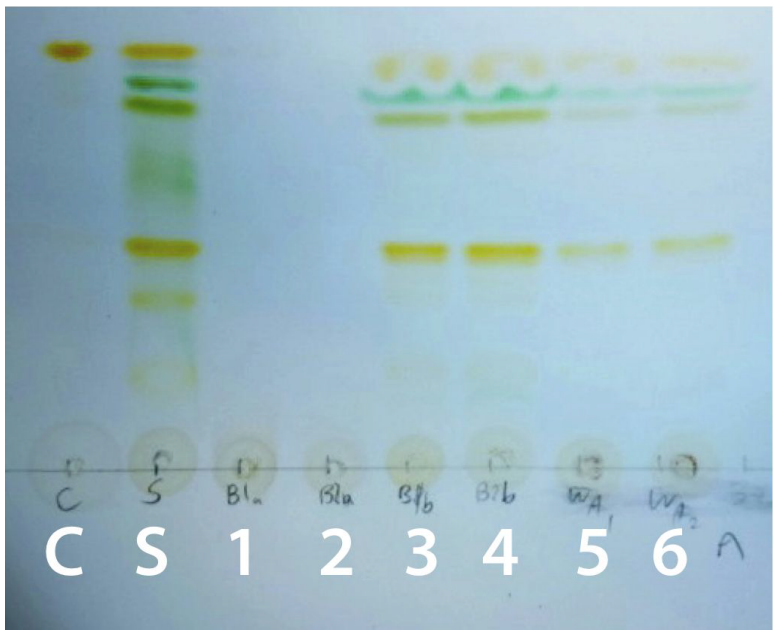

Fig. 2. Example chromatogram showing photosynthetic pigmentation in selected tissues. From left to right: (**C**) Carrot standard was used for β-carotene reference. (**S**) Fresh spinach standard contained all photosynthetic pigment compounds for reference; from top to bottom, these compounds are β-carotene (orange), pheophytin (grey), chlorophyll *a* (blue-green), chlorophyll *b* (green-yellow), and xanthophyll (yellow). Lanes **1** and **2** are orange cork tissues. **3** and **4** are orange cork tissues with cambium and chlorenchyma. **5** and **6** are white cork tissue with chlorenchyma. Not shown are TLC runs in which we sampled only white cork tissues with no chlorenchyma, but all such runs lacked photosynthetic pigments, as in lanes 1 and 2.

silica plates, and the plates were photographed 10 min after removal from the mobile phase chamber.

RESULTS

Cork cambium cells of *Populus tremuloides* were observed in layers resembling a "honeycomb" lattice, with layered cells connected in tangential hexagons. The cork cambium is orange in color and generates cork cells of similar pigmentation outward. Orange cork cells were found closer to the cambium, and white cells were found on the most superficial surface of the bark. No other colors were represented in our cork cell samples.

Cross sections of tissue samples revealed distinct layers of secondary phloem, chlorenchyma, cork cambium, orange cork cells, and white cork cells. The layering of peridermal tissues was consistent throughout samples. White cork cells were not observed without subtending orange cork and cambium cells (Fig. 1).

TLC assays demonstrated the presence of photosynthetic pigments β-carotene, chlorophyll *a*, chlorophyll *b* and xanthophyll in chlorenchyma tissue (Fig. 2) corresponding to known R_f values (λ/nm) of these pigments based on standards described by Wellburn (1994). Photosynthetic pigments were solely in samples that contained chlorenchyma tissue. No photosynthetic pigments were observed in cork or cambium tissues.

DISCUSSION

Our microscopy study showed that the powder tissue remnant characteristic of the surface of aspen trees is sloughed cork cells. Orange cells were found adjacent to the cambium, whereas white cells, if present, were found on the surface of the tree. The cambium was orange in color, supporting the idea that cork cells transition from orange to white. Because no white cork cells were found without orange cork cells subtending them, we assert that orange cells initiated from the cork

cambium lose pigmentation and become white over time due to exposure. The cells accumulate on the surface of the tree and create the familiar powder and observable color typical of aspen bark. Further study would be required to determine the environmental factor that bleaches the cork cells.

In our TLC assay, we found that cork cells did not contain B-carotene or any other any active photosynthetic pigments and therefore appear not to play a role in chemical photosynthesis. We further support the conclusion made by previous authors that bark photosynthesis occurs only in the chlorenchyma tissue subtending the cork cambium. Orange cork cells may serve a photoprotective function for subtending chlorophyll-rich chlorenchyma, akin to that of cuticle tissue on leaf epidermis (Robinson et al. 1993, Bartley and Scolnik 1995). Thus, removal of cork from trees may place them at a higher risk of solar UV-B damage. The structure and color of sloughed bark cells may facilitate efficient bark photosynthesis, but further experimentation would be necessary to demonstrate this, including the isolation, identification, and reflectivity of the pigment responsible for the orange color of aspen cambium tissue.

Different colors of cork cells were found within and between stands of aspen trees. Trees with white bark have thick layers of white powdery cork cells on their surface subtended by the orange cambium. Orange trees have no white cork tissue and reveal the thinner layers of orange cork and cambium beneath. Trees with a greenish appearance have the thinnest layer of accumulated cork; sampling revealed that the verdant chlorenchyma was visible through the cambium and was responsible for the observed coloration. We caution that the above observations were made without consideration of the genetic relatedness of individuals, although the well-known clonal nature of aspen trees (Debyle 1964) suggests that it is likely that most of our samples within a given population were genetically homogenous.

Several other avenues of future research may answer remaining questions about *Populus tremuloides* tree bark. First, the study done by Majer et al. (2004) on *V. xanthophloea* should be repeated on other species that develop powdery cork cells (*E. accedens*, *P. tremuloides*) to support or reject the hypothesis that powdery bark may disrupt locomotion of arthropods navigating the tree trunks. Second, environmental factors that remove loose cork cells from trees and the effects of cork removal on damage caused by solar radiation should be studied in the context of forest management and organismal preservation. Third, research is required to understand whether age, exposure, radiation, or other factors trigger the color transition of the cork cells from orange to white. Finally, field studies across a larger geographic range of aspen, especially those that incorporate a genetic dimension, would help to confirm the findings in the present manuscript as well as explore whether evolutionary relatedness impacts bark color.

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