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An Annotated Bibliography of Thousand Cankers Disease

Including general reports related to this disease, information on the walnut twig beetle, *Pityophthorus juglandis* Coleoptera: Curculionidae (Scolytinae), and the associated fungus *Geosmithia morbida*.

Rachael Sitz [1], Emily Luna [2], Ned Tisserat [3],
and Whitney Cranshaw [3]

An Annotated Bibliography of Thousand Cankers Disease

This annotated bibliography contains references to University theses, publications related to these species, and several related articles of potential interest.

[1] PhD Candidate, Department of Bioagricultural Sciences and Pest Management, Colorado State University
Contact email: rachael.fithian@colostate.edu

[2] Research Associate, Department of Bioagricultural Sciences and Pest Management, Colorado State University

[3] Professor, Department of Bioagricultural Sciences and Pest Management, Colorado State University

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INTRODUCTION

This annotated bibliography is a compilation of sources related to thousand cankers disease. The following citations were obtained by cross checking data base search results with recovered articles. Encompassed references are first organized as articles and books relating to thousand cankers disease, walnut twig beetle, and *Geosmithia morbida*. Then a section with references containing information on related genera are separated into entomological and mycological segments.

Most peer-reviewed citations are followed by a description of the material. Article abstracts were used when possible, but for articles lacking abstracts the author composed brief summaries based on their review. This bibliography is complete as of December 2015.

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Drs. Whitney Cranshaw and Ned Tisserat provided excellent guidance, and a template for this project. My colleagues Emily Luna, Jorge Ibarra Caballero, and Dr. Marcello Zerillo aided in retrieving articles. Drs. Louis Bjostad, Andrew Norton, and Boris Kondratieff provided editorial comments. Also, thank you to all of the researchers who spent countless hours working to better understand this disease complex.

AN ANNOTATED BIBLIOGRAPHY OF THOUSAND CANKERS DISEASE

Articles that Discuss Thousand Cankers Disease

Bostock, R., Tisserat, N.A., Hasey, J., Cranshaw, W., and Seybold, S.J. 2010. Update on thousand cankers disease. Western Pest Diagnostic Network Newsletter, February 2010, 3(1):1. Retrieved October 15, 2012 from http://www.wpdn.org/webfm_send/42

Cranshaw, W., and Tisserat, N. 2011. Plant health: Thousand cankers disease: An unusual paradigm. American Nurseryman Magazine. Retrieved December 16, 2015 from http://www.amerinursery.com/growing/thousand-cankers-disease-an-unusual-paradigm/

Cranshaw, W., and Tisserat, N. 2011. Thousand cankers disease a recently recognized threat to North American black walnut. *The Official Newsletter of the Society of Commercial Arboriculture* 16(1). Retrieved October 8, 2012 from http://sca.isa-arbor.com/PDFs/2011April_SCA_Today.pdf

EPPO [European and Mediterranean Plant Protection Organization]. 2015. Pest risk analysis for thousand cankers disease (*Geosmithia morbida* and *Pityophthorus juglandis*). EPPO, Paris. Retrieved September 29, 2015 from http://www.eppo.int/QUARANTINE/Pest_Risk_Analysis/PRA_intro.htm

Flint, M.L., Graves, A.D., and Seybold, S.J. 2010. Thousand cankers disease of walnuts spreads in California. *CAPCA Advisor Magazine*, June 2010, 8(3): 36–39.

Grant, J.F., Windham, M.T., Haun, W.G., Wiggins, G.J., and Lambdin, P.L. 2011. Initial assessment of thousand cankers disease on black walnut, *Juglans nigra*, in eastern Tennessee. *Forests* 2(3): 741–748.

> In 2010, thousand cankers disease (TCD) was documented in Tennessee, representing the first confirmation of this disease in the native range of black walnut and the first known incidence of TCD east of Colorado. Tennessee Department of Agriculture personnel conducted surveys to determine the extent of TCD in counties in eastern Tennessee. Samples of symptomatic black walnuts were sent to the University of Tennessee for processing. The causative agents, walnut twig beetle, *Pityophthorus juglandis*, and the fungal pathogen *Geosmithia morbida*, were documented on the same trees in four counties. Tree mortality was observed in two counties, and tree decline was observed in at least 10 counties although it may be attributed to previous droughts or to TCD. In 2010, four confirmed counties were quarantined by TDA, and 10 buffer counties were also regulated. Research is underway to further assess the incidence and impact of TCD on black walnut in Tennessee.

Griesser, A. and Neveln, V. 2009. New disease wreaks havoc on western walnut trees. *American Gardener* 88(5): 50.

Griffin, G.J. 2012. Thousand cankers disease: A recently emerging disease of eastern black walnut in the eastern United States [Abstract.] *Phytopathology* 102(7): S159.

> Eastern black walnut (EBW) is an important forest and landscape tree in the eastern U.S. Since the discovery of thousand cankers disease (TCD) in TN in 2010 and subsequently in VA and PA in 2011, the threat of this disease to the EBW resource has been of increasing concern. The rate of

spread of TCD in the East is yet to be defined. Many EBW trees have been killed by this disease in the West, often within a few years. As environmental conditions in the West are quite different than in the East, it is not clear if the progression of this disease in the East will be the same as in the West. EBW is a mesic tree species, native to moist coves and alluvial soils. In contrast, many EBW trees are planted in the East on sites that often experience seasonal drought or other stresses that may affect TCD development. In 2011 in TN, I observed little or no new development of TCD external symptoms (wilt and branch dieback) on EBW trees in research plots, and increase in live crown ratings (recovery) on some trees was observed early in 2011. The rainfall for the growing season was 53% greater in 2011 than in 2010, when severe TCD symptoms occurred. It is possible that TCD on individual EBW trees in the East will not be continuously progressive, as was for chestnut blight, but be intermittently progressive, depending on the occurrence of environmental conditions favorable to disease development. Movement of infested EBW products is of special concern for TCD spread to new areas.

Griffin, G.J. 2013. Progression of thousand cankers disease (TCD) on black walnut in Tennessee in 2010 and 2011 and possible association with drought stress [Abstract.] *Phytopathology* 102(6): S3.

Griffin, G.J. 2015. Status of thousand cankers disease on eastern black walnut in the eastern United States at two locations over 3 years. *Forest Pathol.* 45(3): 203–214.

Thousand cankers disease (TCD), a lethal fungal dieback of eastern black walnut (*Juglans nigra*), caused by *Geosmithia morbida*, and spread by the walnut twig beetle, *Pityophthorus juglandis*, was documented in 2009 to be very destructive in the western United States and was identified in the native range of *J. nigra* at Knoxville, Tennessee, in 2010, and in 2011 at Richmond, Virginia. Beginning late 2010, we studied branch dieback levels (per cent live crown) and new TCD symptom development at these two quarantined locations monthly for 3 years. Of the 106 trees studied (53 at each location), 31 trees had low live crown ratings of 70 to 0% with little change over the 3 years of the study. One per cent of the trees developed new symptoms on a per-year basis. Thus, a moderate level of TCD (mean = 76% live crown) was present in these two locations, and most trees were in a quiescent or dormant TCD condition for 3 years, an important finding not previously reported. We found new TCD symptoms developed in Richmond in 2011 and 2012 when precipitation from January 1 to the end of August was low (60–64 cm), and not when the precipitation in Richmond was higher (99 cm). In late 2012, in Richmond, soil water potential assays indicated that some black walnut trees were under severe physiological stress (-15 bars). In contrast, in 2013, high precipitation levels (99 and 130 cm) and high soil water potentials (-0.1 to -3 bars) at both locations were associated with extensive new foliage and stem growth and recovery from TCD. Further research is needed on water relationships in regard to TCD and black walnut health.

Hasey, J.K., Bostock, R.M., and Michailides, T.J. 2010. Thousand cankers disease of walnut: Status in California. [Abstract.] *Phytopathology* 100(6): S48.

Thousand cankers disease of eastern black walnut (*Juglans nigra*) and related *Juglans* spp. has emerged as a disease of significant concern in the western U.S. The disease is caused by a newly described fungal pathogen, with a proposed name of *Geosmithia morbida*, and is spread from attacks by the walnut twig beetle (WTB, *Pityophthorus juglandis*) with subsequent canker formation in the phloem. These cankers eventually coalesce to girdle the stems and branches. Trees usually die within three years of initial symptom development that include upper crown yellowing and dark bark staining. The thousand cankers disease was first confirmed in CA in June, 2008 in Yolo Co. Since then, the beetle-fungus complex has been confirmed in many counties distributed across the state on four black walnut species, English walnut, and/or seedling Paradox hybrid walnut rootstock. English walnut planted for commercial nut production does not appear to be a preferred host for the beetle. *Botryosphaeria* spp. that can cause cankers and limb dieback on

English walnut have also been isolated from some branches infected with thousand cankers. WTB is a native bark beetle first collected in 1959 in Los Angeles Co., but its association with *Geosmithia* spp. in CA has only recently been documented. What is unclear is why thousand cankers disease has emerged on such a wide scale in California and the western U.S.

Hasey, J.K., and Seybold, S.J. 2010. What's happening with thousand cankers disease of walnut in California. *Diamond Grower News* Summer 2010, 5–6. Retrieved December 18, 2012 from http://www.diamondfoods.com/docs/growers/Grower-News_Summer2010.pdf

Hadziabdic, D., Vito, L.M., Wadl, P.A., Windham, M.T., and Trigiano, R.M. 2013. Genetic diversity of *Geosmithia morbida*, the causal agent of thousand canker disease in the southeastern United States [Abstract.] *Phytopathology* 102(4): S49.

The fungus *Geosmithia morbida* sp. nov, and the walnut twig beetle, *Pityophthorus juglandis*, have been associated with a disease complex of black walnut (*Juglans nigra*) known as Thousand Canker Disease (TCD). Disease is manifested as branch dieback and canopy loss, eventually resulting in tree death. In 2010 the disease appeared in the native range of black walnut including Tennessee, and subsequently in Virginia and Pennsylvania in 2011. This was the first known incident of TCD east of Colorado, where the disease has been established for more than a decade on indigenous walnut species. A genetic diversity study of 54 isolates of *G. morbida* throughout the native range of black walnut was completed using 15 polymorphic microsatellite loci. The mean number of alleles was 4.2 across five locations of *G. morbida*. Genotypic diversity parameter quantified by Shannon's Information Index was 1.12. Moderate genetic diversity (Fst=0.12) and analysis of molecular variance (AMOVA) revealed that the majority of genetic variation was within examined groups (88%). Understanding genetic composition and demography of *G. morbida* can provide valuable insight into understanding factors affecting the persistence and spread of an invasive pathogen, disease progression, and future infestation predictions.

Hadziabdic, D., Vito, L.M., Windham, M.T., and Pscheidt, J.W., Trigiano, R.M., and Kolařík, M. 2013. Genetic differentiation and spatial structure of *Geosmithia morbida*, the causal agent of thousand canker disease in black walnut. *Curr. Genet.* 60(2): 75–87. Online publication. doi:10.1007/s00294-013-0414-x.

The main objectives of this study were to evaluate genetic composition of *Geosmithia morbida* populations in the native range of black walnut and provide a better understanding regarding demography of the pathogen. The fungus *G. morbida*, and the walnut twig beetle, *Pityophthorus juglandis*, have been associated with a disease complex of black walnut (*Juglans nigra*) known as thousand cankers disease (TCD). The disease is manifested as branch dieback and canopy loss, eventually resulting in tree death. In 2010, the disease was detected in black walnut in Tennessee, and subsequently in Virginia and Pennsylvania in 2011 and North Carolina in 2012. These were the first incidences of TCD east of Colorado, where the disease has been established for more than a decade on indigenous walnut species. A genetic diversity and population structure study of 62 *G. morbida* isolates from Tennessee, Pennsylvania, North Carolina and Oregon was completed using 15 polymorphic microsatellite loci. The results revealed high haploid genetic diversity among seven *G morbida* populations with evidence of gene flow, and significant differentiation among two identified genetic clusters. There was a significant correlation between geographic and genetic distance. Understanding the genetic composition and demography of *G. morbida* can provide valuable insight into recognizing factors affecting the persistence and spread of an invasive pathogen, disease progression, and future infestation predictions. Overall, these data support the hypotheses of two separate, highly diverse pathogen introductions into the native range of black walnut.

Hadziabdic, D., Windham, M., Baird, R., Vito, L., Cheng, Q., Grant, J., Lambdin, P., Wiggins, G., Windham, A., Merten, P., and Taylor, G. 2013. First report of *Geosmithia morbida* in North Carolina: the pathogen involved in thousand cankers disease of black walnut. *Plant Dis.* 98(7): 992. Online publication. doi:10.1094/PDIS-06-13-0630-PDN.

In August of 2012, branch samples from TCD symptomatic black walnut trees were collected on the North Carolina side of the Great Smoky Mountain National Park in Cataloochee and near the Big Creek Campground, in Haywood County. Five symptomatic trees near the Big Creek Campground and three from Cataloochee Cove displayed typical TCD signs including progressive crown thinning, branch flagging and branch dieback, however, insect holes were not observed. Fungal isolates were tentatively identified as G*eosmithia morbida* by using culture morphology, and characteristics of conidiophores and conidia. Isolates from Cataloochee Cove were characterized using ITS1 and ITS4 universal primers. The putative *G. morbida* isolate (GenBank Accession No. KC461929) had ITS sequences that were 100% identical to the *G. morbida* type isolate CBS124663 (accession number FN434082.1). To date, all confirmed cases of TCD in the native range of black walnut have been in urban areas, along rural roadsides and/or fence rows. The report in North Carolina is the first finding of *G. morbida*, the causal agent of TCD, in a forest setting.

Hishinuma, S.M., Dallara, P.L., Yaghmour, M.A., Zerillo, M.M., Parker, C.M., Roubtsova, T.V., Seybold, S.J. 2015. Wingnut (Juglandaceae) as a new generic host for *Pityophthorus juglandis* (Coleoptera: Curculionidae) and the thousand cankers disease pathogen, *Geosmithia morbida* (Ascomycota: Hypocreales). *Can. Entomol.* 00: 1–9.

The walnut twig beetle (WTB), *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae), vectors a fungus, *Geosmithia morbida* Kolařík, Freeland, Utley, and Tisserat (Ascomycota: Hypocreales), which colonizes and kills the phloem of walnut and butternut trees, *Juglans* Linnaeus (Juglandaceae). Over the past two decades, this condition, known as thousand cankers disease (TCD), has led to the widespread mortality of *Juglans* species in the United States of America. Recently the beetle and pathogen were discovered on several *Juglans* species in northern Italy. Little is known about the extra-generic extent of host acceptability and suitability for the WTB. We report the occurrence of both the WTB and *G. morbida* in three species of wingnut, *Pterocarya fraxinifolia* Spach, *Pterocarya rhoifolia* Siebold and Zuccarini, and *Pterocarya stenoptera* de Candolle (Juglandaceae) growing in the United States Department of Agriculture-Agricultural Research Service, National Clonal Germplasm Repository collection in northern California (NCGR) and in the Los Angeles County Arboretum and Botanic Garden in southern California, United States of America. In two instances (once in *P. stenoptera* and once in *P. fraxinifolia*) teneral (*i.e.*, brood) adult WTB emerged and were collected more than four months after infested branch sections had been collected in the field. Koch's postulates were satisfied with an isolate of *G. morbida* from *P. stenoptera*, confirming this fungus as the causal agent of TCD in this host. A survey of the 37 *Pterocarya* Kunth accessions at the NCGR revealed that 46% of the trees had WTB attacks and/or symptoms of *G. morbida* infection. The occurrence of other subcortical Coleoptera associated with *Pterocarya* and the first occurrence of the polyphagous shot hole borer, a species near *Euwallacea fornicatus* Eichhoff (Coleoptera: Curculionidae), in *Juglans* are also documented.

Hulcr, J.H., and Dunn, R.R. 2011. The sudden emergence of pathogenicity in insect-fungus symbioses threatens naïve forest ecosystems. *Proc. R. Soc. B.* 278: 431–434.

Invasive symbioses between wood-boring insects and fungi are emerging as a new and currently uncontrollable threat to forest ecosystems, as well as fruit and timber industries throughout the world. The bark and ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) constitute the large majority of these pests, and are accompanied by a diverse community of fungal symbionts.

Increasingly, some invasive symbioses are shifting from non-pathogenic saprotrophy in native ranges to a prolific tree-killing in invaded ranges, and are causing significant damage. In this paper, we review the current understanding of invasive insect–fungus symbioses. We then ask why some symbioses that evolved as non-pathogenic saprotrophs, turn into major tree-killers in non-native regions. We argue that a purely pathology-centred view of the guild is not sufficient for explaining the lethal encounters between exotic symbionts and naive trees. Instead, we propose several testable hypotheses that, if correct, lead to the conclusion that the sudden emergence of pathogenicity is a new evolutionary phenomenon with global biogeographical dynamics. To date, evidence suggests that virulence of the symbioses in invaded ranges is often triggered when several factors coincide: (i) invasion into territories with naive trees, (ii) the ability of the fungus to either overcome resistance of the naive host or trigger a suicidal over-reaction, and (iii) an 'olfactory mismatch' in the insect whereby a subset of live trees is perceived as dead and suitable for colonization. We suggest that individual cases of tree mortality caused by invasive insect–fungus symbionts should no longer be studied separately, but in a global, biogeographically and phylogenetically explicit comparative framework.

Leslie, C.A., Seybold, S.J., Graves, A.D., Cranshaw, W., and Tisserat, N. 2010. Potential impacts of thousand cankers disease on commercial walnut production and walnut germplasm conservation. VIth Intl. Walnut Symposium. *Acta Hort.* ISHS, 861: 431–434.

Thousand cankers disease (TCD) is a recently identified insect/fungal disease complex responsible for mortality of eastern black walnut trees, *Juglans nigra*, planted in non-native areas of the western United States. The presence in California of both the insect vector, the walnut twig beetle, *Pityophthorus juglandis,* and the fungal pathogen, a species of *Geosmithia*, raises concerns regarding the potential impact of the disease on commercial walnut production and the long term health of other walnut species native to California and American Southwest. Movement of the disease eastward could threaten native *J. nigra* populations in hardwood forests of the eastern United States. Identification of both the vector and the fungal pathogen of TCD from trees in the USDA Germplasm Collection near Davis, CA may impact ex situ germplasm management.

Mayfield, A.E., Fraedrich, S.W., Taylor, A., Merten, P., and Myers, S.W. 2014. Efficacy of heat treatment for the thousand cankers disease vector and pathogen in small black walnut logs. *J. Econ. Entomol.* 107(1): 174–184.

Thousand cankers disease, caused by the walnut twig beetle (*Pityophthorus juglandis* Blackman) and an associated fungal pathogen (*Geosmithia morbida* M. Kolarík, E. Freeland, C. Utley, and N. Tisserat), threatens the health and commercial use of eastern black walnut (*Juglans nigra* L.), one of the most economically valuable tree species in the United States. Effective phytosanitary measures are needed to reduce the possibility of spreading this insect and pathogen through wood movement. This study evaluated the efficacy of heat treatments and debarking to eliminate *P. juglandis* and G. *morbida* in *J. nigra* logs 4–18 cm in diameter and 30 cm in length. Infested logs were steam heated until various outer sapwood temperatures (60, 65, and 70°C in 2011; 36, 42, 48, 52, and 56°C in 2012) were maintained or exceeded for 30–40 min. In 2011, all heat treatments eliminated *G. morbida* from the bark, but logs were insufficiently colonized by *P. juglandis* to draw conclusions about treatment effects on the beetle. Debarking did not ensure elimination of the pathogen from the sapwood surface. In 2012, there was a negative effect of increasing temperature on *P. juglandis* emergence and *G. morbida* recovery. *G. morbida* did not survive in logs exposed to treatments in which minimum temperatures were 48°C or higher, and mean *P. juglandis* emergence decreased steadily to zero as treatment minimum temperature increased from 36 to 52°C. A minimum outer sapwood temperature of 56°C maintained for 40 min is effective for eliminating the thousand cankers disease vector and pathogen from walnut logs, and the current heat treatment schedule for the emerald ash borer (60°C core temperature for 60 min) is more than adequate for treating *P. juglandis* and *G. morbida* in walnut firewood.

Mcdermott-Kubeczko, M.E., Juzwik, J., Paschen, M., Klingeman, W.E., and Ginzel, M.D. 2014. Fungal and insect-associated branch damage of eastern black walnut in Indiana and Tennessee. [Abstract.] *Phytopathology* 104(11): 77.

> Thousand Cankers Disease (TCD), caused by interaction of the walnut twig beetle (WTB) and the fungus *Geosmithia morbida*, threatens the health of high-value eastern black walnut. However, other fungi and insects may also cause dieback and death of the species. Baseline data on fungi and insects colonizing the bark was obtained from branches of black walnut in urban areas with (Tennessee) and without TCD (Indiana). A drawknife was used to expose insect and non-insect related damage on 1 girdled (G) and 1 non-girdled (NG) branch from 29 visually healthy trees and 2 NG branches from 3 visually-healthy trees (controls, Indiana only). Branches (NG) from three trees with TCD also were examined. Six types of bark damage (including two insect-related) were characterized for Indiana branches, while seven types (including two insect-related) were described for Tennessee branches. Buprestid-like damage was the only type common to both states. For Indiana, damage frequencies were similar for G and NG branches but lower for NG branches of control trees. Typical *G. morbida* cankers were found on branches of both visually healthy and TCD trees in Tennessee. Fungal isolations were attempted from representatives of all damage types from each state. To date, isolates obtained represent 21 genera. In Tennessee, *G. morbida* was isolated from three non-insect damage types and from > 50% of WTB damage on G branches. Results are of value to diagnosticians receiving TCD-suspect samples.

Montecchio, L., and Faccoli, M. 2013. First record of thousand cankers disease *Geosmithia morbida* and walnut twig beetle *Pityophthorus juglandis* on *Juglans nigra* in Europe. *Plant Dis.* 98(5): 696. Online publication. doi:10.1094/PDIS-10-13-1027-PDN.

> In September 2013, thousand cankers disease was observed in northeastern Italy (Bressanvido, Vicenza, 45°39’N, 11°38’E) in black walnuts of different ages: ca. 80-yrs-old plants growing in a garden, and 17-yrs-old trees belonging to a nearby walnut plantation for timber production. Main symptoms were yellowing, wilting, twig and branch dieback, and a high number of small bark cankers. To our knowledge, this is the first record of thousand cankers disease and *Pityophthorus juglandis* to Europe, where walnut species (*Juglans* mainly *J. regia*, *J. nigra* and their hybrids) are intensively cultivated for timber production.

Nix, K., Lambdin, P., Grant, J., Coots, C., and Merten, P. 2013. Concentration levels of imidacloprid and dinotefuran in five tissue types of black walnut, *Juglans nigra*. *Forests* 4(4): 887–897.

> Black walnut, a valuable economic and environmentally important species, is threatened by thousand cankers disease. Systemic imidacloprid and dinotefuran applications were made to mature black walnut trees to evaluate their translocation and concentration levels in various tissue types including leaf, twig, trunk core, nutmeat, and walnut husk. The metabolism of imidacloprid in plants produces a metabolite, olefin-imidacloprid, which has been documented to have insecticidal properties in other systems. Trunk CoreTect (imidacloprid) soil pellets and a trunk spray of dinotefuran were applied to mature black walnuts in spring 2011. Imidacloprid concentrations were detected in both the lower and upper strata in all tissue types tested and progressively increased through month 12 post-treatment in twig and leaf tissue. Olefin-imidacloprid was detected in the nutmeat and walnut husk. Dinotefuran was only detected in the first sampling period and was found in low concentration levels in leaf and twig tissue types, and was not detected in the trunk, nutmeat or the walnut husk.

Pscheidt, J. 2011. Disease progress of thousand cankers disease in Oregon [Abstract.] *Phytopathology* 101(6): S146.

Thousand cankers disease has spread throughout Oregon since first observed in the early '90's. Symptomatic, mature black walnut (*Juglans* sp.) trees are harvested for valuable lumber with the assumption they will rapidly decline and die. Disease progress was documented for 60 trees from 11 locations in the Willamette Valley from Sep 07 to Jul 10. The walnut twig beetle vector (*Pityophthorus juglandis*) and causal pathogen (*Geosmithia morbida*) were confirmed in each general location. The amount of canopy with dieback symptoms was recorded for each tree in Sep 07, Sep 08, Aug 09 and Jul 10. At the Jul 10 rating 15 trees had higher canopy dieback ratings, 36 had similar ratings and 8 trees had lower dieback ratings when compared to Aug 09. Trees with higher ratings had an average increase of 6.4%, with a range from 5 to 20%. Trees with lower ratings had an average decrease of 6.2% ranging from 5 to 10%. At the Jul 10 rating for trees on which data had been collected in Sep 07, 17 trees had higher canopy dieback ratings, 26 had similar ratings and 8 trees had lower ratings. Trees with higher ratings had an average increase of 17.2%, with a range from 5 to 70%. Trees with lower ratings had an average decrease of 6.3% ranging from 5 to 15%. Although some trees seem to die quickly, the vast majority die back very slowly, if at all. Based on these observations, disease progression in trees with thousand cankers disease is a slow process in Oregon.

Randolph, K.C., Rose, A.K., Oswalt, C.M., and Brown, M.J. 2013. Status of black walnut (*Juglans nigra* L.) in the Eastern United States in light of the discovery of thousand cankers disease. *Castanea* 78(1): 2–14.

Juglans nigra (black walnut) is widely distributed throughout the US eastern forest, with high concentrations occurring in Missouri and the Ohio and Tennessee River basins. It is an extremely desirable tree for wildlife forage and timber production on forest land, and for shade, aesthetics, and wildlife forage in urban areas. Current (2009–2010) estimates from US Forest Service Forest Inventory and Analysis (FIA) data indicated that there were 306 million live black walnut trees in the eastern United States with a live volume totaling 112.76 million cubic meters (m3). This resource is currently threatened by the newly discovered presence of thousand cankers disease (TCD) in Pennsylvania, Tennessee, and Virginia. Thousand cankers disease may have been present in these areas for at least 10 years prior to discovery; however, no evidence of TCD in the forest at large was apparent in the crown condition and mortality data collected by FIA between 2000 and 2010. During this time period black walnut crown conditions were within the range of what is typically considered normal and healthy for hardwood trees and dead black walnut accounted for < 5% of the total number of black walnut trees in 82% of the counties where black walnut occurred. Lack of evidence of TCD in our study could be due to its actual absence or to an inability of the inventory and monitoring system to detect its presence.

Serdani, M. 2012. Butternut- A new host for the walnut twig beetle and thousand cankers disease. NPDN News 7(1):1–2. Retrieved December 2, 2013 from http://extension.psu.edu/plants/green-industry/news/2012/butternut-a-new-host-for-the-walnut-twig-beetle-and-thousand-cankers-disease

Serdani, M., Vlach, J.J., Wallis, K.L., Zerillo, M., McCleary, T., Romero-Severson, J., and Tisserat, N.A. 2013. First report of *Geosmithia morbida* and *Pityophthorus juglandis* causing thousand cankers disease in butternut. *Plant Health Progr.* Online publication. doi:10.1094/PHP-2013-1018-01-BR.

Tisserat, N. 2013. The distribution and impact of thousand cankers disease in walnut species in the western United States [Abstract.] *Phytopathology* 102(7): S158.

Thousand cankers disease (TCD) was likely present in black walnut along the Wasatch Front Range in Utah and areas of the Columbia Gorge and Willamette Valley in Oregon as early as the late 1980's. TCD in black walnut is now distributed in the West as far south as New Mexico, as far north as Idaho and Washington, and as far east as Colorado. Disease severity has intensified in communities where it has been found and in many locations mature black walnuts have been eliminated, or nearly so, by TCD. The walnut twig beetle (WTB) and cankers caused by *Geosmithia morbida* also have been associated with a decline of southern and northern California walnuts in their native range. TCD has not yet significantly impacted the English walnut industry in California, although individual trees have been killed. The WTB and *G. morbida* have not been found in the native range of little walnut, although this species is susceptible to the fungus. Both the WTB and *G. morbida* are widespread in Arizona walnut throughout its range in the southwestern US, but only in association with damaged or senescing branches. The population structure of *G. morbida* in the West is complex. Haplotype diversity in isolates collected thus far from Arizona walnut, the putative native host of the beetle and fungus, is different from isolates collected from black walnut and other walnut species in the West.

Tisserat, N., Cranshaw, W., Leatherman, D., Utley, C., and Alexander, K. 2009. Black walnut mortality in Colorado caused by the walnut twig beetle and thousand cankers disease. *Plant Health Progr.* Online publication. doi: 10.1094/PHP-2009-0811-01-RS.

Since 2001, widespread mortality of black walnut (*Juglans nigra*) has been reported in Colorado, USA. Affected trees initially show a yellowing and thinning of leaves in the upper crown, followed by twig and branch dieback and ultimately tree death. We report that this mortality is the result of a combination of an expanded geographic range of the walnut twig beetle (*Pityophthorus juglandis*), its aggressive feeding behavior on black walnut, and extensive cankering caused by an unnamed *Geosmithia* fungus associated with the beetle. *Geosmithia* was consistently recovered from the bodies of *P. juglandis* and this insect introduces the fungus into healthy trees during gallery formation. This is the first report of *Geosmithia* as a pathogen of black walnut. We propose the name Thousand Cankers to describe this disease because mortality is the result of bark necrosis caused by an enormous number of coalescing branch and trunk cankers. A second pathogen, *Fusarium solani*, was isolated from the margins of elongate trunk cankers during the final stages of decline, but not from cankers surrounding beetle galleries. Thousand Cankers Disease is eliminating black walnut along the Front Range of Colorado and poses a grave risk to this species in its native range in eastern North America should the insect/*Geosmithia* complex be introduced.

Tisserat, N., Cranshaw, W., Putnam, M.L., Pscheidt, J., Leslie, C.A., Murray, M., Hoffman, J., Barkley, Y., Alexander, K., and Seybold, S.J. 2011. Thousand cankers disease is widespread in black walnut in the western United States. *Plant Health Progr.* Online publication. doi: 10.1094/PHP-2011-0630-01-BR.

This article explains the history of thousand cankers disease, how to identify and confirm the disease, along with potential disease severity and spread.

United States Department of Agriculture Forest Service, Northeastern Area State and Private Forestry, Purdue University Department of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center, American Walnut Manufacturers Association, and Walnut Council. 2015. Thousand Cankers Disease Website. Retrieved December 3, 2012 from http://www.thousandcankers.com/

This website is frequently updated and contains a vast amount of information on thousand cankers disease.

Utley, C. 2012. The biology of *Geosmithia morbida* and susceptibility of walnut and hickory species to thousand cankers disease. M.S. Thesis. Colorado State University.

Since 2001 widespread mortality of black walnut (*Juglans nigra*) has been reported in Colorado, USA. Affected trees initially show a yellowing and thinning of leaves in the upper crown, followed by twig and branch dieback and ultimately tree death. We report that this mortality is the result of a

combination of an expanded geographic range of the walnut twig beetle (WTB) (*Pityophthorus juglandis*), its aggressive feeding behavior on black walnut, and extensive cankering caused by a filamentous ascomycete in the genus *Geosmithia* (Ascomycota: Hypocreales). Thirty seven *Geosmithia* strains collected from *J. californica*, *J. hindsii*, *J. major*, and *J. nigra* in eight USA states (AZ, CA, CO, ID, OR, UT, WA) were compared using morphological and molecular methods (ITS rDNA sequences). Strains had common characteristics including a yellowish color of conidia *en masse*, growth at 37°C, and absence of growth on Czapek-Dox agar and belonged to a single species described here as *G. morbida*. *G. morbida* is the first *Geosmithia* species documented as a plant pathogen. We also tested the susceptibility of hickory and walnut species to *G. morbida* in greenhouse and field studies. *Carya illinoinensis*, *C. aquatica*, and *C. ovata* were immune. All walnut species tested, including *J. ailantifolia*, *J. californica*, *J. cinerea*, *J. hindsii*, *J. major*, *J. mandshurica*, *J. microcarpa*, *J. nigra* and *J. regia* developed cankers following inoculation with *G. morbida*. *J. nigra* had the largest cankers, whereas *J. major*, a native host of the WTB and presumably *G. morbida*, had smaller and more superficial cankers. Canker size differed among maternal half-sibling families of *J. nigra* and *J. cinerea*, indicating genetic variability in resistance to *G. morbida*. Our inoculation studies with *G. morbida* have corroborated many of the field observations on susceptibility of hickory and walnut species to thousand cankers disease, although the ability of the WTB to successfully attack and breed in walnuts is also an important component in thousand cankers disease resistance.

Utley, C., Cranshaw, W., Seybold, S., Graves, A., Leslie, C., Jacobi, W., and Tisserat, N. 2009. Susceptibility of *Juglans* and *Carya* species to *Geosmithia*; a cause of thousand cankers disease [Abstract.] *Phytopathology* 99: S133.

Thousand cankers disease of black walnut (*Juglans nigra*) is caused by an unnamed species of *Geosmithia* that is vectored by the walnut twig beetle (*Pityophthorus juglandis*). Black walnut is extremely susceptible to the disease and has been eliminated from several Colorado municipalities. The beetle and cankers caused by *Geosmithia* have been observed on *J. hindsii* and *J. californica* throughout California and are associated with mortality of both species near Sacramento. The disease was found on a single *J. regia* tree in Colorado, although cankers have not yet been observed in *J. regia* orchards in California. Similarly the beetle and fungus were found on shaded, senescing branches of *J. major* in Arizona, but did not appear to be causing tree decline. *Juglans major* is thought to be the native host of the walnut twig beetle. An assessment of susceptibility of these and other species is being conducted by artificial inoculations in greenhouse and field studies. In preliminary studies, cankers caused by *Geosmithia* developed on *J. microcarpa* and *J. mandshurica* but not on *J. cinerea*, *J. ailantifolia*, or *Carya illinoensis*.

Utley, C., Nguyen, T., Roubtsova, T., Coggeshall, M., Ford, T.M., Grauke, L.J., Graves, A.D., Leslie, C.A., McKenna, J., Woeste, K., Yaghmour, M.A., Cranshaw, W., Seybold, S.J., Bostock, R.M., and Tisserat, N. 2013. Susceptibility of walnut and hickory species to *Geosmithia morbida*. *Plant Dis.* 97(5): 601–607.

Thousand cankers disease (TCD) of walnut is a result of feeding in the phloem by the walnut twig beetle (WTB), *Pityophthorus juglandis*, and subsequent canker formation caused by *Geosmithia morbida* around galleries. TCD has caused extensive morbidity and mortality to *Juglans nigra* in the western United States and, in 2010, was discovered in the eastern United States, where the tree is a highly valuable timber resource. WTB and *G. morbida* also have been found in *J. regia* orchards throughout major production areas in California, and the numbers of damaged trees are increasing. We tested the susceptibility of walnut and hickory species to *G. morbida* in greenhouse and field studies. *Carya illinoinensis*, *C. aquatica*, and *C. ovata* were immune. All walnut species tested, including *J. ailantifolia*, *J. californica*, *J. cinerea*, *J. hindsii*, *J. major*, *J. mandshurica*, *J. microcarpa*, *J. nigra*, and *J. regia*, developed cankers following inoculation with *G. morbida*. *J. nigra* was the most susceptible, whereas *J. major*, a native host of the WTB and, presumably, *G.*

morbida, had smaller and more superficial cankers. Canker formation differed among maternal half-sibling families of *J. nigra* and *J. cinerea*, indicating genetic variability in resistance to *G morbida*. Our inoculation studies with *G. morbida* have corroborated many of the field observations on susceptibility of walnut and hickory species to TCD, although the ability of the WTB to successfully attack and breed in walnut is also an important component in TCD resistance.

Van Meter, J. 2012. Dark days for black walnut. Nebraska Department of Agriculture. *Nebraskaland Magazine* June 2012, 47 pp.

Van Sambeek, J., and Juzwik, J. 2010. What's killing my walnuts – how to find help. *Walnut Council Bulletin Notes* 37(1): 10–12.

For the last decade, we have watched as the granulate ambrosia beetle (GAB) formerly the Asian ambrosia beetle spread into the southern region of walnut. Now we are asked to watch for the possible invasion of the thousand canker disease (TCD) complex into the eastern United States assuming we cannot prevent its invasion from the western United States. For both pest problems, we need to watch our walnut trees for symptoms that include leaf flagging or yellow wilted leaves on one or more branches (also symptoms of several, less important problems), dead or dying branches retaining dead leaves, and emergence of epicormic sprouts on large branches and main stem.

Van Sambeek, J.W., and Reed, S. 2013. Thousand Cankers Disease – What Have We Learned. *The Nutshell* 67(2): 15–20. Retrieved December 12, 2013 from http://www.nrs.fs.fed.us/pubs/44203

Warmund, M., and Van Sambeek, J. 2014. Thousand cankers disease. *Geosmithia morbida* spores isolated from a weevil. *Missouri Environment & Garden* 20(6): 3.

Windham, M. 2012. From discovery to regulation: A pathologist's perspective of thousand cankers disease in eastern United States [Abstract.] *Phytopathology* 102(7): S158–159.

Thousand Cankers Disease (TCD), is an insect (*Pityophthorus juglandis*)/ fungal (*Geosmithia morbida*) complex that threatens black walnut (*Juglans nigra*). In early August, 2010, TCD was confirmed in Knox County in east Tennessee and this was the first report of TCD within the native range of black walnut. Since then, TCD has been found in five additional counties in east Tennessee. In 2011, the disease was reported in Virginia and in Pennsylvania. TCD may be hard to detect because the initial symptoms mimic those associated with drought stress. Since much of the eastern U.S. had suffered from drought in the last seven years, many hardwood species have displayed symptoms consistent with drought and this may have allowed TCD to stay hidden for several years in affected areas. To date, all reports of TCD in eastern outbreaks have been in urban areas or in trees along roadways. No trees in a forest environment have had a case of TCD confirmed. Since *P. juglandis* is considered a native insect by APHIS, the disease has not been regulated on a national basis. Regulation has been left to individual states and regulation has not been consistent. For example, in Tennessee, counties surrounding counties with known outbreaks of TCD are regulated. However, when a county with TCD is next to a county in another state, the adjacent county is not regulated since it is outside of Tennessee's jurisdiction. This may make quarantines ineffective.

Yaghmour, M.A., Nguyen, T.L., Roubtsova, T.V., Hasey, J., DeBuse, C., Fichtner, E.J., Hoenisch, R., Seybold, S., and Bostock, R.M. 2013. Thousand cankers disease in California English walnut: Incidence, distribution, and characterization of *Geosmithia morbida* [Abstract.] *Phytopathology* 103(6): S163.

Thousand cankers disease (TCD) is an emerging fungal disease responsible for serious decline and death of eastern black walnut (*Juglans nigra*) in Colorado. In California, TCD occurs on native

Juglans californica and *Juglans hindsii*, and in orchard trees of English walnut (*Juglans regia*). The causal agent, *Geosmithia morbida,* is vectored by the walnut twig beetle (WTB; *Pityophthorus juglandis*). Twelve commercial orchards in six counties in California were surveyed for TCD incidence. Disease incidence ranged from 17% to 65% of the trees in these orchards. One orchard that is bordered by a riparian area with mature *J. hindsii* trees showing WTB damage was surveyed and sampled extensively. The orchard was surveyed in quadrats each containing 5-6 rows. Disease incidence based on symptoms in the 2,217 trees that were examined was 37%. *G. morbida* was isolated from 18% of 61 cankers associated with insect galleries, and was isolated from 36% of WTB collected within the orchard and in the riparian area. Joint-count analysis showed disease aggregation in sections of the field, while disease distribution was random in other parts of the field. Sequence analysis of*EF-1α* of 22 *G. morbida* isolates from different counties revealed little diversity among these isolates at this level of resolution.

Yaghmour, M.A., Nguyen, T.L., Roubtsova, T.V., Hasey, J.K., Fichtner, E.J., DeBuse, C., Seybold, S.J., and Bostock, R.M. 2014. First report of *Geosmithia morbida* on English walnut and its paradox rootstock in California. *Plant Dis.* 98(10): 1441.

Articles that Discuss Walnut Twig Beetle, *Pityophthorus juglandis*

Audley, J. 2015. Assessment of *Pityophthorus juglandis* colonization characteristics and implications for further spread of thousand cankers disease. M.S. Thesis, University of Tennessee.

> The goal of this project was to identify potential pathways by assessing *P. juglandis* colonization of *J. nigra* logs and seedlings, and to identify treatments to prevent colonization in logs. Beetle colonization of logs and lumber treated by phytosanitation measures was tested. When exposed to high colonization pressures and baited with a pheromone lure, beetles attacked steam heated, methyl bromide fumigated, and kiln-dried samples. Colonization in a reduced pressure exposure scenario was inconclusive.
>
> Azadirachtin, borate, and permethrin were tested as a means of preventing *P. juglandis* colonization of black walnut logs. Beetle survival rates over a 120 hour exposure period, and colonization activity was compared. Permethrin was the only insecticide to kill all beetles and to prevent any attacks. A 30% concentration of borate showed some control, reducing survival rate compared to control levels, however, beetles successfully attacked the material, and its use as a control method is questionable. Azadirachtin was not effective in the doses tested.
>
> Finally, *P. juglandis* colonization of *J. nigra* nursery stock seedlings was tested. Seedlings were exposed to beetles in no-choice and choice assays. For no-choice assays, beetles were caged directly onto the stems. In choice assays, seedlings were placed near infested logs, presenting the seedlings to beetles emerging from the infested material. Beetles attacked stems in all diameter size classes tested (0.5 – 2.0 cm), however, showed a preference for larger diameter trees. No attacks were observed in the choice assays and may have been confounded by the method used to introduce beetles to seedlings.

Audley, J., Mayfield, A.E., Myers, S.W., Taylor, A., and Klingeman, W.E. 2015. Phytosanitation methods influence posttreatment colonization of *Juglans nigra* logs by *Pityophthorus juglandis* (Coleoptera: Curculionidae: Scolytinae). Online publication. *J. Econ. Entomol.* doi: 10.1093/jee/tov252.

> Several North American walnut species (*Juglans* spp.) are threatened by thousand cankers disease which is caused by the walnut twig beetle (*Pityophthorus juglandis* Blackman) and its associated fungal plant pathogen, *Geosmithia morbida* M. Kolarík, E. Freeland, C. Utley and N. Tisserat sp. nov. Spread of this disease may occur via movement of infested black walnut (*Juglans nigra* L.) wood. This study evaluated the ability of *P. juglandis* to colonize *J. nigra* wood previously treated with various phytosanitation methods. Steam-heated and methyl bromide-fumigated *J. nigra* logs, as well as kiln-dried natural wane *J. nigra* lumber (with and without bark) were subsequently exposed to *P. juglandis* colonization pressure in two exposure scenarios. Following a pheromone-mediated, high-pressure scenario in the canopy of infested trees, beetles readily colonized the bark of steam-heated and methyl bromide- fumigated logs, and were also recovered from kiln-dried lumber on which a thin strip of bark was retained. In the simulated lumberyard exposure experiment, during which samples were exposed to lower *P. juglandis* populations, beetles were again recovered from bark-on steam-heated logs, but were not recovered from kiln-dried bark-on lumber. These data suggest logs and bark-on lumber treated with phytosanitation methods should not be subsequently exposed to *P. juglandis* populations. Further beetle exclusion efforts for phytosanitized, bark-on walnut wood products transported out of quarantined areas may be necessary to ensure that these products do not serve as a pathway for the spread of *P. juglandis* and thousand cankers disease.

Audley, J., Taylor, A., Klingeman, W.E., Mayfield, A.E., and Myers, S.W. 2015. Insecticide dip treatments to prevent walnut twig beetle colonization of black walnut logs. *Forest Prod. J.* In-Press doi: http://dx.doi.org/10.13073/FPJ-D-15-00038.

The health, sustainability, and commercial viability of eastern black walnut (*Juglans nigra*), is currently under threat from thousand cankers disease. The disease is caused by an invasive bark beetle species, the walnut twig beetle (*Pityophthorus juglandis*), and its associated fungal pathogen, Geosmithia morbida. Range expansion of the beetle and pathogen has likely been facilitated by transport of infested walnut forest products. Preventing colonization of these products is crucial to limiting further spread of thousand cankers disease. This study evaluated three insecticides for their ability to induce walnut twig beetle mortality and prevent colonization of black walnut bolts, 3 - 5 cm diam, following dip treatment applications. Treatments included 0.003% azadirachtin, 15% disodium octaborate tetrahydrate [DOT], 0.5% permethrin, and water in Trial 1, and 0.013% azadirachtin, 30% DOT, 0.5% permethrin, and water in Trial 2. A total of 40 beetles, four beetles per sample, were exposed to treated samples and observed for 120 hours in each trial. Permethrin was the only treatment to achieve 100% mortality and prevent all colonization activity. The 30% DOT treatment increased mortality compared to the control; however, it did not reduce the mean number of attacks or mean gallery length. Azadirachtin was not effective at either concentration. Results suggest that insecticide dip treatments can prevent walnut twig beetles from colonizing cut black walnut logs. Treatments could be used in conjunction with phytosanitation to help prevent further spread of thousand cankers disease while allowing for the continued transport of bark-on walnut forest products.

Blackman, M.W. 1928. The genus *Pityophthorus* Eichh. in North America: A revisional study of the Pityphthori, with descriptions of two new genera and seventy-one new species. Bull. 1 (3-6), Tech. Pub. 25. College Environ. Sci. For., St. Univ. New York. 184 pp.

Blackman outlines the taxonomic descriptions of male and female *Pityophthorus juglandis*. The reports include key identifying eye, antennae, pronotum, elytra, and declivity features (42 p).

Bright, D.E. 1981. Taxonomic monograph of the genus *Pityophthorus* Eichhoff in North and Central America. *Mem. Entomol. Soc. Canada* 113: 1–378.

Bright provides taxonomic descriptions of male and female *Pityophthorus juglandis*. He expands upon features including frons, antennae, pronotum, elytra, and declivity, and adds their length as "1.7-2.0 mm, about 3.0 times as longer than wide." Host (*Juglans* spp.), distribution (Southern California to New Mexico, south into northern Mexico), and collection information on the original specimens is provided. The concluding remarks follow: "adults of *juglandis* are most easily distinguished by the evenly convex elytral declivinity on which interstriae 1 and 3 of the male bear distinct granuals, by the broadly flattened, densely pubescent female frons, by the irregular rows of asperities on the anterior surface of the pronotum, by the close, deep punctures on the posterior portion of the pronotum, by the generally distinct rows of punctures in declivital striae 1 and 2, and by the host" (81-82 pp).

Bright, Jr. D.E., and Skidmore, R.E. 2002. A catalog of Scolytidae and Platypodidae (Coleoptera), Supplement 2 (1995–1999). NRC Research Press, Ottawa, Canada.

This Supplement lists *Pityophthorus juglandis* (408 p) and references page 1005 of Wood and Bright 1992. A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic Index, Volume B. *Great Basin Nat Memoirs.* 13: 1–1553. This Supplement does not contain further information on walnut twig beetle.

Bright, D.E., and Stark, R.W. 1973. The bark beetles and ambrosia beetles of California (Scolytidae and Platypodidae). *Bull. Calif. Insect Survey* 16: 1–169.

The authors provide type specimens, records, and distribution information on the walnut twig beetle. In the discussion, they state, "this species is the only representative of Group II found in

California. It is a recent introduction to the state; the first record is 1959. Adults of *P. juglandis* may be recognized by the fused pronotal asperities that are arranged in unbroken concentric rows by the oblique declivity with distinct, strial punctures, by the frontal setae of the female being equal in length and by the host. The beetle is about 1.8 mm long, reddish-brown in color" (106 p).

Chamberlin, W.J. 1939. The bark and timber beetles of North America north of Mexico. The taxonomy, biology and control of 575 species belonging to 72 genera of the super family Scolytoidea. OSC Cooperative Association, Corvallis, Oregon. 513 pp.

Chamberlin notes that *P. juglandis* looks "very similar to *rhios*; length 1.80 mm.; front finely densely punctured below, with fine moderately long hairs. Declivity oblique, suture wide, elevated scarcely granulate; second striae not impressed. Antennae as illustrated in Figure 245. Type locality: Lone Mountain, New Mexico. Distribution: Arizona and New Mexico. Host: Black Walnut (*Juglans* sp.)" (362 p).

Chen, Y., and Seybold, S. 2014. Crepuscular flight activity of an invasive insect governed by interacting abiotic factors. *PLoS one* 9(8): e105945. doi:10.1371/journal.pone.0105945.

Seasonal and diurnal flight patterns of the invasive walnut twig beetle, *Pityophthorus juglandis*, were assessed between 2011 and 2014 in northern California, USA in the context of the effects of ambient temperature, light intensity, wind speed, and barometric pressure. *Pityophthorus juglandis* generally initiated flight in late January and continued until late November. This seasonal flight could be divided approximately into three phases (emergence: January–March; primary flight: May–July; and secondary flight: September–October). The seasonal flight response to the male-produced aggregation pheromone was consistently female-biased (mean of 58.9% females). Diurnal flight followed a bimodal pattern with a minor peak in mid-morning and a major peak at dusk (76.4% caught between 1800 and 2200 h). The primarily crepuscular flight activity had a Gaussian relationship with ambient temperature and barometric pressure but a negative exponential relationship with increasing light intensity and wind speed. A model selection procedure indicated that the four abiotic factors collectively and interactively governed P. juglandis diurnal flight. For both sexes, flight peaked under the following second order interactions among the factors when: 1) temperature between was 25 and 30uC and light intensity was less than 2000 lux; 2) temperature was between 25 and 35uC and barometric pressure was between 752 and 762 mba (and declined otherwise); 3) barometric pressure was between 755 and 761 mba and light intensity was less than 2000 lux (and declined otherwise); and 4) temperature was ca. 30uC and wind speed was ca. 2 km/h. Thus, crepuscular flight activity of this insect can be best explained by the coincidence of moderately high temperature, low light intensity, moderate wind speed, and low to moderate barometric pressure. The new knowledge provides physical and temporal guidelines for the application of semiochemical-based control techniques as part of an IPM program for this invasive pest.

Costanzo, T.M. 2012. Developing a kiln treatment schedule for sanitizing black walnut wood of the walnut twig beetle. M.S. Thesis. Colorado State University.

The purpose of this research was to determine whether the International Plant Protection Convention (IPPC) International Standards for Phytosanitary Measures (ISPM-15) standards and United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) Treatment T314-a/c regulations are sufficient to kill live beetles in the bark. The thermotolerance of the walnut twig beetles was evaluated by subjecting walnut twig beetle populations at all stages (including eggs, larvae, pupae and adults) to a series of time and temperature regimen. Results from the emergence trials showed that adults were able to survive up to 48°C (118°F) but no survival of any stage of beetle development was detected at 50.1°C (122°F) when wood samples were heated for 30 minutes.

Cranshaw, W.S. 2011. Recently recognized range extensions of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae: Scolytinae), in the western United States. *Coleopterist's Bull*. 65(1): 48–49.

This article compiles our historical records of the walnut twig beetle. It includes information on where it was first described, their associated *Juglans* hosts, a breakdown of its range expansion, and explanations behind the range expansion.

Dallara, P.L., Flint, M.L., and Seybold, S.J. 2012. An analysis of the larval instars of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), in northern California black walnut, *Juglans hindsii*, and a new host record for *Hylocurus hirtellus*. *Pan. Pac. Entomol.* 88(2): 248–266.

By measuring and analyzing larval head capsule widths, we determined that a northern California population of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), has three larval instars. We also developed rules to classify *P. juglandis* larval instars. Overlap in the ranges of widths among consecutive instars was addressed by fitting a distribution model to the data and determining limits for head capsule width classes and probabilities of instar misclassification. Growth ratios for laboratory and field samples of *P. juglandis* were consistent with the Brooks-Dyar rule and with results in the literature for other bark and ambrosia beetle species. As a consequence of branch dissection to retrieve larvae of *P. juglandis*, we established that northern California black walnut, *Juglans hindsii* (Jeps.) Jeps. ex R.E. Sm., is a host for the xylophagous ''California hardwood bark beetle,'' *Hylocurus hirtellus* (LeConte) (Coleoptera: Scolytidae), and that *H. hirtellus* is associated with *P. juglandis* beneath the bark of small diameter branches. It appears that in northern California, univoltine *H. hirtellus* is active in flight from early March through early May, with the majority of the adults dispersing in April. The females are the colonizing sex.

Furniss, R.L., and Carolin, V.M. 1977. Western forest insects. USDA Forest Service Miscellaneous Publication No. 1339.

"*P. juglandis* Blackman in Arizona and California on *Juglans* is one of the few western species attacking a nonconifer" (402 p).

Hadziabdic, D.; Wadl, P.A.; Staton, M.E.; Klingeman, W.E.; Moulton, J.K.; Pscheidt, J.W.; Wiggins, G.J.; Grant, J.F.; Lambdin, P.L.; Windham, M.T.; Faccoli, M.; Merten, P.R.; Trigiano, R.N. 2015. Development of microsatellite loci in *Pityophthorus juglandis*, a vector of thousand cankers disease in *Juglans* spp. *Conserv. Genet. Resour*. 7(2): 431–433.

Using next-generation sequencing, 18 microsatellite loci were developed and characterized for walnut twig beetle, *Pityophthorus juglandis*, a vector of thousand cankers disease (TCD) affecting *Juglans* spp. Although all *Juglans* species are susceptible to TCD infection, native populations of *J. nigra* and *J. cinerea*, which is endangered in Canada, are most susceptible and threatened by habitat loss. Novel primers amplified di-, tri-, and tetra nucleotide repeats and detected 4–14 alleles per locus. Averaged observed and expected heterozygosity was 0.22 and 0.67, respectively. Our results indicate that *P. juglandis* microsatellite loci can be used to investigate genetic diversity and population structure of this vector across a widespread geography. These markers will be useful tools for evaluating genetic structure of *P. juglandis* population outbreaks and developing appropriate conservation strategies.

Lambdin, P., Nix, K., Grant, J., Pausen, D., and Merten, P. 2015. Natural enemies of the walnut twig beetle in Eastern Tennessee. *International Journal*, 2(9): 31–39.

Sixteen species of natural enemies were found to be associated with the walnut twig beetle. Of these, 14 coleopteran species representing five families were collected and identified as potential predators of the walnut twig beetle in eastern Tennessee. Of the six clerid species collected, larvae of three species (*Enoclerus nigripes* (Say), *Madoniella dislocatus* (Say), and *Pyticeroides laticornis* (Say)) were observed to feed on walnut twig beetle larvae within the galleries. Consumption rates for these three clerid species were recorded. Behavioral comparisons for *M. dislocatus* and *P. laticornis* consisted of predator recognition time, time between attacks, total feeding time, and preening behavior. In addition to the 14 coleopteran species, two parasitoid species (*Neocalosoter pityophthori* and *Theocolax* sp. (Cerocephalinae)) emerged from walnut twig beetle infested black walnut bolts. Natural enemies of the invasive walnut twig beetle may be useful as biological control agents of the walnut twig beetle within newly infested areas.

Luna, E.K., Sitz, R.A., Cranshaw, W.S., and Tisserat, N.A. 2013. The effect of temperature on survival of *Pityophthorus juglandis* (Coleoptera: Curculionidae). *Environ. Entomol.* 42(5): 1085–1091.

The walnut twig beetle (*Pityophthorus juglandis* Blackman) vectors *Geosmithia morbida*, the causal agent of thousand cankers disease in *Juglans*, and is particularly damaging to *J. nigra* (black walnut). Native hosts of *P. juglandis* are distributed in the southwestern United States where winter temperatures tend to be higher than those found within the native range of black walnut. To better understand temperature effects on survival of *P. juglandis*, we initiated studies to determine: (1) seasonal variations in cold tolerance, as measured by the supercooling point (SCP), and (2) upper and lower lethal temperatures. In the lower lethal temperature study *Xyleborinus saxeseni* (Ratzeberg) was tested for comparison. Insects were either exposed to increasing or decreasing temperatures and then checked for survival. Upper and lower lethal temperatures (LT) were estimated using a logistic model. For the supercooling point study, data were grouped into seasons. Seasonal mean SCPs were highest in summer (-15.4°C) and lowest in fall (-18.1°C). The upper lethal limit estimations required to kill 99% of the population (LT_{99}) for adults and larvae were 52.7°C and 48.1°C respectively and lower limit LT_{99} estimations for adults and larvae were -18.1°C and -18.7°C respectively. The lower median lethal temperature (LT_{50}) of *X. saxeseni* was -24.7°C. These studies, as well as beetle survival in infested Colorado trees where temperatures reached -29°C in February 2011, suggest *P. juglandis* could survive the winter in much of the native range of black walnut, but may be limited in trees where temperatures regularly exceed the lower lethal temperatures.

Luna, E., Cranshaw, W., and Tisserat, N. 2014. Attraction of walnut twig beetle *Pityophthorus juglandis* to the fungus *Geosmithia morbida*. *Plant Health Progr*. 15(3): 135–140.

Geosmithia morbida causes thousand cankers disease of *Juglans nigra* and it is transmitted by the walnut twig beetle (WTB, *Pityophthorus juglandis*). Along with WTB, an ambrosia beetle, *Xyleborinus saxeseni*, is commonly associated with thousand cankers disease in the later stages of *J. nigra* decline, although *X. saxeseni* is not a known vector of *G. morbida.* We initiated studies to determine whether WTBs or *X. saxeseni* were attracted to volatiles produced by *G. morbida* and other bark fungi in a laboratory choice-test setting. There was no difference between the numbers of emerged WTB adults that were collected in tubes containing potato dextrose agar colonized by *G. morbida* and those in tubes containing agar only. More adult *X. saxeseni* were collected in tubes containing agar only compared with *G. morbida.* Walnut twig beetle larvae migrated more frequently toward an agar plug colonized by *G. morbida* and *Fusarium solani* compared with an un-colonized agar plug. No larval preference was observed when agar plugs colonized by *F. solani* and *G. morbida,* or *G. morbida* and *Penicillium solitum,* were placed in the same petri dish. These results suggest that WTB larvae are attracted to bark fungi in general, but not specifically to *G. morbida*.

McEwen, C. 2015. A new species of *Theocolax* Westwood (Hymenoptera: Pteromalidae: Cerocephalinae) reared from *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae: Scolytinae). *P. Entomol. Soc. Wash.* 117(2): 162–178.

> The new species *Theocolax americanus* McEwen, n. sp. (Hymenoptera, Pteromalidae, Cerocephalinae), is described from the United States of America. A description is provided with a diagnosis and comparison to similar species. A key to differentiate the *Theocolax* species recorded in the USA is included and notes given on new morphological features. *Theocolax americanus* is thought to attack the walnut twig beetle (*Pityophthorus juglandis* Blackman), which transmits thousand cankers disease. Sequencing of CO1 for the new species, other *Theocolax* species found in the U.S.A., *Neocalosoter pityphthori* Ashmead (Hymenoptera, Pteromalidae, Cerocephalinae), and *P. juglandis* is reported.

Mackes, K., Costanzo, T., Coleman, R., Eckhoff, M., and Vaughan, D. 2015. A protocol for heat treating black walnut wood infested with walnut twig beetle. Online publication. *Forest Prod. J.* In Press doi: http://dx.doi.org/10.13073/FPJ-D-14-00082.

> Black walnut (*Juglans nigra*) is one of the most important tree species in the U.S. for producing lumber and other forest products. However, a recent outbreak of thousand cankers disease vectored by the walnut twig beetle (*Pityophthorus juglandis*) threatens the population of black walnut in the U.S. Infected walnut trees are typically removed to prevent spread of the vector, resulting in large quantities of potential sawlogs that must be sanitized. The objective of this study was to identify the temperature and time combination necessary to heat treat infested materials to 100% beetle mortality. Testing was done on infested sample blocks that contained sapwood and bark. The specimens were heated to various temperatures and examined, both through emergence chambers and destructive sampling, for the presence of P. juglandis at any life stage. Results of the study indicate that heat treating black walnut products to 50.1Â°C at a depth of 3.8 cm (1.5-in) for 30 minutes will result in 100% beetle mortality. As a side-product, this study also produced 3,000 board feet of rough-cut lumber. These boards were heat treated and sold, following the protocol developed in this study. This study demonstrates that wood from black walnut trees infected with thousand cankers disease can be effectively heat sterilized and utilized, reducing the need to chip, landfill, or otherwise disposed of the material without economic return.

Nix, K.A. 2013. The life history and control of *Pityophthorus juglandis* Blackman on *Juglans nigra* L. in eastern Tennessee. M.S. Thesis, University of Tennessee.

> The primary objective of this study was to determine the life history of WTB in eastern Tennessee using field-infested black walnuts bolts. From this data, it was determined that WTB has at least three larval instars. Gallery structures of the different WTB life stages were determined. Secondly, a survey was initiated to identify native predators and parasitoids for use as potential biological control agents of WTB. The consumption rates of WTB by the collected the predators were recorded and a potential listing of native predators was developed. From this survey, three clerid species were observed to feed on WTB. Nine additional coleopteran species and two parasitoid species were found in association with WTB infested logs. Concentration levels and translocation of two systemic insecticides (imidacloprid and dinotefuran) were studied in mature *J. nigra* and tissue types affected were identified. Concentrations of all the chemicals were determined using high pressure liquid chromatography (HPLC). Imidacloprid concentrations were detected in all tissue types tested including nutmeat. Dinotefuran was only detected in trace amounts in the first sampling period.

Peachey, E. 2012. Studies on the walnut twig beetle (WTB), *Pityophthorus juglandis*, in relation to its association with *Geosmithia morbida*, its survival in felled logs, and its sensitivity to temperature extremes. M.S. Thesis. Colorado State University.

To better understand walnut twig beetle (WTB), studies were initiated to: 1) determine if WTB is attracted to *Geosmithia morbida*, or other bark fungi, when given a choice; 2) determine whether clear plastic tarping or applications of bifenthrin, permethrin, and biodiesel to felled logs affected the persistence of the walnut twig beetle or altered the suitability of logs for future breeding by the insect; and 3) establish upper and lower lethal temperature (LT) limits of the beetle as well as determine supercooling point (SCP) and seasonal variation of SCP of walnut twig beetle.

Peachey, E. Cranshaw, W., and Tisserat, N. 2011. Persistence of the walnut twig beetle in black walnut logs as influenced by chemical and cultural treatments. [Abstract.] *Phytopathology* 101(6): S138–139.

The walnut twig beetle (WTB, *Pityophthorus juglandis*) is the vector of *Geosmithia morbida* and a causal agent of thousand cankers disease (TCD) of black walnut (*Juglans nigra*). In addition to aggressive feeding by WTB, damage to walnut occurs from canker development around beetle galleries due to *G. morbida*. Cankers coalesce and girdle the branches and trunk eventually causing mortality. To better understand WTB, studies were initiated to: 1) determine if WTB is attracted to *G. morbida*, or other bark fungi, when given a choice; 2) determine whether clear plastic tarping or applications of bifenthrin, permethrin, and biodiesel to felled logs affected the persistence of the walnut twig beetle or altered the suitability of logs for future breeding by the insect; and 3) establish upper and lower lethal temperature (LT) limits of the beetle as well as determine supercooling point (SCP) and seasonal variation of SCP of walnut twig beetle.

Ploetz, R.C., Hulcr, J., Wingfield, M.J., and De Beer, Z.W. 2013. Destructive tree diseases associated with ambrosia and bark beetles: Black swan events in tree pathology? *Plant Dis.* 95(7): 856–872.

This article gives an overview of ambrosia and bark beetles that cause tree damage, and explains the life history changes in organisms that lead to increased disease development. Thousand cankers disease is used as an example where a twig-beetle changes behavior to attack the main trunk, the associated pathogen changes in virulence, and there is a marked host shift.

Popa, V., Morneau, L., Piché, C. Deshaies, A., Bauce, E., and Guertin, C. 2013. Occurrence of species of the genus *Pityophthorus* Eichhoff (Coleoptera, Curculionidae, Scolytinae) in the province of Quebec, Canada. *ZooKeys* 348: 97–124.

Pityophthorus juglandis is mentioned in association with *Geosmithia morbida* as the causal agents of thousand cankers disease. Currently, the presence of thousand cankers disease has not been documented in Quebec.

Reed, S.E., Juzwik, J., English, J.T., and Ginzel, M.D. 2015. Colonization of artificially stressed black walnut trees by ambrosia beetle, bark beetle, and other weevil species (Coleoptera: Curculionidae) in Indiana and Missouri. *Environ. Entomol.* 46(4): 1455–1464.

Thousand cankers disease (TCD) is a new disease of black walnut (*Juglans nigra* L.) in the eastern United States. The disease is caused by the interaction of the aggressive bark beetle *Pityophthorus juglandis* Blackman and the canker-forming fungus, *Geosmithia morbida* M. Kolarik, E. Freeland, C. Utley & Tisserat, carried by the beetle. Other insects also colonize TCD-symptomatic trees and may also carry pathogens. A trap tree survey was conducted in Indiana and Missouri to characterize the assemblage of ambrosia beetles, bark beetles, and other weevils attracted to the main stems and crowns of stressed black walnut. More than 100 trees were girdled and treated with glyphosate (Riverdale Razor Pro, Burr Ridge, Illinois) at 27 locations. Nearly 17,000 insects were collected from logs harvested from girdled walnut trees. These insects represented 15 ambrosia beetle, four bark beetle, and seven other weevil species. The most abundant species included *Xyleborinus saxeseni* Ratzburg, *Xylosandrus crassiusculus* Motschulsky, *Xylosandrus* *germanus* Blandford, *Xyleborus affinis* Eichhoff, and *Stenomimus pallidus* Boheman. These species differed in their association with the stems or crowns of stressed trees. Multiple species of insects were collected from individual trees and likely colonized tissues near each other. At least three of the abundant species found (*S. pallidus*, *X. crassiusculus*, and *X. germanus*) are known to carry propagules of canker-causing fungi of black walnut. In summary, a large number of ambrosia beetles, bark beetles, and other weevils are attracted to stressed walnut trees in Indiana and Missouri. Several of these species have the potential to introduce walnut canker pathogens during colonization.

Reed, S., Juzwik, J., and English, J. 2013. Fungi isolated from four ambrosia beetle species emerged from stressed black walnut [Abstract.] *Phytopathology* 103(6): S120.

Attacks of black walnut by walnut twig beetles (*Pityophthorus juglandis*) can introduce *Geosmithia morbida* into entry tunnels and egg galleries in the inner bark. Thousand Cankers Disease forms when numerous bark cankers caused by *G. morbida* coalesce. It is not known what other bark and ambrosia beetles may vector *G. morbida* or other deleterious fungi. Consequently, we collected bark and ambrosia beetles from stressed black walnut trees and characterized their fungal associates. More than 100 trees in Indiana and Missouri were girdled and treated with glyphosate. Stem and branch sections were placed in insect emergence buckets. Over 16,000 beetles representing 15 ambrosia and 4 bark beetle species were collected upon emergence. The most abundant beetle species collected were *Xyleborinus saxesenii*, *Xylosandrus crassiusculus*, *Xylosandrus germanus*, and *Xyleborus affinis*. No *P. juglandis* was detected. Fungi carried by these beetle species were isolated by serial dilution plating of macerated beetle suspensions. Between 44 and 56 fungal taxa were associated with each assayed beetle species, and included ascomycetes, basidiomycetes and zygomycetes. Putative tree pathogens associated with > 10% of each beetle species included *Fusarium solani* and *Phaeoacremonium* spp. The blue-stain fungus, *Graphium penicilloides* and a red stain fungus, *Scytalidium cuboideum* were also commonly isolated. Two beetle species yielded *Geosmithia pallida*, a close relative of *G. morbida*.

Rugman-Jones, P.F., Seybold, S.J., Graves, A.D., and Stouthamer, R. 2015. Phylogeography of the walnut twig beetle, *Pityophthorus juglandis*, the vector of thousand cankers disease in North American walnut trees. *PLoS one* 10(2): e0118264. doi:10.1371/journal.pone.0118264.

Thousand cankers disease (TCD) of walnut trees (*Juglans* spp.) results from aggressive feeding in the phloem by the walnut twig beetle (WTB), *Pityophthorus juglandis*, accompanied by inoculation of its galleries with a pathogenic fungus, *Geosmithia morbida*. In 1960, WTB was only known from four U.S. counties (in Arizona, California, and New Mexico), but the species has now (2014) invaded over 115 counties, representing much of the western USA, and at least six states in the eastern USA. The eastern expansion places TCD in direct proximity to highly valuable (> $500 billion) native timber stands of eastern black walnut, *Juglans nigra*. Using mitochondrial DNA sequences, from nearly 1100 individuals, we examined variation among 77 samples of WTB populations across its extended range in the USA, revealing high levels of polymorphism and evidence of two divergent lineages. The highest level of genetic diversity for the different lineages was found in the neighboring Madrean Sky Island and Western New Mexico regions, respectively. Despite their proximity, there was little evidence of mixing between these regions, with only a single migrant detected among 179 beetles tested. Indeed, geographic overlap of the two lineages was only common in parts of Colorado and Utah. Just two haplotypes, from the same lineage, predominated over the vast majority of the recently expanded range. Tests for *Wolbachia* proved negative suggesting it plays no role in "driving" the spread of particular haplotypes, or in maintaining deep levels of intraspecific divergence in WTB. Genotyping of ribosomal RNA corroborated the mitochondrial lineages, but also revealed evidence of hybridization between them. Hybridization was particularly prevalent in the sympatric areas, also apparent in all invaded areas, but absent from the most haplotype-rich area of each mitochondrial lineage. Hypotheses about the specific status of WTB, its recent expansion, and potential evolutionary origins of TCD are discussed.

Seybold, S.J. 2012. Life history of the walnut twig beetle, *Pityophthorus juglandis*, and its association with *Geosmithia morbida*, causal agent of thousand cankers disease in the United States. [Abstract.] *Phytopathology* 102(7): S159.

The walnut twig beetle (WTB) is a native phloeophagous (phloem-feeding) insect that has recently been associated with the newly described fungus, *Geosmithia morbida*. This insect-fungal complex is known as thousand cankers disease. In northern California male WTB colonize *Juglans* branches and stem material by tunneling into the phloem where they produce an aggregation pheromone that attracts females and more males. The pioneer males are joined by two or more females and together they construct a largely transverse gallery system in the phloem. Both sexes carry the conidia for *G. morbida* and the pathogen appears to be introduced into host *Juglans* phloem during gallery construction. The pheromone has been used to demonstrate that WTB flies nearly year round with trap catches absent in December and very low in January. Peak flights occur in May/June and August/September; another major flight occurs in March/April when overwintering adults emerge. Overwintering larvae develop in spring and likely emerge in May. In the summer, most of the beetles fly between 1800 and 2000 h (crepuscular flight), but this diurnal pattern appears to shift as temperatures cool in the fall. The bulk of the WTB responding to pheromone-baited traps are females (0.59 to 0.83 relative to total number of beetles captured). An analysis of larval head capsule widths showed that WTB has three larval instars. The efficacy of the pheromone for detecting WTB was demonstrated over a range of population densities in eastern and western states.

Seybold, S.J., Coleman, T.W., Dallara, P.L., Dart, N.L., Graves, A.D., Pederson, L.A., and Spichiger, S. 2012. Recent collecting reveals new state records and geographic extremes in the distribution of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), in the United States. *Pan. Pac. Entomol.* 88(2): 277–280.

This article establishes the recent broadened geographic distributions of walnut twig beetles in the United States.

Seybold, S.J., Dallara, P.L., Nelson, L.J., Graves, A.D., Hishinuma, S.M., and Gries, R. 2011. Methods of monitoring and controlling the walnut twig beetle, *Pityophthorus juglandis*. Provisional Patent filed with the U.S. Patent and Trademark Office, U.S. Dept. of Commerce, July 15, 2011, U.S. Provisional Application No. 61/508.441.

Seybold, S.J., King, J.A., Harris, D.R., Nelson, L.J., Hamud, S.M., and Chen, Y. 2012. Diurnal flight response of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), to pheromone baited traps in two northern California walnut habitats. *Pan. Pac. Entomol.* 88(2): 231–247.

The diurnal flight response of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), was assessed during two seasonal periods at two sites in northern California. Males and females flew primarily at dusk in response to aggregation pheromone-baited traps during late June/early July, and the percentage of beetles that flew between sunrise and late afternoon was positively correlated with the temperature at 6 AM of that day. Between late August and early November, the diurnal flight pattern was more varied and generally bimodal, though approx. 42 to 55% of the beetles still flew during the dusk period. During either period of the season, there was very little evidence that any flight occurred during the night. Between late August and late September, diurnal flight of both sexes during two-hour intervals was associated with temperature in a Gaussian manner with a threshold at 17–18 uC (62.6–64.4 uF) and peak activity at 23–24 uC (73.4–75.2 uF). The proportion of females in the catches of P. juglandis that

responded to pheromone-baited traps ranged from 0.43 to 0.84 but did not vary throughout the day within a seasonal period.

Sitz, R.A. 2013. Management options for the walnut twig beetle, *Pityophthorus juglandis* Blackman, vector of the fungal canker pathogen *Geosmithia morbida*. M.S. Thesis. Colorado State University.

To better understand and manage thousand cankers disease, studies were initiated to (1) determine monthly variations in *P. juglandis* cold tolerance, (2) develop effective management tools for infested felled logs, and (3) determine suitable fungicides to treat thousand canker diseased trees and techniques to create *Geosmithia*-free beetle populations.

Turcotte, R.M., Martin, D.K.H., Smith, P.M., Kasson, M.T., and Rhodes, D. 2013. A case study: Walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae: Scolytinae) in Bucks County, Pennsylvania. *Entomol. News* 123(4): 311–314.

The Pennsylvania WTB were identified as the same WTB that are present in CA. "It is believed that the beetles arrived on infected Claro (*J. hindsii*) walnut wood shipped to the sawmill owner in 2001 or 2008. The objectives of this project were to determine: 1) if WTB was established in Bucks County, 2) the distribution of WTB within Bucks county, 3) the spatial distribution of black walnut and WTB around the introduction site, 4) if distance, dbh and other abiotic and biotic factors affect infestation by WTB, and if this information could be used in developing a potential sampling plan for the detection of WTB." An association was found between infestation and distance from an infected tree along with mortality and distance from an infected tree. "WTB was trapped an average of 5.4 km from the introduction site. Although none of the original material or any of the cut infested material has been moved from the site, local walnut logs and firewood have passed through the site over the years, raising the question of whether the captured beetles have spread by "natural" dispersal or through the movement of infested wood products (logs and firewood). Based on these results, it is recommended that baited funnel traps used for detection be placed within 500 meters of potential introduction sites (i.e., mills) and near the base of the largest diameter walnut trees possible."

Wiggins, G.J., Grant, J.F., Lambdin, P.L., and Merten, P., Nix, K.A., Hadziabdic, D., Windham, M.T. 2014. Discovery of walnut twig beetle, *Pityophthorus juglandis*, associated with forested black walnut, *Juglans nigra*, in the Eastern U.S. *Forests* 5(6): 1185–1193.

Wood, S.L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Nat. Memoirs* 6: 1–1359.

"This species is distinguished by having four to six concentric rows of pronotal asperities, by the dull (shagreened) desclivital surface, and by other characters described below." The listed characteristics include information on male and female eye, antennae, pronotum, elytra, and declivity qualities. It also mentions distribution (S. California and New Mexico to Chihuahua), hosts (*Juglans californica, J. major, J. nigra*), and biology (1123 p).

Wood, S.L. 2007. Bark and ambrosia beetles of South America (Coleoptera, Scolytidae). Brigham Young University, M.L. Bean Life Science Museum, Provo, Utah.

Wood provides a key to the genera within the tribe Corthylini (541 p), and a key to species within the genus *Pityophthorus* (625 p), but *P. juglandis* is not mentioned. It references page 1005 of Wood and Bright 1992. A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic Index, Volume B. *Great Basin Nat. Memoirs* 13: 1–1553.

Wood, S.L., and Bright, Jr. D.E. 1992. A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic Index, Volume B. *Great Basin Nat. Memoirs* 13: 1–1553.

(Scolytidae, Corthylini, *Pityophthorus*)
"*Juglandis* Blackman 1928b: 42 Holotype ♀; Lone Mountain, New Mexico [USA]; USNM, Washington. Distribution: North America (Chihuanhua in Mexico/ Arizona, California, New Mexico in USA). Hosts: *Juglans californica, J.major*, *J. nigra*, References: (by)…" (1005 p).

Articles that Discuss *Geosmithia morbida*

Daniels, D.A., Lord, R.J., Wadl, P.A., Nix, K.A., Vito, L.M., Merten, P.R., Windham, M.T., Lambdin, P.L., Grant, J.F., Wiggins, G.J., and Hadziabdic, D. 2014. To the vector go the spoils: Identifying fungal pathogens associated with walnut twig beetle, a vector of thousand cankers disease complex. [Abstract.] *Phytopathology* 104(11): 30.

> The fungus, *Geosmithia morbida,* vectored by the walnut twig beetle (WTB), *Pityophthorus juglandis,* causes mortality in black walnut (*Juglans nigra*) known as Thousand Cankers Disease (TCD). Infected trees exhibit symptoms similar to drought, making disease identification difficult. Early detection and confirmation of the pathogen is feasible using molecular markers. In 2010, TCD was discovered in Tennessee and has since been detected in four more eastern U.S. states within the native range of black walnut. Our objective was to identify fungal pathogens associated with WTB infestation of black walnut in Tennessee. WTB (n=180) were collected from two symptomatic trees in Knoxville, TN, separated by sex, divided into 3 treatment groups (decapitation, washing, or direct plating), and placed onto antibiotic amended 10% potato dextrose agar medium. Fungal isolates were identified based on morphology and confirmed by sequencing ITS1 and ITS4 regions. Additionally, microsatellite loci were used to detect the presence of *G. morbida*. Statistically significant associations between pathogen presence and direct plating method (P=0.026), and between male WTB and *G. morbida* (P=0.044) were determined. Although four species of *Fusarium,* including *F. solani*, were isolated from both female and male WTB, the findings were not significant with respect to sex. It is vitally important to fully understand TCD complex and the role of secondary pathogens in TCD pathogenicity.

Fisher, J.R., McCann, D.P., and Taylor, N.J. 2013. *Geosmithia morbida*, thousand cankers disease of black walnut pathogen, was found for the first time in southwestern Ohio. *Plant Health Prog*. Online Publication. doi:10.1094/PHP-2013-1201-01-BR.

> *Geosmithia morbida* is established in the Ohio landscape and has likely been present longer than initially suspected since the fungus was isolated from mature declining black walnut trees in a wooded residential environment nearly three miles from the initial WTB catches. The veneer mill where the WTB was first discovered has been importing walnut material from California for over a decade. The fungal conidiophore and spore morphology, and the colony morphology on ¼ PDA++ agar are consistent with those reported for *G. morbida* (Kolarik et al 2011; Tisserat et al 2009). Furthermore, the ITS1-4 and ITS 4-6 DNA sequence data is 100% identical to isolates from California, Colorado, and Oregon, including the *G. morbida* type isolate (accession number FN434082.1) (Hadziabdic et al 2014, Tisserat et al 2009). It is important to emphasize that we isolated the fungus from discolored phloem tissue resembling small cankers that were not associated with WTB galleries or exit holes, and were much smaller than TCD cankers reported in the literature (Tisserat et al 2009).

Frascella, A., Bettini, P.P., Kolařík, M., Comparini, C., Pazzagli, L., Luti, S., Scala, F., and Scala, A. 2014. Interspecific variability of class II hydrophobin GEO1 in the genus *Geosmithia*. *Fungal Biol.* 118(11): 862–871.

> The genus *Geosmithia* Pitt (Ascomycota: Hypocreales) comprises cosmopolite fungi living in the galleries built by phloeophagous insects. Following the characterization in *Geosmithia* species 5 of the class II hydrophobin GEO1 and of the corresponding gene, the presence of the geo1 gene was investigated in 26 strains derived from different host plants and geographic locations and representing the whole phylogenetic diversity of the genus. The geo1 gene was detected in all the species tested where it maintained the general organization shown in *Geosmithia* species 5, comprising three exons and two introns. Size variations were found in both introns and in the first exon, the latter being due to the presence of an intragenic tandem repeat sequence corresponding to

a stretch of glycine residues in the deduced proteins. At the amino acid level the deduced proteins had 44.6 % identity and no major differences in the biochemical parameters (pI,GRAVYindex, hydropathy plots) were found. GEO1 release in the fungal culture medium was also assessed by turbidimetric assay and SDS-PAGE, and showed high variability between species. The phylogeny based on the geo1 sequences did not correspond to that generated from a neutral marker (ITSrDNA), suggesting that sequence similarities could be influenced by other factors than phylogenetic relatedness, such as the intimacy of the symbiosis with insect vectors. The hypothesis of a strong selection pressure on the geo1 gene was sustained by the low values (<1) of non synonymous to synonymous nucleotide substitutions ratios (Ka/Ks), which suggest that purifying selection might act on this gene. These results are compatible with either a birth-and-death evolution scenario or horizontal transfer of the gene between *Geosmithia* species.

Freeland, E. 2011. Intraspecific variability of *Geosmithia morbida* the causal agent of thousand cankers disease, and effects of temperature, isolate and host family (*Juglans nigra*) on canker development. M.S. Thesis. Colorado State University.

Since the mid-1990's widespread mortality of Black Walnut (*Juglans nigra*) in the western United States has been noted. This mortality is the result of aggressive feeding of the Walnut twig beetle (*Pityophthorus juglandis)* and subsequent canker development caused by the newly named fungus *Geosmithia morbida*. Thousand canker disease (TCD) has been confirmed in Oregon, Washington, Idaho, Utah, California, Colorado, New Mexico, Arizona and recently in the native range of *J. nigra*, in Tennessee, Pennsylvania, and Virginia. Intraspecific variability of isolates was determined using rDNA ITS partial sequences and partial beta tubulin sequences. Nested clade phylogeographic analysis was used to look for correlations between haplotype trees and geography of isolates collected in screenings for the disease. Patterns of restricted dispersal by distance were found for both markers and high variability was found in isolates from single locations. Indicating that the populations causing disease throughout the western United States are not the result of recent point introductions. *G. morbida* isolate and *J. nigra* family had inconsistent effects on canker development, while temperature had a consistent effect. At higher temperatures (32°C) canker development was reduced compared to 25°C. Several genetically different *G. morbida* isolates were compared and repeated differences in pathogenicity were produced.

Freeland, E., Cranshaw, W., and Tisserat, N. 2011. Temperature and fungal isolate influence canker development in black walnut caused by *Geosmithia morbida.* [Abstract.] *Phytopathology* 101: S55.

Thousand cankers disease of black walnut (*Juglans nigra*) is the result of aggressive feeding by the walnut twig beetle (*Pityophthorus juglandis*) and subsequent canker formation around galleries caused by *Geosmithia morbida*. We studied temperature effects and *G. morbida* isolate aggressiveness on canker development in black walnut. One-year-old trees were placed in growth chambers maintained at 20°C at night and either 25 or 30°C during the day. Each tree was inoculated with four different haplotype isolates (based on rDNA ITS sequence data) positioned on stems in a Latin square design. There was no effect ($P > 0.10$) of inoculation position on canker development but cankers were larger ($P < 0.05$) at 25°C compared to 30°C six weeks after inoculation. All isolates tested caused cankers, although in two experiments an isolate collected from Arizona walnut (*J. major*) in Arizona resulted in slightly smaller ($P < 0.05$) cankers than the other isolates collected from black walnut. Furthermore, all canker areas were smaller in experiments in which trees were entering dormancy or were fully dormant (i.e. they had defoliated by late fall in the greenhouse) prior to inoculation in growth chambers. These data suggest that black walnuts are more susceptible to canker development when they are actively growing.

Freeland, E., Cranshaw, W., and Tisserat, N. 2012. Effect of *Geosmithia morbida* isolate and temperature on canker development in black walnut. Online Publication. *Plant Health Progr.* doi: 10.1094/PHP-2012-0618-01-RS.

Thousand cankers disease of black walnut (*Juglans nigra*) is the result of aggressive feeding by the walnut twig beetle (*Pityophthorus juglandis*) and extensive cankering around beetle galleries caused by the fungus *Geosmithia morbida*. We developed a consistent, reproducible inoculation technique to screen black walnut trees for their reaction to canker development following inoculation with *G. morbida*. Canker areas in one-year-old trees were not affected by the location on the stem that inoculations were made. Differences in aggressiveness of *G. morbida* isolates, representing different rDNA ITS haplotype groups, to black walnut were observed in some experiments. However, these differences were small and evidence indicates that a single, highly aggressive haplotype is not responsible for the current TCD epidemic. Cankers formed in black walnut at all temperatures tested, but they were consistently smaller at 32/20°C day/night temperatures compared to 25/20°C. Although *G. morbida* is thermotolerant, higher temperatures may not enhance canker development.

Freeland, E., Kolarik, M., Utley, C., Cranshaw, W., Tisserat, N. 2009. The *Geosmithia* causing thousand cankers disease of walnut is a new species. [Abstract.] *Phytopathology* 99: S137.

Thousand cankers disease of black walnut (*Juglans nigra*) is caused by an unnamed species of *Geosmithia* that is vectored by the walnut twig beetle (*Pityophthorus juglandis*). *Geosmithia* isolates collected from walnut cankers differ from previously described *Geosmithia* species and operational taxonomic units (OTU's) isolated from bark beetles associated with conifers and hardwoods. Differences include growth habit and color of isolates in culture and morphological characteristics of the conidiophores and conidia. We sequenced the rDNA ITS region of 37 walnut isolates collected from *Juglans* species in Arizona, California, Colorado, Idaho, Oregon, Utah and Washington. Sequences from these isolates were distinct from other described *Geosmithia* species and OTU's based on parsimony and Bayesian analyses and supported morphological observations indicating this is a new species. Nevertheless, at least seven different ITS sequences have been identified among the walnut isolates. This variability was not correlated with geographic sites or hosts from which isolates were collected. Thus the walnut *Geosmithia* population in the western United States appears to be diverse and complex.

Hadziabdic, D., Wadl, P.A., Vito, L.M., Boggess, S.L., Scheffler, B.E., Windham, M.T., and Trigiano, R.N. 2012. Development and characterization of sixteen microsatellite loci for *Geosmithia morbida,* the causal agent of thousand canker disease in black walnut (*Juglans nigra*). Online publication. *Springer* doi: 10.1007/s12686-011-9526-0.

Sixteen polymorphic microsatellite loci were identified from the fungal pathogen *Geosmithia morbida*. Loci were characterized for 13 different isolates collected in 2010 from symptomatic black walnut trees in Tennessee. A total of 77 loci were tested and 16 of those were optimized, screened and selected for diversity studies of *G. morbida*. Number of alleles per locus ranged from 3 to 8. These microsatellite loci will be useful for rapid disease diagnostic, population genetic analyses on a global scale as well as further epidemiological studies of *G. morbida*.

Juzwik, J., Banik, M.T., Reed, S.E., English, J.T., and Ginzel, M.D. 2015. *Geosmithia morbida* found on weevil species *Stenomimus pallidus* in Indiana. Online publication. *Plant Health Progr.* doi: 10.1094/PHP-RS-14-0014.

The canker pathogen *Geosmithia morbida* is known to be transmitted to *Juglans* species by the bark beetle *Pityophthorus juglandis*, and to lead to development of thousand cankers disease. In an Indiana-wide trap-tree survey of ambrosia and bark beetles and weevils colonizing stressed *Juglans nigra*, *G. morbida* was detected on three *Stenomimus pallidus* weevils emerged from two trees on one site. This is the first report of the pathogen in Indiana and the first report of the fungus from an insect species other than *P. juglandis.*

Kolařík, M., Freeland, E., Utley, C., and Tisserat, N. 2011. *Geosmithia morbida* sp. Nov., a new phytopathogenic species living in symbiosis with the walnut twig beetle (*Pityophthorus juglandis)* on *Juglans* in USA. *Mycologia* 103(2): 325–332.

Widespread morbidity and mortality of *Juglans nigra* has occurred in the western USA over the past decade. Tree mortality is the result of aggressive feeding by the walnut twig beetle (*Pityophthorus juglandis*) and subsequent canker development around beetle galleries caused by a filamentous ascomycete in genus *Geosmithia* (Ascomycota: Hypocreales). Thirty-seven *Geosmithia* strains collected from *J. californica, J. hindsii, J. major* and *J. nigra* in seven USA states (AZ, CA, CO, ID, OR, UT, WA) were compared with morphological and molecular methods (ITS rDNA sequences). Strains had common characteristics including yellowish conidia en masse, growth at 37 C and absence of growth on Czapek-Dox agar and belonged to a single species described here as *G. morbida*. Whereas *Geosmithia* are common saprobes associated with bark beetles attacking hardwoods and conifers worldwide, *G. morbida* is the first species documented as a plant pathogen.

Montecchio, L., Faccoli, M., Short, D., Fanchin, G., Geiser, D., and Kasson, M.T. 2015. First report of *Fusarium solani* phylogenetic species 25 associated with early stages of thousand cankers disease on *Juglans nigra* and *Juglans regia* in Italy. Online publication. *Plant Dis.* doi: 10.1094/PDIS-01-15-0103-PDN.

Montecchio, L., Fanchin, G., Berton, V., and Scattolin, L. 2015. Vegetative incompatibility and potential involvement of a mycovirus in the Italian population of *Geosmithia morbida*. Online publication. *Phytopathol. Mediterr.* doi: 10.14601/Phytopathol_Mediterr-16376.

Studies carried out during an Italian outbreak of the Thousand Cankers Disease of walnut, demonstrated that non-coalescing cankers on host plants, separated by equidistant uninfected zones, were associated with incompatible strains of *Geosmithia morbida*. Confirmation of the vegetative incompatibility of paired fungal isolates, randomly collected from black walnuts, was obtained from observations of a clear separation zones and the absence of anastomoses. Pairing tests with two incompatible monoconidial strains indicated differences in morphology and growth rates. Electron microscopy revealed the presence of icosahedral mycovirus-like particles in one of the monoconidial strains that demonstrated low degrees of virulence *in planta* compared with a particle-free monoconidial strain. The occurrence of a vegetative incompatibility system in recently introduced populations of *G. morbida* has considerable implications for fungal biology. Incompatibility in *G. morbida* and potential direct or indirect roles of the observed virus-like particles have potential ecological and epidemiological consequences.

Reed, S.E., English, J.T., Juzwik, J. 2014. Pathogenicity of fungi from walnut branch cankers and fungi isolated from ambrosia beetles colonizing stressed walnut trees. [Abstract.] *Phytopathology* 104(11): 96.

Thousand Cankers Disease (TCD) of eastern black walnut (*Juglans nigra*) results from mass attack by the walnut twig beetle and coalescing of numerous cankers caused by the insect vectored fungus *Geosmithia morbida*. An array of fungi has been isolated from cankers on black walnut branches and from insects that had colonized stressed *J. nigra*. The potential for selected taxa of these fungi to contribute to decline of TCD trees was evaluated. Two 15-week-long pot trials were conducted in shade houses to assess pathogenicity of representative isolates on stems of 1-year-old *J. nigra*. In June and July 2013, five seedlings were inoculated per fungal isolate. Lengths of resulting stem lesions or of xylem stains were measured and morphological characteristics noted. In both trials, stem cankers resulted from inoculations with *Fusarium solani* obtained from the ambrosia beetle *Xylosandrus crassiusculus* and with *Fusarium*-like isolates obtained from walnut branch cankers. In addition, stained woody tissue resulted from inoculations with *Phaeoacremonium angustius, P.*

rubrigenum, *P. mortoniae, Penicillium sumatrense* and *Graphium penicilloides*. Neither cankers nor stain were found on sterile agar plug inoculated stems. All canker and stain-causing fungi were recovered from the fungus-inoculated stems. These results confirm the pathogenicity of *F. solani* and other *Fusarium*-like isolates obtained from branch cankers and from ambrosia beetles colonizing *J. nigra*.

Reed, S., Juzwik, J., and English, T. 2014. Fungi isolated from four ambrosia beetle species emerged from stressed black walnut. [Abstract.] *Phytopathology* 103(6): 120.

Attacks of black walnut by walnut twig beetles (*Pityophthorus juglandis*) can introduce *Geosmithia morbida* into entry tunnels and egg galleries in the inner bark. Thousand Cankers Disease forms when numerous bark cankers caused by *G. morbida* coalesce. It is not known what other bark and ambrosia beetles may vector *G. morbida* or other deleterious fungi. Consequently, we collected bark and ambrosia beetles from stressed black walnut trees and characterized their fungal associates. More than 100 trees in Indiana and Missouri were girdled and treated with glyphosate. Stem and branch sections were placed in insect emergence buckets. Over 16,000 beetles representing 15 ambrosia and 4 bark beetle species were collected upon emergence. The most abundant beetle species collected were *Xyleborinus saxesenii*, *Xylosandrus crassiusculus*, *Xylosandrus germanus*, and *Xyleborus affinis*. No *P. juglandis* was detected. Fungi carried by these beetle species were isolated by serial dilution plating of macerated beetle suspensions. Between 44 and 56 fungal taxa were associated with each assayed beetle species, and included ascomycetes, basidiomycetes and zygomycetes. Putative tree pathogens associated with > 10% of each beetle species included *Fusarium solani* and *Phaeoacremonium* spp. The blue-stain fungus, *Graphium penicilloides* and a red stain fungus, *Scytalidium cuboideum* were also commonly isolated. Two beetle species yielded *Geosmithia pallida*, a close relative of *G. morbida*.

Tylová, T., Kolařík, M., and Olšovská, J. 2011. The UHPLC-DAD fingerprinting method for analysis of extracellular metabolites of fungi of the genus *Geosmithia* (Acomycota: Hypocreales). Online publication. *Springer-Verlag.* doi: 10.1007/s00216-011-4982-7.

A new simple ultra-high-performance liquid chromatography method with diode array detection (UHPLC-DAD) was developed for chemical fingerprinting analysis of extracellular metabolites in fermentation broth of *Geosmithia* spp. The SPE method employing Oasis MCX strong cation-exchange mixed-mode polymeric sorbent was chosen for extraction of the metabolites. The analyses were performed on an Acquity UPLC BEH C18 column (100×2.1 mm i.d.; particle size, 1.7 μm; Waters) using a gradient elution program with an aqueous solution of trifluoroacetic acid and acetonitrile as the mobile phase. The applicability of the method was proved by analysis of 38 strains produced by different species and isolated from different sources (hosts). The results revealed the correlation of obtained HPLC-DAD fingerprints with taxonomical identity.

Veselská, T., and Kolařík, M. 2015. Application of flow cytometry for exploring the evolution of *Geosmithia* fungi living in association with bark beetles: The role of conidial DNA content. *Fungal Ecol.* 13: 83–92.

Geosmithia belongs among fungi living in symbiosis with phloem-feeding bark beetles. Several species have altered their ecology to that of obligatory symbiosis with ambrosia beetles, which has led to a shift in their phenotype and caused formation of large spherical conidia. In this study, we pose the following questions; (1) Is the conidial DNA content of *Geosmithia* correlated with conidial volume?; (2) Is the DNA content of *Geosmithia* related to the degree of mutual dependence between *Geosmithia* and their vector? There was a positive and strong correlation between conidial DNA content and conidial volume in *Geosmithia.* Also species more narrowly associated with the vector tend to have a larger conidial DNA content and volume than less narrowly associated species. Ambrosia fungi achieved the biggest conidial DNA content and volume compared to other species. We suppose that polyploidisation occurred during the evolution of ambrosia species in the genus *Geosmithia*.

Vito, L., Grant, J., and Windham, M. 2012. A comparison of two isolation techniques for G*eosmithia morbida*, the causal agent of thousand cankers disease. [Abstract.] *Phytopathology* 102(7): 128.

Thousand cankers disease of black walnut is caused by the fungus *Geosmithia morbida* and is vectored by the beetle, *Pityophthorus juglandis. Geosmithia morbida* has killed thousands of black walnut trees in the western part of the United States where they are grown for landscape and nutmeat purposes. The disease was first confirmed in Tennessee (within the native range of black walnut) in August, 2010 and since then, a large number of limb samples from suspect trees have been submitted for fungal screening. The fungus may be isolated from cankers and/or beetles, and this study was conducted to determine which method was more efficient at isolating pure cultures. Beetles were individually processed by placing them in sterile micro-centrifuge tubes, rinsing them with 200µl sterile water for 1 min, then transferring the water to petri dishes containing 1/10 PDA. Cankers were processed by removing thin sections of suspect tissue with a scalpel and placing them directly onto the same medium. Samples were checked daily for emerging hyphae which were transferred to fresh medium. The cultures were confirmed to be *Geosmithia* by microscopic identification. Twelve cultures were obtained from 88 beetles (14%), whereas 28 cultures were obtained from 98 canker samples (29%). Direct plating of diseased tissue is about twice as efficient for obtaining fungal cultures as beetle rinsing, has fewer input requirements, and is more feasible at this time.

Zerillo, M.M., Caballero, J.I., Woeste, K., Graves, A.D., Hartel, C., Pscheidt, J.W., Tonos, J., Broders, K., Cranshaw, W., Seybold, S., and Tisserat, N. 2014. Population structure of *Geosmithia morbida*, the causal agent of thousand cankers disease of walnut trees in the United States. *PloS one* 9(11): e112847. doi:10.1371/journal.pone.0112847.

The ascomycete *Geosmithia morbida* and the walnut twig beetle *Pityophthorus juglandis* are associated with thousand cankers disease of *Juglans* (walnut) and *Pterocarya* (wingnut). The disease was first reported in the western United States (USA) on several *Juglans* species, but has been found more recently in the eastern USA in the native range of the highly susceptible *Juglans nigra*. We performed a comprehensive population genetic study of 209 *G. morbida* isolates collected from *Juglans* and *Pterocarya* from 17 geographic regions distributed across 12 U.S. states. The study was based on sequence typing of 27 single nucleotide polymorphisms from three genomic regions and genotyping with ten microsatellite primer pairs. Using multilocus sequence-typing data, 197 *G. morbida* isolates were placed into one of 57 haplotypes. In some instances, multiple haplotypes were recovered from isolates collected on the same tree. Twenty-four of the haplotypes (42%) were recovered from more than one isolate; the two most frequently occurring haplotypes (H02 and H03) represented 36% of all isolates. These two haplotypes were abundant in California, but were not recovered from Arizona or New Mexico. *G. morbida* population structure was best explained by four genetically distinct groups that clustered into three geographic regions. Most of the haplotypes isolated from the native range of *J. major* (Arizona and New Mexico) were found in those states only or present in distinct genetic clusters. There was no evidence of sexual reproduction or genetic recombination in any population. The scattered distribution of the genetic clusters indicated that *G. morbida* was likely disseminated to different regions at several times and from several sources. The large number of haplotypes observed and the genetic complexity of *G. morbida* indicate that it evolved in association with at least one *Juglans* spp. and the walnut twig beetle long before the first reports of the disease.

Zerillo, M.M., Woeste, K., Freeland, E., Seybold, S., Cranshaw, W., and Tisserat, N. 2011. Population structure of *Geosmithia morbida* in the United States is complex. [Abstract.] *Phytopathology* 101(6): S201.

Thousand Cankers Disease of black walnut (*Juglans nigra*) was first described in 2009, but was likely present in Utah and Oregon since at least the early 1990's based on walnut twig beetle (WTB) collections and historical records of walnut mortality. *Geosmithia morbida*, the causal agent, is now widely distributed in the western United States on several walnut species and was recently found in Tennessee. The origin of the WTB and *G. morbida* has not been proven, although both are likely native to Arizona walnut (*Juglans major*) in southwestern USA. We sequenced the rDNA internal transcribed spacer (ITS) region and the beta-tubulin gene of 100 *G. morbida* isolates from a broad geographic area and from different walnut species. At least nine ITS and three beta tubulin haplotypes have been identified indicating the population is complex and diverse. Haplotype variability was not correlated with geographic sites or hosts from which isolates were collected. In some cases more than one haplotype was collected from cankers on individual branches. Four haplotypes were identified from 9 isolates collected in Tennessee. To further elucidate the relationship among isolates, five housekeeping genes are being used in a multilocus sequence typing (MLST) analysis. We have also constructed a DNA library enriched for microsatellites. Seventeen candidate loci for polymorphism are currently been screened in a panel of 10 *G. morbida* isolates from different geographical locations.

Zerillo, M.M., Woeste, K., Snelling, J., and Tisserat, N. 2011. Genome and transcriptome analysis of *Geosmithia morbida*. [Abstract.] *Phytopathology* 101: S201.

Thousand cankers disease of black walnut is the result of aggressive feeding by the walnut twig beetle (*Pityophthorus juglandis*) and canker formation around galleries caused by *Geosmithia morbida*. The disease is widespread in the western United States and was detected in the native range of black walnut in Tennessee in 2010. *G. morbida* is the first phytopathogenic species reported in this genus and in the Bionectriaceae. We started a genomic and transcriptomic analysis of *G. morbida* to better understand its gene complement and for comparison of its genome to other fungal pathogens in the Hypocreales. Total DNA of isolate CBS124663 was sequenced in a half plate reaction using 454 GS FLX (Roche) sequencing technology. The other half was used to sequence the transcriptome from *G. morbida* growing under six different environmental conditions including nutrient and temperature differences and in the yeast phase. Genome sequencing resulted in 779,553 reads that assembled into 27,933 contigs. This represented 16 million of nonredundant base pairs, or approximately 1/3 of the predicted genome size. A total of 15,254 sequences (7,697 contigs and 7,557 singletons) were automatically annotated using the Blast2GO similarity tool (http://www.blast2go.org/). Another 3.4 Mbp of non-redundant sequences of the fungus transcriptome were assembled into 5,773 contigs and that were subsequently mapped into 6,867 contigs of the *G. morbida* genome.

Related Articles of Potential Interest

Entomological Articles

Amman, A.G., Amman, S.L., and Amman, G.D. 1974. Development of *Pityophthorus confertus*. *Environ. Entomol.* 3(3): 562–562.

> *Pityophthorus confertus* development is described from observations using inner bark samples of lodgepole pine. The article includes information on nuptial chambers, egg galleries, brood development time, niche spacing, and larval instars.

Bonello, P., McNee, W.R., Storer, A.J., Wood, D.L., and Gordone, T.R. 2001. The role of olfactory stimuli in the location of weakened hosts by twig-infesting *Pityophthorus* spp. *Ecol. Entomol.* 26(1): 8–15.

> Senescing, shade-suppressed, or broken branches of Monterey pine *Pinus radiata* are infested by twig beetles in the genus *Pityophthorus* (Coleoptera: Scolytidae). The studies reported here tested whether twig beetles can discriminate between healthy and pitch canker-diseased branches, whether diseased branch tips produce more ethylene than undamaged controls, and whether ethylene and other volatiles, produced by the plant in response to tissue damage, are utilized by twig beetles in host location. 2. Significantly greater numbers of twig beetles were reared from pitch canker symptomatic than from pitch canker-asymptomatic branches of Monterey pine collected in the field. 3. Needles of Monterey pine branches inoculated with the pitch canker fungal pathogen *Fusarium circinatum* produced significantly higher levels of ethylene than needles of control branches, and this was evident just prior to, and during, symptom expression. 4. In trapping studies in which pheromone production was prevented, there was no evidence of attraction of twig beetles to a source of ethylene alone, to cut host branches, or to cut branches treated with the ethylene-releasing compound, ethephon. The results suggest that twig beetles identify weakened branches after landing.

Bright, Jr. D.E., and Skidmore, R.E. 1997. A Catalog of Scolytidae and Platypodidae (Coleoptera), Supplement 1 (1990–1994). NRC Research Press, Ottawa, Canada. 376 pp.

> This Supplement does not contain information on walnut twig beetle (*Pityophthorus juglandis*), but it provides supplements to Wood and Bright 1992.

Ciesla, W.M. 2015. The role of human activities on forest insect outbreaks worldwide. *Int. For. Rev.* 17(3): 269–281.

> Human activities, including international trade and travel, certain forest management policies and practices, and actions that contribute to global climate change, can create conditions favourable for development of forest insect outbreaks. International trade and travel provides numerous opportunities for insects to expand their ranges and become damaging in new locations. Forest management actions leading to fire exclusion can change the character of forests in fire prone ecosystems and increase their susceptibility to insect outbreaks and fires of increased severity. Establishment of plantations of exotic trees provides an abundance of host material for both introduced insects and indigenous species that can adapt to the new hosts. Special classifications, such as national parks and wilderness, which preclude timber harvesting or pest management, can, over time, result in large areas of mature/overmature forests, susceptible to outbreaks. Global climate change can lead to range expansions of forest insect pests accompanied by new host associations, occurrence of outbreaks in new locations, outbreaks of increased severity and of longer duration and changes in outbreak cycles.

Dallara, P.L., Seybold, S.J., Meyer, H., Francke, W., and Wood, D.L. 2000. Semiochemicals from three species of *Pityophthorus* (Coleoptera: Scolytidae) in California: Identification and field response. *Can. Entomol.* 132(6): 889–906.

Analyses of pentane extracts of frass, whole beetles, and volatiles trapped on Porapak-Q from *Pityophthorus* Eichhoff spp. fed on *Pinus radiata* D. Don demonstrated that (*E*)-pityol [2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran] was produced by male *Pityophthorus carmeli* Swaine, female *Pityophthorus nitidulus*(Mannerheim), and female *Pityophthorus setosus* Blackman. (*E*)-(−)-Conophthorin) [(5*S*,7*S*)-(−)-7-methyl-1,6-dioxaspiro[4.5]decane] was produced by male *P. carmeli* and male *P. nitidulus*. Only the (2*R*,5*S*)-(+) stereoisomer of (*E*)-pityol was produced by male *P. carmeli* and female *P. setosus*. In field bioassays in central coastal California, *P. setosus* was attracted to (*E*)-(+)-pityol, whereas *P. carmeli* responded only to a combination of (*E*)-(−)-conophthorin and (*E*)-(+)-pityol. Male *P. setosus* and female *P. carmeli* responded to these treatments with larger numbers than opposite-sex conspecifics. (*E*)-(−)-Conophthorin alone did not attract species of *Pityophthorus*but significantly reduced catches of *P. setosus* to (*E*)-(+)-pityol. *Lasconotus pertenuis* Casey (Coleoptera: Colydiidae) and *Ips mexicanus* (Hopkins) (Coleoptera: Scolytidae) were attracted to a combination of (*E*)-(−)-conophthorin and (*E*)-(+)-pityol, and showed a trend for attraction to all (*E*)-(−)-conophthorin-containing treatments. (*E*)-(−)-Pityol was neither attractive nor interruptive for any taxon. (*E*)-(+)-Pityol is shown to be an aggregation pheromone component for *P. carmeli* and *P. setosus*. (*E*)-(−)-Conophthorin functions as a pheromone component for *P. carmeli* and may also function as a synomone that decreases competition of *P. carmeli* and *P. nitidulus* with *P. setosus* and as a kairomone for *L. pertenuis*. These semiochemicals have been useful in studying relationships among twig insects and the pathogen *Fusarium circinatum* (Nirenberg and O'Donnell), causal agent of pitch canker disease in *P. radiata*.

Doane, R.W., Van Dyke, E.C., Chamberlain, W.J., and Burke, H.E. 1936. Forest insects. McGraw-Hill Book Co. Inc.: New York.

This book describes the radiate or star shaped tunnels produced by multiple bark beetle genera including *Pityophthorus* (70 pp). They state it is not uncommon for coniferous twig feeding beetles in the genus *Pityophthorus* to attack trunks and larger limbs. In most cases "the male occupies the central nuptial chamber and assists with the construction of the galleries, and the larvae upon hatching work through the cambium of the twig's bark and finally pupate at the ends of the larval mines. Upon changing to adults they eat their way directly to the surface and fly to attack other twigs. The number of generations varies with the different species and with different localities, but usually there are two or more generations each year (112 pp). Blackman 1928 is referenced for expanding descriptions of *Pityophthorus* species (113 pp).

Jacobi, W.R, Hardin, J.G., Goodrich, B.A., and Cleaver, C.M. 2012. Retail firewood can transport live tree pests. *J. Econ. Entomol.* 105(5): 1645–1658.

Several invasive native or exotic forest insects, including the walnut twig beetle, pose threats to North American forests if they are transported in or on unprocessed wood such as firewood.

López, S., Quero, C., Iturrondobeitia, J.C., Guerrero, A., and Goldarazena, A. 2011. Evidence for (E)-pityol as an aggregation pheromone of *Pityophthorus pubescens* (Coleoptera: Curculionidae: Scolytinae). *Can. Entomol.* 143: 447–454.

We present evidence favoring the use of (E)-pityol as an aggregation pheromone in *Pityophthorus pubescens* (Marsham). (E)-Pityol was detected in effluvia of male and female *P. pubescens*, and antennae of both sexes responded to (E)-(+)-pityol in electroantennogram assays. In two-choice olfactometer tests, males significantly preferred (E)-(+)-pityol and (E)-(±)-pityol to blank controls at doses of 1, 10, and 100 ng, whereas females only showed a preference for (E)-pityol at the 1 ng dose.

Piskorski, R., and Dorn, S. 2011. How the oligophage codling moth *Cydia pomonella* survives on walnut despite its secondary metabolite juglone. *J. of Insect Physiol.* 57: 744–750.

Besides apple, its primary host, the codling moth *Cydia pomonella* uses walnut as a secondary host. Abundance of toxic naphthoquinones, among which juglone prevails, does not restrain this economically important pest insect from infesting walnut, but processes underlying the suitability of this host were yet unknown. Larvae feeding on an artificial diet supplemented with juglone at naturally occurring concentrations survived to adulthood at a similarly high proportion as those in the juglone-devoid control. However, their development time was prolonged, their weight gain was reduced, and adult sex ratio was distorted. Results from the natural system with walnut and apple fruits were in line with data gained on artificial diet. Remarkably, a twofold increase of the maximal juglone content reported from the walnut husk was lethal to the larvae. Chemical analyses showed that larvae feeding on the artificial diet supplemented with juglone concentrations present in walnut contained 1,4,5-trihydroxynaphthalene and excreted it in their frass, whereas the hemolymph contained neither detectable amounts of juglone nor the product of its reduction. Hence, effective metabolism of juglone in the intestinal system of the larvae underlies their survival on host plants containing this defensive compound.

Piskorski, R., Ineichen, S., and Dorn, S. 2011. Ability of the oriental fruit moth *Grapholita molesta* (Lepidoptera: Tortricidae) to detoxify juglone, the main secondary metabolite of the non-host plant walnut. *J. Chem. Ecol.* 37(10): 1110–1116.

Many plant species produce toxic secondary metabolites that limit attacks by herbivorous insects, and may thereby constrain insect expansion to new hosts. Walnut is a host for the codling moth *Cydia pomonella*, which efficiently detoxifies the main walnut defensive compound juglone (5-hydroxy-1,4-naphthoquinone). The oriental fruit moth *Grapholita molesta*, which also belongs to the tribe Grapholitini, does not feed on walnut. We tested the performance of *G. molesta*, a highly invasive species, on artificial diets containing juglone at levels mimicking those found in walnut over the growing season. Juglone-fed *G. molesta* survived relatively well to adulthood, but larval and adult body weights were reduced, and larval developmental time was prolonged in a dose-dependent fashion. Chemical analysis of frass from larvae that had been fed a juglone-containing diet suggests that *G. molesta* reduces juglone to non-toxic 1,4,5-trihydroxynaphthalene in its gut. This unexpected tolerance of *G. molesta* to high levels of juglone may facilitate expansion of the host range beyond the current rosacean fruit trees used by this invasive pest.

Storer, A.J., Wood, D.L., and Gordon, T.R. 2004. Twig beetles, *Pityophthorus* spp. (Coleoptera: Scolytidae), as vectors of the pitch canker pathogen in California. *Can. Entomol.* 136(5): 685–693.

Twig beetles in the genus *Pityophthorus* Eichhoff are known to be associated with the pitch canker pathogen, *Fusarium circinatum*, in California. Phoresy of the pathogen on these species has been reported to occur when insects emerge from diseased branches and when they infest disease-free, cut branch tips. To demonstrate that twig beetles can vector the pathogen, studies of phoresy and transmission were conducted in a native Monterey pine, *Pinus radiata* D. Don (Pinaceae), forest. Phoresy was confirmed for both *Pityophthorus setosus* Blackman and *Pityophthorus carmeli* Swaine, and *P. setosus* was shown to vector the pitch canker pathogen when contaminated with fungal spores and caged onto Monterey pine branches. When attractive baits were used to increase visitation to Monterey pines by *P. setosus*, baited trees were more likely to develop pitch canker than unbaited trees even though the beetles did not tunnel into the host to develop egg galleries.

Therefore, twig beetles are competent as vectors of the pitch canker pathogen, and their vectoring activity, though requiring a wound, does not require that they establish egg galleries in the host.

Related Articles of Potential Interest

Mycological Articles

Bettini, P.P., Frascella, A., Kolařík, M., Comparini, C., Pepori, A.L., Santini, A., Scala, F. and Scala, A. 2014. Widespread horizontal transfer of the cerato-ulmin gene between *Ophiostoma novo-ulmi* and *Geosmithia* species. *Fungal Biol.* 118(8), 663–674.

> Previous work had shown that a sequence homologous to the gene encoding class II hydrophobin cerato-ulmin from the fungus *Ophiostoma novo-ulmi*, the causal agent of Dutch Elm Disease (DED), was present in a strain of the unrelated species *Geosmithia* species 5 (*Ascomycota*: *Hypocreales*) isolated from *Ulmus minor* affected by DED. As both fungi occupy the same habitat, even if different ecological niches, the occurrence of horizontal gene transfer was proposed. In the present work we have analysed for the presence of the *cerato-ulmin* gene 70 *Geosmithia* strains representing 29 species, isolated from different host plants and geographic locations. The gene was found in 52.1% of the strains derived from elm trees, while none of those isolated from nonelms possessed it. The expression of the gene in *Geosmithia* was also assessed by real time PCR in different growth conditions (liquid culture, solid culture, elm sawdust, dual culture with *O. novo-ulmi*), and was found to be extremely low in all conditions tested. On the basis of these results we propose that the *cerato-ulmin* gene is not functional in *Geosmithia*, but can be considered instead a marker of more extensive transfers of genetic material as shown in other fungi.

Čížková, D., Šrůtka, P., Kolařík, M., Kubátová, A., and Pažoutová, S. 2005. Assessing the pathogenic effect of *Fusarium, Geosmithia* and *Ophiostroma* fungi from broad-leaved trees. *Biol. Control* 50(1): 59–62.

> Phytopathogenic effect of *Geosmithia pallida*, *G. langdonii*, *Ophiostoma grandicarpum*, *O. querci*, two isolates of *O. piceae*, and two isolates of *Fusarium solani* was compared using plant growth test (stem and root length of garden cress plants seeded on mycelium-covered potato carrot agar); *Ophiostoma* spp. and *F. solani* were isolated from oak, *Geosmithia* spp. from galleries of *Scolytus intricatus* on beech. All fungi inhibited more the root elongation than that of stems. *F. solani* led to plant collapse after briefly stimulating the growth of stem and in one case also root. *G. langdonii* inhibited stem and root growth to 20 % and led to plant collapse. *G. pallida* inhibited root growth to 25 % whereas stem growth was almost unimpaired. *Ophiostoma* spp. reduced stem growth to ≈60–80 % and root growth to 25–60 %. *O. piceae* and *O. querci* caused plant collapse after 15–20 d.

Dori-Bachash, M., Avrahami-Moyal, L., Protasov, A., Mendel, Z., and Freeman, S. 2015. The occurrence and pathogenicity of *Geosmithia* spp. and common blue-stain fungi associated with pine bark beetles in planted forests in Israel. *Eur. J. Plant Pathol.* 143(4): 627–639.

> Fungi associated with the bark beetles *Orthotomicus erosus*, *Tomicus destruens* and *Pityogenes calcaratus* were sampled in various pine forests throughout Israel. Three ophiostomatoid fungi, *Ophiostoma ips*, *Graphilbum rectangulosporium* and *Leptographium wingfieldii*, and a fourth non-ophiostomatoid fungus, *Geosmithia* sp. 24, were identified by using morphological characteristics and molecular genetic analyses. *O. ips*, the most common fungus, was mainly isolated from *O. erosus*. The least common fungus, *G. rectangulosporium*, was frequently isolated from all three studied scolytids, while *L. wingfieldii* was almost exclusively associated with *T. destruens*. The fourth fungus, *Geosmithia* sp. 24, was isolated from both *O. erosus* and *P. calcaratus*. This is the first time that an association between *O. erosus* and a *Geosmithia* sp. has

been reported. Our findings also suggest that *Geosmithia* sp. 24 can be separated into two distinct sub-groups by molecular analyses. Pathogenicity was demonstrated only for *L. wingfieldii*, both on Aleppo and brutia pine, exclusively under controlled conditions (25 ± 5°C) but not at elevated temperatures.

Jankowiak, R., Kolařík, M., and Bilański, P. 2014. Association of *Geosmithia* fungi (Ascomycota: Hypocreales) with pine-and spruce-infesting bark beetles in Poland. *Fungal Ecol.* 11: 71–79.

Bark beetles (Coleoptera, Scolytinae) are known to be associated with fungi, particularly species of the orders Ophiostomatales and Microascales. However, very little is known about other ectosymbionts of phloeophagous bark beetles on Pinaceae. In this study, we examined the *Geosmithia* species associated with eight bark beetle species infesting *Picea abies* and *Pinus sylvestris* branches in Poland. Fungi were isolated from 1 731 samples collected from 14 study sites. We identified a total of 653 isolates that were sorted into nine taxa based on their phenotypic similarity and phylogeny of their ITS-LSU regions of rDNA, b-tubulin, elongation factor 1a and the second-largest subunit of the RNA polymerase II gene. They represented nine species without formal names. There were large quantitative and qualitative differences in the composition of *Geosmithia* communities between *P. sylvestris* and *P. abies* trees. The proportion of samples infested with *Geosmithia* species suggests that this association is more widespread among bark beetles infesting branches of *P. sylvestris*. In addition, these beetles were vectors of different *Geosmithia* species compared with than the beetles that colonize *P. abies*. In mixed-conifer forests, the *Geosmithia* communities were more diverse and richer than in pure spruce or pine stands, where the insects *Pityogenes chalcographus* and *Pityophthorus pityographus* with low host-specificity play a distributing role for various *Geosmithia* species. Among eight bark beetle species examined, only *P. bidentatus*, *P. pityographus*, *P. chalcographus* and *Polygraphus poligraphus* acted as effective vectors for *Geosmithia* species. The following hypothesis emerges from these studies: changes in the composition of ectosymbionts of pine- and spruce-infesting bark beetles in Central Europe run along a gradient of thickness of the wood substrata preferred by insects.

Kessler Jr, K.J. 1974. An apparent symbiosis between *Fusarium* fungi and ambrosia beetles causes canker on black walnut stems. *Plant Dis. Rep.* 58: 1044–1047.

A stem girdling canker of black walnut was found in plantations in Illinois, Indiana, Missouri, and Iowa. The canker is thought to be caused by *Fusarium*-ambrosia beetle complex. The problem may be initiated by buildup of beetle populations on pruning and/or dead trees and branches allowed to remain in plantations. Dieback around increment borer wounds on black walnut may be caused by invasion of the wounds by *Fusarium* species.

Kolařik M., Hulcr J., Kirkendall L.R. 2015. New species of *Geosmithia* and *Graphium* associated with ambrosia beetles in Costa Rica. *Czech Mycol*. 67(1): 29–35.

Geosmithia cnesini sp. nov. is a dominant symbiont of the ambrosia beetle *Cnesinus lecontei* collected from *Croton draco* in Costa Rica. This fungus is characterised by whitish colonies and penicillate conidiophores with extraordinary large catenate conidia. *Graphium scolytodis* sp. nov. is described here from the galleries of ambrosia beetle *Scolytodes unipunctatus* collected from the trunk of a fallen *Cecropia angustifolia* tree in Costa Rica. This species does not seem to be a nutritional mutualist but rather a stable associate of unknown function. It produces mononematic conidiophores only and is related to *Graphium penicillioides*.

Kolařík, M., and Kirkendall, L.R. 2010. Evidence for a new lineage of primary ambrosia fungi in *Geosmithia* Pitt (Ascomycota: Hypocreales). *Fungal Biol.* 114(8): 676–689.

Geosmithia is a genus of mitosporic filamentous fungi typically associated with phloeophagous bark beetles world-wide. During this study, the fungal associates of ambrosia beetles *Cnesinus lecontei, Eupagiocerus dentipes,* and *Microcorthylus sp.* from Costa Rica, were studied using morphology and DNA sequences. Fungal associates belonged to four undescribed *Geosmithia* species. *Geosmithia eupagioceri* sp. nov.and *G. microcorthyli* sp. nov. are evidently primary ambrosia fungi of their respective vectors *E. dentipes* and *Microcorthylus* species. They both have convergently evolved distinct morphological adaptations including the production of large, solitary and globose conidia, and yeast-like cells. Tunnels of *C. lecontei* contained an undescribed *Geosmithia* species, but its nutritional importance for its vector is unclear. An auxiliary ambrosia fungus, *Geosmithia rufescens* sp. nov., was found associated with both *G. eupagioceri* and the *Geosmithia* species associated with *C. lecontei*. *G. microcorthyli* is genetically quite similar to the phloem-associated *Geosmithia* sp. 8 from Europe. Large differences in morphology between these two species suggest the rapid co-evolution resulting from the close symbiosis of the former with its beetle host. The ITS rDNA sequences of *G. microcorthyli* and *Geosmithia* sp. 8 were not diagnostic, suggesting that alternative markers such as EF-1a, IGS rDNA or b-tubulin should be used, together with morphological and ecological data, for species delimitation in this genus. The primary ambrosia fungi described here are derived from phloem-associated ancestors, and represent two independent lineages of ambrosia fungi in the Hypocreales and a new ecological strategy within *Geosmithia*.

Kolařík, M., Kostovčík, M., and Pažoutová, S. 2007. Host range and diversity of the genus *Geosmithia* (Ascomycota: Hypocreales) living in association with bark beetles in the Mediterranean area. *Mycol. Res.* 111(11): 1298–1310.

Geosmithia spp. (*Ascomycota*: *Hypocreales*) are dry-spored fungi that occur in galleries built by many phloeophagous bark beetles. This study mapped the diversity, host spectrum and area of distribution of *Geosmithia* spp. occurring in galleries of bark beetle species with a Mediterranean distribution. Eighty-six wood samples of 19 tree species infested by 18 subcortical insect species were collected from across the Mediterranean Basin during the years 2003–2006. *Geosmithia* spp. were found in 82 samples of angiosperms and two host trees from the family *Juniperaceae* infested by 14 bark beetles and the bostrichid *Scobicia pustulata,* suggesting that the association of *Geosmithia* and phloeophagous bark beetles is very widespread in the Mediterranean. *Geosmithia* isolates were sorted into 13 operational taxonomic units (OTUs) based on their phenotype similarity and phylogeny of their ITS regions of rDNA (ITS1–5.8S–ITS2). The OTUs represent five known species (*G. flava, G. langdonii, G. lavendula, G. pallida, G. putterillii*) and seven undescribed taxa. Most of the bark beetles were associated with on average 1–2.5 OTUs per sample. *G. lavendula,* considered very uncommon in nature, was found as a common associate of bark beetles. Six out of 13 OTUs were found to be distributed in the Mediterranean but not in neighbouring areas of temperate Europe suggesting that *Geosmithia* spp. have a geographically limited distribution, probably due to their dependency on the geographically limited area of their vectors. The proportion of generalists and specialists among *Geosmithia* spp. was smaller compared with data from temperate Europe. A possible explanation is the effective dispersal of *Geosmithia* by polyphagous bostrichids across the niches defined by mutually exclusive bark beetles.

Kolařík, M., Kubátová, A., Cepicka, I., Pažoutová, S., and Srutka, P. 2005. A complex of three new white-spored, sympatric, and host range limited *Geosmithia* species. *Mycol. Res.* 109(12): 1323–1336.

All hypocrealean species of the genus *Geosmithia* are anamorphic fungi with connections to bark beetles. *G. fassatiae, G. langdonii* and *G. obscura* are described as new sympatric species associated with *Scolytus carpini, S. intricatus* and *S. rugulosus* in Central Europe. The species represent a complex of three sister taxa with affinities to *G. flava* that may be distinguished by differences in morphology, unique RAPD patterns and by sequences of ITS region rDNA.

Intraspecific variability and habitat specificity of new species is described and discussed. The high morphological, genetic and ecological uniformity suggest that these *Geosmithia* spp. are recently derived. A key to all accepted hypocrealean species of the genus is provided.

Kolařík, M., Kubátová, A., Hulcr, J., and Pažoutová, A. 2008. *Geosmithia* fungi are highly diverse and consistent bark beetle associates: Evidence from their community structure in temperate Europe. *Microbial Ecol.* 55(1): 65–80.

Geosmithia spp. (Ascomycota: Hypocreales) are little-studied, dry-spored fungi that occur in galleries built by many phloeophagous bark beetles. This study mapped the distribution and environmental preferences of *Geosmithia* species occurring in galleries of temperate European bark beetles. One hundred seven host tree samples of 16 tree species infested with 23 subcortical insect species were collected from across Europe during the years 1997–2005. Over 600 *Geosmithia* isolates from the beetles were sorted into 17 operational taxonomic units (OTUs) based on their phenotype similarity and phylogeny of internal transcribed spacer (ITS) region of rDNA (ITS1-5.8S-ITS2). The OTUs represent six known species and eight undescribed taxa. Ninety-two samples infested with subcortical insects were characterized by the presence/absence of OTUs and the similarity among the samples was evaluated. Geographically distant populations of the same beetle species host relatively uniform *Geosmithia* communities across large geographic areas (ranging from southern Bulgaria to the Czech Republic). This suggests effective dispersal of *Geosmithia* spp. by bark beetles. Clustering of similar samples in ordination analysis is correlated predominantly with the isolation source (bark beetles and their respective feeding plant), but not with their geographical origin. The composition of the *Geosmithia* OTU community of each bark beetle species depends on the degree of isolation of the species' niches. Thus, *Geosmithia* communities associated with regularly co-occurring bark beetle species are highly similar. The similarity decreases with decreasing frequency of beetle species' co-occurrence, a pattern resembling that of entomochoric ophiostomatoid fungi. These findings suggest that: 1) communities of *Geosmithia* spp. are vector-specific; 2) at least in some cases, the association between *Geosmithia* OTUs and bark beetles may have been very stable and symbioses are likely to be a fundamental factor in the speciation of *Geosmithia* fungi; and 3) that even nonsticky spores of *Geosmithia* are suitable for maintaining an insect–fungus association, contrary to previous hypotheses.

Kolařík, M., Kubátová, A., and Srutka, P. 2004. Morphological and molecular characterization of *Geosmithia putterillii*, *G. pallida* comb. nov. and *G. flava* sp. nov., associated with subcorticolous insects. *Mycol. Res.* 108(9): 1053–1069.

Geosmithia putterillii is an anamorphic fungus with connections to bark beetles. Genetic variability of 89 isolates traditionally grouped in *G. putterillii* and *G. lavendula* isolated from different geographical regions from subcorticolous insects and from other unspecific substrata was assessed using RAPD, sequencing of the ITS region (ITS1-5.8SrDNAITS2) and morphological characters. RAPD analysis revealed eight distinct groups. One group was represented by *G. lavendula* type strain and showed no relations to any other isolate. Five RAPD-types with similar ITS sequences and phenotype were related to the ex-type strain of *Penicillium pallidum* (generally given as a synonym of *G. putterillii*). Because of unique phylogenetic position and a phenotype markedly different from *G. putterillii*, the new combination *G. pallida* is made here. For another group of isolates formerly identified as *G. putterillii* the new species *G. flava* is described based on a characteristic RAPD-type, a unique ITS sequences and a different phenotype. These newly recognized species are stable in culture and with worldwide distribution.

Kolařík, M., Stodulková, E., Kvresinová, Z., Kuzma, M., Sulc, M., Man, P., Novák, P., Marsík, P., Landa, P., Olsovská, J., Chudícková, M., Pazoutová, S. Cerný, J., Bella, J., and Flieger, M. 2009. Hydroxylated anthraquinones produced by *Geosmithia* species. *Folia Microbiol.* 54(3): 179–187.

Geosmithia fungi are little known symbionts of bark beetles. Secondary metabolites of lilac colored species *G. lavendula* and other nine *Geosmithia* species were investigated in order to elucidate their possible role in the interactions of the fungi with environment. Hydroxylated anthraquinones (yellow, orange, and red pigments), were found to be the most abundant compounds produced into the medium during the submerged cultivation. Three main compounds were identified as 1,3,6,8-tetrahydroxyanthraquinone (1), rhodolamprometrin (1-acety1-2,4,5,7-tetrahydroxyanthraquinone; 2), and 1-acety1-2,4,5,7,8-pentahydroxy-anthraquinone (3). Compounds 2 and 3 (representing the majority of produced metabolites) inhibited the growth of G super(+)-bacteria *Staphylococcus aureus* and *Bacillus subtilis* with minimum inhibitory concentration of 64-512 mu g/mL. Anti-inflammatory activity detected as inhibition of cyclooxygenase-2 was found only for compound 3 at 1 and 10 mu g/mL. Compound 2 interfered with the morphology, compound 3 with cell-cycle dynamics of adherent mammalian cell lines.

Kubátová, A., Kolařík, M., Prasil, K., and Novotný, D. 2004. Bark beetles and their galleries: Well-known niches for little known fungi on the example of *Geosmithia*. *Czech Mycol.* 56: 1–18.

The oak bark beetle (*Scolytus intricatus*, *Scolytidae*, Coleoptera) was studied during the years 1997- 2003 with respect to the occurrence of microscopic fungi on the surface of its body. Samples were collected in eight localities in the Czech and Slovak Republics. The investigation was focused on all different stages of the beetle´s life cycle: eggs, larvae, adults before emergence, adults in generation and maturation feeding (nearly 600 samples), and also on galleries (400 samples). The most frequent fungi associated with *S. intricatus* were yeasts, *Geosmithia* spp. and *Penicillium* spp. Ophiostomatoid fungi were isolated, too. Great attention was paid to the occurrence of *Geosmithia* spp., which were so far recorded rarely. They were frequently found in all stages of the life cycle of *Scolytus intricatus*, except for males in maturation feeding. The ecology of *Geosmithia* spp. in feedings of phloem inhabiting insects is discussed for their negative cellulase production and the ecology of associated insect species. Trees infested with *Scolytus intricatus* represent a major and still little explored niche of *Geosmithia* spp.

Ogawa, H., Yoshimura, A., and Sugiyama, J. 1997. Polyphyletic origins of species of the anamorphic genus *Geosmithia* and the relationships of the cleistothecial genera: Evidence from 18S, 5S and 28S rDNA sequence analyses. *Mycol.* 89(5): 756–771.

The anamorphic genus *Geosmithia*, with the type species *G. lavendula*, includes species strictly lacking a teleomorph as well as species associated with the teleomorphs *Talaromyces* and *Chromocleista*. Our 18S rDNA sequence-based tree, inferred from 1586 alignable sites from 57 selected taxa within the Ascomycota and using two basidiomycetes as out- groups, clearly demonstrates that *Geosmithia* is a poly- phyletic taxon with evolutionary affinities to at least three groups of the euascomycete lineage within the Ascomycota: (1) *Geosmithia lavendula*, the type of the genus, *G. putterilli*, and the hypocrealean fungi, *Gliocladium*-producing *Hypocrea lutea* and *Verticillium/ Sepedonium*-producing *Hypomyces chrysogenum* within the pyrenomycete lineage, appear as a monophyletic group with 100% bootstrap support; (2) *G. cylindrospora* and the *Geosmithia*-producing *Talaromyces* species (i.e., *T. bacillisporus, T. ebruneus*, and *T emerson*- ii) group with the *Penicillium*-producing *T. flavus* var. *macrosporus* of the Trichocomaceae within the plectomycete lineage in 57% of bootstrap replicates; (3) *G. namyslowskii*, the *Geosmithia*-producing *Chromocleista malachitea*, the *Penicillium*-producing *Eupenicillium crustaceum* and the *Merimbla*-producing *Talaromyces avellaneus* (= *Hamigera avellanea*) of the same higher categories appear to be a monophyletic group in 74% of bootstrap replicates. The boot- strapped NJ and MP analyses, using 1706 sites of the same gene only from plectomycetes and pyrenomycetes, demonstrate similar phylogenetic relationships. The bootstrapped NJ and MP analyses, based on 70 alignable sites of 5S rDNA, support the results from 18S rDNA sequence analyses. Within the *Hypocreales*, *G. lavendula* and *G. putterillii* group with the hypocrealean fungi, cleistothecial,

Acremonium- producing *Mycoarachis inversa* and *Emericellopsis terricola*, and the strictly anamorph species of *Acremonium* in 66% or greater of the bootstrapped NJ and MP trees derived from 28S rDNA partial (580 sites) sequences. Phylogenetic considerations are also presented for several cleistothecial taxa.

Pepori, A.L., Kolarik, M., Bettini, P.P., Vettraino, A.M., and Santini, A. 2015. Morphological and molecular characterisation of *Geosmithia* species on European elms. *Fungal Biol.* 119(11): 1063–1074.

Species of the genus *Geosmithia* are associated with insect species, mainly bark beetles. On *Ulmus* spp., the same beetles are also vectors of *Ophiostoma ulmi* s.l., the agent of Dutch elm disease (DED), a worldwide elm disease. Aim of this paper is to characterise *Geosmithia* species associated with elms and/or elm beetles in Europe. Seventy-two strains representative of all morphological taxonomic units were used to build a phylogenetic tree based on ITS, β-tubulin and elongation factor 1-α gene regions. On the basis of molecular and morpho-physiological traits, seven taxonomic entities were identified. In addition to the species previously known our results assigned strains previously identified as *Geosmithia pallida* to two separate taxa: *Geosmithia* sp. 2 and *Geosmithia* sp. 5. Two new species, *Geosmithia omnicola* and *Geosmithia ulmacea*, are described. Two strains were assigned to the partially described species *Geosmithia* sp. 20. *Geosmithia* species living on *Ulmus* do not discriminate between elm species, but between different environments. The association between *Ulmus* and *Geosmithia* is common, stable, and seems to be related to specific vectors. The relationship between *Geosmithia* and *Ophiostoma* would deserve further investigation, as these fungi share the same vectors and habitat for a significant part of their life cycles.

Pitt, J.I. 1979. *Geosmithia*, gen. nov. for *Penicillium lavendulum* and related species. *Can. J. of Bot.* 57(19): 2021–2030.

Geosmithia Pitt is erected to accommodate species, previously placed in *Penicillium*, the following combination of characters: colonies with conidia in colors other than grey-blue or grey-green. penicilli with all elements roughened, and with both phialides ant1 conidia cylintlroidal. *Geosmithia lavendula* (Raper & Fennell) Pitt, *G. putterillii* (Thorn) Pitt, *G. namyslowskii* (Zalcski) Pitt. *G .cylindrospora* (G. Smith) Pitt. *G*. a*rgillacea* (Stolk, Evans & Nilsson) Pitt. et *G. emersonii* (Stolk) Pitt are described as new combinations; *G. swiftii* is a new species for the anamorphic state of *Talaromyces baci1lisporus* (Swift) C. R. Benjamin. A key is provided.