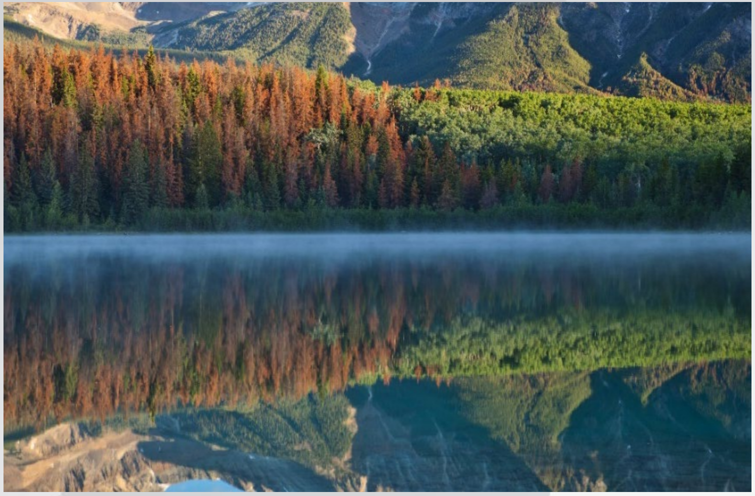

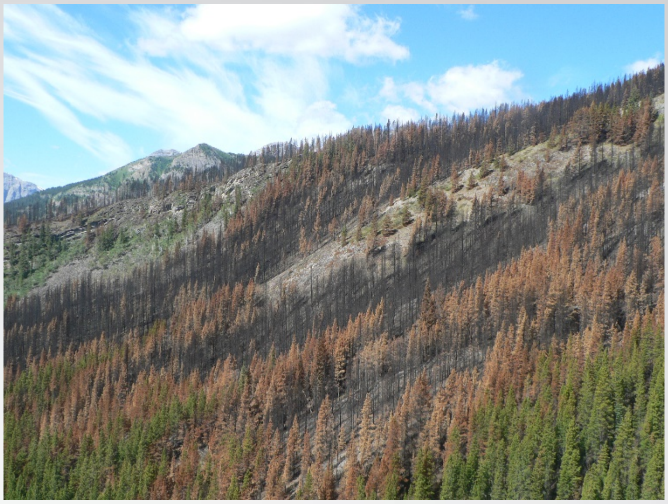

Annotated Bibliography for the TRIA Network

TRIA Network

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CONTEXT

The recent mountain pine beetle (MPB) epidemic has had unprecedented impacts on western Canadian forests, devastating over 1 billion cubic meters of mature pine trees. The resulting social, economic, and ecological implications to the landscape and its communities have been a key management challenge for policy makers and forest managers. Conducting research and developing the necessary tools to better understand MPB spread and its effects is critical to address these impacts. The “Turning Risk Into Action Network” (TRIA-Net) was developed to address these concerns and knowledge gaps, by leveraging the experience and innovation of participating scientists, government, not-for-profit, and industry organizations.

In 2013, the Natural Science and Engineering Council (NSERC), Strategic Network Grants program, funded the TRIA-Net to facilitate research aimed at protecting Canadian forests through science-based strategies to control spread of the MPB. The network builds off several years of collaborative research that began as the TRIA Project in 2007 which was originally funded by Genome Canada, Genome Alberta, and Genome BC. The original TRIA Project focused on the interactions between mountain pine beetle, host pine trees, and symbiotic fungi—the three core elements of the outbreak system.

Together, this work has brought together multiple perspectives and fields of study to provide new insights into the MPB outbreak. The range of expertise and research ranges from entomology to functional genomics, to landscape ecology.

The TRIA-Net research team is comprised of 18 multidisciplinary co-investigators from:

- University of Alberta
- University of British Columbia (Vancouver and Okanagan campuses)
- Université Laval
- Université de Montreal
- University of Northern British Columbia
- Natural Resources Canada – Canadian Forest Service
- Alberta Innovates Technology Futures, and
- University of Minnesota.

To ensure the research outcomes effectively inform decision-making, the team partnered with various government, not-for-profit, and industry organizations:

- Alberta Agriculture and Forestry,
- fRI Research,
- Manitoba Conservation and Water Stewardship,
- Millar Western,
- Natural Resources Canada – Canadian Forest Service,
- Northwest Territories Department of Environment and Natural Resources,

- Ontario Ministry of Natural Resources and Forestry,
- Saskatchewan Ministry of Environment,
- West Fraser, and
- Weyerhaeuser.

As a result, an exceptionally large body of new knowledge has been developed that is improving our understanding of the MPB system, with over 100 publications from the TRIA-Net and earlier TRIA Project.

To bring this work together, this annotated bibliography was created to provide concise summaries of a large sample of this research. The summaries have been compiled in this accessible format to promote knowledge transfer between project partners. Seventy-eight of these research publications, ranging from the years 2009-2019, were selected for the annotated bibliography with a priority placed on the most recent research. The document is intended to be a 'living document', in that future reports and research can be added to this annotated bibliography. Each summary provides a 0.5-1 page overview of the publication highlighting the key findings, methods, and implications. The summaries are ordered by publication year, starting with the most recent publications. To access the publications that were not included in this annotated bibliography, a complete list is available through the NSERC TRIA-Net website.

FUNDING AND SUPPORT FOR THE ANNOTATED BIBLIOGRAPHY

The authors wish to thank the Forest Resource Improvement Association of Alberta (FRIAA) and its members and fRI Research through their Mountain Pine Beetle Ecology Program (MPBEP) for the funding and support of this annotated bibliography. Thanks go to Tom Daniels, West Fraser and member of the Mountain Pine Beetle Activity Team at fRI Research; Keith McClain and fRI Research for taking initiative to secure funding for this work; and staff at Fuse Consulting for compiling the Annotated Bibliography.

EXECUTIVE SUMMARY

The TRIA-Net is a collaborative research initiative that has supported research related to MPB outbreaks in western Canada for over a decade. Together, this work has resulted in a large volume of interconnected and multidisciplinary research using genomics, molecular analyses, population genetics, systematics, ecology, population dynamics, and modeling to provide new insights about MPB and host relationships and the factors influencing spread. The findings from this research can help forest managers and decision-makers develop and improve the necessary tools for monitoring and managing MPB spread and its impacts. This annotated bibliography was created to bring this body of research together to facilitate information transfer between project partners.

Summary of Key Outcomes to Date from the TRIA Network

Sequencing the Beetle Genome

Sequencing the Mountain pine beetle's (MPB) genome must be recognized as one of the TRIA Network's most significant contributions. While there are over 400,000 beetle species, many of which are critical pests in agriculture sector, the MPB is only the second beetle genome to be fully sequenced. The MPB genome includes 12 pairs of chromosomes and approximately 13,000 genes. The genome sequencing of MPB has led to a better understanding of beetle physiology including that related to the complexities of pheromone synthesis, antennae olfaction, detoxification, overwintering development, and dispersal by flight. Understanding the genome has furthered understanding of the structure and natural variation of MPB populations throughout Alberta and beyond. It has also shed light on how the MPB is able to adapt to novel hosts, potentially expand its geographical range, and potentially adapt to a new and everchanging climate.

Sequencing the Fungi Genome

Sequencing the genome of the MPB's principal fungi *Grosmannia clavigera* is also a major accomplishment. This genome includes some 8,314 protein-coding genes and provides the basis for understanding the molecular mechanisms that allow this species to overcome the multiple chemical defense mechanisms of pine host trees. These adaptive mechanisms have been acquired through the process of evolution and define the interactions between host tree, the fungal pathogen, and its beetle vector. It is worth highlighting here that both the fungi and the beetle have an evolutionary advantage over their pine hosts because of the short life cycles of the beetle and fungi.

Biochemical Response and Genomics

Sequencing the genomes of the MPB and its principal fungi has led to an extensive effort by the TRIA Network to characterize genes. This has been largely accomplished by defining the functional role of genes in both the production of, and mitigation of, secondary metabolite defenses. Research has also related genes to the production of cryoprotectants, to quality of host tree, and to overwintering survival. This extensive body of new knowledge has created a foundation for research to further clarify the complex relationships between MPB, its host species and its principal fungi.

Origin of Epidemic

The distinct genetic signatures of individual beetle populations may provide an approach to track MPB populations as they spread eastward. Knowing these genetic structures can also help identify key populations that are contributing to the spread. For example, recent research has revealed the current MPB in Jasper National Park is comprised of both northern and southern MPB populations. This hybrid population may result in the creation of novel genetic material and increased adaptive potential for the beetle throughout Alberta. As such, genetic markers may potentially inform management strategies.

Jack Pine Resistance

There is growing evidence that MPB can both survive and sustain their populations in jack pine forests. This is of major concern as the leading edge of MPB has reached eastern Alberta where the forest composition shifts from lodgepole to jack pine through a hybrid zone. Susceptibility of jack pine may facilitate the spread of the MPB throughout the boreal forest of Canada.

There has long been concern that MPB may be able to take advantage of the "*evolutionary naivety*" of jack pine and exploit this species more effectively than lodgepole pine with which they have coevolved. However, jack pine response to MPB and its principal fungi is complex. For example, monoterpene response in jack pine indicates that it has a low capacity to inhibit early stages of beetle colonization, leaving jack pine trees open to mass attacks. But research has revealed that jack pine invests resources into xylem terpenoid defenses and the development of vertical xylem resin ducts. These investments hinder the principal fungi from inhibiting water uptake in jack pine trees. There is also evidence of gene flow between jack pine and lodgepole pine, which may provide western populations of jack pine with genes beneficial to defending against the MPB. Despite these key advances in knowledge, there is a continual need for study into the complexities of jack pine defenses and how they interact with climate.

Drought Implications

There is evidence that water stress does appear to impede the defense mechanisms of the host pine species. Studies also revealed the relationship between tree defense responses and drought is inherently complex at the molecular level. The observed reductions in the strength of defense mechanisms may, in part, be related to the effect of drought stress on photosynthesis and the production of carbohydrates. There is also evidence that drought increases the body size of emerging larva and is therefore beneficial to MPB. Periods of severe drought may facilitate the spread of MPB beyond its natural zone and is of concern in the context of climate change.

Future of Genomics

The TRIA Network has demonstrated that significant advances have been achieved in the field of genomics over the past 20 years. This body of research has demonstrated not only the complexities of the interactions between trees, insects and fungi found within Alberta's forests, but also how much work remains. There can be little doubt that advances in tree genomics will continue to accelerate and play a central role in helping to understand interactions between, and the functional characteristics of, a wide array of genes. These in-depth understandings are now more important than ever as provinces face an ever-changing environment. Success of the TRIA Network, and in particular the wide array of methodologies developed by the team of research partners, should facilitate further genomics investigations. As costs associated with gene sequencing continue to decline, genomics will expand to not only inform ecological process but also contribute to tree improvement and genetic conservation. Genomics is also anticipated to inform the emerging field of chemical ecology.

Implications and Applications

The TRIA-Net program has made considerable advances in understanding MPB ecology and how the outbreak system functions. The diversity of the TRIA-Net research team enabled perspectives to be generated on topics related to entomology, functional genomics, and landscape ecology. New knowledge from these domains has had a real impact on how the outbreak is managed in both the historical and expanded ranges of the MPB. The accumulated scientific knowledge of the program has enhanced the determination of risk and risk management related to mountain pine beetle, and this has in turn informed the allocation of resources.

Specific areas of application of this information have been in assessing stand susceptibility, predicting spread, and developing better monitoring approaches. These outcomes have helped managers determine key areas of threat and take management actions to control the spread of the mountain pine beetle. Key learnings have also helped assess the susceptibility of jack pine to the spread of MPB, and to inform national strategies and partnerships to slow the spread of the MPB in Alberta. Overall, the TRIA-Net program has been a core example of how basic science is needed to help understand novel systems and to inform effective and lasting management outcomes.

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INTRODUCTION

Since the mountain pine beetle (MPB) began to spread at unprecedented rates, beyond its historic range, the impacts have been devasting to western Canadian pine forests. Understanding the various dynamics of MPB populations and their spread is critical to mitigating the risks of spread and manage the extent of its damage. To address impacts related to the MPB epidemic, the TRIA Network (Turning Risk into Action for the Mountain Pine Beetle Epidemic) has supported a large volume of interdisciplinary research aimed at filling knowledge-gaps to help better understand MPB and control its spread. The collaborating research team has also partnered with members of government, industry, and not-for-profit organizations to bring this research to life and into operational and policy decisions.

This annotated bibliography brings this research together in an accessible and useful format for project partners to understand the key outcomes and contributions of the TRIA Network and to inform future planning. Overall, the research provides new insights and tools to enable decision-makers to develop the best strategies for monitoring, controlling, and addressing the impacts of MPB Spread.

METHODS

Peer-reviewed publications related to projects funded through the TRIA Network between 2009 and 2019 were reviewed, and 0.5—1 page summaries were developed for each document. Each summary provides a concise and accessible synthesis of the publications, highlighting key outcomes and implications. The summaries are ordered by publication year, starting with the most recent publications.

ANNOTATED BIBLIOGRAPHY

2019

C. C. CHIU, C. I. KEELING AND J. BOHLMANN. 2019. CYTOCHROMES P450 PREFERENTIALLY EXPRESSED IN ANTENNAE OF THE MOUNTAIN PINE BEETLE. J. CHEM. ECOL. 45:178-186. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- “P450” enzymes are of significant interest to researchers in the field of physiology because these enzymes can metabolize and detoxify numerous substrates and they also perform multiple oxidative reactions. They are found in all insects. The MPB genome contains 86 different P450 genes but, despite their importance, the functions assigned to each are largely unknown.
- This study was designed to identify and analyze transcript expression profiles of P450 that may be involved in olfaction, pheromone biosynthesis, and detoxification of host defense chemicals. Understanding the biochemical system of olfaction in antennae was of high priority to these researchers as olfaction is involved in host selection and the detection of very low concentrations of pheromones.
- This study identified six P450 enzymes thought to have a role in olfaction. This body of research remains in its infancy, though, and further study is needed before any applications can be made to managing the MPB epidemic.

Approach:

- The research team’s primary objective was for this investigation to serve as a “foundation for future research” in the area of cytochromes P450, with an overall goal of better understanding MPB physiology.
- Tree bolts were collected from infected trees located at a site near Whistler, British Columbia in 2014.
- Three types of MPB beetles were tested: 1) fourth-star larvae collected from bolts by dissecting bark under laboratory conditions; 2) adult beetles that had emerged from bolts several weeks later; and 3) adult beetles that had emerged and which were then introduced to unaffected bolts for several days to simulate early colonization phase.
- Male and female beetles were separately dissected into antennae, heads without antennae, midguts and fat bodies. Transcriptome analysis was undertaken to identify P450 enzymes in MPB with specific focus on those in beetle antennae.

Key Findings and Implications:

- Transcript expression of the different P450s varied significantly with tissue type and stage of development.
- Differential transcript abundance suggested specific and overlapping roles of P450s in olfaction, pheromone biosynthesis and in detoxification. Six of the P450s were assigned a role in olfaction, three were assigned a role in detoxification, and two assigned to a role in pheromone biosynthesis.
- These results lay the foundation for future work pertaining to the remaining 79 P450s to determine their specific substrates, products and roles in MPB physiology.

K. L. Jones, V. A. Shegelski, N. G. Marculis, A. N. Wijerathna and M. L. Evenden. 2019. Factors influencing dispersal by flight in bark beetles (Coleoptera: Curculionidae Scolytinae): from genes to landscapes. Can. J. For. Res. 49: 1024-1041. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Despite the ecological and economic impacts of MPB, significant knowledge gaps remain in modelling beetle dispersal. This publication prepared a literature review to provide guidance to entomologists on research priorities and outlined a call for further investments into predictive modelling which can aid forest managers.

Approach:

- This research paper represented a detailed review of the literature that focused on the morphological, physiological, biotic and abiotic factors that influence dispersal.

Key Findings and Implications:

- Development of robust predictive models for MPB dispersal requires superior efforts in the integration and parameterization of beetle physiology, morphology, genetics and the role of abiotic and biotic factors that define the beetle environment.
    - Abiotic factors affecting dispersal include light, humidity, landscape features, temperature and wind.
    - Biotic factors include population dynamics, endoparasites, stand density and composition, and ectoparasites.
        - Morphological factors include sex, sexual size and dimorphism, wing size, mass and flight muscles.
        - Physiological factors include amino acids, lipids, proteins, genetics and carbohydrates.
- Understanding factors that influence species establishment and speed of dispersal are critical to defining how to best respond to beetle outbreaks. This includes knowing the minimum population levels needed to sustain a population.
- Superior models are increasingly needed in the face of a rapidly changing climate, as small changes in the physical environment can trigger extreme outbreaks.

R. GONZALES AND L. PARROTT. 2019. HOW STAKEHOLDERS STRUCTURE THEIR COLLABORATIONS TO ANTICIPATE AND TACKLE THE THREAT OF MOUNTAIN PINE BEETLE IN THE JASPER-HINTON (ALBERTA, CANADA) AREA. CAN. J. FOR. RES. 49:480-490. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study used network theory to understand the level of collaboration and exchange of information between stakeholders dealing with the MPB outbreak in the Jasper and Hinton regions.
- The structure of a stakeholder network was largely influenced by geographic, institutional, economic and ideological factors. This strong clustering effect played an important role in restricting information flow, resource sharing and trust, and in possibly introducing bias in policy decision making processes.
- The authors argued that more investments are required to forge and build lasting relationships among a wider range of stakeholders. They further emphasized that an effort to include the interests of a wider range of stakeholders could lead to the development and adoption of more sustainable forest management practices and policies to deal with the current beetle outbreak.
- Finally, the authors noted that such clusters are not uncommon among stakeholders, but the latter must acknowledge that such an arrangement is certainly not ideal and may lead to a level of fragility within the network as a whole.

Approach:

- A questionnaire was sent to a range of players in the Hinton and Jasper area. These included municipal, provincial and federal departments, fRI Research, academics, First Nations and local community groups.
- Responses to the questionnaire were solicited through a series of in-person interviews, telephone interviews and an on-line questionnaire. A total of 90 responses were received though none of these were from First Nations groups. The lack of participation by First Nation communities was identified as a major shortcoming of the study.
- Data was analyzed using a combination of network mapping and graph theory. The latter facilitates the identification of clusters and outliers.

Key Findings and Implications:

- The most dominant flow of information was that between Hinton Wood Products, the Department of Agriculture and Forestry and fRI Research. These tight linkages were attributed to the shared goal of enhancing the development of the local economy. As a result, information sharing was greatest within this small cluster. The authors were careful to point out that the strength of this cluster may be detrimental as other stakeholders may feel alienated.
- In sharp contrast, the Municipality of Jasper was isolated from the rest of the knowledge network. This isolation was attributed to the conservation mandate of Parks Canada. Further, the Municipality was connected to the rest of the network only through the work of a limited number of individuals associated with the Canadian Forest Service and Parks Canada.
- Academics were present in the network, the majority of which were researchers with the University of Alberta. Interestingly, respondents separated these academics into two groups: researchers studying genetics and the researchers studying ecology and entomology. The academics were found at the edges of the network, meaning they were relied for information and not as information connectors.
- The authors argued that more efforts are required to build lasting relationships among a wider range of stakeholders. An effort to include the interests of a wider range of stakeholders could lead to the development and adoption of more sustainable forest management practices and policies.

V. A. SHEGELSKI, M. L. EVENDEN AND F. A. H. SPERLING. 2019. MORPHOLOGICAL VARIATION ASSOCIATED WITH DISPERSAL CAPACITY IN A TREE-KILLING BARK BEETLE DENDROCTONUS PONDEROSAE HOPKINS. AGRICULTURE AND FOREST ENTOMOLOGY. 21: 79-87. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Female beetles that were heavier and had larger wing size flew longer distances when using a flight mill. However, flight in these beetles was not solely a function of morphological features, but rather may be much more complex, making the development of dispersal models more challenging.

Approach:

- This study compared pre-flight body weight and wing size of female MPB to flight distance using a flight mill.
- Four lodgepole pine bolts were collected from trees infested with MPB and subsequently stored in laboratory conditions. A total of 124 female beetles emerged and were used in this study.
- Flight distance, duration and frequency data were collected using a flight mill using well accepted methodologies. Data was collected over a 22-hour span. Beetle weights were measured pre- and post-flight.
- Beetles were subsequently dissected for morphological measures. This included detailed measurements of their wings and muscular structures.

Key Findings and Implications:

- Flight data results were highly variable. The average flight distance was approximately 5.5 km with a maximum flight distance of 28.8 km. Forty-four beetles did not fly at all.
- Long distance and short distance fliers had different morphological features. For example, long distance fliers had greater pre-flight body weight and body density. This observation is consistent with that of other studies, implying that heavier beetles have greater lipid contents, and hence more fuel for long distance flight.
    - Other studies have found that bark beetles with large fat reserves were less responsive to aggregation pheromones and more aggressive in seeking out new hosts. The possible relationship between olfactory sensitivity and fat contents could be linked to the success of colonizing a new host and subsequent reproduction.
- Larger wing size could be an advantage in colder climates where muscle function is impaired due to low temperatures. Large wing size may also have enhanced the spread of beetles over the Rocky Mountains, but this remains totally speculative.
- The lack of flight in 44 beetles might suggest that something was wrong with these beetles; however, when they were tested on the flight mill they opened their wings passively but did not flap them. This may suggest that this population of beetles were programmed for “passive dispersal” representing a “drifting behavior.” The authors suggested that this behavior was consistent with beetles being adapted to utilize updrafts for dispersal, and that the failure to beat their wings was a strategy to reduce energy consumption.
- While the wide range in flight distances (0–28.8 km) may offer significant population-wide advantages, it does pose a significant barrier to modelers in predicting population-wide dispersal. In this study, less than 20% of variation in flight distance could be explained by morphological features. Indeed, the results of this paper indicate quite clearly that many of the factors that define beetle flight remain unidentified.

A. WIJERATHNA, C. WHITEHOUSE, H. PROCTOR AND M. EVENDEN. 2019. TESTING FOR TRADE-OFFS BETWEEN FLIGHT AND REPRODUCTION IN THE MOUNTAIN PINE BEETLE (COLEOPTERA: CURCULIONIDAE) ON TWO PINE (PINACEAE) HOSTS. THE CANADIAN ENTOMOLOGIST, 151(3): 298-310. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This is the first study of the effect of dispersal activity on subsequent reproduction in different host species.
- Results revealed, for the first time, that MPB possess a physiological trade-off between flight and reproductive success. Long-distance dispersal may decrease beetle fitness yet aid in the search for high quality hosts and mating opportunities with previously unrelated beetles.
- This study confirmed that jack pine is a suitable host for MPB, though offspring emerging from jack pine had slightly inferior body condition than those which emerged from lodgepole pine.
- Aerial dispersal, which may be local in nature (i.e., within a stand) or may take place over vast landscapes, is energetically costly. This energetic investment may come at a significant cost in terms of lower pheromone production in males and production of smaller eggs.

Approach:

- This study investigated the effect of adult beetle dispersal activity on their reproduction in pine hosts.
- Bolts were collected from three MPB-infested lodgepole pine stands near Grande Prairie, Alberta and affected with MPB. Bolts were also collected from non-affected lodgepole and jack pine stands near Edson.
- Bolts from the infested lodgepole pine stand were managed in the laboratory until beetle emergence. Beetles were either weighed and then tethered to a flight mill for a period of 23 hours, or assigned to a no-flight control group.
- Bolts collected from the unaffected lodgepole and jack pine stands were inoculated with beetles from both the flight and control pools. Emerging beetles were counted, sexed, measured (pronotum width and body length), weighed, and their fat content measured.

Key Findings and Implications:

- Female beetles flew for longer times and longer distances than male beetles. As expected, beetles that were flown showed significant weight loss, illustrating the trade-off of dispersal and energetics.
- Significantly more offspring were produced in jack pine bolts. Pre-flight resulted in a significant reduction in offspring production and this effect was greatest in jack pine.
- Pre-flight did not affect the body size of offspring. This would suggest that fat lost in pre-flight was made up for in subsequent host feeding.
- Beetles that emerged from jack pine bolts had the highest fat content, but overall body condition was slightly higher in beetles that emerged from lodgepole pine bolts. Further studies of this nature are needed.

2018

L. C. Horianopoulos, C. K, Boone and G. D. N. G. Samarasekera, G. K. Kandola and B. W. Murray. 2018. Selection of the sex-linked inhibitor of apoptosis in mountain pine beetle (*Dendroctonus pondersosae*) driven by enhanced expression during early overwintering. Ecology and Evolution. 8: 6253-6264. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- MPB has spread in recent years to novel, colder habitats that have included high elevations in the United States and forests in northern Alberta. Identification of genes that have allowed the beetle to expand its range into colder habitats may provide important insights as to how the beetle has adapted to overcome defenses of its host species.
- Early expression of the 162-bp allele may give these larvae a competitive advantage by allowing them to have enhanced survival during early cold periods.

Approach:

- Beetles were sampled from the Grande Prairie, Mackenzie and Houston areas, and Banff (“northern sample”) and three locations in southern British Columbia (“southern sample”).
- A second sample of beetles was collected from 52 known populations across the western United States. This sample was used to identify gene-linked microsatellite for IAP-bp (inhibitor of apoptosis).

Key Findings and Implications:

- The authors hypothesized that a sex-based allele identified as 162-bp may be important in providing local populations with a protective mechanism against harsh cold climates.
- No differences in IAP expression were found between male and female beetles. However, the overall expression of IAP increased between Oct. 3–10, which corresponded with a cooling period.
- Further research was recommended into the evolution of sex-based genes and their role in adapting to local climates.

S. A. L. Trevoy, J. K. Janes and F. A. H. Sperling. 2018. Where did mountain pine beetle populations in Jasper Park come from? Tracking beetles with genetics. The Forestry Chronicle. 94(1): 20-24. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The source of MPB in the Jasper National Park area is debated. The infestation may be the result of a slow spread southeast of the northern population of beetles from the Grande Prairie area, which had exceptionally high populations on beetles in 2009, or it may be from Robson Provincial Park in the immediate west.
- Current beetle populations in the Jasper National Park area of Alberta comprise mixed individuals of both the northern and southern populations.
- Genetic markers can be used to identify the origin of novel populations, facilitate precise monitoring of beetle expansions and potentially inform management strategies.

Approach:

- A total of 175 MPB were collected from 33 sites in BC and Alberta between 2007 and 2015.
- Beetles were also collected from an area south of Grande Prairie and near Canmore. These collections were used as breeding pairs to create artificially hybridized individuals of north and south descent. A total of 13 offspring were bred.
- Standard genome-wide sampling of DNA markers and analysis were carried out on this population of 188 beetles.

Key Findings and Implications:

- Northern and southern beetle populations had clear genetic differences. However, the populations from Jasper Park and the crosses were more similar to each other than to either the southern or northern populations.
- Beetles in the Jasper National Park area appear to originate from central BC. In sharp contrast, MPB collected in Yellowhead County east of Jasper National Park were most similar genetically to the northern population, strongly suggesting that the Grande Prairie region was the source of these beetles.
- The intermediate nature of the MPB population in the Jasper Park area is noteworthy. Hybrid populations may result in the creation of novel genetic material and increased adaptive potential for the beetle throughout Alberta.
- The authors argued that if left unchecked, the current infestation in the park may lead to increased adaptive potential of the Jasper National Park population.
- The distinct genetic signatures of individual beetle populations may provide an approach to tracking populations as they spread eastward and identify key areas that are contributing to that spread.
- Efforts to decrease the genetic diversity of populations may have some utility in controlling beetle spread. This approach is the opposite of conventional genetic conservation efforts.

C. H. McAllister, C. E. Fortier, K. R. St. Onge, B. M. Sacchi, M. J. Nawrot, T. Locke and J. E. K. Cooke. 2018. A novel application of RNase H2-dependent quantitative PCR for detection and quantification of *Grosmannia clavigera*, a mountain pine beetle fungal symbiont, in environmental samples. Tree Physiology. 38: 485-501. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Lesion length in lodgepole pine does not accurately reflect the extent of fungal colonization of *G. clavigera* along the stem, nor does it reflect the quantity of fungal growth within the stem. An alternative PCR approach is offered which is both sensitive and cost effective.
- This new approach should enable more detailed investigations of the relationship between fungal species, including *G. clavigera* and their pine hosts.

Approach:

- The research team relied on tissue samples collected from a range of studies including field-based trials and laboratory trials.
- Generally, PCR techniques (used to amplify a minute sample of DNA sufficiently for detailed study) are used for the identification of fungal species. They have not previously been used to quantify fungal species in a host.
- The objective of this study was to develop a new PCR test that could be used to quantify a fungal species such as *G. clavigera.* The methodology focused in on an RNase H2-dependent PCR.

Key Findings and Implications:

- The study suggests that lesion length does not provide an accurate measure of the true extent to which a fungal agent such as *G. clavigera* penetrates radially into the tree's sapwood. This important finding underscores that the relationship between lesion formation and the fungal colonization is not as well understood as previously thought.
- The ability to quantify a pathogen's growth within a host is critical to understanding host and fungal interactions. Lesion length has often been used to measure pathogen virility within a host as well as a relative measure of the tree's resistance to the invader.
- The DNA type diagnostic techniques used in this experiment offer a new and exciting approach to study a wide range of host and pathogen factors. Further, the study's results suggest previous estimates of pathogen virility using lesion length alone may be inaccurate.

A. Dhar, L. Parrott and S. Heckbert. 2018. Large scale biotic damage impacts on forest ecosystem services. Scand. J. of For. Res. 33(8): 741-755. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Insect outbreaks have significant impacts to timber supply, but may actually benefit other ecosystem services such as biodiversity. Careful management of residual volume within affected stands is key to the maintenance of long-term ecosystem services and can mitigate the impact of beetle outbreaks.
- When considering a holistic approach to economic impacts, the authors suggest the economic impacts on social issues in rural communities are too often left out.
- Local decision makers are recommended to use an ecosystem service-based approach when developing sustainable forest management plans to address large scale insect outbreaks such as MPB.

Approach:

- The objectives of this paper were to: (1) identify which ecosystem values could be spatially mapped using readily available data, (2) understand how impacts to natural goods and services could enhance our appreciation of the social impacts of infestations, and (3) draw conclusions as to how a natural goods and services approach can inform or assist policy decision makers.
- This is thought to be the first study of its kind to map the spatial extent and distribution of large impacts to natural goods and services from a large insect outbreak.
- The study area consisted of 15.5 million hectares in the interior of British Columbia corresponding to the majority of the pine dominated forests impacted by the current outbreak. No portion of Alberta was included in the study.
- Three categories of ecosystem values were considered: timber and water, climate regulation, carbon storage.
- British Columbia's Vegetation Resources Inventory was adopted to inform disturbance history. Hydrological data consisted of streamflow data collected from 196 active hydrometric stations located within the study area.

Key Findings and Implications:

- The study confirmed the current MPB outbreak in interior BC both directly and indirectly influenced timber, stream water peak flow, and carbon storage in dead pine trees.
- Timber losses were assessed at 805 million m^3. Current and future timber production, and concomitant levels of allowable cut, will be significantly curtailed. The economic impacts of these timber losses are beyond the scope of this study but will heavily impact communities that are forest dependent.
- While many stands lost 100% of their volume, the majority of stands in the study area had significant residual volume (1735 million m^3). This implies the impact of the MPB on ecosystem services may be short-lived so long as management actions encourage regrowth of this residual volume. Stands that have little residual volume will require rapid intervention to restore ecosystem services.
- Insect outbreak and the combined effects of large-scale salvage logging together had significant impacts on peak water flow. Changes in timing of snow melt may lead to soil water deficits at higher elevations and during late summer months. MPB impacts on water quality remain debatable, and some argue outbreaks would result in increased water treatment costs by downstream communities.
- Vegetation diversity was higher in impacted stands than originally expected, with diversity increasing in the northern regions of the study areas.

M. Kunegel-Lion, R. L. McIntosh and M. A. Lewis. 2018. Management assessment of mountain pine beetle infestation in Cypress Hills, SK. Can. J. For. Res. 49: 154-163. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Current detection and control strategies designed to combat MPB are both effective and cost efficient, though small increases to the surveyed area and the integration of predicted model outputs could have added value.

Approach:

- The Cypress Hills area of southwestern Saskatchewan has been infested with MPB for several years and active survey measures have been in place since early days of the current epidemic. Current control strategies call for a “zero tolerance” policy as there is concern that this region may serve as a “stepping stone” for the spread of MPB eastward.
- The objective of this study was to utilize machine learning to compare current strategies with a range of management approaches.
- The study area was divided into over 18 000 cells, each measuring 100 by 100 m. Each cell was populated with variables related to topography, weather, vegetation and beetle pressure. Data was used for the time period 2007–2015. The precise location of red trees was known because of detailed survey data completed by the Canadian Forest Service during the 2011–2013.
- Management control efficiency (the number of infested trees controlled in the park divided by the number of infested trees) was determined in relation to the net survey area. Detailed statistical modeling included a generalized boosted classification model.

Key Findings and Implications:

- Modelling results show that though the current detection strategy (i.e., using 50 m search plot radius) is efficient, a larger survey area and a different strategy would improve efficiency. This is attributed to the fact that MPB dispersal range is often greater than 50 m.
- The current results also confirm that increasing the area surveyed is more important than investing in increasing effectiveness. This is due in part to the fact that beetle density is a critical factor predicting infestation success and beetle spread.
- Short distance dispersal seems to remain the dominant form of beetle spread in this region. Thus, the local search strategy around infested trees was superior to a purely random approach.
- The current modeling exercise includes several assumptions that are mainly related to the restricted number of years that data was available.
- The researchers recommend further analysis to refine both cost and control efficacy.

C. C. CHIU, C. I. KEELING AND J. BOHLMANN. 2018. MONOTERPENYL ESTERS IN JUVENILE MOUNTAIN PINE BEETLES AND SEX-SPECIFIC RELEASE OF THE AGGREGATION PHEROMONE *TRANS*-VERBENOL. PNAS. 115(14): 3652-3657. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- It has long been known that trans-verbenol is the most critical aggregation pheromone produced and released by female MPB to coordinate mass attack. This pheromone is synthesized by female beetles using the monoterpene α-pinene, a primary defense mechanism of host species, when the female first colonizes the host.
- This paper is the first to report that female beetles begin to synthesize trans-verbenol during earlier stages of their life and accumulate this hormone in their midgut. This "reservoir" of pheromones may serve an important role as females coordinate the mass attack of new host trees.
- Females are now known to have the ability to release pheromones calling for mass attack once they have landed on a host tree and, most noteworthy, before they are even exposed to the tree defense chemical monoterpene α-pinene.
- Implications of this discovery in pheromone production to beetle outbreaks remains unknown.

Approach:

- Tree bolts were collected from infected trees located at a site near Whistler, BC in 2016.
- Four types of MPB were collected, including larvae, pupae, teneral and emerging adults. A phloem sample was also collected from the bolts. Male and female beetles were separately dissected into heads, thorax and abdomen.
- Emerging male and female beetles were treated with α-pinene, hormones and other monoterpenes to test for the production of trans-verbenol.

Key Findings and Implications:

- Concentrations of verbenyl esters—primarily verbenyl and myrtanyl esters—were present in both male and female beetles as early as the first instar of larvae development. However, by the time that male beetles emerged from the tree bolts, these chemicals dropped to low concentrations. In sharp contrast, concentrations of verbenyl esters remained high in female beetles and were found in the each of the three major body parts.
- Live male and female beetles exposed to the monoterpene α-pinene produced trans-verbenol, cis-verbenol and myrtenol. Levels of trans-verbenol also increased with exposure to hormones.

2017

J. L. BURKE AND A. L. CARROLL. 2017. BREEDING MATTERS: NATAL EXPERIENCE INFLUENCES POPULATION STATE-DEPENDENT HOST ACCEPTANCE BY AN ERUPTIVE INSECT HERBIVORE. PLOS ONE 12(2): E0172448. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Nutritional quality of food resources available to reproducing female MPB influence the health and fecundity of their offspring.
- Although the shift between endemic and epidemic behaviors of MPB is related to changes in population density, the precise mechanisms by which the beetle determines the appropriate behaviors and strategies has not been well investigated. A clear understanding of the constraints that define endemic and epidemic phases of MPB is required to better predict and mitigate impending outbreaks.
- In this study, beetle behavior was strongly dependent on the conditions under which they were reared, including phloem quality and monoterpene concentrations. These results demonstrate potential mechanisms that control population dynamics of MPB and allow for more accurate predictions of their continued impact and spread.

Approach:

- This study tested the relationship between the rearing conditions of female MPBs (simulating endemic vs epidemic conditions) and the host selection behaviours of their offspring (i.e., selection of well or poorly defended substrates).
- Beetles were reared in the laboratory under conditions that simulated both endemic (smaller-diameter, low vigor lodgepole pine bolts) and epidemic (larger diameter bolts) conditions.

Key Findings and Implications:

- Beetles reared in poorly defended bolts (i.e., endemic conditions) had offspring that were more likely to select poorly defended hosts, suggesting that in nature, offspring from impaired hosts will continue to select similar hosts and maintain the epidemic phase.
- Beetles reared in well-defended bolts (i.e., epidemic conditions) had offspring that were more likely to reject poorly defended substrates and pursue a well-defended, nutritionally optimal host under most conditions. This represents a positive feedback loop to maintain the epidemic phase and, if optimal hosts are abundant, will result in mass attacks in the following year leading to stand level outbreaks. Further, if optimal hosts are present in nearby stands then beetles dispersing into these stands will take with them their epidemic behaviors even if they are the first beetles to arrive in such stands.
- Once the supply of optimal host trees is extinguished, the population of epidemic beetles, which show a strong preference for these hosts, will likely collapse.

J. L. Lewis, J. Bohlmann and A. L. Carroll. 2017. Consequence of distributional asymmetry in a warming environment: invasion of novel forests by the mountain pine beetle. Ecosphere. 8(4) Article e01778. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Forests north or east of the historical range of the MPB have been under no known evolutionary pressure from previous MPB attacks. The expansion of the MPB into these areas represents an invasion into novel habitats.
- Weakly coevolved defenses in novel habitats may explain the high rate of spread and high mortality in newly invaded forests. However, the authors caution that the unprecedented size of the current outbreak would likely enable the MPB to overcome even the strongest tree defenses.
- Climate change will likely exacerbate the spread of the MPB into forests of western Canada that lack any co-evolution with the beetle.

Approach:

- This study assessed the relationship between prolonged (i.e., historical) exposure to MPB attack and monoterpene production.
- Four sampling locations with different historic climate suitability classes for beetle (very low, low, moderate, and high) were selected: Tumbler Ridge in northern BC; Hinton in central Alberta; Merritt in southern BC; and Baldy Mountain in southern BC. Two stands in each location were sampled for a total of eight sampling locations.
- Treatments included inoculations of *G. clavigera* in wounds of 11 trees per site. The authors used methyl jasmonate injections to account for between tree variability.
- Monoterpene concentrations were determined in the laboratory using chemical digests of phloem samples collected from the field.

Key Findings and Implications:

- Monoterpene concentrations were highest in samples collected from the population that had the highest historic climatic suitability, suggesting that enhanced and rapid production of monoterpenes was associated with the degree of historic exposure to the MPB.
- The authors noted that invasive species are expanding their range across the world, and further, that novel habitats often have missing or ineffective population defenses or population regulators due to a lack of coevolution. These observations may be applicable to the expansion of the MPB.

C. C. CHIU, C. I. KEELING AND Y. BOHLMANN. 2017. TOXICITY OF PINE MONOTERPENES TO MOUNTAIN PINE BEETLE. *SCI REP* 7, 8858 (2017). [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- A mixture of complex volatile monoterpenes, which are toxic to a wide range of insects, compose a significant defense system in conifers and are produced when trees are under stress. Interestingly, the strength of this tree defense mechanism differs strongly between species and among populations and individuals.
- Co-evolution of MPB and monoterpenes has resulted in complex chemical and ecological interactions. MPB can use monoterpenes as an indicator of suitable host trees and can incorporate monoterpenes to produce pheromones for mass attack to overcome host defense. These variations are attributed to genomic differences in terpene synthesis genes.
- Perhaps reflecting the complex evolutionary interaction between MPB and host trees, the most abundant monoterpenes were not the most toxic to MPB and, further, the most toxic monoterpenes were not the most abundant in host trees. Limonene was found to be the most toxic.
- These results are critical to understanding the efficacy of chemical defense in lodgepole pine and to understanding the complex evolutionary relationships between host and MPB.

Approach:

- Researchers quantitatively tested the toxicity of monoterpene volatiles that are abundant in lodgepole pine using a dose-mortality experiment. Beetles were sourced from beetle infested stands located near Whistler and Mount Baldy, BC.
- Ten monoterpene volatiles were used to individually test for toxicity following a 24-hour exposure. Effects of monoterpenes via either physical contact or ingestion were not assessed.

Key Findings and Implications:

- Monoterpene concentration had a significant positive relationship with mortality for each of the ten monoterpenes tested.
- Mortality decreased with increasing body weight of individual beetles, which was consistent with related studies reported in the literature. Beetle sex had no effect on mortality.
- Limonene was found to be the most toxic, yet it comprised only 1-5% of the monoterpene profile of lodgepole pine.

D. J. O. Alayon, C. K. M. Tsui, N. Feau, A. Capron, B. Dhillon, Y. Zhang, S. M. Alamouti, C. K. Boone, A. J. Carroll, J. E. K. Cooke, A. D. Roe, F. H. Sperling and R. C. Hamelin. 2017. Genetic and genomic evidence of niche partitioning and adaptive radiation in mountain pine beetle fungal symbionts. Mol. Ecol. 26: 2077-2091. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The three fungi involved in a multipartite relationship with MPB have different responses to environmental drivers such as temperature. Each of these species possesses its own set of genetic and phenotypic characteristics that has facilitated relationships over the course of evolutionary time and a range of environmental conditions.
- MPB appears to have evolved a strategy of being associated with fungal associates that possess a range of different yet complementary characteristics. Hence the beetle reduces its chances of being left without a fungal associate and its concomitant benefits of association.
- The research team noted that by maintaining a multipartite relationship, the MPB may be more successful in colonizing new habitats and surviving a wide range of harsh environmental conditions and habitats.

Approach:

- This investigation compared the inter- and intraspecific population genetic and phenotypic characteristics of *Grosmannia clavigera*, *Leptographium longiclavatum* and *Ophiostoma montium*. The adaptation of fungi to a wide range of environmental conditions was of primary interest and whether it would lead to species-specific signatures of natural selection.
- The research involved genomic analysis at the landscape scale in an attempt to identify loci involved in local adaptation and environmental factors driving selection.
- Samples of the three fungal species were collected at 35 separate locations in Canada and the United States. Seventeen of these locations supported all three fungi.
- Landscape genomics was investigated in detail using a variety of approaches including the use of genotype-environment association analysis. Growth rates of fungi species was assessed in the lab across a range of six different temperatures.

Key Findings and Implications:

- The continued relationship between beetle and fungus hence raises the question as to why this symbiotic relationship has remained stable over the course of evolution. One explanation is that these three fungal species may occupy ever so slightly different niches, or what the researchers refer to as “niche space”.
- Niche space may have arisen as the fungal species differ in their ability to cause disease (i.e., their pathogenicity), their tolerance for a range of temperatures, and their variation in response to changes in host water potential. Also of note, the time that the fungal species colonize the host is slightly offset with that of the life cycle of the beetle, a sort of temporal niche. Finally, these species of fungus are found to vary with respect to their location on the landscape and this may be indicative of different adaptive responses within and among the three species.
- Population structure analysis revealed three distinct structures which were related to geography or epidemiological events. The first cluster consisted of populations from sites that had not been attacked by MPB before 2006 (i.e., north of 52°N latitude). The second cluster were populations within the historical range of MPB in both Alberta and British Columbia. The final cluster consisted of populations from the United States, located south of the 48°N latitude.
- *Grosmannia clavigera* had the highest level of within-population diversity.

- Landscape genomics revealed that environmental factors such as precipitation and temperature were important drivers in the three populations, but their impacts varied among the three fungal species.
- A study of genes generally attributed to environmental patterns revealed both gene categories that were shared by the three species, and several that were unique to each species. This observation was consistent with the conclusion that many genes were under selective pressure.
- Growth patterns under laboratory conditions suggested differential temperature adaptations between the three species. For example, *Ophiostoma montium* had the best tolerance for high temperatures, which may offer this species a unique niche.
- Observations support the conclusion that an exceptionally wide range in environmental factors (temperature, drought, cold stress and precipitation) across the historical range of MPB defined the genomes of these three species with contrasting outcomes.
- Results from this study also support the conclusion that heterogeneity in the environmental conditions across the wide range of study sites was the result of local selective pressures and that fungal species occupy slightly different roles.

J. A. Cale, M. Muskens, A. Najar, G. Ishangulyyeva, A. Hussain, S. S. Kanekar, J. G. Klutsch, S. Taft and N. Erbilgin. 2017. Rapid monoterpene induction promotes the susceptibility of a novel host pine to mountain pine beetle colonization but not to beetle-vectored fungi. Tree Phyiol. 37: 1597-1610. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Monoterpene response in jack pine indicates that this species has a low capacity to inhibit early stages of beetle colonization, leaving this species open to mass attacks. This is most likely the result of jack pine having evolved in the absence of MPB pressures, in contrast to lodgepole pine, which most certainly co-evolved with MPB.

Approach:

- The following research questions were investigated: (1) do lodgepole and jack pine differ in their ability to rapidly synthesis monoterpenes? (2) does this induction respond to increasing densities of MPB attack? (3) does rapid induction in phloem of each species differentially affect the growth and reproduction of fungi? and (4) how do these responses relate to the relative susceptibility of jack pine to MPB colonization and fungal infection?
- A combination of field and laboratory-based studies were adopted.
- For the field-based study, a lodgepole pine stand near Hinton, and a jack pine stand located near Lac La Biche were chosen. Treatments consisted of seven different densities of *G. clavigera* inoculation in the stem. Two phloem samples, each containing both infected and non-infected phloem, were collected from each tree seven days following treatment. Monoterpene concentrations were extracted from phloem tissues using standard analytical procedures.
- The laboratory study consisted of growing *G. clavigera* on artificial media. The media was treated with additions of myrcene, limonene and α-pinene to test their effects of growth and reproduction of the fungus. These three monoterpenes were selected based upon results of the field experiment.

Key Findings and Implications:

- Induction of α-pinene was between 18 and 77 times greater in jack pine than in lodgepole pine and was unaffected by inoculation density. This finding is significant as female beetles use this monoterpene to produce pheromones.
- Induction of myrcene was higher in jack pine than in lodgepole pine phloem. Like α-pinene, myrcene is linked to increased production of pheromones in female beetles and may contribute to mass attack.
- Limonene concentrations were 80% lower in jack pine compared to lodgepole pine trees seven days after inoculation. This monoterpene is toxic to beetles and represents one of the core defense mechanisms against the beetle.
- In the laboratory study, α-pinene, myrcene and limonene had different impacts on growth and reproduction of *G. clavigera*. High myrcene and α-pinene levels, such as those found in the jack pine phloem, favored fungus growth but not reproduction. Higher concentrations of limonene depressed fungus growth and reproduction, but low concentrations stimulated growth instead.
- Overall, the researchers concluded that the threat of *G. clavigera* to jack pine was likely similar to that in lodgepole pine. The researchers called for additional research to further our understanding of the precise mechanisms that define the interaction between host induced responses and fungal growth.

J. D. Fraser, T. R. Bonnett, C. J. Keeling and D. P. W. Huber. 2017. Seasonal shifts in accumulation of glycerol biosynthetic gene transcripts in mountain pine beetle, *Dendroctonus ponderosae* Hopkins (*Coleoptera Curculionidea*), larvae. PeerJ 5:e3284. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study was the first of its kind to investigate and report the precise pathways for glycerol production over a period of 35 weeks by assessing changes in gene expression.
- These findings may inform future studies that attempt to more fully understand levels of internal regulation during changes in external temperatures.

Approach:

- This study documented the mechanisms and timing by which MPB larvae produce glycerol by determining changes in transcription levels for genes involved in glycerol synthesis over seven months.
- The genes involved in glycogenolysis and lipolysis were studied in detail, because glycogen is the most important carbohydrate reserve in overwintering insects and triglycerides are the most dominant lipid store. As overwintering insects do not feed, they must have tight control on the allocation of scarce internal reserves to maintain basic metabolic functions and the production of glycerol. Accordingly, the genes involved in glycogenolysis and lipolysis were studied in detail.
- Overwintering larvae were live-collected from eleven trees and flash frozen using liquid nitrogen, over ten collection dates from mid-Sept, 2008 to mid-May, 2009. Each tree was equipped with a data logger to measure and record ambient air temperature.

Key Findings and Implications:

- There were no changes in the transcripts associated with lipolysis during any time period, confirming that lipids are not an energy source for glycerol production.
- In contrast, significant increases in transcripts associated with glycogenolysis were observed in the fall as temperatures cooled, and then decreased in the spring as temperatures warmed—suggesting that glycerol was produced from glycogen. Further analysis indicated that gluconeogenesis was a secondary mechanism for glycerol production, and further, that this metabolic pathway activates several weeks after glycogenolysis and may serve as a sort of back-up mechanism.
- Findings from this study confirmed that glycerol was not converted back to glycogen in the spring as had been speculated in the literature.

J. L. Lewis, J. Bohlmann and A. L. Carroll. 2017. Consequence of distributional asymmetry in a warming environment: invasion of novel forests by the mountain pine beetle. Ecosphere 8(4) e01786. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Forests north or east of the historical range of the MPB have been under no known evolutionary selection pressure from previous MPB attacks and that expansion of beetle into these areas represents an invasion into novel habitats.
- Weakly coevolved defenses in novel habitats may explain the high rate of spread and high mortality in newly invaded forests. However, the authors have cautioned that the unprecedented size of the current outbreak would likely enable the MPB to overcome even the most heavily defensible tree.
- Climate change will likely exacerbate the spread of the MPB into forests of western Canada that lack any co-evolution with the beetle.

Approach:

- The authors hypothesized that prolonged exposure of MPB attacks would lead to intensive selective pressure to attack. They predicted that the production of monoterpenes would be greatest in populations that had historic exposures to MPB attacks.
- Four sampling locations were identified based on their historic climate suitability class for MPB (very low, low, moderate, and high). These locations included Tumbler Ridge in northern British Columbia; Hinton, central Alberta; Merritt, southern British Columbia; and Baldy Mountain, also located in southern British Columbia. Two stands in each location were sampled for a total of eight sampling locations.
- Treatments included inoculations of G. clavigera in wounds of 11 trees per site. Of particular note, the authors also used methyl jasmonate injections to account for between tree variability.
- Monoterpene concentrations were determined in the laboratory, rather than the field, using chemical digests of phloem samples collected from the field.

Key Findings and Implications:

- The authors noted that invasive species are expanding their range across the world, and further, that novel habitats often have missing or ineffective population defenses or population regulators due to a lack of coevolution. These observations may be highly applicable to the expansion of the MPB.
- Concentrations of monoterpenes was highest in samples collected from the population that had the highest historic climatic suitability. That is to say that enhanced and rapid production of monoterpenes was associated with the degree of historic exposure to the MPB.

2016

J. A. Holliday; S. N. Aitkin; J. E. K. Cooke; B. Fady; S. C. Gonzalez-Martinez; M. Heuertz; J. Jaramillo-Correa; C. Lexer; M. Staton; R. W. Whetten; and C. Plomion. 2016. Advances in ecological genomics in forest trees and applications to genetic resources conservation and breeding. Mol. Biol. 26(3): 706-713. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Tree species are known to possess extensive genetic variation both between and within populations. Genomic studies offer a unique opportunity to peer into the processes that shape this variation and facilitate adaptation.
- Exciting new advances in tree genomic methodologies are expected to accelerate the understanding of genomes and their interaction with environments to an extent not thought possible even from that of a few years ago.

Approach:

- An international conference titled “Genomics and Forest Tree Genetics” was held in 2016 in France. The conference attracted experts in tree genetics from around the world to discuss how genomics data could be applied to new efforts in genomic-based breeding and related conservation efforts.
- The conference had three broad themes: (1) the democratization of genomic studies across a wide spectrum of species; (2) further the understanding of neutral and adaptive processes within and amongst species, particularly in the context of climate change; and (3) translating gains in genomics to informing conservation and sustainability.
- The conference heard of new discoveries and, of equal importance, how new genomic based approaches could be utilized in basic and applied studies in tree biology and forest ecology.

Key Findings and Implications:

- A key goal of genomic investments must remain combining conventional genetic studies of phenotypic variation with genomic data to define the genomic underpinnings of adaptive traits and their relationships with the environment.
- While significant gains have been achieved in the field of genomics over the past 20 years, trees species of interest remain a difficult and complex genetic system to understand. Consequently, trees species remain underrepresented among known genomic sequences, though new studies are underway.
- Forested ecosystems are under considerable biotic stressors including climate change, and introduction of exotic species, and range expansion in pests and diseases. There is an urgent need to understand mechanisms of climate resilience.
- Genomic studies not only provide a more complete understanding of adaptation, but also may lead to the identification of genes for additional functional investigation. New genomic resources and approaches for conifers were reported and represent new opportunities to address existing ecological and genetic questions.
- The conference was also reminded of the importance of genomics to address a host of questions related to conservation. While this field of science remains in its early stages, and much caution needs to be exercised in terms of methodology, there remains significant promise that this approach may inform the management of genetic resources.
- Recent and anticipated gains in knowledge are expected to significantly inform breeding programs in the 21st century and the identification of methodologies, targets and priorities.

P. D. Batista, J. K. Janes, C. K. Boone, B. W. Murray and F. A. H. Sperling. 2016. Adaptive and neutral markers both show continent-wide population structure of mountain pine beetle (Dendroctonus ponderosae). Ecology and Evolution. 6(17): 6292-6300. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study clearly demonstrated there are significant advantages to including "outlier" loci when determining the population structures of MPB. Indeed, outlier loci may be critical in identifying genetic differences that define cellular or metabolic processes.
- This new and important finding challenges both the long-held convention that only neutral genetic markers should be used to assess populations and, further, that outlier markers should be removed.

Approach:

- Beetles were collected from a total of 62 sites located in Canada and the United States. DNA was extracted from late instar larvae and adults.

Key Findings and Implications:

- No outliers were detected in either the United States population or beetles collected from northern Alberta. However, 12 outlier loci were found in beetles collected from southern Alberta.
- Outlier detection revealed several SNPs (single nucleotide polymorphism which refers to a variation of a single nucleotide between individuals) were thought to be associated with DNA and RNA processing proteins, cellular transport and production of cold shock proteins.
- Results of this analysis confirmed that outlier loci can provide important information for determining population structures of MPB across its entire range. Removal of these outliers prior to analysis, as is common practice, may prevent the opportunity to identify what the authors defined as "fine-scale" structures.

Dhar, L. Parrott and C. D. B. Hawkins. 2016. Aftermath of Mountain Pine Beetles outbreak in British Columbia: stand dynamics, management response and ecosystem resilience. Forest. 7(8): 171. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Residual stand structures within beetle-killed stands of BC are much greater than had been first predicted. This residual growing stock may positively contribute to short-term timber supply and contribute to non-timber values such as ecological complexity at both the stand and landscape levels.

Approach:

- This synthesis explores the ramifications of the MPB infestation in BC on mid-term timber supplies, vegetation diversity, ecosystem resilience, structure and composition, forest growth, forest fire and climate change.

Key Findings and Implications:

- Though most stands impacted by MPB appear to be "devastated," non-salvaged stands often have a high component of residual trees of commercial species. Recruitment in these stands is highly variable but a pulse is often observed 10 to 20 years following infestation. Species composition of regeneration in non-salvaged stands shifted to more shade tolerant species and from being even-aged to being uneven-aged.
- The current MPB epidemic in BC has killed approximately 752 million m^3 of merchantable timber. Annual allowable cuts have been increased to salvage as many dead trees as possible before wood quality degrades. The harvest levels will have to be adjusted downwards at some point and the wood supply in the Prince George area is predicted to fall 75% from preharvest levels.
- There are now numerous reports that beetle-killed stands with healthy, well-spaced advanced residuals can yield between 200–300 m^3 within the next 25 to 40 years. This future yield may outweigh current harvesting plans that salvage smaller amounts of beetle-killed timber but destroy residual stand structures.
- Current salvage operations should avoid damaging residual structure by using selection harvesting systems. Clear-cutting beetle killed stands with residuals may accentuate mid-term wood supply issues.
- There remains debate in the literature as to the short- and long-term effects of MPB on fire risk. Some results indicate that fire spread will be greater in beetle killed stands and some have reported a reduction in fire likelihood. These contrasting opinions may owe to the fact that wildfire is exceptionally complex. What remains clear is that the primary driving factor for wildfire likelihood and severity is climate.
- The impacts of MPB infestation on carbon dynamics in British Columbia are not clear. Some studies produced by the federal government indicate that beetle-killed stands will be a significant source of greenhouse gases. However, direct field measurements have not supported these findings and some researchers have predicted that beetle-killed stands could quickly become a sink due to increased growth of residual growing stock. While debate continues, it is becoming clearer that forest carbon dynamics will not be as severely impacted as once feared.
- Climate change projections have for some time predicted a species shift northward and to higher elevations. It has been hypothesized that the MPB epidemic may facilitate this shift by opening canopies and increasing stand recruitment processes.
- Loss of canopy trees to MPB had either a direct or indirect effect on wildlife. For example, some papers have reported that mortality rates of less than 60% had little effect on bird populations and diversity but

losses greater than 60% had significant impacts. Increases in growth of residual trees and the understory had positive impacts on forage amounts and wildlife species. Salvage logging though, and the associated impacts to residual structures, will negatively impact biodiversity.

- MPB-caused mortality increases species diversity and complexity at both the stand and forest level. In sharp contrast, aggressive salvage logging has the risk of creating simplistic landscapes and simplistic stand structures. Forest management strategies to deal with beetle-killed stands should seek to promote landscape and stand complexity.

D. W. Goodsmann, D. Koch, C. Whitehouse, M. L. Evenden, B. J. Cooke and M. A. Lewis. 2016. Aggregation and a strong Allee effect in a cooperative outbreak insect. Ecol. Appl. 26(8): 2623-2636. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study used field data to develop a new MPB dynamic model that took into consideration aggregation, mass-attack, a critical attack threshold and beetle dispersal. This new model may have exceptional value to forest managers for prioritizing stands for beetle treatment.

Approach:

- Beetle dispersal and infestation processes were studied in an area within the Willmore Wilderness Park located in northeast Alberta. Infestations in this area have occurred in both 2007 and 2008 and local site productivity and inventory data for lodgepole pine was well documented.
- Beetles were collected using standard bark disk collections. The number of beetle life stages and the number of entrance holes were counted in the laboratory to determine an R-value.
- The authors used the field data to modify an existing insect x host model by incorporating considerations of aggregation, mass-attack, a critical attack threshold and beetle dispersal.

Key Findings and Implications:

- Species are adversely affected when their populations are very low, and they aggregate to overcome this effect. This observation is consistent with the “Allee effect”.
- The Allee effect is a phenomenon which holds that at very low densities, an individual benefits from the presence of others beyond that expected from mere reproduction. This is contrary to the expectation that at low densities individuals should benefit because of an abundance of resources.
- This study demonstrated that a low ratio of MPB per susceptible tree within a given stand will lead to the MPB overcoming a tree’s defense system.
- Aggregation in MPB is inherently complex as female beetles have chemical-based mechanisms to both attract and repel other beetles, depending on the attack stage of the host and the strength of its defense mechanisms. Aggregation is further complicated by the stage of the outbreak with the selection of the host highly dependent on the size of the beetle population.
- A female productivity threshold of 4.5 is proposed, below which the population is expected to collapse. This information is valuable to forest managers in the prioritization of pine stands for treatment.

J. S. LANDRY AND L. PARROTT. 2016. COULD THE LATERAL TRANSFER OF NUTRIENTS BY OUTBREAKING INSECTS LEAD TO CONSEQUENTIAL LANDSCAPE-SCALE EFFECTS? ECOSPHERE. 7(3) E01265. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The authors have presented a hypothesis that large outbreaks of insects may transfer significant amounts of nutrients across vast landscapes as their bodies typically contain high concentrations of both nitrogen (N) and phosphorus (P). Further, the authors speculated that these lateral transfers of nutrients may be significant enough in major outbreak years to affect landscape level processes. Dedicated research remains to be undertaken to test this hypothesis.

Approach:

- This paper represents a review of the literature. The authors have presented no new research but rather lay out a new hypothesis to stimulate a new line of research.

Key Findings and Implications:

- Primary nutrient cycles take place at local scales and involve nutrient transfer between plants, microbes and soil, and thus represent a one-dimensional model of nutrient fluxes. Little attention has been focused on the transfer of nutrients across landscapes by insects, a so-called two-dimensional approach.
- The long-distance transfer of nutrients via insects is based on three key factors: (1) high concentrations of nutrients, including N and P, in the bodies of insects, (2) often dramatic changes in total population biomass, and (3) long-distance transport of insects across vast and varied landscapes.
- A literature review suggests that nutrients transfer via insect outbreaks may exceed decades of annual atmospheric deposition of nutrients.
- The exact fate of insect-bound nutrients remains unknown but should influence biological processes unless a strong nutrient sink exists on a specific site.

J. Landry, D. T. Price, N. Ramankutty, L. Parrott and H. D. Matthews. 2016. Implementation of a Marauding Insect Module (MIM, version c1.0) in the Integrated Biosphere Simulator (ISIS, version 2.6b4) dynamic vegetation-land surface model. Geosci. Model Dev. 9: 1243-1261. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The research team developed a Marauding Insect Module (MIM) version 1.0 that is capable of quantifying, in the Integrated Biosphere Simulator (IBIS), effects of large-scale infestations on biogeochemical and biophysical fluxes.
- Process-based modelling tools such as the MIM should be of significant value in furthering the understanding of the widespread impacts of insect-induced plant damage on climate processes.
- Presented in this paper is the first version of the MIM; valuable refinements are expected in the future to enhance the value of simulated results.

Approach:

- The MIM model is distinctive from other models in that it can simulate damage from defoliators of broadleaf species, defoliators of conifers, and bark beetles that feed on conifers.
- MIM simulates the effect of insect activity on plant functional types and land-atmosphere exchanges in water, energy and carbon.

Key Findings and Implications:

- Development of climate models that simulate, in detail, the effects of widespread insect damage has not received the attention that fire has received in recent years. This is even though there are currently catastrophic impacts of MPB infestations.
- The MIM model was applied to the MPB outbreak in British Columbia. Modelled predictions were consistent with field-based measurements.

J. Landry, L. Parrott, D. T. Price, N Ramankutty and H. D. Matthews. 2016. Modelling long-term impacts of mountain pine beetle outbreaks on merchantable biomass, ecosystem carbon, albedo, and radiative forcing. Biogeosciences 13: 5277-5295. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study suggests the current MPB outbreak in British Columbia had only a minor effect on global temperatures. However, some modelling scenarios predicted the MPB outbreak was equivalent to upwards of 50 years of British Columbia's normal greenhouse gas emissions. The authors cautioned that a more detailed modelling assessment was required.
- Carbon storage within impacted stands was more resilient than previously thought because of the growth release of residual plants and trees. This effect could be negated if salvage logging does not protect residuals.

Approach:

- This study modelled the impacts of MPB outbreaks on variables pertinent to the forest sector, global climate change, and changes in carbon and energy storage and fluxes.
- The Integrated Biosphere Simulator (IBIS) was coupled with the recently developed Marauding Insect Module. Four main variables were studied: merchantable lodgepole pine biomass, ecosystem carbon, surface albedo, and radiative forcing (a measure of the difference between solar irradiance absorbed by the Earth and that energy radiated back to space).

Key Findings and Implications:

- Non-target vegetation has a major influence on ecosystem carbon storage and net climate impact following MPB outbreaks. Previous studies that failed to take into account the often-significant growth release of non-target species, and residual understories, may have overestimated the impacts of MPB outbreaks on ecosystem carbon.
- Results indicate that dead standing trees could facilitate the growth of residuals by warming the surrounding air.
- More definitive modelling results are dependent on empirical measurements across a range of site types and over extended time periods.

I. LUSEBRINK, N. ERBILGIN AND M. L. EVENDEN. 2016. THE EFFECT OF WATER LIMITATION ON VOLATILE EMISSION, TREE DEFENSE RESPONSE, AND BROOD SUCCESS OF *DENDROCTONUS PONDEROSAE* IN TWO PINE HOSTS, LODGEPOLE, AND JACK PINE. FRONTIERS IN ECOLOGY AND EVOLUTION. 4(2). [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This field-based study tested the effect of water stress on tree defense mechanisms of both lodgepole and jack pine trees, with and without inoculation by *G. clavigera*. This study was the first of its kind to assess volatile emissions of trees under natural conditions.
- Jack pine trees contain the necessary precursors for the production of pheromones post inoculation.
- Jack pine trees might support a higher attack density and protect beetles from harsh winters because they have thicker bark than lodgepole pine trees. These factors could help facilitate the spread of MPB throughout the northern boreal region.

Approach:

- This field-based experiment included a jack pine stand near Smoky Lake, Alberta, and a lodgepole pine stand near Hinton, Alberta. Water treatments included no water additions (control) and a water deficit treatment wherein tarps were placed to intercept and divert rainfall. Biological treatments included inoculating physical wounds on trees with *G. clavigera*.
- Volatile compounds were collected from individual trees. Concentrations of defense compounds in phloem, needles and lesions were determined.
- Beetle condition was assessed to understand the possible effect of water treatment and inoculation on beetle weight and beetle fat content. Bolts were collected from all trees and transported to the lab where they were each inoculated with four pairs of live MPB. Following a period of five weeks to allow for gallery development, half of these bolts were subjected to a cold period to simulate overwintering.

Key Findings and Implications:

- A total of 12 monoterpenes were detected in either one or both species. Water treatment did not have any effect on monoterpene production. In contrast, fungal inoculation significantly increased volatile emissions and these increases were much more pronounced in lodgepole pine treatments.
- The primary compound found in phloem and tissue was b-phellandrene in lodgepole pine trees and (+)-@-pinene in jack pine trees. A third of jack pine trees did not show any increase in (+)-@-pinene following either treatment.
- Water treatments had no clear effect on lesion length, but lesions were longer in the lodgepole pine trees. Carbon content in lesions was higher than surrounding phloem tissues for both species.
- Female beetles reared in lodgepole pine bolts had the highest weights in those trees inoculated with *G. clavigera*. Female beetles that emerged from jack pine bolts had greater fat content, despite jack pine having thinner phloem and lower nitrogen concentrations than lodgepole pine trees. This might suggest that other nutrients might be more important than nitrogen, or alternately, that beetles need to spend less energy to detoxify chemical defenses in jack pine.

A. DHAR, L. PARROTT AND S. HECKBERT. 2016. CONSEQUENCES OF MOUNTAIN PINE BEETLE OUTBREAK ON FOREST ECOSYSTEM SERVICES IN WESTERN CANADA. CAN. J. OF FOR. RES. 46(8): 987-999. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Forest pest outbreaks of the scope and scale of the current MPB epidemic have impacts beyond timber losses. These include losses associated with environmental, economic, and social services.
- This review outlines that forest pest outbreaks, of the scope and scale of the current MPB epidemic, have impacts that include more than just timber losses and include those associated with environmental, economic, and social services.
- While salvage logging MPB attacked stands allows for short-term timber volume gains, it may not be the best option if achieving a range of ecosystem services is desired. Forgoing salvage logging to retain residual stems may augment mid-term timber supplies and provide a range of habitat and ecosystem benefits at a time when these are needed most post epidemic.
- Management of MPB-impacted forests should adopt more of a systems approach to decision making and be informed by science and best available data obtained through robust monitoring. Management decisions should also consider the multiple processes that support a range of natural goods and services. Consideration of both local knowledge and professional expertise will be key to achieving positive outcomes.

Approach:

- This paper represents a review of the literature pertaining to the effect of the MPB epidemic in western Canada on ecosystem goods and services. As such, it adopts more of a holistic approach that considers the interconnections among impacts, ecological functions, and ecosystem services.

Key Findings and Implications:

- Ecosystem services can be broadly grouped into four categories. The first category, and the easiest to identify, refers to provisioning services such as timber, food and water. The second category refers to regulating services and includes fire, carbon sequestration and water quality. The third category refers to supporting services and includes nutrient cycling and habitat. The final category consists of cultural services and includes non-material benefits such as recreational or cultural.
- If forest management decisions focus solely on the recovery of timber post-MPB attack, then there becomes a potential for the loss of supporting, regulating and provisioning ecosystem services. The latter may include biodiversity, water quality, aesthetic qualities, aquatic habitats, carbon sequestration. There is also potential that both the tourism and recreation economies may be impacted.
- Given the large spatial extent of the current MPB epidemic, losses in ecosystem services can be significant. Therefore, adopting a systems and ecosystem approach that recognizes the complexity of these forested ecosystems is critical.
- MPB impacted stands that are not salvage-logged can “recover” their ecosystem services faster than their harvested counterparts as significant portions of their ecosystems remain intact. There is also evidence that non-salvage logged stands are more resilient than logged stands because of their structural complexity.

A. Arango-Velez, W. E. Kayal, C. C. J. Copeland, L. I. Zaharia, I. Lusebrink and J. E. K. Cooke. 2016. Differences in defense responses of *Pinus contorta* and *Pinus banksiana* to the mountain pine beetle associate *Grosmannia clavigera* are affected by water deficit. Plant, Cell and Environment 39: 726-744. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study represents a detailed physiological investigation into how lodgepole and jack pine differ in their responses to *G. clavigera* and how these mechanisms may be affected by drought. A key strength of this paper is the integration of physiological and histological approaches.

Approach:

- This study compared the effectiveness of jack pine and lodgepole pine defenses against *G. clavigera* colonization and evaluated whether water deficits in either species compromised defense mechanisms. Both greenhouse and field studies were conducted.
- In the greenhouse study, one-year old seedlings were subjected to one of two watering regimes, well-watered and simulated drought conditions. A subset of seedlings was either inoculated with *G. clavigera,* mechanically wounded, or untreated (control group). Response variables included gas exchange, hydraulic conductivity, lesion lengths, hormonal analysis and monoterpene analysis.
- The field study was carried out at the Alberta Tree Improvement and Seed Centre. Planted lodgepole and jack pine trees (c. 40 and 64 years old, respectively) were assigned to one of two treatments: water deficits (wherein tarpaulins prevented rainfall penetration into the soil) or artificial watering (watered using an artificial water source and driplines). Half of the trees in each treatment were inoculated with *G. clavigera.* Five weeks following inoculation, bark and phloem samples were collected for hormone quantification. Response variables were like those in the greenhouse study (above).

Key Findings and Implications:

- Lodgepole and jack pine differed strongly in their response to inoculation by *G. clavigera.* Lodgepole pine invested more of its resources into monoterpene production within its phloem tissues. Jack pine invested more resources into xylem terpenoid defenses and greater development of vertical xylem resin ducts. This is an interesting finding and is no doubt related to the fact that jack pine is a novel species for *G. clavigera* whereas lodgepole pine is an evolutionarily co-evolved host.
- Inoculated lodgepole and jack pine both lost hydraulic conductivity in their stems but the effect was more pronounced in lodgepole pine. This effect was attributed to the radial penetration of *G. clavigera* into the sapwood. Histological analysis failed to report fungal hyphae in tracheids, which may support the hypothesis that reductions in stem conductivity were the result of xylem embolisms. More research pertaining to this aspect is needed.
- Total bark concentrations of monoterpenes were greatest in lodgepole pine trees and the specific monoterpene profiles for each species differed. Most notable was the production of α-pinene in jack pine, which has been well reported in the literature.
- *G. clavigera* inoculations in both lodgepole and jack pine resulted in the increased synthesis of jasmonic acid (JA), most certainly in response to the fungi consuming host tissues. Jasmonic acid is an important plant hormone that regulates cellular defense mechanisms. This study is believed to be the first of its kind to report this relationship. Understanding the hormonal impacts of infection would likely shed more light on defense mechanisms and their complexities over time.
- Elevated concentrations of JA were reported not only in lesions but also in phloem tissues near lesions, which reveals that defense mechanisms are not confined to lesions. Further, concentrations in JA were greatest in lodgepole pine, which may be indicative of this species having co-evolved with *G. clavigera*.

- Small spherical structures which remained unstained were found attached to the cell walls of tracheid lumens. This effect was seen in seedlings that had been inoculated with *G. clavigera* but was oddly absent in seedlings that were not inoculated. While the researchers did not definitively identify these structures, the histological approach adopted by these researchers does help to showcase the complexity of tree defenses and responses to insect attack.
- The water deficit treatment in the field component of this study was not severe enough to simulate drought conditions and had little effect on the physiological parameters studied.

T.D. Ramsfield. 2016. Evolving symbioses between insects and fungi that kill trees in Canada: new threats associated with invasive organisms. Can. Entomol. 148: 160-169. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- A review of case studies relevant to Canada including the brown spruce longhorn beetle, sirex woodwasp and the mountain pine beetle.
- Impacts of insect-fungi symbioses are generally restricted to weak or dying trees when these agents are restricted to their native habitats. Global trade has resulted in the movement of pests to new, novel habitats, including Canada, with devastating impacts. This reality calls to action regulatory agencies that are responsible for keeping such pests from entering Canada.
- Similarly, a changing climate is also thought to have created a situation where pests, such as the MPB, are encountering novel habitats consisting of trees that are thought to never have been exposed to these new symbiotic partners.
- An emerging threat is that of a new insect and its fungal symbiont developing within the range of closely related native species leading to interactions between insects and fungi. Dutch elm disease was cited as one devastating example which then initiates a new evolutionary trajectory, one that may very well have a competitive advantage over other species.

Approach:

- Literature review focusing on tree death in Canada caused by symbiotic relationships between fungi and insects.

Key Findings and Implications:

- Native and invasive insect species may possess high flexibility and adaptive capacity that appears to be inherent within symbiotic relationships. This flexibility and adaptive capacity can facilitate the survival and spread of new insects.
- Flexibility of the MPB is illustrated in its ability to partner with three different fungi and to shift its fungal association to that which facilitates the survival of the beetle under changing environmental conditions. This mechanism appears to be relevant as the MPB movers into colder climates.
- Flexibility in invasive species is also illustrated by the westward spread of the Dutch elm disease. The fungi associated with Dutch elm disease is spread by an insect species that is similar to its traditional host insect. Similarly, the spruce longhorn beetle has been detected in Halifax after arriving in Canada via shipping containers. In this case, the beetle is morphologically similar to an existing insect, uses the same mating system, but attacks red spruce which is a novel host for the new arrival.
- In the context of MPB, the author raises concern over long-term range expansion and possibility of new fungi associations owing to the adaptive capacity of exotic pests to respond to new environments. The possibility for such a development, and its outcomes, is not currently known but this is thought to be the first paper to raise this concern.

P. M. A. JAMES, J. K. JANES, A. D. ROE AND B. J. COOKE. 2016. MODELING LANDSCAPE-LEVEL SPATIAL VARIATION IN SEX RATIO SKEW IN THE MOUNTAIN PINE BEETLE (COLEOPTERA: CURCULIONIDAE). ENV. ENTOMOL. 45(4) 790-801. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Bias in sex ratios that favour MPB females is the result of higher mortality rates in male beetles during development.

Approach:

- This study evaluated: (1) the effect of tree size, climate and stage of outbreak on sex ratio, (2) which environmental factors can be used to predict sex-ratio skew, and (3) how environmental determinants of sex ratio vary between larval and adult beetles.
- The study area spanned 34 stands in Alberta and British Columbia. Larvae and adult beetles were collected from each stand on a continual basis between September 2007 and April 2008. A total of 2400 beetles were collected and sexed, and the diameter at breast height (dbh) of each tree from which beetles were collected was measured. Stands were classified by year of outbreak.

Key Findings and Implications:

- Skews in the sex ratio were found in both larvae and adult beetle collections but was much more pronounced in the latter, suggesting that male beetles have higher mortality rates during their development. If the ratio were defined at the time of oviposition, it would be similar between larvae and adults.
- Higher mortality rates in male beetles may be due to their smaller size and lower fat reserves, and because they are thought to be less tolerant to environmental stress such as low temperatures.
- A range of sex ratios (female:male) was observed among the sampling locations, ranging generally from 0.8:1 to 5.5:1. Most notably, adult beetles had a sex ratio of 22:1 in a stand in Cypress Hills yet the sex ratio of larvae was only 2:1.
- The probability of finding female beetles increased as both latitude and longitude increased. This increased probability is in alignment with the current expansion of the beetle. The cause for this effect remains unclear.
- Based on these results, an increase in the female:male ratio may be observed as the beetle moves eastward and northerly into novel habitats that have smaller trees and lower temperatures.

B. H. AUKEMA, F. R. MCKEE, D. L. WYTRYKUSH AND A. L. CARROLL. 2016. POPULATION DYNAMICS AND EPIDEMIOLOGY OF FOUR SPECIES OF *DENDROCTONUS* (COLEOPTERA CURCULIONIDAE): 100 YEARS SINCE J. M. SWAINE. CAN. ENTOMOL. 148: S82-S110. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This review of bark beetle life histories and population dynamics stresses the importance of field-based research, the value of considering each bark beetle outbreak individually and in the context of local conditions, and that control measures should be led by competent entomologists.

Approach:

- This detailed review of the literature focused on life histories and population dynamics of four bark beetle species prevalent in North America. Only limited reference was made to mountain pine beetle.
- This paper also noted and celebrated the research of J. M. Swaine, who has made historical contributions to the field of entomology.

Key Findings and Implications:

- The review of Swaine's scientific accomplishments highlights the importance of extensive field-based research and exceptional concern for accuracy and detail. The value of investments in field-based research is easily overlooked given rapid advancements in genomics, artificial intelligence, etc.
- The authors of this journal article noted that much of the scientific knowledge accumulated in the earlier part of the 20th century was never properly published or has been lost due to federal budget cuts. These scientific accomplishments are now generally thought to have been lost to time.

J. L. Burke and A. L. Carroll. 2016. The influence of variation in host tree monoterpene composition on secondary attraction by an invasive bark beetle: implications for range expansion and potential host shift by the mountain pine beetle. For. Ecol. Mgmt. 359: 59-64. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Beetles were attracted to bolts with elevated concentrations of α-pinene in their phloem. Given that hybrid and jack pine trees typically have 3-4 times the concentration of α-pinene than lodgepole pine trees, these higher concentrations of α-pinene may facilitate the spread of the beetle into novel habitats.
- Elevated α-pinene contents may offset the barriers to spread such as jack pine stands of lower volume and low pine connectivity.

Approach:

- The research team tested whether α-pinene concentrations will influence the secondary attraction of MPB during an active infestation. They predicted that higher concentrations of α-pinene in infested bolts would be more attractive to foraging beetles.
- Fresh bolts were collected from a lodgepole pine stand, a jack pine stand, and a hybrid stand, all located near Slave Lake, AB and not previously exposed to MPB. The bolts were transported to a mature lodgepole pine stand near Oliver, BC, within the historical range of MPB and experiencing epidemic MPB infestation at the time. Bolts were hung from trees to facilitate beetle inoculation, and were additionally inoculated with female beetles collected from an infested stand.

Key Findings and Implications:

- MPB were significantly more attracted to bolts with higher concentrations of α-pinene. The relationship between attack and relative α-pinene content was not linear but rather was best explained using an exponential curve, indicating that trans-verbenol production rapidly increases as more beetles arrive and begin to colonize the bolts.
- Total monoterpene contents were highest in the phloem collected from lodgepole pine trees, but total contents were not related to number of beetle attacks. α-pinene comprised 80% of the total monoterpene in phloem collected from the hybrid bolts but only 24% of the total content in native lodgepole pine bolts.
- Approximately 75% of all attacks were focused on the hybrid pine bolts.
- While the authors called for caution in extrapolating the findings of their research to the landscape level, the high levels of α-pinene in novel habitats could be a major factor contributing to the rapid spread of the beetle throughout the boreal forest.

N. Erbilgin, J. A. Cole, I. Lusebrink, A. Najar, J. G. Klutsch, P. Sherwood, P. Bonello and M. L. Evenden. 2016. Water-deficit and fungal infections can differentially affect the production of different classes of defense compounds in two host pines of mountain pine beetle. Tree Physiol. 37: 338-350. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study investigated phenolic and monoterpene concentrations in phloem and needles in jack and lodgepole pine, and how these concentrations might be affected by periods of drought and inoculation with *G. clavigera*.
- The results confirm that monoterpenes are the principle chemical defense mechanism against *G. clavigera*. Phenolics do not appear to have a critical defensive role.
- In the context of jack pine, a better understanding of the full suite of chemical defenses available to this species would help to predict future spread of beetles in eastern forests, particularly during periods of drought.

Approach:

- This field-based experiment included a jack pine stand near Smoky Lake, AB and a lodgepole pine stand near Hinton, AB. Water treatments included no water additions (control) and water deficit treatment wherein tarps were placed to intercept and divert rainfall. Approximately 5 weeks after the water treatments commenced, trees were either inoculated with *G. clavigera* or not inoculated (control).
- Ten weeks after inoculation, ten trees were felled per treatment for a total of 40 trees. Tissue samples were collected from foliage, phloem of non-inoculated trees, and phloem from both inside lesions of inoculated trees and nearby un-affected phloem, and analyzed for both phenolic and monoterpenes.

Key Findings and Implications:

- Water limitation inhibited synthesis of phenolics in both species but had no effect on the synthesis of monoterpenes. Simulated fungal infection had no effect on the synthesis of either monoterpenes or phenolics in jack pine. In sharp contrast, simulated fungal infection significantly increased the synthesis of monoterpenes, and reduced the synthesis of phenolics, in lodgepole pine. Phenolic concentrations were lower in lesions of both species when compared to non-lesion phloem.
- These findings support the conclusion that monoterpenes are the principal form of chemical defense against MPB and *G. clavigera.* The results reveal, for the first time, that phenolics do not play an important defensive role in either species to either of these agents.
- The failure of jack pine to up-regulate monoterpene production following infection by *G. clavigera* is consistent with similar studies. This is to be expected as these two species have shown no evidence of co-evolution, unlike lodgepole pine and *G. clavigera.*

A. Robert, T. Bonnett, C. Pitt, L. J. Spooner, J. Fraser, M. M. S. Yuen, C. I. Keeling, J. Bohlmann and D. P. W. Huber. 2016. Gene expression analysis of overwintering mountain pine beetle larvae suggests multiple systems involved in overwintering stress, cold hardiness, and preparation for spring development. PeerJ. 2016; 4: e2109. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Results of this study reveal that larvae are under significant physiological stress in the autumn and possess complex physiological response mechanisms. The upregulation of a host of enzymes and physiological pathways between September and November indicates the larvae preparation for overwintering is much more complex than previously thought and extends much beyond the mere production of glycerol.

Approach:

- Cold-induced mortality has been well recognized as a critical feature limiting the historical spread of MPB. Understanding the cold tolerance mechanisms of MPB has now become a high priority to inform predictive spread models.
- The research team utilized RNA-seq analysis to monitor transcript profiles in larvae at four points in their development: early-autumn, late-autumn, early-spring and late-spring. It was hypothesized that changes in transcript profiles over this time period would shed light on the precise physiological mechanisms that contribute to overwintering success.
- Overwintering larvae were live-collected from eleven trees and flash frozen using liquid nitrogen. Each tree was equipped with a data logger to measure and record ambient air temperature. RNA extractions and analysis followed standard laboratory procedures.

Key Findings and Implications:

- It is widely known that MPB larvae suffer high mortality if they are not fully acclimated to the cold environment through the production of anti-freeze compounds such as glycerol. However, this study revealed that its synthesis differs from what some other researchers have reported.
- Of particular note, results revealed a significant increase in the expression of immune response transcripts (i.e., stress response genes) occurring between September and November. For example, several gene transcripts associated with heat-shock proteins, important for protein stabilization and produced during a period of stress and temperature changes, were significantly upregulated during this period. These results reveal that larvae are responding to a toxic environment at the same time as they are preparing for winter temperatures. The authors suggested the physiological stress may have been due to the possible presence of pathogens in the galleries, low nutrient status of the host tissue, and/or strong host chemical defenses.
- In the autumn months there was a significant upregulation in the genes thought to be associated with the detoxification of metabolites produced by the host. For example, eleven genes associated with the cytochrome 450, well documented to be associated with detoxification of host tissues, was upregulated between September and November.
- Stress response gene transcripts were downregulated in late-instar larvae during the spring whereas those associated with larvae development were significantly upregulated.
- A significant number of other gene transcripts were found to increase by over 100-fold between March and May but their function is not currently understood. This finding confirms that many developmental processes in larvae remain to be identified.

D. W. GOODSMAN AND M. A. LEWIS. 2016. THE MINIMUM FOUNDING POPULATION IN DISPERSING ORGANISMS SUBJECT TO STRONG ALLEE EFFECTS. METHODS ECOL. EVOL. 7(9):1100-1109 [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Dispersal can push population density below the threshold needed for a founding population to survive. Therefore, the minimum founding population size that allows a species to establish can be different from the Allee threshold for isolated populations of dispersing species, like MPB.
- This study proposes an idealized 'integrodifference model' for a dispersing organism (like, MPB) subject to a strong Alee effect, and generates a mathematical solution for minimum founding populations.
- Calculating the minimum founding population sizes is useful to predict invasion success (based on propagule size) and to maximize species reintroduction success (when strong Allee effects are present).

Approach:

- We derive an expression for the minimum founding population size for a general integrodifference equation model of a dispersing population with a strong Allee effect.
- The model was used to estimate minimum founding populations of MPB.

Key Findings and Implications:

- While the minimum founding population is driven by a strong Allee threshold, it is not directly comparable.
- This paper defined the minimum founding population for organisms with strong Allee effects as the minimum population size which will eventually grow and expand after a point introduction.
- When strong Allee effects are present, calculating the minimum founding population using models can be useful to predict and control invasive or desirable species success.

2015

K. F. Raffa, B. H. Aukema, B. J. Bentz, A. L. Carroll, J. A. Hicke and T. E. Kolb. 2015. Response of tree-killing bark beetles to a changing climate. *In.* C. Bjorkman, & P. Niemela (Eds.), Climate Change and Insect Pests (pp. 173-201). Oxfordshire, UK: CAB International. [URL].

Document Type: Book Chapter

Core Messages:

- A conceptual model was developed to define the constraints and drivers of dramatic changes in beetle populations. These constraints were: the availability and quality of the host; the quality or strength of the host defense mechanisms; and the effect of temperature. The authors propose that competitors and predators play an essential role, especially when the host resource is limited. Climatic factors such as a warming have a dramatic effect since they affect numerous constraints and operate across vast scales.
- When evaluating the possible impacts of a changing climate on MPB spread, it is important to consider different areas in which the beetle may establish: (1) areas that historically had only occasional exposures; (2) areas that have host tree species but little or no historical exposures; and (3) novel habitats that have susceptible species but zero previous exposure. These novel habitats may constrain MPB populations if they have less homogeneous forests and lower host nutritional value.
- There remains significant uncertainty in predicting future beetle dynamics in a changing climate.

Approach:

- A conceptual model developed from a literature review.

Key Findings and Implications:

- There is substantial evidence that climate change has impacted temperature-dependent constraints and increased both the frequency and magnitude of outbreaks. MPBs are surviving better at high elevations which may have historically had little exposure to MPB and hence, the host species have poorly developed defense mechanisms.
- Periods of severe drought are known to reduce both tree vigour and defense mechanisms.
- There is currently a lack of information to predict how MPB populations will behave in *novel* habitats (i.e., those with zero historical exposure to MPB). Numerous factors will be at play and the precise impacts of a changing climate are difficult to predict.

2014

M. L. Evenden, C. M. Whitehouse and J. Sykes. 2014. Factors influencing flight capacity of the mountain pine beetle (Coleoptera Curculionidae: Scolytinae). Environmental Entomology. 43(1): 187-196. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- MPB body size is a critical factor in dispersal capacity, and dispersal capacity declines with the age of MPB post-emergence.
- Heavier beetles flew longer distances, flew for the longest duration, and were more likely to initiate flight than beetles of low weight. The relationships between MPB weight (i.e., fat and lipid reserves) and flight parameters has implications for understanding population dynamics. These results indicate that the beetles with the largest lipid reserves will be able to colonize hosts the furthest distance away.

Approach:

- MPB must disperse to search for new hosts but aspects of dispersal remain poorly understood. This laboratory-based study assessed the effect of sex, age and weight of beetles on flight potential.
- Beetles were collected from a stand located near Grande Prairie, AB. Three age groups were tested to study the effect of age of flight: young beetles (1–3 days post emergence), middle-aged beetles (5–7 days post emergence) and old beetles (9–11 days post emergence).
- A total of 148 beetles served as controls and 174 beetles were flown on a mill apparatus, and their energetic condition assessed by measuring lipid extractions.

Key Findings and Implications:

- Heavier beetles flew longer distances, flew for the longest duration, and were more likely to initiate flight than beetles of low weight. Sex had no effect on flight parameters.
- Flight distance averaged between 2.1 and 6 km and the longest flight measured c. 24 km. The authors did caution these results may be an overestimate as the beetles were supported somewhat by the flying apparatus.
- Dispersal capacity was affected by the age of the beetles. Beetles that flew the longest distances had a mean age of 2 days post-emergence; this age effect was attributed to a lack of time for fat metabolism in younger beetles.
- Flight velocity was not affected by age, sex or beetle weight.

C K. Tsui, L. Farfan, A. D. Roe, A. V. Rice, J. E. K. Cooke, Y. El-Kassaby and R. C. Hamelin. 2014. Population structure of mountain pine beetle symbiont *Leptographium longiclavatum* and the implication on the multipartite beetle-fungi relationships. PLOS ONE. 9:e105455. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Molecular ecology has grown significantly during the past two decades and has become an invaluable tool for understanding symbiotic relationships between organisms, and how theses relationships have been shaped through evolutionary processes.
- Since fungal symbionts can affect beetle performance, understanding their biology, ecology and population structures can help to understand how MPB became such a successful species attacking pine over vast landscapes.
- The findings of this study are consistent with the hypothesis that fungi symbionts and the MPB are very specialized, rather than being merely opportunistic. This specialization has evolved and arisen in more than one fungal species that have shared close ecological niches and close-evolutionary relationships.

Approach:

- This study assessed the population genetic structures of the fungal symbiont *Leptographium longiclavatum,* within both its historical distribution and newly colonized range.
- A total of 241 fungal isolates were collected from 17 sample locations within British Columbia and Alberta.
- DNA extractions, PCR amplifications, and genotyping were carried out using standard procedures. Genetic clustering of *Leptographium longiclavatum* was determined through Bayesian algorithms and migration patterns were similarly tested via the GENECLASS model.

Key Findings and Implications:

- Three genetic cluster populations of *L. longiclavatum* were detected: British Columbia, the southern Rocky Mountains, and a northern population including the Peace River and Grande Prairie regions. These findings confirm that geographic distance has affected population structuring.
- The distinct population structures between British Columbia and the southern rocky mountains of Alberta are thought to have originated from restricted gene flow due to distance and topographical barriers. These disjointed populations may have remained isolated when MPB populations were at endemic levels and only interacted when outbreaks were of historic proportions.
- The weak genetic differentiation of the northern population, currently thought to be outside the normal range of the MPB, may be due to the mixing of various fungi populations caused by long distance dispersal known to have occurred during the past decade. This population likely originated from a mixture of the British Columbia and southern rocky mountain clusters.
- Although *L. longiclavatum* and *G. clavigera* are closely related, *L. longiclavatum* is far more abundant in northern areas of Alberta and British Columbia. This may be due to it being more adapted to colder climates.
- Given the expansion of MPB to northern latitudes and higher elevations, future research should investigate the extent to which *L. longiclavatum* and *G. clavigera* differ in their cold tolerance.

Y. Wang, L. Lim, L. Madilao, L. Lah, J. Bohlmann and C. Breuil. 2014. Gene discovery for enzymes involved in limonene modification or utilization by the Mountain Pine Beetle-associated pathogen *Grosmannia clavigera*. Appl. Envir. Biol. 80 (15) 4566-4576. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The fungi *Grosmannia clavigera* is strongly associated with MPB and responsible for tree death. It has a wide array of mechanisms to detoxify monoterpenes, the primary defense mechanism of trees against both the fungi and the MPB. *G. clavigera* is also able to use monoterpenes as a carbon source.

Approach:

- Various fungi strains associated with MPB were tested, including *G. clavigera.* Young mycelia were exposed to (+)-limonene for three days. To assess the ability of the fungi to use monoterpenes as a sole energy source, the fungi were grown on a medium that only contained monoterpenes.
- Standard genomics procedures were carried out with a specific goal to identify gene functions.

Key Findings and Implications:

- Addition of (+)-limonene to the growth medium reduced mycelia growth by 20–40% in fungi associated with MPB. In contrast, the monoterpenes completely killed fungi associated with elm trees. This finding clearly demonstrated that many fungi may not have developed mechanisms to overcome monoterpene-based defenses.
- Mycelia of *G. clavigera* and of fungi associated with Jeffery pine were able to exploit monoterpenes as an energy source, whereas other fungi species were not.
- Previous work by these authors showed that one the mechanisms that *G. clavigera* had developed to defend against monoterpenes was the induction of an ABC efflux transporter. This transporter excretes monoterpenes from fungi cells to maintain low concentrations of monoterpenes within the physio-active portions of cells.
- The current study identified a second mechanism possessed by *G. clavigera* to overcome the monoterpene defense: the direct modification or degradation of monoterpenes. Two separate genes were identified and hypothesized to govern this mechanism.
- Additional defense mechanisms are thought to be possessed by *G. clavigera* but will require further investigation.

D. W. GOODSMAN, B. COOKE, D. W. COLTMAN, M. A. LEWIS. 2014. THE GENETIC SIGNATURE OF RAPID RANGE EXPANSIONS: HOW DISPERSAL, GROWTH AND INVASION SPEED IMPACT HETEROZYGOSITY AND ALLELE SURFING. THEORETICAL POPULATION BIOLOGY. 98: 1-10. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The authors developed a new two-dimensional model that reaffirmed that deme (i.e., a local group of individuals that interbreed with each other and share a gene pool) interconnectedness is of critical importance to defining genetic diversity within the expanding population of the MPB.

Approach:

- The primary purpose of this investigation was to examine MPB population diversity arising from the rapid range expansion. Both population dynamics and spread models were considered.
- Mathematical modeling of MPB populations is desirable but exceptionally difficult owing to spatial and temporal effects which are inherently difficult to empirically measure. The most common type of model to explain spread of populations, and patterns of genetic diversity, across space and time is called the stepping stone model. In the approach, the population moves from one unoccupied to area at a time. But for some populations such as the MPB the spread, or dispersal, may be less uniform.
- The purpose of this investigation was to examine the population diversity arising from the rapid range expansion. Integrodifference equations were used as the basis for the study into genetic signatures following range expansion.

Key Findings and Implications:

- Current models that attempt to connect demography (size, structure, and movements of populations over space and time) with dispersal and genetics are thought to be of limited value when applied to the current issue of MPB. This is in part due to the rapid range expansion of the MPB in novel habitats and the dispersal nature of the MPB.
- Modeled results predicted low probabilities of a mutant allele occurring in high enough numbers for it to become relevant.
- Heterozygosity, or genetic variation within a population, was found to decline along the axis of spread when the latter was assumed to be at either a constant or reduced rate. That is to say that variation was lowest at the farthest point of spread.
- Heterozygosity was maintained when the range expansion was accelerated by using an exponential kernel function and when spread rate was driven by dispersal.
- Mutants were able to survive within the populations with increasing rates of spread and when the primary spread was by means of dispersal.
- Model simulations indicate that heterozygosity of MPB populations should be highest in the directions of faster spread. This finding has significant implications for genetic studies attempting to measure MPB heterozygosity. The authors did caution though that factors such as landscape heterogeneity, movement corridors or lake thereof, may obscure some predictions.

C. Pitt, J. A. Robert, T. R. Bonnett, C. I. Keeling, J. Bohlmann and D. P. W. Huber. 2014. Proteomics indicators of the rapidly shifting physiology from whole mountain pine beetle, *Dendroctonus ponderosae* (Coleoptra: Curculionidae), adults during early host colonization. PLOS ONE. 9(10): e110673. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Proteomic profiles were developed for MPBs collected immediately upon emerging from their host and compared to that of MPB that were given the opportunity to colonize a fresh host for a period of 24 hours.
- These results add to the growing database of studies that attempt to explore and understand the short colonization phase of the MPB life cycle.

Approach:

- Lodgepole pine bolts were collected from an infested stand near Penticton, BC. Emerging beetles were either immediately frozen in liquid nitrogen (control) or placed within freshly collected bolts to feed on fresh phloem. The latter were left to feed for 24 hours and then frozen using liquid nitrogen.
- Protein extractions were analyzed for male and female beetles in the control and treatment groups.

Key Findings and Implications:

- A total of 757 and 739 proteins were identified for female and male beetles, respectively. Male and female beetles shared 463 of these proteins, with the rest being unique to each sex.
- In total, 23 and 29 proteins in females and male beetles (respectively) shifted following feeding on the host material. The shifts were generally much more pronounced in female beetles.
- Significant accumulations of chaperone proteins in the treatment beetles are indicative of chemical defenses that the beetles face immediately after colonizing the host.
- Changes in proteins within the female beetles indicated their physiology was changing within hours of infesting the host, to that of a reproductive stage.
- During colonization, male beetles down-regulated protein synthesis thought to be associated with wing muscles, likely to access stored energy.

N. ERBILGIN, C. MA, C. WHITEHOUSE, B. SHAN, A. NAJAR AND M. EVENDEN. 2014. CHEMICAL SIMILARITY BETWEEN HISTORICAL AND NOVEL HOST PLANTS PROMOTES RANGE AND HOST EXPANSION OF THE MOUNTAIN PINE BEETLE IN A NAIVE HOST ECOSYSTEM. NEW PHYTOL. 201: 940-950. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study confirms that MPB can survive and sustain their populations in jack pine forests. Further, the results have conclusively shown the chemical similarities of secondary compounds between lodgepole and jack pine has facilitated range expansion to naïve jack pine forests of Alberta.

Approach:

- There has long been concern MPB, like that of some other herbivores, may be able to take advantage of the "*evolutionary naivety*" of novel host species, such as jack pine, and exploit them more effectively than native hosts, such as lodgepole pine, with which they have coevolved. One of the hypotheses that makes colonization of novel species more possible is if the secondary defenses of the novel species are similar to that of the host species.
- The primary purpose of this research was to further the understanding of the adaptation potential of MPB and thus its potential to spread to novel habitats.
- More precisely, this study investigated the role of a host of secondary compounds on pheromone production by MPB and beetle attraction to a historical (lodgepole pine) and novel (jack pine) host species. The research team also set out to test if MPB can produce pheromones in jack pine and if the quality or quantity of that produced was similar to that in lodgepole pine. Finally, they further tested the suitability of jack pine for beetle reproduction.
- Volatile chemicals emitted from logs of both lodgepole and jack pine were assessed in a laboratory environment. Five log bolts were collected from the field and inoculated with both male and female beetles. Volatiles emitted from small holes were continuously measured for a four-hour period and analyzed using a gas chromatography and mass spectrometer.
- The amount of pheromones and monoterpenes emitted varied between the two tree species. The differences of these on beetle attraction was tested in the field using a lodgepole pine stand located near Hinton. Attempts were made to replicate this treatment in a jack pine stand but no suitable stands could be located.
- Quality of host substrate on MPB colonization and brood quality was assessed, again using bolts collected from the field. Beetles emerging from these bolts were sexed, weighed and then stored for use in a subsequent flying experiment.
- Flight capacity of the beetles that had previously emerged from jack and lodgepole pine bolts was measured using a standard flight mill. Measurements included flight distance flown, changes in beetle weight, and changes in lipid concentrations pre and post flight.

Key Findings and Implications:

- This is believed to be the first study of its kind to confirm pheromone production in jack pine. In addition, pheromone emissions differed only slightly between the two species. Female beetles reared in jack pine bolts emitted twice the concentration of *trans*-verbenol.
- Lodgepole pine trees emitted significantly more monoterpenes than that of jack pine. However, of note, jack pine emitted higher concentrations of α-pinene. This is most significant as the beetle uses α-pinene as a precursor form in pheromone production.
- MPB reproduction and brood weight and success supported the conclusion that jack pine stands are suitable hosts for MPB. Indeed, female beetles emerging from jack pine bolts had greater weights, which can have effects on flight and subsequent egg production.

- The highest number of beetles were found to be attracted to field traps baited with pheromones plus 3-carene and mimicking jack pine trees. Pheromones mimicking lodgepole pine trees attracted the fewest beetles.
- Total distance flown did not differ between tree species though, as expected, heavier beetles did fly longer distances.

N. KOLOSOVA, C. BREUIL AND J. BOLHMANN. 2014. CLONING AND CHARACTERIZATION OF CHITINASES FROM INTERIOR SPRUCE AND LODGEPOLE PINE. PHYTOCHEMISTRY. 101: 32-39. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Chitinases are enzymes that break down chemical bonds in chitin. The latter is typically a component of the cell walls of fungi and can also be found in the exoskeletal elements of some insects. As such, they may constitute an important defense mechanism within both angiosperms and gymnosperms.
- Inoculation of lodgepole pine seedlings with *G. clavigera* and *L. abietinum*, two fungi associated with MPB, resulted in a significant induction of the chitinases defense mechanism. The precise impact of chitinases in impeding fungi inoculation remains elusive.
- The induction noted above illustrates the complexities of the defense mechanisms deployed by lodgepole pine to defend itself against both *G. clavigera* and *L. abietinum.*

Approach:

- Ten chitinases were cloned from 3-year-old interior spruce and lodgepole pine seedlings. Chitinases were then “expressed” in *Escherichia coli* in a subsequent *in vivo* lab experiment.

Key Findings and Implications:

- Gene-specific transcript analysis revealed that *G. clavigera* strongly induced the production of chitinases in lodgepole pine. This may suggest that chitinases in lodgepole pine are an important defense mechanism against colonization by fungi.

S. M. Alamouti, S. Haridas, N. Feau, G. Robertson, J. Bohlmann and C. Breuil. 2014. Comparative genomics of the pine pathogens and beetle symbionts in the genus *Grosmannia*. Mol. Biol. Evol. 31(6): 1454-1474. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This keystone paper identified biogeographically structured lineages of the *Grosmannia* genus and one additional species not previously known to exist, using new advances in genomics.
- Significant variations in genes were found within the genus *Grosmannia.* These genes were attributed to a host of metabolic functions and chemical detoxification strategies. This finding is indicative of strong adaptive selection within fungal populations to the specific chemistries of hosts and their geographic location.

Approach:

- The primary goal of the research team was to identify *G. clavigera* genes that showed evidence of selective pressure and relate these variations to differences in fungal ecology and biology. A secondary objective was to identify a new research approach that could be adopted by future researchers interested in landscape population genetics.
- Studies to date suggest there are two distinct species of *G. clavigera*, one associated with *P. contorta* (Gs) and one with either *P. jeffreyi* or *P. ponderosae* (Gc). This study used the reference Gs genome to identify possible evolutionary divergence in different populations of Gs and Gc.
- The research team hypothesized that genes involved in host-pathogen interactions may have diverged to a much larger extent than other genes in response to selective pressures that exist in different host environments.

Key Findings and Implications:

- Seven genes that indicated strong selective pressures were identified and thought to be affiliated with a wide range of metabolic functions including metabolic production, ion transporters, membrane proteins, and various enzymes.
- This new genomics approach should help to inform future research teams interested in the evolution of other species of fungi and their evolutionary relationships with different hosts.

E. L. CLARK, C. PITT, A. L. CARROLL, B. S. LINDGREN AND D. P. W. HUBER. 2014. COMPARISON OF LODGEPOLE PINE AND JACK PINE RESIN CHEMISTRY: IMPLICATIONS FOR RANGE EXPANSION BY THE MOUNTAIN PINE BEETLES, *DENDROCTONUS PONDEROSAE* (COLEOPTERA: CURCULIONIDAE). PEERJ. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Jack pine may be easier to attack and colonize due to a combination of low levels of toxic terpenes and higher levels of terpenes thought to be important for pheromone production.
- Terpene concentrations varied considerably between lodgepole and jack pine. Concentrations of α-pinene, used by beetles to produce pheromones, were highest in jack pine. This terpene may accelerate mass attack of individual trees and increase colonization success. In sharp contrast, concentrations of toxic terpenes were consistently higher in lodgepole pine.
- Differences in terpene concentrations may play a significant role in the spread of beetle within jack pine forests, particularly if climate suitability in this new range increases with climate change. The lack of toxic terpenes in jack pine raises concern that these forests represent "*defense free space*".

Approach:

- This investigation compared terpene defenses of lodgepole and jack pine in northern and central Alberta to better understand the beetle's ability to locate hosts, attract other beetles, and ultimately to reproduce.
- Resin chemistry and induced responses to simulated beetle attacks were compared across 12 sites along a south-north transect from lodgepole pine growing in southern BC (historical range) to northern BC (novel habitat) and jack pine growing in northern and central Alberta (novel habitat).
- Terpene concentrations were measured following wounding of stems in two lodgepole pine stands, one near Chetwynd (where the beetle was outside of the historical range) and one near Kelowna.
- Wounding responses were measured in a jack pine stand near Fort McMurray, AB, at three time intervals: initial, two days and fourteen days post treatment.

Key Findings and Implications:

- Terpene concentrations differed between the two species for 23 of 26 terpenes measured. Lodgepole pine had the highest terpene concentrations, and jack pine had the highest levels of α-pinene.
- Terpene concentrations in wounded trees varied with time. The southern BC population synthesized limonene, a highly toxic terpene, more quickly than either the northern lodgepole pine stand or the jack pine stands. This more rapid synthesis was observed within the historical range of the beetle and may be due to selective pressures. The southern population was also able to synthesize α-pinene more quickly.
- The ability of trees to quickly produce terpenes in response to beetle attack, simulated here by wounding, is of paramount importance as beetles will abandon a tree if defense mechanisms are formidable.

J. K. Janes, Y. Li, C. I. Keeling, M. M. S. Yuen, C. K. Boone, J. E. K. Cooke, J. Bohlmann, D. P. W. Huber, B. W. Murray, D. W. Coltman and F. A. H. Sperling. 2014. How the mountain pine beetle (*Dendroctonus ponderosae*) breached the Canadian Rocky Mountains. Mol. Bio Evol. 31(7): 1803–1815. [URL]

Document Type: Non-Refereed Publication

Core Messages:

- The current expansion of MPB populations arose from multiple sources.
- The spread of MPB beyond its historical range may be in part due to its ability to adjust both its cellular and metabolic functions, enabling populations to withstand colder temperatures and facilitate longer dispersal distances. This is the first study to link changes at the molecular level to rapid range expansion in an insect.

Approach:

- The research team hypothesized the range expansion of MPB was due to regulatory changes in selected genes that could affect flight, cold tolerance, pheromone production and detoxification.
- Single nucleotide polymorphisms (a variation at a single position in a DNA sequence among individuals and known as SNPs) were sampled across the genome to understand genetic diversity, structure, connectivity and detection of outliers. The latter were in turn used to understand the source of the current populations of MPB and whether genomic adaption is occurring and facilitating range expansion.
- A total of 27 populations from Alberta, British Columbia, and South Dakota were studied.

Key Findings and Implications:

- Genomic data confirmed that the most likely dispersal routes of the beetle were: (1) expansion from southwestern toward northwestern BC (from Manning Park to Houston), and (2) slower expansion in all directions from Crowsnest Pass, AB, reaching Valemount, BC. Most importantly, significant changes in genomic compositions were observed between southern and northern populations.
- Most interestingly, the researchers reported that "*signatures of selection exist within MPB populations*" supporting the hypothesis above.
- This study found no evidence of an "epicenter" for the recent expansion, contrary to speculation.
- Populations near Manning Park and Whistler, BC had low genetic diversity, attributed to their isolated locations, low density forests and low dispersal. In contrast, a high level of genetic diversity and connectivity was observed in the Valemount and Wells Gray populations. This may be the result of gene flow from both more northerly and southerly populations.
- SNPs that were identified as outliers were generally linked to three key metabolic functions: (1) cholesterol/sterol production, (2) ion transport, and (3) actin contraction. Shifts in these functions may provide beetles with the ability to adapt to novel environments.
- The proportion of SNPs that could be attributed to a metabolic role or function was fairly low in this study, simply owing to the fact that little is known of the relationship between precise SNPs and function in the beetle. Expansion of similar research may provide more clarity around the role of molecular changes in function and range expansion.

D. I. OJEDA, B. DHILLON, C. K. M. TSUI AND R. C. HAMELIN. 2014. SINGLE-NUCLEOTIDE POLYMORPHISM DISCOVERY IN *LEPTOGRAPHIUM LONGICLAVATUM*, A MOUNTAIN PINE BEETLE-ASSOCIATED SYMBIOTIC FUNGUS, USING WHOLE-GENOME RESEQUENCING. MOL. ECOL. RES. 14: 401-410. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This paper reports the development of a new strategy for designing and developing SNP platforms using whole-genome resequencing for the MPB fungi *Leptographium longiclavatum.* This new methodology was found to be both robust and accurate, providing researchers with a new approach to investigating MPB fungal agents. As such, this paper represents an important contribution to basic research.

Approach:

- Single nucleotide polymorphisms (SNPs) are polymorphisms that are caused by point mutations that give rise to different alleles containing alternative bases at a given position of nucleotide within a locus. Their application is becoming increasingly popular in ecological and evolutionary studies as sequencing costs have decreased dramatically in recent years and a wide array of bioinformatics tools have been recently developed.
- This study involved the development of a new genotyping platform for *L. longiclavatum*, a fungus commonly associated with MPB in northern Alberta, and the identification of SNPs involved in host detoxification and pathogenicity.

Key Findings and Implications:

- The research team developed a new strategy for designing and developing SNP platforms using whole-genome resequencing. The new approach included illumina sequencing, genome assembly, gene annotation, SNP discovery, SNP selection and validation and illumina technology.
- Protein sequences for *L. longiclavatum* were similar (97%) to those of *G. clavigera*. Only 241 protein genes were unique to *L. longiclavatum.* Further studies are required to understand the role and function of these protein genes.
- The team did not find significant differences in the SNPs between different populations of *L. longiclavatum,* indicating a rather simple population structure.

A. Ham K. P. Bleiker, M. R. O'Brien, G. D. Smith and A. L. Carroll. 2014. Characterization of attacks made by the mountain pine beetle (Coleoptera: Curculionidae) during its endemic population phase. Can. Entomol. 146: 271–284. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- MPB typically exists in an endemic phase where populations are low, and beetles colonize weakened trees and are regulated by host tree defenses.
- However, little is known about MPB attack behavior and offspring development during the endemic phase which may impact outbreak triggers.
- This study investigates the life history and characteristics of MPB attack during its endemic phase.
- In general, MPB reproductive success at endemic levels is low. However, a high-quality host tree that has recently suffered abrupt stress could facilitate a rapid increase in MPB populations.

Approach:

- Eight trees were selected in 2009 for intensive sampling from two study sites in north-central Alberta, in areas where endemic MPB-attacks were previously identified.
- Log-bolts were extracted from each tree and researchers recorded the height of the attack, total gallery length, and cardinal direction of attack. Egg gallery characteristics were also recorded to determine the reproductive status of female beetles (mated vs. unmated) that initiated attacks.

Key Findings and Implications:

- Egg galleries were more concentrated on the lower ~3 m of the bole, but they did not favour a particular aspect. During mass attacks, MPB typically favours cooler and shadier aspects, but the weakened trees they attack at endemic levels may already grow in shadier areas.
- The vast majority (99%) of galleries were created by **mated** female beetles. This finding indicates that MPB at endemic levels had ample mating opportunities and likely mated at the host, not natal trees.
- Reproductive success was generally low as beetles rarely matured into pupae.
    - However, in one of the trees, egg galleries were three times the average length (25 cm) and with 123 pupae (60 eggs is the average for MPB). This tree was an example of a very favorable, but rare, host with little defenses and minimal competitors to MPB.
    - This finding reveals that under certain conditions an endemic population of MPB could rapidly grow in their host.
- While low reproductive success is typical for MPB at low/endemic levels, a high-quality host that has recently suffered abrupt stress could cause high population growth rates. Understanding these factors is important to know what could trigger a transition of MPB from endemic to outbreak population levels.

2013

A. Hammerbacher, A. Schmidt, N. Wadke, L. P. Wright; B. Schneider; J. Bohlmann, W. A. Brand, T. M. Fenning, J. Gershenzon and C. Paetz. 2013. A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway Spruce. Plant Phyiol. 162: 1324-1336. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Norway spruce is a dominant species in European forests and is frequently attacked by bark beetles. These beetles typically introduce fungal pathogens into the host, such as the virulent blue-staining ascomycete *C. polonica* which commonly affects Norway spruce.
- Norway spruce is thought to protect itself against insect and fungal attacks through the production of terpenoid resins, but their effectiveness against *C. polonica* is unclear. Stilbenes are a common phenolic compound found in the bark of spruce trees, reported to have antifungal properties.
- *C. polonica* is exceptionally well-adapted to overcome stilbene production, one of the tree's primary defense mechanisms, even when the production of stilbenes is upregulated in infected trees. This is somewhat surprising given the well-known antifungal properties of stilbenes.
- *C. polonica* was also able to metabolize stilbenes through a multitude of chemical pathways, and was even able to rely on stilbenes as their sole source of carbon.

Approach:

- The research team tested the effectiveness of stilbenes, a form of phenolic compounds that possess antifungal properties, in defending against the spruce bark beetle's primary fungal associate.
- Norway spruce saplings were inoculated with the fungus *C. polonica* and the activity of several genes were assessed. The possible effect of fungal metabolism on stilbene was also tested *in vitro* using cultures grown in Petri dishes.
- To test whether the fungus could reply solely on stilbenes as their carbon source, fungal isolates were grown in sealed containers on medium containing different concentrations of ^{13}C enriched caffeic acid, which has a similar carbon structure as stilbene. Carbon isotope analysis of the air in the headspace of the sealed containers was conducted to assess $^{13}Co_2$ – $^{12}CO_2$ ratios.

Key Findings and Implications:

- During initial infection with the fungus, stilbene biosynthesis was upregulated as a direct result of both elevated transcript levels and of stilbene synthesis genes. Unexpectedly, overall concentrations of stilbene **declined** during this period of fungal infection. The fungus was able to metabolize stilbene, and higher levels of metabolism were associated with higher levels of virulence of the fungus.
- Though not clear from these results, the authors speculated that the fungus may be able to utilize stilbenes as a source of both nutrients and energy, which would then facilitate growth rate of the fungus and facilitate further infection of the host.
- The authors also hypothesized the degradation of stilbenes in the host may directly benefit bark beetle larvae by increasing the quality of feeding substrate.

C. I. CULLINGHAM, J. E. K. COOKE, S. DANG AND D. W. COLTMAN. 2013. A SPECIES-DIAGNOSTIC SNP PANEL FOR DISCRIMINATING LODGEPOLE PINE, JACK PINE, AND THEIR SPECIFIC HYBRIDS. *TREE GENETICS & GENOMICS* 9: 1119-1127. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The authors have developed a unique and cost-effective approach to discern jack and lodgepole pine from their interspecific hybrids. This new approach may have significant applications to seed collection in areas of Alberta where these species are known to hybridize.

Approach:

- Microsatellites provide reliable and informative genomic data, but at a high cost. A less expensive—and thus more operationally feasible—approach is the use of SNP (single nucleotide polymorphism). SNPs are locations within the human genome where the type of nucleotide present (A,T,G or C) can differ between individuals.
- The objective of this study was to develop a reliable, cost effective and repeatable SNP approach that could discern jack and lodgepole pine from their interspecific hybrids.
- Xylem, bark, needles and roots of two-year old seedlings were collected for DNA testing. These seedlings had been previously subjected to a variety of treatments including water stress and wounding. Tissues were also collected from approximately 1900 pine samples from British Columbia, Alberta Saskatchewan and Ontario. The latter were also utilized in hybrid identification and markers sets.

Key Findings and Implications:

- Unexpectedly, the authors have identified SNP markers they believe have more discriminatory power than those identified in the microsatellite approach.
- The authors have identified two practical applications of their work. The first application is the identification of ancestry of seed stock used in reforestation. The second application is the refinement of hybridization zones within Alberta that, in turn, may help inform investigations relevant to the spread of MPB. A further, though less practical application, is the transfer of these findings to other genomics studies involving these species and their hybrids.

M. N. Andersson, E. Grose-Wilde, C. I. Keeling, J. M. Bengtsson, M. M. S. Yuen, M. Li, Y. Hillbur, J. Bohlmann, B. S. Hansson, and F. Schlyter. 2013. Antennal transcriptome analysis of the chemosensory gene families in the tree killing bark beetles, *Ips typographus* and *Densroctonus ponderosae* (Coleoptera: Curculionidae: Scolytinae). *BMC Genomics* 14, 198 (2013). [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Bark beetle olfactory detection sense is of critical importance to fitness. However, there is currently little information pertaining to the molecular aspects of odor detection.
- The research team has identified the precise receptor proteins in the antennae of MPB involved in olfactory detection. These findings may offer future researchers a new and novel approach to integrated pest management strategies.

Approach:

- This study reported on the analysis of antennal transcriptomes (i.e., set of all RNA transcripts) in two major bark beetle species and reported upon six major gene families that encode proteins for roles in chemoreception.

Key Findings and Implications:

- This research effort identified 31 putative odorant binding proteins, 11 chemosensory proteins, 3 sensory neuron membrane proteins, 49 odorant receptors, 2 gustatory receptors, and 7 ionotropic receptors in MPB. Similar results were found for the European bark beetle, though subtle differences were found.
- Identification of the precise receptor proteins in the antennae of MPB may offer future researchers a new and novel approach to integrated pest management strategies. Further, understanding the molecular basis for odor detection may aid in linking physiological and ecological relationships and processes.

J. A. ROBERT; C. PITT; T. R. BONNETT; M. M. S. YUEN; C. I. KEELING; J. BOHLMANN AND D. P. W. HUBER. 2013. DISENTANGLING DETOXIFICATION: GENE EXPRESSION ANALYSIS OF FEEDING MOUNTAIN PINE BEETLE ILLUMINATES MOLECULAR-LEVEL HOST CHEMICAL DEFENSE DETOXIFICATION MECHANISMS. PLOS ONE 8(11): E77777. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Colonization of host trees and tissues is fraught with challenges, including the need to overcome several toxic host defense mechanisms. Tissues of host conifers are saturated with potentially toxic metabolites that insects and their larvae must either tolerate or detoxify to successfully reproduce. The ability of mountain pine beetle to overcome these chemical defense mechanisms is thought to be a key factor contributing to their reproductive success.
- This molecular-based study on the metabolism of host metabolites provides new information on the ability of mountain pine beetle to overcome host defense mechanisms and may ultimately help predict, or explain, the spread of mountain pine beetles to other host species.
- The research team hypothesized that metabolic changes in beetles which occur during the initial period of colonization are an indicator of stress in beetles and the chemical priming of detoxification mechanisms.

Approach:

- The research team used RNA-sequencing analysis to monitor gene expression patterns in adult male and female beetles during early colonization, to study potential molecular-level host chemical detoxification mechanisms.
- Treatments included adult beetles that were starved for a period of 24 hours, and male and female pairs that were fed susceptible host material for a similar length of time. Lodgepole pines bolts were collected near Penticton, British Columbia and RNA extraction utilized standard methodologies.

Key Findings and Implications:

- The team found transcript changes in numerous groups of enzymes thought to be key in the detoxification mechanism. These included cytochromes, glucosyl transferases and glutathione S-transferases, esterases, alcohol dehydrogenases, and ABC transporters. Several gene transcripts thought to be important for immune system responses, reproduction, pheromone flux and digestion processes were also identified.
- Male and female beetles had different responses. Female beetles, which are the first to colonize trees and construct galleries, showed high levels of oleoresin. Males, in contrast, had the largest increase in CYP6DE4.

C. L. Keeling, M. M. Yuen, N. Y. Liao, T. R. Docking, S. K. Chan, G. A. Taylor, D. L. Palmquist, S. D. Jackman, A. Nguyen, M. Li, H. Henderson, J. K. Janes, Y. Zhao, P. Pandoh, R. Moore, F. A. H. Sperling, D. P. W. Huber, I. Birol, S. J. M. Jones and J. Bohlmann. 2013. Draft genome of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a major forest pest. *Genome Biol* 14, R27. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This is a landmark paper that is the first to sequence the genome of the MPB. While there are over 400,000 beetle species, many of which are critical pests in both the forestry and agriculture sectors, this is only the second beetle genome sequence reported.
- Genome sequencing of MPB should further understanding of the structure and natural variation of MPB populations throughout North America, and shed light on how they adapt to novel hosts, new geographical ranges, and new and ever changing climates.
- Researchers identified a near identical gene, associated with sucrose-6-phosphate hydrolase, in both MPB and in enterobacteria. This exciting finding suggests that bacterium-insect-transfer-gene-transfer likely occurred over the course of evolution. This gene may help MPB metabolize carbohydrates.

Approach:

- MPB pupae were collected from trees felled near Prince George, BC. Genomic DNA for each sex of beetle was extracted using standard lab procedures.

Key Findings and Implications:

- This landmark paper reports on the finding of a horizontally transferred gene, the identification of genome sequencing representing the sex chromosomes, and gene families associated with host colonization.
- Costs associated with gene sequencing continue to drop significantly, opening the door to aggressively increase the understanding of insect functions and their relationships with hosts and symbionts. This paper, and its detailed methodology, should facilitate further genomics investigations.

D. E. HALL, P ZERBE, S. JANCSIK, A. L. QUESADA, H. DULLAT, L. L. MADILAO, M. YUEN AND J. BOHLMAN. 2013. EVOLUTION OF CONIFER DITERPENE SYNTHASES: DITERPENE RESIN ACID BIOSYNTHESIS IN LODGEPOLE PINE AND JACK PINE INVOLVES MONOFUNCTIONAL AND BIFUNCTIONAL DITERPENE SYNTHASES1[W](OA). PLANT PHYSIOL. 161: 600-616. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Lodgepole pine and jack pine trees produce several complex mixtures of diterpenoid metabolites as part of a broad chemical defense strategy against both insect and pathogen attacks.
- Detailed analysis of biosynthesis pathways has revealed, for the first time, that diterpene resin acids are present in both lodgepole pine and jack pine.

Approach:

- Tissues were collected from two-year-old lodgepole pine and jack pine seedlings. Tissue analysis was extensive and included RNA and DNA analysis, enzyme assays, diterpene and metabolite extractions, computational structure analysis and finally phylogenic analysis.

Key Findings and Implications:

- Eleven new diterpene synthases (diTPS) were discovered in both lodgepole and jack pine trees. Seven of these syntheses were functionally characterized. The eleven newly discovered diTPS included the discovery of four mono-functional diTPSs of DNR biosynthesis.
- These findings demonstrate that in-depth mining and functional analysis of plant transcriptomes can reveal new biosynthetic pathways, even when all essential enzymes of the pathway were thought to be known.

A. Arango-Velez, L. M. Gonzalez, M. J. Meents, W. E. Kayal, B. J. Cooke, J. Linsky, I. Lusebrink and J. E. K. Cooke. 2013. Influence of water deficit on the molecular responses of *Pinus contorta* x *Pinus banksiana* mature trees to infection by the mountain pine beetle fungal associate, *Grosmannia clavigera*. Tree Physiol. 34(11):1220-1239. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Water deficits reduced monoterpene levels, protein-based defense mechanisms, phenolic biosynthesis and cell wall biosynthesis in lodgepole x jack pine hybrid trees. These reductions in tree defense mechanisms may have been partly the result of reduced photosynthesis, which would have impaired the ability of host trees to produce both carbon and nitrogen-based defenses.
- The identification of *G. clavigera* response genes provide a superior method for assessing defense mechanisms in lodgepole x jack pine hybrids than physical measurement of lesion lengths. Some of these genes were enhanced with moisture stress and some were attenuated, revealing the tree response to water stress and infection is complex at the molecular level and needs further study.
- Lodgepole x jack pine hybrids may not be well adapted to defend against *G. clavigera* infections, and the threshold for successful MPB attack may be lower in these maladapted trees. Periods of severe drought in northern and eastern Alberta may facilitate the spread of MPB beyond its natural zone.

Approach:

- Researchers examined the effect of drought on the molecular defense responses of mature lodgepole x jack pine hybrid trees in central Alberta to the MPB fungal associate *G. clavigera*.
- The study was in a 60-year-old stand north of Whitecourt, AB. One treatment group of trees was well watered, and the other had artificial drought conditions induced using tarps that restricted rainfall penetration into the ground.
- Trees were inoculated with *G. clavigera* in July. Phloem was collected five weeks later from outside and inside the lesion area for gene expression analysis. The vertical limits of lesions were measured.
- Stomatal conductance and photosynthesis were measured, and transcript abundance profiles were developed for 18 genes belonging to the families generally associated with both biotic and abiotic stressors.

Key Findings and Implications:

- Lesion length was greatest in well-watered trees, which the research team argued was a potential indicator that these trees were able to deploy stronger defense mechanism to mitigate infection.
- Several genes affiliated with tree response to both biotic and abiotic stress showed significant changes in response to *G. clavigera* infection. These changes were thought to be general in nature and not specific to *G. clavigera* per se, as the hybrids tested were beyond the historical range of the fungus.
- Most of the *G. clavigera* response genes showed the same transcript abundance in phloem tissues sampled (1) within lesions and (2) near lesions. This suggests the production of phenolic- and terpenoid-based compounds occurs some distance from the lesions and is transported to the site of infection. The researchers speculated that a mechanism to upregulate the production of defense compounds in the immediate vicinity of infection could result in a timelier response to attack.

C. K Tsui, S. DiGuistini, Y. Wang, N. Feau, B. Dhillon, J. Bohlmann and R. C. Hamelin. 2013. Unequal recombination and evolution of the mating-type (MAT) loci in the pathogenic fungus *Grosmannia clavigera* and relatives. G3 Genes Genomes Genetics 3(3): 465–480. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study confirms that *G. clavigera* and relatives can reproduce sexually, disproving the previously held thought they were asexual.
- The ability to sexually reproduce increases their genetic diversity and could significantly enhance their fitness when introduced to novel ecological niches. This finding has significant implications given the spread of MPB beyond its historical range.

Approach:

- Thirty-four fungal isolates belonging to nine species of Ohiostomatales (fungi commonly associated with many bark beetles) were sampled.
- DNA extraction, polymerase chain reaction amplification and gene sequencing were carried out. The MAT locus received detailed scrutiny including from the perspective of evolutionary processes.

Key Findings and Implications:

- Detailed genetic analysis has confirmed these fungi species are indeed capable of sexually reproducing in nature.
- Heterothallic fungi benefit from recombination by increased genetic variability which could enhance the fitness of these fungal pathogens in new ecological niches.

D. E. HALL, M. M. S. YUEN, S. JANCSIK, A. L. QUESADA, H. K. DULLAT, M, LI, H. HENDERSON, A. ARANGO-VELEZ, N. Y. LIAO, R. T. DOCKING, S. K. CHAN, J. E. K. COOKE, C. BREUIL, S. J. M. JONES, C. I. KEELING AND J. BOHLMANN. 2013. TRANSCRIPTOME RESOURCES AND FUNCTIONAL CHARACTERIZATION OF MONOTERPENES SYNTHESES FOR TWO HOST SPECIES OF THE MOUNTAIN PINE BEETLE, LODGEPOLE PINE (*PINUS CONTORTA*) AND JACK PINE (*PINUS BANKSIANA*). BMC PLANT BIOL. 13, 80 (2013). [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- One of the primary defense mechanisms of both lodgepole and jack pine is the production of terpenoid oleoresins, which act as both a physical and chemical defense. Despite their importance, prior to this study the genes responsible for terpene biosynthesis in lodgepole pine and jack pine were unknown.
- The researchers have identified, for the first time, genes responsible for terpene biosynthesis in jack and lodgepole pine.
- Genomic investigations have identified 34 unique terpenoid pathway genes in lodgepole pine, and 38 similar genes in jack pine. These gene discoveries provide valuable insights into intra- and interspecific variations in the defense mechanisms of these species.

Approach:

- This investigation into genes involved Sangor, Roche 454 and Illumina sequencing technologies to assemble transcriptomes for tissues collected from jack pine and lodgepole pine seedlings.

Key Findings and Implications:

- Monoterpenes were determined in various tissues including apical buds, stem, foliage and roots of both species. Monoterpenes were found in all tissues but the type and concentration varied significantly between the two species.
- The dominant monoterpenes in lodgepole pine tissues were phellandrene and (-)-β-phellandrene. In sharp contrast, a wider mixture of terpenes were found in jack pine tissues with (+)-α-pinene being dominant.
- Interestingly, genes that produce (+)-α-pinene have only been detected in pine species, suggesting this defense mechanism evolved in pine following their evolutionary separation from spruce and fir species.

Y. Wang, L. Lim, S. DiGuistini, G. Robertson, J. Bohlmann and C. Breuil. 2013. A specialized ABC efflux transporter GcABC-G1 confers monoterpenes resistance to *Grosmannia clavigera*, a bark beetle-associated fungal pathogen of pine trees. New Phytol. 197(3):886-98. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study conclusively shows that *G. clavigera* has developed, over the course of co-evolution with pine trees, an efflux transporter ("GcABS-G1") that effectively regulates the concentrations of monoterpenes within the fungal cells.
- Strong inducible expressions of GcABS-G1 suggest this transporter is an "*efflux pump*" that removes toxic monoterpenes from cells and should therefore provide pine trees with an adaptive advantage.

Approach:

- Species affected by MPB have complex oleoresin-based chemical defenses against both pests and pathogens. The most dominant form of oleoresin in pine trees is monoterpenes, which essentially serve as fungicides, yet little is known how *G. clavigera* colonizes these monoterpene-rich growth environments.
- This study sought to identify *G. clavigera*'s mechanisms for dealing with chemical toxins by assessing the response of various genes to a range of monoterpene concentrations. Common mechanisms or strategies to deal with chemical toxins in the environment include compartmentalization of the toxin to vacuoles or detoxifying the toxin.

Key Findings and Implications:

- A total of 39 ABC transporters were identified in *G. clavigera*. The gene found to be most strongly induced by monoterpenes was GcABS-G1. Expression of this gene seemed to be exclusive to the presence of monoterpenes and could not be induced by other stress treatments.
- GcABS-G1 confers tolerance to monoterpenes. This was confirmed by deleting the GcABS-G1 gene and creating a mutant. The latter increased the sensitivity of the fungus to the effect of monoterpenes. Further, development of fungal symptoms in young lodgepole pine trees was delayed when the trees were inoculated with a mutant not containing this gene.
- These results confirm that this gene contributes to the ability of *G. clavigera* to overcome host defense chemicals.

C. I. KEELING, H. HENDERSON, M. LI, H. K. DULLAT, T. OHNISHI AND J. BOHLMANN. 2013. CYP345E2, AN ANTENNA-SPECIFIC CYTOCHROME P450 FROM THE MOUNTAIN PINE BEETLE, *DENDROCTONUS PONDEROSA* HOPKINS, CATALYZES THE OXIDATION OF PINE HOST MONOTERPENE VOLATILES. INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY. VOLUME 43(12): 1142-1151. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- CYP345E2 is a type of P450, where the latter represents a sort of super family of enzymes, that plays a critical role in the antenna of the MPB by modifying the majority of (though not all) monoterpenes emitted by host conifers. This role enables the MPB to quickly deactivate odors so its olfactory system does not become overloaded and instead remains responsive to new odors.

Approach:

- While MPB must be able to quickly identify and respond to pheromones, they must also be able to quickly clear these compounds from their antennae so they can remain responsive to new odors. This means the antennae must possess odorant-degrading and odorant-modifying enzymes in addition to odorant binding enzymes involved in odor detection and processing.
- Previous research has indicated that CYP345E2 is the most highly expressed transcript in the MPB antennae CDNA library. To date, however, the precise function role of CYP345E2 is unknown, as is that of other P450s involved in olfaction.
- The research team tested the hypothesis that CYP345E2 is an odorant-modifying enzyme and, moreover, that it acts on the volatile monoterpenes emitted from its conifer hosts.

Key Findings and Implications:

- CYP345E2 used several monoterpenes as substrates in an *in vitro* study including limonene, α-pinene, β-pinene, carene and terpinolene, which were either epoxidized or hydroxylated. Several other monoterpenes were not found to be related to CYP345E2, including α-phellandrene and frontalin. Myrcene had only a weak relationship. Several P450s may have a similar though more minor role as an odorant-modifying enzyme.
- CYP345E2 is the first P450 to be functionally characterized as it pertains to insect antenna.

C. I. KEELING, C. C. CHIU, T. AW, M. LI, H. HENDERSON, C. TITTIGER, H. WENG, G. J. BLOMQUIST AND J. BOHLMANN. 2013. FRONTALIN PHEROMONE BIOSYNTHESIS IN THE MOUNTAIN PINE BEETLE, *DENDROCTONUS PONDEROSAE*, AND THE ROLE OF ISOPRENYL DIPHOSPHATE SYNTHASES. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, 110(47) 18838-18843. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study explored the biosynthesis of the frontalin pheromone. Detailed analysis revealed that this pheromone originates from a 20-carbon isoprenoid precursor. This finding, very technical in nature, provides new insights that should form the basis for additional study.

Approach:

- The frontalin pheromone was first identified forty years ago. Female MPB use a combination of frontalin, trans-verbenol and verbenone to attract other male and female beetles. The trans-verbenol and the verbenone intensify the attracted character of (-)-frontalin.
- The precise mechanism overseeing the synthesis of frontalin is not fully understood.
- A combination of genome and transcriptional analysis, RNA interference, transcript and metabolite analysis and the functional characterization of up-regulated proteins was adopted to elicit an understanding of frontalin synthesis.

Key Findings and Implications:

- The possible role of 15-farnesyl diphosphate or 10-carbon geranyl diphosphate as possible precursors to frontalin was disproven.

A. S. ADAMS, F. O. AYLWARD, S. M. ADAMS, N. ERBILGEN, B. H. AUKEMA, C. R. CURRIE, G. SUEN AND K. F. RAFFA. 2013. MOUNTAIN PINE BEETLES COLONIZING HISTORICAL AND NAIVE HOST TREES ARE ASSOCIATED WITH A BACTERIAL COMMUNITY HIGHLY ENRICHED IN GENES CONTRIBUTING TO TERPENE METABOLISM. APPL. ENVIR. MICRO. 79(11): 3468-3475. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This paper investigates the possible role of bacterial communities commonly associated with MPB and host conifers in overcoming the chemical defense mechanisms of conifer hosts.
- The results confirmed, for the first time, that microbes associated with MPB have numerous genes involved in terpenoid degradation and have either a direct or indirect role in the MPB's ability to attack live or otherwise healthy conifers. These results confirm that bacteria associated with the MPB are very well adapted to metabolize the toxins produced by host species.

Approach:

- The purpose of this investigation was to assess the potential role of MPB-associated bacterial communities during the colonization of both lodgepole and hybrid pines. The research team tested the hypothesis that bacteria play a role in detoxification of tree host defenses by analyzing genes involved in terpene degradation.
- Samples of MPB adults, their galleries and phloem were collected from sites spanning a wide geographical range (Mackenzie, Grande Prairie, Slave Lake, Whitecourt, Athabasca and Peace River). Samples were collected from both MPB-affected and -unaffected stands.
- Metagenomic analysis was used to identify genes thought to be associated with detoxification and already publicly available in sequence databases.

Key Findings and Implications:

- Numerous bacterial genes associated with the degradation of diterpenes, limonene and pinene were identified in the samples. The number of these genes found in MPB associated bacteria was much higher than that found in non-associated bacteria.
- The research team found no significant differences in the broader bacterial community between Alberta and British Columbia. This relative stability of the bacterial communities across the range of the MPB indicates the rapid expansion of the MPB will not be limited by lack of suitable bacterial communities.
- The researchers were careful to conclude that further research is needed to quantify the benefit provided by bacteria.

L. LJERKA, S. HARIDAS, J. BOHLMANN AND C. BREUIL. 2013. THE CYTOCHROMES P450 OF *GROSMANNIA CLAVIGERA*: GENOME ORGANIZATION, PHYLOGENY, AND EXPRESSION IN RESPONSE TO PINE HOST CHEMICALS. FUNGAL GENET. BIOL. 50: 72-81. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Within fungi including *Grosmannia clavigera*, the cytochromes P450 have an important role in detoxifying chemical defense mechanisms of host species and accessing the carbon and nutrients necessary for hyphae growth.
- *G. clavigera* had fewer cytochromes P450 genes than expected, likely owing to evolutionary pressures that favoured their role in detoxification and accessing carbon and nutrients.
- Identification and characterization of cytochromes in *G. clavigera* represents an important step in further deciphering the metabolic relationships between host, beetle and fungi.

Approach:

- MPB and its associated fungi have developed complex detoxification mechanisms to overcome the effects of toxic compounds within their host environments. In the case of lodgepole pine, these defense compounds include monoterpenes, phenolics and terpenoids.
- This paper reports the identification and phylogenetics (i.e., evolutionary pathways) of the cytochromes of G. clavigera.
- To discover which cytochromes had a role in detoxification of pine defense chemicals, the team analyzed the expression profiles of cytochromes from mycelia grown on a medium treated with a mono- and diterpene blend.
- To reveal which cytochromes might be involved in the utilization of different carbon sources, the team analyzed cytochrome gene expression profiles of *G. clavigera* grown on media treated with three different carbon sources for a period of 10 days.

Key Findings and Implications:

- Fifty-four cytochromes P450 genes were identified—far less than a historical ancestor, which had 81 such genes, suggesting that the cytochrome family contracted within *G. clavigera* over evolutionary time. However, though the number of cytochromes were low, evolution may have favoured those that have a role in tolerance of host defense chemicals.
- Most cytochromes were down-regulated during the 12 hours following inoculation on media treated with defense chemicals (mono- and diterpene blend). However, the profile changed significantly after 36 hours of exposure: ten cytochromes were significantly up-regulated and six were down- regulated. Genes were further expressed after an additional 24 hours of exposure, with one having a 118-fold increase.
- Eleven cytochromes were up-regulated and four were down-regulated when the carbon source was monoterpenes. One cytochrome was up-regulated by a factor of 430. Similar results were also found when the carbon source was either triglycerides or oleic acid.
- These findings support the argument that cytochromes are involved in both detoxification and accessing carbon and nutrients required for *G. clavigera* to develop its hyphae network.

I. LUSEBRINK, N. ERBILGIN AND M. L. EVENDEN. 2013. THE LODGEPOLE X JACK PINE HYBRID ZONE IN ALBERTA, CANADA: A STEPPING STONE FOR THE MOUNTAIN PINE BEETLE ON ITS JOURNEY EAST ACROSS THE BOREAL FORESTS? J. CHEM. ECOL. 39, 1209-1220 (2013). [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This in-depth, largely field-based study demonstrates that drought increases the body size of emerging larva and is therefore beneficial to MPB.
- The ability of hybrid jack pine to defend against MPB was intermediate when compared to that of lodgepole and pure jack pine.
- The phloem chemistry of hybrid trees resembled a mixture of both lodgepole and jack pine trees, and may therefore provide the "*perfect stepping stone*" to enable future range expansion by MPB.

Approach:

- The objectives of this field-based study were to: (1) assess the profile of volatile compounds in hybrid trees, (2) evaluate the effect of drought on volatile compounds and monoterpene concentrations in tree tissues, and (3) determine if brood success is affected by simulated drought.
- The study was carried out in a stand near Whitecourt, AB in an area thought to support hybridized pine. Trees received one of two water treatments: well-watered using slow-release watering bags attached to the bole of each trees, and a drought treatment imposed by the use of tarpaulins.
- Five weeks following the imposition of the watering treatments, four biological treatments were imposed: (1) control, (2) mechanical wounding only, (3) mechanical wounding followed by inoculation with *G. clavigera*, and (4) mechanical wounding, inoculation with *G. clavigera* and the addition of dead beetles (live beetles were not permitted in the area).
- Volatile collections were collected from trees one day prior to treatment and six times over the nine weeks post treatment. After nine weeks, the trees were harvested, lesion length was measured and the phloem between the lesions was sampled for chemical analysis.
- Finally, bolts were collected from each tree and inoculated in the lab with live beetles. Beetles were retained in the bolts for five weeks to allow for instar development. After five weeks, emerging beetles were sexed, weighed, and their fat contents determined, and gallery size was measured.

Key Findings and Implications:

- Trees that experienced water deficits emitted more total monoterpenes than well-watered trees.
- Control trees emitted lower monoterpene concentrations than treated trees. Mechanically wounded trees had lower monoterpene concentrations than trees that were wounded and inoculated with *G. clavigera.*
- Monoterpene concentrations varied greatly with time, showing a dramatic increase in synthesis by the 4th day following treatments. Concentrations began to decline by the 5th week of measurement.
- The types of monoterpenes produced by lodgepole and jack pine trees differed widely. For example, the dominant monoterpene emitted by lodgepole pine was β-phellandrene but this form was almost undetectable in jack pine trees. In contrast, α-pinene was the dominant monoterpene in jack pine.
- The hybrid trees had a wider range of monoterpenes but were most similar to the profile for lodgepole pine. The authors attributed this similarity to the fact that the ancestry of hybrids is favored towards that of lodgepole pine.
- A total of 329 beetles emerged from the bolts. Neither biological treatment nor watering treatment had any effect on number of beetles that emerged, their sex or number of larvae galleries. However, female beetles from trees that had imposed water deficits had significantly higher body weight and fat content. These female beetle attributes are expected to aid in beetle dispersal, colonization and reproduction.

2012

C. I. Keeling, H. Henderson, M. Li, M. Yuen, E. L. Clark, J. D. Fraser, D. P. W. Huber, N. Y. Liao, T. R. Docking, I. Birol, S. K. Chan, G. A. Taylor, D. Palmquist, S. J. M. Jones and J. Bohlmann. 2012. Transcriptome and full-length cDNA resources for the mountain pine beetle, *Dendroctonus ponderosa* Hopkins, a major insect pest of pine forests. Insect Biochem. Mol. Biol. 42(8):525-36. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This significant study reports, for the first time, a large-scale genomics resource of transcriptome sequences for the MPB. This new body of knowledge provides a new and exciting platform for additional research into gene discovery and both functional and population genomics.

Approach:

- To date, the genome sequence has only been published for one species of beetle – the red flour beetle. The importance of developing a comprehensive resource of sequence information for MPB cannot be understated for understanding the biology of this devastating insect.
- MPB were collected from a site near Prince George, British Columbia at various times between 2006 and 2008. Both adult and larva beetles were collected, sexed, and dissected for analysis.
- A complex array of genomic tools were deployed. In addition to the identification of transcriptome sequences the team also placed a priority on the identification of cytochrome P450s.

Key Findings and Implications:

- Over 14,000 transcripts were identified unique to MPB.
- DNA libraries were established for stages of MPB (larva, pupa and adult) and for specific beetle parts (antennae, head and mid guy tissues).
- Some 78 P450's were identified, which places MPB in a mid-range for the number of P450's reported for other species.
- The most frequently sequenced transcript in antennae tissues was a new cytochrome gene identified as CYP345E2. Interestingly, it was only found in the antennae. Additional P450's appeared to be localized to the mid gut indicating they may have an exclusive role in digestion, pheromone biosynthesis, or detoxification of host defenses. Some transcripts were unique to larvae stage of development.

C. CULLINGHAM, P. M. A. JAMES, J. E. K. COOKE AND D. W. COLTMAN. 2012. CHARACTERIZING THE PHYSICAL AND GENETIC STRUCTURE OF THE LODGEPOLE PINE X JACK PINE HYBRID ZONE: MOSAIC STRUCTURE AND DIFFERENTIAL INTROGRESSION. EVOL. APPLIC. 5(8): 879-891. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The hybrid zone for lodgepole and jack pine is more spatially complex (i.e., patchier) and extensive than previously thought. Hybrid pine was identified in northern British Columbia for the first time.
- This study found evidence of gene flow between the two species, which may provide western populations of jack pine with genes beneficial to defending against MPB. Further study is required to assess the magnitude of this benefit, particularly with respect to spread rates of MPB eastward.

Approach:

- The objective of this study was to delineate the area of lodgepole and jack pine hybridization in Alberta, understand the role of the environment on hybridization, and understand the extent of patterns of gene flow and ancestry.
- Genomic DNA was determined using foliage samples collected from 662 lodgepole pine, jack pine and hybrid trees. Samples were collected from British Columbia, Alberta, Saskatchewan and Minnesota. Additional tissues were obtained from young germinants so that additional sites in British Columbia and Alberta could be included in the study.
- Various genetic-based software was used to define species classes, hybrid ancestor and gene flow between parental species.

Key Findings and Implications:

- The zone for lodgepole and jack pine hybrids in north-central Alberta was previously poorly defined.
- Newly defining this zone may have important ramifications for reforestation decision making.
- Lodgepole pine and jack pine hybrids can be difficult to identify in the field because they often resemble one or the other of the pure species. Molecular markers can now be utilized to identify hybrids of the two species.
- The hybrid ancestor was biased towards lodgepole pine though evidence of gene flow between the two pure species was found to be generally equal.

C. J. CULLINGHAM, A. D. ROE, F. A. H. SPERLING AND D. W. COLTMAN. 2012. PHYLOGEOGRAPHIC INSIGHTS INTO AN IRRUPTIVE PEST OUTBREAK. ECOLOGY AND FUNCTION. 2(5): 908–919. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Phylogeography is the study of the historical processes that may be responsible for the geographic distributions of individuals or specific populations. Use of phylogeography has expanded in recent years with the increasing amount of information becoming available from the field of population genetics.
- Defining the source and spread of an outbreak using population genetics can aid in the delineation of boundaries that can be used for control purposes.
- The most northerly population of MPB had the lowest genetic diversity and level of population structure. However, genetic signatures throughout this region were weaker than expected, which complicates the identification of source populations.
- This study has identified limitations of using a phylogeography approach in the study of a highly dispersive species that has a rapidly changing population.

Approach:

- The authors used a phylogeographic approach to shed light on the origins of the current MPB outbreak. The objectives of this specific study were to: (1) understand genetic diversity across the geographic distribution of the MPB; (2) determine if future spread can be predicted based on recent population dynamics; and (3) determine if genetic information can be used to identify the precise source of current outbreak.
- Live beetles were collected from 26 locations in British Columbia, Alberta and South Dakota. These sites were divided into three distinct populations based on historical data up to 1970 to allow for lineage diversification in relation to geography. The South Dakota dataset was included to represent populations south of the Cordilleran ice sheet. A second grouping of sample sites was obtained from the northern range of the current infestation and a third grouping represented sampling sites in southern Alberta and southern British Columbia.
- DNA extraction and testing followed standard laboratory processes.

Key Findings and Implications:

- Mitochondrial diversity in the British Columbia and Alberta populations revealed a poorly defined phylogeography structure with limited genetic differentiation.
- Analysis of microsatellite markers indicated the outbreaks in southern Alberta and British Columbia represented a single population with a weak genetic structure owing to size and the rate of expansion.

T. R. BONNETT, J. A. ROBERT, C. PITT, J. D. FRASER, C. I. KEELING, J. BOHLMANN AND D. P. W. HUBER. 2012. GLOBAL AND COMPARATIVE PROTEOMIC PROFILING OF OVERWINTERING AND DEVELOPING MOUNTAIN PINE BEETLE, *DENDROCTONUS PONDEROSAE* (COLEOPTERA: CURCULIONIDAE), LARVAE. INSECT BIOCHEM. MOL. BIOL. 42(12): 890-901. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study identifies changes in proteins in overwintering and developing larvae. As such, it provides an important basis to enable further studies that detail physiological changes in larvae development over the course of time and changes in the larvae's environment.

Approach:

- Overwintering larvae were collected from a lodgepole pine stand on four sampling dates (26 Sept. 2008, 7 Nov. 2008, 25 March 2009 and 27 May 2009) and quick frozen. Protein extracts were determined at the proteomics laboratory at the University of Victoria using the iTRAQ approach. The latter allows the concurrent identification and relative quantification of hundreds of proteins.

Key Findings and Implications:

- Larvae collected in the autumn contained 1140 proteins, and those collected in the spring contained c. 1050 proteins. Of note, 456 proteins were exclusive to the autumn-sampled beetles and 368 were exclusive to the spring-sampled beetles. This highlighted the fact that season had a significant effect on proteome composition.
- Accumulations of 33 proteins changed between September and November. These proteins were related to energy metabolism and defense against toxins. These results were evidence that the beetles were under considerable stress during this time—namely, low temperatures and hypoxia.
- Between the two spring samples, 473 proteins changed; this large change was attributed to rapid changes in larvae development.

J. BOHLMANN. 2012. PINE TERPENOID DEFENSES IN THE MOUNTAIN PINE BEETLE EPIDEMIC AND IN OTHER CONIFER PEST INTERACTIONS: SPECIALIZED ENEMIES ARE EATING HOLES INTO A DIVERSE, DYNAMIC AND DURABLE DEFENSE SYSTEM. Tree Physiol. 32(8): 943–945. [URL]

Document Type: Commentary, Tree Physiology, 2012

Core Messages:

- A short but powerful commentary in which the author reflects on the immense and complex systems of tree defenses against insects and fungi, and the molecular-level ecological and physiological adaptions by beetle and fungi to not only overcome but to benefit from these defenses.
- Owing to these complexities, the author argues that a tree-centric approach to defense physiology and ecology is now out of date and contends that we must consider the interactions between host, beetle and fungi together.

Approach:

- The author compiled various reflections and provided these through a commentary piece.

Key Findings and Implications:

- The author notes the formidable defense systems that have evolved in conifers such as lodgepole pine. These include physical defenses such a thick bark, synthesis of large amounts of resins which are directed to insect wounds, development of specialized parenchyma cells, and traumatic resin ducts. Chemical defenses include production of oleoresins and phenolics.
- While the conifer defense system is indeed formidable, dynamic and durable, the author stresses that it is now readily apparent these same defenses can be utilized by the MPB for food and for signaling.
- The author noted the much shorter generation times of MPB, compared to that of conifers, may have provided the MPB the advantage of time to develop molecular-based mechanisms to overcome and even benefit from conifer defense mechanisms.
- The final conclusion was that the high flammability of terpene hydrocarbons may increase the fire risk of sawmills processing large amounts of beetle-infected wood. This note comes several months after the large and devastating sawmill explosion in Burns Lake, BC.

L. KHADEMPOUR, V. LEMAY, D. JACK, J. BOHLMANN AND C. BREUIL. 2012. THE RELATIVE ABUNDANCE OF MOUNTAIN PINE BEETLE FUNGAL ASSOCIATES THROUGH THE BEETLE LIFE CYCLE IN PINE TREES. MICROB. ECOL. 64, 909-917. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The MPB is closely associated with the fungal species *Grosmannia clavigera, Leptographium longiclavatum, Ophiostoma montium* and *Ceratocystiopsis*. The relative abundance of these fungi in MPB galleries changed significantly over the course of the MPB's life cycle. These changes may enhance the overall fitness of the MPB.

Approach:

- The primary goal of this study was to identify and compare the relative abundance of these fungal associates through the life cycle of the MPB. It was thought that a better understanding of potential changes in abundance through time may lead to a better understanding of the ecological roles of each species of fungi.
- The team hypothesized that faster-growing fungi would be associated with mass attack and host colonization, and slower-growing fungi and less pathogenic fungi would be associated with development of larvae and teneral adults. This pattern of fungi being associated with specific stages of the beetle life cycle was thought to enhance the fitness of the beetle.
- The experiment was conducted in a mature lodgepole pine stand near Merritt, BC. Thirty trees were baited with pheromone in mid-July of 2009. Three baited trees were randomly selected and felled at a time thought to coincide with four stages of beetle development: 24 Jul. 2009, 7 Aug. 2009, 5 Jul. 2010 and 28 Jul. 2010. Intact galleries were identified in the laboratory and the phloem was sampled for DNA extraction and PCR analysis.

Findings and Implications:

- *Grosmannia clavigera* and *Leptographium longiclavatum* colonized the phloem rapidly. Colonization by these fungi was followed by the less pathogenic *Ophiostoma montium* and *Ceratocystiopsis,* which were more abundant during the pupae and teneral adult stage.
- *Ceratocystiopsis* was present at very low abundance in the beetle gallery during the colonization phase and was more abundant at the pupal stage of beetle development. This finding is thought to be significant as this fungi is often ignored in the literature.
- The increased abundance of *Ceratocystiopsis* during the pupae and teneral stages of MPB development may have been the result of the presence of meconium, a waste product produced by the larvae. This waste product is high in nitrogen and may stimulate fungi growth. Of note, *Ceratocystiopsis* does not produce any pigment and this quality, combined with its high nitrogen content, may make it a favoured food for the pupae.
- This study confirms that qPCR analysis is of value in quantifying changes in fungal abundance and is a superior method to fungi identification based on morphology.
- The changes in relative abundance of fungi species in galleries may contribute to the overall fitness of the MPB.

R. C. HAMELIN. 2012. CONTRIBUTIONS OF GENOMICS TO FOREST PATHOLOGY. CAN. J. PLANT PATHOL. 34:20-28. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Forest pathogens are major causes of forest disturbances around the world, with the ability to reduce economic revenues and endanger host species and their ecosystems.
- Characterizing and comparing pathogen genomes at individual and population levels helps identify common and unique regions of the genome. This provides a better understanding of mechanisms of pathogen-host interactions, as well as their evolution.
- Identified genes can be used to develop pest detection and monitoring assays.

Approach:

- This research paper represented a detailed review of the literature that focused on the innovative technologies used to characterize organisms at the species or population level useful for pathogen characterization.
- Most of the review focused on advances in poplar rust research, since both *Populus trichocarpa* Torr. & A. Gray and *Melampsora larici-populina* Kleb. (poplar leaf rust) genomes are sequenced.

Key Findings and Implications:

- Individual protein families in biotrophic fungi have undergone genome expansion, suggesting a specific role in host-pathogen interactions. In contrast, some biotrophic fungi display a reduced number of genes in certain functional categories, such as hydrolytic enzymes (e.g., cell wall-degrading enzymes). Additionally, some pathogens also show a reduction in RXLR effectors and other secreted proteins (host-pathogen recognition system proteins).
- The poplar leaf rust genome displayed a decrease in glycoside hydrolases, and a genome expansion via a proliferation of transposable elements (a driving factor for pathogenicity factor evolution) and the expansion of some gene families, which may reveal lineage-specific novel function evolution.
- The rust haustoria, with a role in establishing the host interaction and facilitating nutrient acquisition and influencing host metabolism and defence responses, delivers numerous secreted proteins. A large portion of this secretome is novel, and many of these are effectors that facilitate host infection, such as cysteine-rich proteins. However, few effectors have been characterized, as better rust transformation systems are needed to perform knockout studies.
- Short regions of DNA sequences for a large number of individuals and populations provide barcodes, with the internal transcribed spacer (ITS) region of rRNA genes providing the best barcoding for identification. Barcoding studies have been useful for species recognition, identifying past species hybridizations and identifying potential alternate hosts.
- Genomic resources have identified single nucleotide polymorphisms (SNPs) and microsatellite markers for high-throughput genotyping. These allow outlier region identification and the identification of purifying, balancing and positive selection, as well as allow demographic, epidemiological and population structure analysis.
- Genome sequencing advances have made it possible to improve forest pathogen detection and monitoring. The technology can be applied to quarantine services, to prevent novel pathogen introduction, and allow the health certification of export and import material. Assays can be developed for pathogen population surveillance, to indicate potential for infection and to predict the evolution of new pathogen races or host shifts.

N. G. Samarasekera, N. V. Bartell, B. S. Lindgren, J. E. K. Cooke, C. S. Davis, P. M. A. James, D. W. Coltman, K. E. Mock and B. W. Murray. 2012. Spatial genetic structure of the mountain pine beetle (*Dendroctonus Ponderosae) outbreak in western Canada*: historical patterns and contemporary dispersal. Mol. Ecol. 21: 2931-2948. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Environmental change has a variety of ecological consequences, including species range expansion and extinction. As many insects respond rapidly to climatic changes, continuing changes in climate could facilitate poleward and elevational range expansions for various insect pests.
- MPB is one of the most destructive forest pests in North America, and a large area of contiguous, high-density trees vulnerable to MPB attack exists. Combined with a northern shift in climatic suitability, ideal conditions exist for MPB expansion northward and eastward.
- Very distinct north-south MPB genetic relationships and diversity gradients were identified, which illustrated a rapid range-wide response of MPB to the removal of climatic constraints, and the potential for range expansion of a regional MPB population.

Approach:

- The goal of the research was to determine the MPB spatial genetic variation found among the outbreak population, allowing genetic structure and MPB dispersal patterns to be inferred.
- Beetles from 49 sampling locations throughout the outbreak area in western Canada were analysed using 13 microsatellite marker loci.
- Marker data for the various locations were used to assess population equilibrium, genetic diversity, population structure, genetic differentiation and gene flow.

Key Findings and Implications:

- Significant north-south population structure was identified by: (i) Bayesian-based analyses; (ii) north-south genetic relationships and diversity gradients; and (iii) a lack of isolation-by-distance in the northernmost cluster
- Genetic diversity, as reflected by declining mean heterozygosity values and allelic richness per sample location, declined from south to north latitudes.
- Two geographically distinct population clusters were identified representing northern and southern clusters. The southern cluster could be subdivided into a southeastern and southwestern subclusters, and the northern cluster could be subdivided into northern upper and northern lower subclusters.
- The primary barrier to gene flow was between the northern and southern clusters, while a secondary barrier to gene flow corresponded to the boundary between the two northern subclusters
- The north-south MPB population structure was attributed to the processes of postglacial colonization and recent climate-driven changes in population dynamics.
- The data supported the hypothesis of multiple sources of origin for the current MPB outbreak, and that the MPB range expansion across the Rocky Mountains into Alberta was from a Northern British Columbia origin.
- No loss of genetic variability was identified in these populations, suggesting that the adaptive potential for MPB has not been reduced, and continued range expansion potential is high.

H. -M. C. de la Giroday, A. L. Carroll and B. H. Aukema. 2012. Breach of the northern Rocky Mountain geoclimatic barrier: initiation of range expansion by the mountain pine beetle. J. Biogeo. 39(6):1112-1123 [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The recent expansion of MPB into the eastern boreal forest could potentially threaten pine trees in eastern Canada where MPB has not historically existed.
- This study examined the historical breach of the geoclimatic barrier of the Rocky Mountains by MPB to investigate potential mechanisms for MPB expansion.
- MPB is likely to continue expanding eastward so long as enough susceptible host trees are available.

Approach:

- This study used a spatial point process model, based on annual survey maps (years 2004-2006) of MPB infestation locations, to compare MPB spread patterns with different dispersal hypotheses.

Key Findings and Implications:

- Aerial deposition along a strong north-west to south-west gradient likely caused the initial MPB invasion. This was coupled with more aerial deposition and localized dispersal from persisting populations in the following years.
- After the initial invasion, in a single year, areas of highest intensity MPB infestation advanced up to 25km north-east towards jack pine habitat.
- Human-caused movement of infested material (i.e., road transport or mill storage of potentially infested logs) did not appear to facilitate MPB expansion across the Rocky Mountains.

P. E. REYES, J. ZHU AND B. H. AUKEMA. 2012. SELECTION OF SPATIAL-TEMPORAL LATTICE MODELS: ASSESSING THE IMPACT OF CLIMATE CONDITIONS ON A MOUNTAIN PINE BEETLE OUTBREAK. J. AGRIC. BIOL. ENVIRON. STAT. 17(3):508-525. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Insects, like MPB, are a key indicator of climate change and even small changes in climate conditions can impact their outbreak success.
- This study evaluated the impacts of temperature, precipitation, and elevation on the tree-killing ability of MPB in B.C. forests.
- This is likely the first study to employ penalized methods to spatial-temporal lattice models that enables covariates and spatial-temporal dependence structures to be selected together.

Approach:

- This study used a spatial-temporal linear regression model and a new statistical method that performs model selection and parameter estimation together through a spatial-temporal adaptive Lasso.
- Data from an MPB outbreak in B.C. from 1977-1986 was used as a case study to evaluate which features and factors led to outbreaks.

Key Findings and Implications:

- In comparison to other methods, the resulting estimates have good asymptotic and finite sample properties that other ad-hoc procedures might not have.
- Alternative approaches (i.e., two-step model selection procedure) often require a lot of trial and error and appear to have unstable results.

C. M. Tsui, A. D. Roe, Y. A. El-Kassaby, A. V. Rice, S. M. Alamouti, F. A. H. Sperling, J. E. K. Cooke, J. Bohlmann and R. C. Hamelin. 2012. Population structure and migration pattern of a conifer pathogen, Grosmannia clavigera, as influenced by its symbiont, the mountain pine beetle. Molec. Ecol. 21:71-86. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- *Grosmannia clavigera*, or blue-stain fungi, is the fungal symbiont of MPB and plays a key role in the beetle's reproductive success.
- This study investigates the population structure of blue-stain fungus to better understand MPB-fungal dynamics.
- Understanding the ecological and evolutionary dynamics of this fungus-beetle association is important for modelling and predicting MPB outbreaks.

Approach:

- 19 populations of MPB were sampled in western North America. Individuals from these populations were genotyped with eight microsatellite markers.

Key Findings and Implications:

- Four genetic clusters corresponded to four major geographic regions suggesting the epidemic emerged from multiple sources.
- A genetic cluster north of the Rocky Mountains, where MPB has recently established, experienced a population bottleneck, likely from the recent range expansion.
- Based on the patterns of diversity and individual assignment of tests of the fungal associates, MPB likely migrated across the Rocky Mountains through a northeastern corridor. This finding agreed with meteorological patterns and MPB movement data.
- While isolation by distance was identified as a possible barrier to gene flow, the spread of MPB and its fungal associates does not appear to be limited by biological obstacles, like competitors.

2011

P. M. A. James, D. W. Coltman, B. W. Murray, R. C. Hamelin and F. A. H. Sperling. 2011. Spatial genetic structure of a symbiotic beetle-fungal system: toward multi-taxa integrated landscape genetics. PLoS ONE 8(10). [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This study examined how spatial heterogeneity affects genetic variation in MPB and its fungal associate *G. clavigera.* The study developed a new integrated landscape genetic framework to investigate genetic influences between species.
- Genetic variation in beetles was greatest within a northern population, whereas the fungi showed no such variation.
- These results reinforce that the management of host connectivity is of critical importance in managing beetle outbreaks and that this connectivity may need to be managed at large landscape scales.

Approach:

- Beetles were collected from sites in Alberta and BC during two time periods at three scales: landscapes, sites within landscapes, and individual trees within sites. In total, the researchers collected 2943 beetles and 174 fungal isolates.
- The genetic structure of the Alberta and BC populations was characterized using 13 microsatellite loci for MPB and seven for *G. clavigera*.
- Genetic similarities were assessed among the different regions to understand how spatial heterogeneity (i.e., landscape features) might affect gene flow in both the beetle and the fungi.
- Mountains were assumed to have the highest resistance to movement and high-volume pine stands were assumed to have the lowest. High climate suitability values were assumed to facilitate the greatest movement across the landscape.

Key Findings and Implications:

- MPB had a very strong north-south gradient in genetic structure, though this was not observed in the fungi. In the north, Euclidean distance and pine volume were the best predictors of genetic connectivity, with climate being a weak contributor. This regional differences between northern and southern beetle populations were attributed to the younger nature of the northern population (i.e., it is largely an emerging population inhabiting a relatively novel habitat).
- Sites at the leading edge of the current MPB outbreak, and sites separated by least resistance in terms of climate, tended to have similar genetic structures. This was not the case for fungi populations.
- The authors noted their findings may be affected by immigration from northern BC, and further confounded by time period of association, which could influence both ecological and evolutionary processes.

S. HARIDAS, C. BREUILL, J. BOHLMANN AND T. HSIAN. 2011. A BIOLOGIST'S GUIDE TO *DE NOVO* GENOME ASSEMBLY USING NEXT-GENERATION SEQUENCE DATA: A TEST WITH FUNGAL GENOMES. J. MICROBIOLOGICAL METHODS. 86(3): 368-375. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Genomics have the potential to rapidly advance the field of biological sciences, and genomic technologies and associated software are increasingly affordable. But the complexities in these technologies, and the use of challenging terminologies, represent a barrier to the adoption of genomics by some laboratories.
- This article provides a practical guide for genome sequencing and assembly written in a simplified way for biologists.

Approach:

- The purpose of this guide was to: (1) serve as a practical guide in genomics; (2) define commonly used terminologies; (3) provide some practical examples of genome sequence data for four strains of *G. clavigera*; and (4) provide a flowchart that starts with DNA preparation through to the processing and assembly of raw sequence reads using freely available software.

Key Findings and Implications:

- One of the most valuable aspects of this paper is its practical glossary, which provides needed definitions to commonly used genomics terminologies. As such, this paper should be viewed as a critical reference to assist those not well versed in genomics.
- The field of genomics and the development of associated technologies is progressing at an exponential rate. This will no doubt further reduce the costs associated with these emerging technologies and expand their application to an ever-increasing range of ecological issues.
- A simplified discussion of laboratory procedures was outlined from the very initial stages of DNA extraction through to final data processing.
- The authors provide recommendations regarding available software and computer hardware.

I. Lusebrink, M. L. Evenden and F. G. Blanchet and J. E. K. Cooke. 2011. Effect of water stress and plant defense stimulation on monoterpene emission from a historical and new pine host of the mountain pine beetle. J. Chem. Ecol. 37: 1013-1026. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Jack and lodgepole pine trees differ significantly in their production of monoterpenes in response to MPB, and this response was mitigated by drought in both species. These results would be expanded upon in subsequent studies.
- Longer and more severe droughts due to climate change, and the resulting stress to these tree species, will reduce carbohydrate production and likely limit their ability to produce defense mechanisms against the MPB and its fungal associate.

Approach:

- This study measured jack and lodgepole pine trees monoterpene production and tested the effects of a range of treatments, including water stress, on production.
- Two-year-old seedlings of each species were grown for 6 weeks in a greenhouse under one of two water treatments: well-watered and water deficit. At the end of this growing period, seedlings were subjected to three treatments consisting of mechanical wounding, mechanical wounding followed by fungal inoculation, and control.
- A subset of seedlings for each treatment and watering scenario were randomly selected for measurement of volatile emissions, photosynthetic rates, stomatal conductance and soil water content. A second subset of seedlings was subject to destructive analysis for volatile content in tree issues.

Key Findings and Implications:

- Jack pine seedlings produced α-pinene while the dominant monoterpene in lodgepole pine seedlings was β-Phellandrene. Notably, β-Phellandrene was absent in jack pine.
- When subject to water stress, seedlings of both species emitted lower overall quantities of volatile organic compounds. This finding has important implications for models that predict tree defenses and that typically rely on a simple input for air temperature.
- Stomatal conductance values confirmed that jack pine trees are more adapted to water stress.

A. S. ADAMS, C. K. BOONE, J. BOHLMANN AND K. F. RAFFA. 2011. RESPONSES OF BARK BEETLE-ASSOCIATED BACTERIA TO HOST MONOTERPENES AND THEIR RELATIONSHIP TO INSECT LIFE HISTORIES. J CHEM. ECOL. 37, 808–817. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Bacteria isolated from the MPB survived and grew in the presence of many monoterpenes (i.e., defense chemicals) produced by the host conifer. The precise extent and role that these bacteria may play in the success of MPB remains unknown.

Approach:

- This study tested how different bacteria isolated from two beetle species with contrasting host defense tolerances (MPB and the red turpentine beetle) are affected by an array of monoterpenes. MPB attacks its host conifer through mass attack whereas the red turpentine beetles lives within its host without causing mortality.
- Bacterial isolates were obtained from MPB collected near Grande Prairie, AB and red turpentine beetles collected in Wisconsin. Five bacterial species were identified using rDNA analysis for each of the two beetle species.
- Monoterpenes were added to bacterial cultures and their effects monitored over 48 hours. Monoterpenes tested included α-pinene, β-pinene, limonene, myrcene and 3-carene. The monoterpene β-phellandrene was also tested with MPB bacterial isolates. No reason was given why this monoterpene was not tested with isolates from the red turpentine beetle.

Findings and Implications:

- Overall, bacteria collected from MPB were more strongly inhibited by exposure to the range of monoterpenes than bacteria collected from the red turpentine beetle. This difference was attributed to the differences in the life histories of the two beetle species: the MPB mass attacks its host and depletes its monoterpenes, while the red turpentine beetle attacks its hosts at low densities and completes its life cycle in the presence of monoterpenes.
- Four of the five bacteria species collected from MPB had impaired growth in the presence of α-pinene and β-phellandrene. This response was largely absent in bacteria associated with red turpentine beetle. All bacteria grew in the presence of β-pinene, myrcene and limonene.
- The antibacterial activity of 3-carene may be a mechanism that assists conifer resistance to the MPB. However, this monoterpene did not affect the growth of bacteria isolated from the red turpentine beetle. Bacteria associated with red turpentine beetle may have developed a tolerance to this monoterpene.
- Additional research is recommended to understand the metabolic roles these bacteria may play in relation to the current MPB epidemic.

U. HESSE-ORCE, S. DIGUISTINI, C. I. KEELING, Y. WANG, M. LI, H. HENDERSON, T. R. DOCKING, N. Y LIAO, G. ROBERTSON, R. A. HOLT, S. J. M. JONES, J. BOHLMANN AND C. BREUIL. 2010. GENE DISCOVERY FOR THE BARK BEETLE-VECTORED FUNGAL TREE PATHOGEN *GROSMANNIA CLAVIGERA*. BMC GENOMICS 11, 536. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Two clusters of genes, which were functionally related, were identified to have important roles in facilitating *G. clavigera* to overcome the chemical defense mechanisms of host species such as lodgepole pine.

Approach:

- Fungi are known to have a variety of different physiological based strategies to defend against chemical defense mechanisms of their host. However, the molecular and biochemical mechanisms involved have remained elusive. Simply put, at the time of this paper, it remained unknown which genes *G. clavigera* might possess for the detoxification of these chemical defenses.
- The primary objective of this study was to ascertain which genes are critical to *G. clavigera* overcoming the host's antifungal strategies including the synthesis of terpenoid and phenolics.
- A total of eight strains of *G. clavigera* were isolated from lodgepole and ponderosa pine. These eight fungal isolates were grown on five media where the latter varied with respect to nitrogen and carbon content. The isolates were grown for a total of four days under laboratory conditions and subsequently harvested for RNA analysis.
- Gene expression was determined in response to a "lodgepole pine phloem extract" which was manufactured using phloem extracted from a freshly collected bolt. The phloem was frozen and ground to ultimately produce a supernatant for testing.
- Key to this study was the determination of expressed sequence tags (ESTs) are relatively short DNA sequences which provide an alternative to full-length cDNA sequencing. Determination of ESTs is also cost effective and common approach to facilitate gene discovery.

Findings and Implications:

- The authors noted that the upregulation of genes following addition of phloem extracts suggested that a fungal oxidative stress response was induced by the defense compounds synthesized by the host.
- Two, closely related clusters of genes, were found to be upregulated following treatment with the phloem extract. In this regard, the authors noted that clustering of functionally related genes in fungi is common and thought to be critical to niche adaptation.
- The first cluster contained a total of five genes and were attributed to varying physiological roles in the detoxification of defense compounds. The second, and smaller cluster of genes, were thought to be associated with specific enzymes that degraded aromatic compounds such as phenolics.
- Further research was recommended to focus on the precise metabolic pathways associated with the fungal defense strategies.
- An extensive DNA library was developed for each of the eight strains of *G. clavigera* which should form the basis for additional studies.

L. Khadempour, S. M. Alamouti, R. Hamelin, J. Bohlmann and C. Breuil. 2010. Target-specific PCR primers can detect and differentiate ophiostomatoid fungi from microbial communities associated with the mountain pine beetle Dendroctonus ponderosae. Fungal Biology. 114: 825-833. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- A new methodology involving polymerase chain reaction (PCR) was developed to distinguish fungi commonly associated with the mountain pine beetle. This new approach is accurate, fast and inexpensive and has applications for the importation and export of timber, as well as for ecological purposes.

Approach:

- MPB is associated with several different fungi species including *Grosmannia clavigera*, *Leptographium longiclavatum* and *Ophiostoma montium.*
- The conventional approach to identifying fungi is by culturing them on a growth media. However, this is highly problematic as it is very hard to distinguish fungi solely on the basis of their morphological features. Failure to be able to rapidly and cost effectively identify fungi at the species level impedes our ability to understand fungi abundance and their specific and complex relationships with the beetle and host.
- The goal of this research trial was to develop a genomics approach, including PCR amplification, that could quickly and accurately identify major fungal species associated with mountain pine beetle. Such an approach is of value not only to ecological studies but would also have immense value in confirming absence or presence of fungi in timber destined for export markets abroad.
- Polymerase chain reaction (PCR) has become a commonly used technology to make multiple copies of a specific segment of DNA. It is a prized technique because it provides precise information and is able to rapidly amplify DNA sequences.
- Substrate for this study consisted of fungi collected from mountain pine beetle bodies, beetle galleries, and inoculated pine phloem. To ensure robustness of the methodology, the sub samples were collected from a wide range of sites located both in western Canada and the United States.

Findings and Implications:

- The PCR approach proved to be vastly superior in fungi identification particularly when fungi abundance was very low in samples and also when there was a high level of contamination by other organisms such as yeast.
- Of note, the research team was successful in developing a primer set specific for *O. montium* which has been historically very difficult to differentiate from the fungi *Ophiostoma ips*. The latter is a common fungi and not generally associated with a beetle.
- The approach to distinguish the closely related *G. clavigera* and *L. longiclavatum* fungi relied upon focusing on a single gene whose function remains unknown and which was identified through DNA libraries.

S. DiGuistini, Y. Wang, N. Y. Liao, G. Taylor, P. Tanguay, N. Feau, B. Henrissat, S. K. Chan, U. Hesse-Orce, S. M. Alamouti, C. K. M. Tsui, R. T. Docking, A. Levasseur, S. Haridas, G. Robertson, I. Birol, R. A. Holt, M. A. Marra, R. C. Hamelin, M. Hirst, S. J. M. Jones, J. Bohlmann and C. Breuil. 2010. Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont *Grosmannia clavigera*, a lodgepole pine pathogen. Proc. Natl. Acad. Sci. U S A. 108(6): 2504–2509. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This landmark paper reports the full sequencing of the *Grosmannia clavigera* fungi—a major research achievement towards understanding not only the biological interactions between fungi, host and beetle but also the evolutionary adaptations of this fungi.
- Terpenoids induced a strong molecular response in *G. clavigera,* indicating that it has a strong role in detoxifying a critical tree defense mechanism.

Approach:

- A wide array of genomic techniques was deployed, including what the authors defined as “next generation” sequencing data.

Findings and Implications:

- The research team has developed a fundamental genome and identified molecular resources for *Grosmannia clavigera.* Its size (29.8-Mb) and gene collection (8 314) are similar to those of other saprophytic and pathogenic fungi. This dataset opens the door for a host of genomic based studies of how the fungi responds to its environment and its relationship with host and beetle.
- When the fungi was treated with terpenoids, growth was impaired during the first twelve hours and there was evidence that the treatment triggered transcriptome reprogramming. Genes affiliated with amino acid biosynthesis were upregulated at this time.
  - Gene expression was markedly different thirty-four hours post treatment. Notably, genes affiliated with fatty acid metabolites were strongly induced. This was thought to be a first step towards terpenoid detoxification, which would represent a significant evolutionary adaptation.
- Sequencing has confirmed that *G. clavigera* does not produce the enzymes required to hydrolyze lignin in cell walls but does produce those that facilitate the colonization of the sapwood.
- Results have confirmed that *G. clavigera* is capable of sexual reproduction, which would enable mutations, though it has seldom been documented under field conditions.

J. BOHLMANN. 2011. TERPENOID SYNTHESES-FROM CHEMICAL ECOLOGY AND FOREST FIRES TO BIOFUELS AND BIOPRODUCTS. STRUCTURE 7;19(12):1730-1. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This paper drew attention to the growing importance of the newly emerging field of "*chemical ecology*" which pertains to chemical interactions between living organisms. These interactions may involve as many as ten thousand primary, secondary metabolites or small molecules produced by a host of different metabolite systems or pathways.

Approach:

- This paper took the form of a short note to emphasis the role of chemical ecology and the role of terpenoids.

Findings and Implications:

- Various phenolics and terpenoids are the most prominent metabolites. Terpenoids are a highly diverse group of specialized chemicals. They have a variety of functions in the large number of "*chemical – ecological*" interactions between living organisms. While these chemicals may not serve any purpose related to the growth and development of plants, they can have critical roles in defense against an array of herbivores and pathogens.
- The production of a vast array of chemicals and small molecules by plant systems is of growing interest to the biofuels and pharmaceutical industry. This is owing to the growing desire to identify and produce chemicals using synthetic systems. In particular, the production of terpenoids has been of significant interest owing to their low molecular weights.

C. K. BOONE, B. H. AUKEMA, J. BOHLMANN, A. L. CARROLL AND K. F. RAFFA. 2011. EFFICACY OF TREE DEFENSE PHYSIOLOGY VARIES WITH BARK BEETLE POPULATION DENSITY: A BASIS FOR POSITIVE FEEDBACK IN ERUPTIVE SPECIES. CAN. J. FOR. RES. 41: 1174-1188. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Host tree defenses were major constraints when MPB populations were low, but were of little importance once stand-level beetle population densities increased above a certain threshold.
- In low beetle densities, trees that exuded more resin and accumulated higher monoterpene concentrations in response to a simulated attack escaped natural beetle attacks. However, these defenses were ineffective once higher beetle densities were achieved.
- Larger diameter trees had better defenses against beetles than smaller diameter trees, with smaller trees preferentially attacked under endemic populations. However, as MPB populations increased, the beetles attacked the larger trees containing stronger defenses.
- When beetle populations were low, they preferentially attacked trees weakened by lower stem insects.
- These points illustrated that beetle behavioural plasticity allowed them to persist at endemic levels until conditions changed, and their behaviour shifted to target larger, stronger defended trees.

Approach:

- This research evaluated the ability of constitutive and inducible defenses to protect trees and restrict MPB reproduction across endemic, transitory and eruptive phases.
- Lodgepole pines in six 12-18 ha stands were examined annually for 3-6 years for beetle attack and for the progression of beetle densities through the various population densities.
- The trees were assayed for a number of physiological and chemical attributes, which were then related to subsequent beetle attacks during that same year.

Key Findings and Implications:

- Beetle attack success was strongly impacted by beetle population size; the proportion of beetle attacks that were successful increased as the stand-level beetle densities increased. As the population shifted from endemic to transitory-eruptive phases, attack success rapidly increased.
- Constitutive and induced resin flow rates were higher in non-attacked trees in endemic phase beetle populations, but in the eruptive phase, these higher resin flow rate trees were attacked.
- Regarding monoterpenes, the concentration of induced limonene, one of the most active against the bark beetle-fungal complex, was always higher in non-attacked trees. Attacked trees at endemic and transitory population phases were higher in α-pinene, used by MPB for pheromone biosynthesis. However, this relationship disappeared during the eruptive phase.
- Larger trees with more defensive capabilities were targeted in the eruptive population phase.
- Endemic beetle populations cull weaker trees, and as beetle populations rise, surviving hosts are larger and better defended. However, these hosts will support more larvae if they can be successfully attacked, illustrating the tradeoff that exists between host tree defense vs. nutritional quality as beetle populations increase.

C. I. CULLINGHAM, J. E. K. COOKE, S. DANG, C. S. DAVIS, B. J. COOKE AND D. W. COLTMAN. 2011. MOUNTAIN PINE BEETLE HOST-RANGE EXPANSION THREATENS THE BOREAL FOREST. MOL. ECOL. 20: 2157-2171. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The current epidemic of MPB, has caused significant losses of lodgepole pine, and associated economic impacts.
- The leading edge of the epidemic has extended into Alberta, where forest composition switches from lodgepole pine to jack pine, with an associated hybrid zone.
- There is concern over the susceptibility of jack pine to MPB, but there has been no confirmation of expansion into jack pine, due to the difficulty in distinguishing between the two species.
- A panel of microsatellite markers optimized for both species was tested for the ability to distinguish lodgepole pine, jack pine, and lodgepole pine-jack pine hybrids.
- These markers allowed an accurate classification of the two species and their hybrids, and confirmed that MPB had successfully attacked jack pine and the hybrids, raising the spectre of a potential eastward expansion of MPB into the boreal forest.

Approach:

- Foliage samples were collected from 10 lodgepole pine locations in British Columbia, 14 jack pine locations in Ontario and Minnesota and one location in Saskatchewan, and from lodgepole pine, jack pine and putative hybrids from 14 locations in Alberta. Both MPB attacked and non-attacked trees were sampled from BC and Alberta.
- Twelve microsatellite loci were used for genotyping of 678 samples in order to resolve species identity for the two pure pine species, and their hybrids.
- The assignment of species identity was then compared against MPB attacked versus non-attacked status.

Key Findings and Implications:

- Putative jack pine samples, classified based on visual phenotypes, displayed signs of MPB attack and successful reproduction on trees, suggesting that MPB can attack jack pine.
- Based on genotyping results, genetic diversity across all locations was high, with higher diversity in lodgepole pine. Genetic differentiation across locations and between species was high, but genetic differentiation within locations was low.
- Using pairwise comparisons of differentiation, there was some significant differences noted, especially for jack pine, with all locations differentiated from those in Ontario/Minnesota.
- The two species could clearly be delineated, but the resolving power for this declined with increasing generations
- The final breakdown of the marker analysis resolved 301 jack pine, 290 lodgepole pine and 87 hybrids, with the ancestry of the hybrids being predominantly lodgepole pine.
- Comparing the genetic background information with MPB attack data, eight pure jack pine and 19 hybrids were identified as having been attacked, which confirmed the susceptibility of jack pine and hybrids to MPB within the hybrid zone.
- These results indicated that MPB has expanded its host repertoire to include jack pine, raising the question of jack pine being able to sustain eruptive MPB populations and expand eastward.

S. M. ALAMOUTI, W. Y. WANG, S. DIGUISTINI, D. L. SIX, J. BOHLMANN, R. C. HAMELIN AND C. BREUIL. 2011. GENE GENEALOGIES REVEAL CRYPTIC SPECIES AND HOST PREFERENCES FOR THE PINE FUNGAL PATHOGEN *GROSMANNIA CLAVIGERA*. *MOL. ECOL.* MOL. ECOL. 20: 2581-2602. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- MPB is commonly associated with the wood colonizing fungus *Grosmannia clavigera*, a tree pathogen that forms a symbiotic relationship with MPB.
- To understand the ecology and biology of this relationship, it is important to be able to clearly identify and resolve *G. clavigera* from other closely related fungal species, and to describe the fungal association with the pine beetle symbionts.
- Using fungal genome sequences and gene annotations, identified nucleotide variation was used to determine if the fungal isolates from two beetle species inhabiting different pine species in epidemic and non-epidemic beetle regions represented different genetic lineages
- Two sibling phylogenetic species, Gc and Gs, were identified. While Gs was exclusively associated with MPB and mainly on the primary host lodgepole pine, it was also on other pine species in current epidemic areas. In contrast, Gc was found on ponderosa and Jeffrey pine infested with local populations of their respective beetles.
- Gs should be classified as a new species, distinct from the original *Grosmannia clavigera* (Gc)
- Host-tree species and beetle population dynamics may contribute to the genetic divergence and diversity of fungal partners in beetle-tree ecosystems.

Approach:

- Isolates (166) of *G. clavigera* and eight additional isolates of closely related species were tested, with *G. clavigera* isolated from MPB and JPB (Jeffrey pine beetle) and their host trees at different sites in Canada and the USA.
- Nucleotide polymorphism was characterized in 67 genomic regions, with 15 selected for phylogenetic analysis.

Key Findings and Implications:

- This study produced the first comprehensive analysis of protein-coding gene variability for *G. clavigera.*
- In contrast to the current simple taxonomic classification, this study revealed *G. clavigera* species diversity and ecological complexity with respect to host species.
- The sequences that had been used for phylogenetic inference for ophiostomatoid fungi prior to this study were inadequate for defining species and inferring evolutionary relationships in *Grosmannia*. This study revealed that sequencing more genomic regions was effective for determining species boundaries.
- Two distinct lineages, Gc and Gc, were identified in *G. clavigera*, with recent species divergence.
- The Gc lineage occurs on both beetle vectors (MPB and JPB) infesting ponderosa pine and Jeffrey pine, respectively. In contrast, Gs was exclusively associated with MPB.
- In the future, Gc genetic variation will evolve slowly while Gs will experience further genetic variation. A future outbreak may see Gs fungal population and genetic diversity increase.
- Another scenario could see the current MPB population establish in a new host-tree species with its fungal symbionts, and adapt to the new physical and chemical host environment.

A. D. ROE, A. V. RICE, D. W. COLTMAN, J. E. K. COOKE AND F. A. H. SPERLING. 2011. COMPARATIVE PHYLEOGRAPHY, GENETIC DIFFERENTIATION, AND CONTRASTING REPRODUCTIVE MODES IN THREE FUNGAL SYMBIONTS OF A MULTIPARTITE BARK BEETLE SYMBIOSIS. MOL. ECOL. 20: 584-600. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- MPB is a common host for multiple symbionts. These multipartite symbioses are complex symbiotic relationships with multiple interacting partners.
- Some of the best-known MPB-fungal symbionts are the blue-stain fungi *Grosmannia clavigera*, *Leptographium longiclavatum*, and *Ophiostoma montium*. While *G. clavigera* is the primary fungal symbiont, interactions between all three and MPB will impact MPB fitness and outbreaks.
- Phylogenetic, population genetic and demographic approaches were used to characterize the phylogeographic patterns of the three fungi, and revealed significant population differentiation with northern and southern populations.

Approach:

- MPB adults, larvae and gallery wood were sampled from 42 stands of lodgepole pine and three stands of lodgepole x jack pine hybrids representing 12 landscapes in northern and southern BC and Alberta.
- Fungal isolates were characterized by amplification and sequencing of four-five gene regions. Sequences were then used for alignments, haplotype analysis and determination of phylogenetic relationships, genetic diversity and population differentiation, comparative population structuring, population demographics and evidence of recombination.

Key Findings and Implications:

- A total of 143 *O. montium*, 155 *G. clavigera* and 169 *L. longiclavatum* were sequenced.
- *O. montium* showed the most diversity of the three species, with few haplotyes shared between the northern and southern populations. In contrast, *G. clavigera* and *L. longiclavatum* displayed lower haplotype diversity, although with several haplotypes unique to the southern population.
- Higher levels of diversity were noted in all three southern fungal populations, which may have represented earlier MPB outbreaks in western Canada.
- The population structuring of *G. clavigera* and *L. longiclavatum* was equal, while it was not for *O. montium*, which may have been due to the high levels of haplotype diversity of *O. montium*.
- The haplotype characteristics were indicative of populations that had undergone recent expansion, which may have been related to the current MPB outbreak in BC and Alberta.
- Evidence for recombination was consistently observed for *O. montium*, while it was rarer for the other two species. Recombination provides an advantage for purifying selection and the production of highly adapted genotypes. How this may impact the role of *O. montium* in the multipartite symbioses and the overall impact on MPB fitness needs to be explored.
- The population structure differences may also be due to the additional transmittance *of O. montium* on the beetle exoskeleton in addition to mycangial transport, while the other two species are transmitted only in the mycangia. This additional transmittance capacity may contribute to the higher levels of *O. montium* genetic diversity.
- This study provides a better understanding of the complexity and evolution of this symbiotic system.

K. R. SAMBARAJU, A. L. CARROLL, J. ZHU, K. STAHL, R. D. MOORE AND B. H. AUKEMA. 2011. CLIMATE CHANGE COULD ALTER THE DISTRIBUTION OF MOUNTAIN PINE BEETLE OUTBREAKS IN WESTERN CANADA. ECOGRAPHY. 35:211-223. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Since temperature directly influences MPB development and population success, climate change and extreme whether events can impact MPB biology, population ecology, and spatial patterns. However, few landscape-level models have studied the relationship between extreme cold weather events and MPB outbreak occurrences.
- The goal of this study was to examine spatial outbreak pattern changes with changing thermal regimes, when underlying MPB outbreak behaviour stays consistent.
- Overall, extreme winter weather patterns (i.e., cold snaps) are very likely to impact MPB outbreaks. However, warmer temperatures from climate change could increase the risk of MPB outbreaks at higher elevations and in northern B.C. and Alberta.

Approach:

- This study examined the relationship between temperature and elevation in areas with MPB outbreaks in western Canada from 1992 to 2007 using a spatial-temporal logistic regression model.
- As a case study, spatial outbreak patterns of MPB were then studied under four simulated climate change and two climatic variability scenarios.

Key Findings and Implications:

- Large temperature drops (>10°C) or extreme winter minimum temperatures reduces the likelihood of MPB outbreak.
- Climate change-induced temperature increases could increase the risk of future outbreaks at higher elevations and northern latitudes in B.C. and Alberta. Outbreak risk will likely be strongest with an increase in average temperature from 1°C to 4°C at higher elevations. These effects will likely be accompanied by a decline in outbreaks at low-mid elevations in southern B.C.
- Low variability in mean temperature increases likely will not significantly impact outbreak risk.

H. -M. C. DE LA GIRODAY, A. L. CARROLL, B. STAFFAN LINDGREN AND B. H. AUKEMA. 2011. INCOMING! ASSOCIATION OF LANDSCAPE FEATURES WITH DISPERSING MOUNTAIN PINE BEETLE POPULATIONS DURING A RANGE EXPANSION EVENT IN WESTERN CANADA. LAND. ECOL. 26:1097-1110. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Landscape features can play an important role in affecting how insects, like MPB, spread, establish, and persist.
- This study examined the influence of landscape features and characteristics on MPB in northeastern B.C.
- Predicting the potential spread of new infestations based on landscape features and their orientation, particularly low-lying valleys, alongside landscape scale spread modeling can help prioritize management areas.

Approach:

- This study used spatial point process regression models to investigate the association of variables (i.e., landscape features and their orientations, habitat suitability, elevation and treatment efforts) with MPB infestations in 2004, 2005 and 2006.

Key Findings and Implications:

- While specific landscape features are associated with the establishment of MPB, MPB infestations primarily established in canyons and valleys before moving into more open-sloped areas.
- The orientation of landscape features also influences the likelihood of continued MPB spread and establishment. Infestations were most intense on southwestern slopes of midslope ridges and small hills, southwest facing open slopes and valleys that run northeast—southwest.
- Following the initial invasion event, continued MPB spread may be facilitated by low-elevation valleys alongside the dominant wind direction providing suitable MPB habitat.

A. D. Roe, P. M. A. James, A. V. Rice, J. E. K. Cooke and F. A. H. Sperling. 2011. Spatial Community Structure of Mountain Pine Beetle Fungal Symbionts Across a Latitudinal Gradient. Microb. Ecol. 62:347-360. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- MPB is associated with at least three well-known fungal symbionts which are important to MPB survival and reproduction.
- This study looked at the role of latitude and elevation on three species of fungus associated with MPB to better understand MPB—fungus associations at the northern and eastern edges of MPB outbreak over time and space.
- This study provides insight to landscape-level processes and symbiont community dynamics. It is one of the first large-scale surveys of symbiont composition patterns in natural MPB populations.

Approach:

- Fungal samples were taken at 50 sites from eastern B.C. to western Alberta over the course of two MPB flights. The samples were analyzed to determine the amount and composition of the three fungal species (*O. montium, L. longiclavatum* and *G. clavigera)*.

Key Findings and Implications:

- Fungal communities responded more strongly to latitude and less to elevation:
  - Latitude strongly affected the abundance of *L. longiclavatum* and *G. clavigera,* but not *O. montium.*
  - Elevation did not affect variation in *O. montium* abundance, but weakly affected *G. clavigera* in combination with latitude.
- *L. longiclavatum* gradually increased with increasing latitude, whereas an opposite change was observed for *G. clavigera.* This suggests that *L. longiclavatum* is replacing *G. clavigera* as latitude increases. *O. montium* also decreased with increasing latitudes, but surprisingly, at a lower rate. This suggests that *O. montium* is more cold-tolerant than expected.
- Competition or coexistence between the three fungal species likely determines the fungal community composition. Maintaining fungal diversity could be critical to MPB population stability and persistence across a geographic range.

2010

C. K. M. Tsui, B. Wang, L. Khadempour, S. M. Alamouti, J. Bohlmann, B. W. Murray and R. C. Hamelin. 2010. Rapid identification and detection of pine pathogenic fungi associated with mountain pine beetles by padlock probes. J. Microbiol. Methods. 83(1) 26-33. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- A new genomics-based methodology was created to accurately distinguish *G. clavigera* and *L. longiclavatum*. This is an important accomplishment as differentiating these two closely related fungi using morphological and growth characteristics has been unreliable. Alternative methods that were rapid, reliable and cost effective were urgently needed.

Approach:

- The research team tested 96 fungal strains including 34 strains of *G. clavigera*, 25 strains of *L. longiclavatum* and 9 strains of *L. terebrantis.* The technique was employed to see if it could detect fungi in 31 MPBs.
- The padlock probes for *G. clavigera* and *L. longiclavatum* were designed to recognize specific SNPs in the rDNA.

Findings and Implications:

- The research team has developed a genomics approach to fungi species identification based on ligase-mediated single nucleotide discrimination, complete with padlock probe technology.
- This new approach was exceptionally accurate in distinguishing *G. clavigera* and *L. longiclavatum* in all samples. These results were consistent with those obtained using more detailed and time-consuming approaches.
- This approach should help facilitate future research concerning the ecological aspects of these important fungi. Identification of fungi using often inaccurate morphological observations should no longer be required.

T. AW, K. SCHLAUCH, C. I. KEELING, S. YOUNG, J. C. BEARFIELD, G. J. BLOMQUIST AND C. TITTIGER. 2010. FUNCTIONAL GENOMICS OF MOUNTAIN PINE BEETLE (*DENDROCTONUS PONDEROSAE*) MIDGUTS AND FAT BODIES. *BMC GENOMICS* 11, 215. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The genes involved in the synthesis of the pheromones exo-brevicomin and frontalin were tentatively identified. Understanding the metabolic pathways and the various enzymes that may be involved in their synthesis could be valuable in the production of new, chemical-based management strategies.

Approach:

- Recent advances in proteomic technologies were used to identify sequence and gene expression databases. These in turn aided in the identification of pheromone biosynthesis and potential resin detoxification genes.
- Beetles were collected from a field site in Nevada. Various treatments were imposed including bark feeding and the use of juvenile hormones. Beetles were dissected to remove their midguts for extensive genomic investigations.

Key Findings and Implications:

- There are thought to be three key pheromone components: (1) trans-verbenol, which is produced by pioneering females as they attack a new host; (2) exo-brevicomin, which is produced by males before they join with females in the tree; and (3) frontalin, which serves as an important anti-aggregation signal.
- Gene induction varied significantly between stages of pupae development, diet and sex.
- The identification of genes thought to catalyze the synthesis of exo-brevicomin and frontalin, and the development of microarray data, should be valuable in spurring additional work in the synthesis of pheromones.

A. D. ROE, A. V. RICE, S. E. BROMILOW, J. E. K. COOKE AND F. A. H. SPERLING. 2010. MULTILOCUS SPECIES IDENTIFICATION AND FUNGAL DNA BARCODING: INSIGHTS FROM BLUE STAIN FUNGAL SYMBIONTS OF THE MOUNTAIN PINE BEETLE. MOL. ECOL. 10: 946-959. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Accurate species identification and delimitation of species boundaries is a necessity for understanding fungal diversity, ecology and functionality. As such, there is wide interest in applying molecular marker techniques to facilitate this.
- The use of DNA sequence (DNA barcoding) information is popular, but it has been difficult to determine which particular genes are the most informative, and how many loci are required for accurate identification and classification.
- A group of closely related blue stain fungi associated with MPB were compared using between one and five different markers (loci) to assess the success of single-locus species identification.
- Single loci varied in their utility for identifying the six fungal species examined, but the use of multiple loci was consistently successful, leading to the recommendation that a multi-locus barcoding approach be used for the development of fungal community-wide molecular identification system.

Approach:

- Fungal sampling was performed in pine beetle-attacked stands in Alberta and eastern British Columbia. Fungal cultures were obtained from MPB adults and larvae, as well as from wood samples taken from beetle galleries.
- Five gene regions were used for the analysis: actin, elongation factor 1 alpha (EF1α), beta-tubulin (Btub), an anonymous nuclear locus (UFM) and the partial 5.8S + internal transcribed spacer 2 + partial 28S (ITS2) ribosomal DNA sequence.
- DNA sequences were used to determine unique haplotypes for each locus, and used in phylogenetic analyses, with single locus and multilocus data sets compared for the ability to resolve species.

Key Findings and Implications:

- Single locus analyses revealed that ITS2 had several haplotypes shared between species of the Grosmannia clade, while no haplotypes were shared between species of the other loci (actin, EF1 α, Btub, UFM). Monophyly of individual species was supported where species were represented by multiple haplotypes per a single locus.
- When the five loci were used together as a single data set, no haplotypes were shared between species, with monophyly of the *Grosmannia* clade strongly supported.
- Multilocus analyses using multiple fungal genera and species revealed that species identification success increased up to 100% with the inclusion of additional loci, but the number of loci needed varied between genera.
- This study, as well as a literature survey, indicated that the use of multiple loci, including those used in this study, are essential for successful fungal genotyping. The number of additional loci required to achieve high levels of successful identification varied between fungal groups.

Y. WANG, S. DIGUISTINI, T. C. WANG, J. BOHLMANN AND C. BREUIL. 2010. ACROBACTERIUM-MEDIATED GENE DISRUPTION USING SPLIT-MARKER IN GROSMANNIA CLAVIGERA, A MOUNTAIN PINE BEETLE ASSOCIATED PATHOGEN. CURR. GENET. 56: 297-307. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- *Grosmannia clavigera* is a fungal symbiont of MPB and a potent plant pathogen. In addition to its contribution to the devastation of Canadian conifer forests, the fungus also produces a dark melanin pigment that discolors sapwood, reducing its value.
- The genome of G. clavigera has been sequenced, with the anticipation that this will help identify the genes controlling various aspects of fungal development, growth and pathogenicity.
- Gene functional characterization, for *G. clavigera* needs efficient gene disruption methods.
- Using an Agrobacterium-mediated transformation system, in conjunction with linear or split-marker deletion cassettes, containing 1.5 – 3 kb DNA regions flanking the target site, efficient gene replacement was obtained.
- The functionality of this system was confirmed through the disruption of two genes in the melanin biosynthetic pathway.

Approach:

- *G. clavigera* wild type strain Kw 1407 was used for gene disruption experiments, and was transformed using *Agrobacterium tumefaciens* strain GV3101.
- Gene disruption cassettes were constructed using the hygromycin β-phosphotransferase (hph) gene, combined with 1.5 – 3 kb flanking DNA regions on both sides of the gene targeted for disruption, deduced from the available genome sequence.
- Three cassettes were generated: a linear cassette (5' flank-*hph*-3' flank) and two split cassettes (5' flank-2/3 *hph* upstream sequence and 2/3 hph downstream sequence-3' flank). Each cassette was introduced into *Agrobacterium* and used to transform *G. clavigera* germinated spores, and the selection of transformant strains by growth on hygromycin.

Key Findings and Implications:

- Two genes in the DHN-melanin pathway were chosen to test the gene disruption protocol, polyketide synthase (*PKS* – 6.7 kb long) and scytalone dehydratase (*SD* – 0.905 kb long).
- Protocol optimization was performed using the linear cassettes, with an optimum of 20 h incubation of Agrobacterium with 3-5 x 10^5 spores/plate.
- Linear cassettes containing 1.5 or 3 kb of flanking DNA were tested for both PKS and SD, and while the 3 kb construct yielded fewer transformants, the rate of gene disruption was higher than with the 1.5 kb flanking DNA constructs.
- The split-marker cassettes were tested using the 3 kb flanking regions. When compared against the linear cassette constructs, while the split-marker system gave lower transformation efficiencies, the rate of targeted disruptions was higher (*PKS* – 82%, *SD* – 65%) compared with the linear system (*PKS* – 53%, *SD* – 39%).
- Analysis of the *PKS* and *SD* mutants indicated color phenotype changes (*PKS* – albino; *SD* – red), but no other marked differences with wild type *G. clavigera*.
- This paper reported the successful use of an *Agrobacterium*-mediated, split-marker system for targeted gene disruption in *G. clavigera*, allowing future studies on *G. clavigera* gene function.

K. G. ZULAK AND J. BOHLMANN. 2010. TERPENOID BIOSYNTHESIS AND SPECIALIZED VASCULAR CELLS OF CONIFER DEFENSE. J. INTEGR. PLANT. BIOL. 52: 86-97. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Conifer defense-related terpenoid biosynthesis involves specialized anatomical structures that allow them to cope with a variety of pest and pathogen attacks.
- Conifers contain both constitutive and inducible terpenoid defenses, providing the capacity to synthesize several hundred different monoterpenes, sesquiterpenes and diterpenes.
- The arrays of compounds are generated from the general isoprenoid pathway through the activity of large gene families encoding terpene synthases and CYP720B cytochrome P450-dependent monoxygenases.
- Most of the terpene synthases generate multiple products, while the CYP720B P450 enzymes catalyze multiple oxidations along multiple series of diterpenoids from a range of substrates.
- Genes encoding terpene synthases and CYP720B enzymes respond to actual or simulated insect attack with transcript up-regulation, increased protein abundance and increased enzyme activity.
- Conifer defense oleoresin terpenoids accumulate in preformed cortical resin ducts and xylem trauma-associated resin ducts. These resin ducts can form *de novo* in the cambium and developing xylem in response to insect attack or methyl jasmonate treatment.

Approach:

- This research paper represented a detailed review of the literature dealing with terpenoid biosynthesis and specialized vascular cells associated with conifer defense.

Key Findings and Implications:

- DMAPP and IPP are the precursors to all isoprenoid compounds.
- The enzymes leading to terpenoid production are the Prenyltransferases, the Terpenoid Synthases (TPS), and the Cytochrome P450-Dependent Monooxygenases.
- The Prenyltransferases catalyze condensation reactions involving DMAPP and IPP, several of which have been cloned and characterized.
- The TPS generate a large amount of the chemical diversity in the oleoresin composition, with mono-, sesqui- and diTPS catalyzing mono- (C_{10}), sesqui- (C_{15}) and diterpene (C_{20}) compounds.
- Mono- and sequiterpenes in conifer oleoresin are not biochemically modified, while the diterpene compounds are further oxidized by multisubstrate, multifunctional Cytochrome P450 enzymes, creating diterpenoid resin acids. Hence, a small number of TPS and P450 enzymes create much of the oleoresin chemical diversity.
- Insect attack (white pine weevil) upregulates TPS genes and the accumulation of mono-, sesqui- and diterpenes, and insect attack is much more inductive than methyl jasmonate application.
- Methyl jasmonate (MeJA) is the volatile methylester of the defense compound jasmonic acid. It is a good mimic of insect feeding without the associated tissue damage and oleoresin release. The treatment of conifers with MeJA has enabled defense response characterization.
- MEP pathway enzymes catalyzing DMAPP and IPP synthesis are induced by MeJA, as are mono- and diTPS enzymes. IPP synthases are a possible regulatory control point for diterpenoid resin acid accumulation.

Y. ZHENG AND B. H.AUKEMA. 2010. HIERARCHICAL DYNAMIC MODELING OF OUTBREAKS OF MOUNTAIN PINE BEETLE USING PARTIAL DIFFERENTIAL EQUATIONS. ENVIRONMETRICS. 21:901-816. [URL].

Document Type: Peer-Reviewed Publication

Core Messages and Approach:

- This study considers a spatial–temporal generalized linear mixed model (GLMM) as an alternative approach to modeling spatial–temporal binary data.
- Using the model, the effects of environmental factors were considered while accounting for the potential influence of space and time on MPB outbreaks.
- The methodology was used to analyze MPB outbreak data in Chilcotin Plateau, B.C.—from its start to collapse (1972-1986).

Key Outcomes:

- The model considered constant diffusion and convection rates and incorporated the effect of environmental factors directly into modelling the spatial-temporal process of MPB spread.
- A diffusion-convection PDE was used to describe outbreak dynamics, however the modeling framework can accommodate different types of PDE dynamics (e.g., can apply a reaction-diffusion models if combing insect movement with population dynamics and multispecies interactions).
- This study showed the potential of generalized linear mixed models in as a potential tool for modelling MPB outbreak events.

2009

S. DiGuistini, N. Y. Liao, D. Platt, G. Robertson, M. Seidel, S. K. Chan, T. R. Docking, I. Birol, R. A. Holt, M. Hirst, E. Mardis, M. A. Marra, R. C. Hamelin, J. Bohlmann, C. Breuil and S. J M. Jones. 2009. De novo genome sequence assembly OF A FILAMENTOUS FUNGUS USING SANGER, 454 AND ILLUMINA SEQUENCE DATA. GENOME BIOL. 10, R94 (2009). [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- This paper reports on the results of a new, hybrid methodology for genome sequencing that combines three different technologies: Sanger PE, 454 SE and Illumina PE sequence data.
- A new assembly procedure was developed to generate data of higher quality than could have been developed using one standalone technology. This hybrid approach could be well suited to account for future changes in tools and methods.

Approach:

- The research team developed a genome sequencing methodology that combines Sanger PE, 454 SE and Illumina PE sequence data. They then applied this new approach to develop a genome sequence for *Grosmannia clavigera,* which generated 32.5 Mb of data. Processes for integrating the data generated from the three technologies were tested and recommended.

Key Findings and Implications:

- Sequencing technologies have become less costly in recent years, but they remain expensive undertakings for generating high quality data. It is predicted that this hybrid approach will further reduce sequencing costs and expand genomics research in fungi.

S. M. ALAMOUTI, C. K. M. TSUI AND C. BREUIL. 2009. MULTIGENE PHYLOGENY OF FILAMENTOUS AMBROSIA FUNGI ASSOCIATED WITH AMBROSIA AND BARK BEETLES. MYCOL. RES. 113: 822-835. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- Bark and ambrosia beetles serve as vectors for diverse fungi that colonize wood. In conifers, these fungi tend to be filamentous ascomycetes (ophiostomatoid fungi), with species involved in tree disease.
- Most ambrosia fungi are a heterogeneous group of ophiostomatoids of the genera *Ambrosiella*, *Raffaelea* and *Dryadomyces*, whose taxonomy are complicated by poorly descriptive features.
- The accurate ability to classify these fungi will improve our understanding of the complexity of the beetle-fungal symbioses impacting forest health and productivity.
- Phylogenetic analyses using a multigene dataset from 67 taxa provided a higher resolution for species phylogeny and clarified relationships for the *Ambrosiella* associates of beetles and the closely related species of genera *Raffaelea* and *Dryadomyces*.
- These molecular analyses enhance the ability for phylogenetic classifications compared to the difficulties using conidiophore morphology and conidiogenesis for classification.
- This study revealed a correlation between the molecular classification of *Ophiostomatales*-related species *Ambrosiella* and *Raffaelea* and their ecological niches.

Approach:

- Twenty-two strains from the genera *Ambrosiella*, *Raffaelea* and *Dryadomyces*, three undescribed *Ambrosiella* strains and an undescribed species isolated from *Trypodendron* ambrosia beetles were used for analyses.
- Fruiting structures were used for culture morphological studies, while phylogenetic analyses were performed using a multigene dataset (nSSU and nLSU - nuclear small and large subunitx ribosomal RNA genes; β-tubulin gene) from 67 taxa, including the three above genera and representatives related to ophiostomatoid fungi in the *Ophiostomatales* and Microascales, as well as representative *Xylariales* and *Hypocreales*.

Key Findings and Implications:

- This report clarified the phylogenetic classification of *Ambrosiella* species isolated from ambrosia and bark beetles, and of the *Raffaelea* and *Dryadomyces* fungi of ambrosia beetles, as well as their relationships with other ophiostomatoid fungi.
- Species of the genus *Ambrosiella* are distributed across several distinct phyologenetic groups, such that they could be reassigned to different genera. In addition, the genus *Raffaelea* should be revised.
- No morphological characteristics strictly supported the monophyletic groups reported from the molecular data for the genera *Ambrosiella* and *Raffaelea*, but these groups were associated with the feeding behaviour of their beetle vectors.
- *Ambrosiella* from scolytid bark beetles represented a monophyletic group in the genus *Ophiostoma*, while fungi from scolytid and platypodid ambrosia beetles represented separate lineages related to the genus *Grosmannia*.
- Additional morphological and ecological trait characterization from an expanded collection of ambrosia is needed to provide additional support for the suggested monophyletic relationships.

C. S. DAVIS, K. E. MOCK, B. J. BENTZ, S. M. BROMILOW, N. V. BARTELL, B. W. MURRAY, A. D. ROE AND J. E. K. COOKE. 2009. ISOLATION AND CHARACTERIZATION OF 16 MICROSATELLITE LOCI IN THE MOUNTAIN PINE BEETLE, *DENDROCTONUS PONDEROSAE* HOPKINS (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE) MOL. ECOL. RESOUR. 9: 1071-1073. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- While MPB has caused significant environmental and economic impact, little is known regarding the genetic structure of this species. Population genetic studies will provide information regarding the dynamics and origins of eruptive populations.
- In order to carry out population genetic studies, MPB-specific genetic markers are needed.
- To develop markers, 16 polymorphic microsatellite loci were isolated from MPB, and the protocol was developed to amplify these markers in multiplexed reactions.
- A number of alleles were identified for each locus across two sampled populations.
- In one population, three loci deviated from Hardy-Weinberg equilibrium, and one of these loci may be sex-linked.
- These markers will be of use in helping to understand the population structure of this forest pest.

Approach:

- Genomic DNA libraries were constructed at two locations, Savannah River Ecology Laboratory (SREL) and the University of Alberta (UA). SREL DNA samples were isolated from thorax tissue of adult beetles, while UA DNA samples were from flight muscle tissue.
- Primer sets for 16 polymorphic loci were used, and multilocus genotypes were determined for 80 individuals from two locations in Alberta (Canmore – 41 beetles; Fairview – 39 beetles).
- Genetic diversity statistics were calculated, in addition to disequilibrium tests.

Key Findings and Implications:

- A total of 36 candidate loci were identified from SREL samples and 134 from UA samples.
- Only 5/36 and 11/134 primer sets yielded reliable polymorphic banding patterns that were specific to MPB.
- These 16 loci were used to genotype 80 individuals representing two different Alberta MPB populations.
- Three to 14 alleles were detected per locus across the two populations.
- Observed and expected heterozygosities ranged from 0.000 – 0.092, and from 0.100 – 0.830, respectively.
- Three loci showed significant heterozygote deficiency, deviating from Hardy-Weinberg equilibrium.
- One locus (Dpo520) appeared to be sex-linked, amplifying regions on both the X (odd length alleles) and the Y (even length alleles) chromosome. Even length alleles were never homozygous within an individual, were never found in female beetles, and were found in three male beetles.
- Locus Dpo486 displayed significant heterozygote deficiency in the Canmore population.
- These loci will serve as a useful tool to genotype populations, and to examine MPB population dynamics and eruptive population origins.

A. V. Rice and D. Langor. 2009. Mountain pine beetle-associated blue-stain fungi in lodgepole x jack pine hybrids near Grande prairie, Alberta (Canada). For. Path. 39: 323-334. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- MPB serves as a vector for three species of blue-stain fungi (*Grosmannia clavigera*, *Leptographium longiclavatum*, *Ophiostoma montium*) which are pathogenic and contribute to the success of the beetle.
- MPB larvae and galleries in a variety of lodgepole x jack pine hybrid stands were sampled during autumn, winter and spring during 2006-2007, to assess if these fungi persisted through the winter in MPB-attacked trees in the field.
- Following a cold winter with temperatures below -30°C, all three fungi were present, although most of the MPB larvae had died, suggesting the overwintering mortality of the fungi is not a problem, and will not limit the persistence and spread of MPB.

Approach:

- Several stands of lodgepole x jack pine hybrid trees near Grande Prairie, Alberta were sampled during autumn (October-November 2006), winter (January 2007) and spring (March 2007).
- A total of 38 gallery systems were sampled in the autumn, including 13 with parental galleries but no larval galleries. A total of 82 gallery systems were sampled in the winter, including 19 without live or intact cold-killed larvae. During spring sampling, a total of 175 galleries with live or cold-killed larvae were sampled.
- Fungi were subcultured and identified, and blue-stain fungi were identified to morphological species based on cultural and microscopic morphology.
- Only gallery systems from which fungi were isolated were used in analyses.

Key Findings and Implications:

- Blue-stain fungi were isolated from all 38 galleries in the autumn, including wood samples from 95% of gallery systems and larval samples from 92% of gallery systems. In the winter, fungi were isolated from 96% of gallery systems and 98% of larval samples. In the spring, fungi were isolated from 85% of wood samples and 94% of larval samples (Stand 1), 94% of wood and larval samples (Stand 2) and 99% of wood and larval samples (Stand 3).
- There were no significant differences in prevalence between the three fungi at any of the sampling points, but *O. montium* was isolated from more gallery system at each sampling period than the other two.
- The fungi differed in prevalence between the two substrates, and overall *O. montium* was isolated from more larval samples than either *L. longiclavatum* or *G. clavigera*. For wood samples, *L. longiclavatum* was isolated from more samples than *G. clavigera* or *O. montium*.
- Most gallery systems contained more than one fungal species. It was common to have three species in autumn and winter, and two in the spring. For gallery systems with two fungal species, not all pairwise combinations occurred at the same frequency, with the *G. clavigera* and *L. longiclavatum* combination less common than either of the pairwise combinations with *O. montium*.
- All of the fungi were present following a cold winter, suggesting that the overwintering mortality of the fungi will not be an issue and will not limit the persistence and spread of MPB.

C. K. M. Tsui, N. Feau, C. E. Ritland, S. M. Alamouti, S. Diguistini, L. Khadempour, J. Bohlmann, C. Breuil and R. C. Hamelin. 2009. Characterization of microsatellite loci in the fungus Grosmannia glavigera, a pine pathogen associated with mountain pine beetle. Mol. Ecol. Resour. 9: 1500-1503. [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- MPB and its fungal associates has caused the largest pest epidemic in Canadian history.
- MPB is closely associated with several fungi, including *Grosmannia clavigera*, which is pathogenic to pines and an important contributor to the MPB epidemic.
- Previous amplified fragment length polymorphism (AFLP) studies indicated that populations from the current epidemic were genetically variable.
- The aim of this study was to identify and characterize polymorphic satellite markers, to supplement AFLP data and to enhance our understanding of the population structure and migration patterns of MPB fungal associates.
- Using 53 isolates from two populations in British Columbia, eight loci were genotyped, revealing a number of alleles per locus. Most of these loci also showed length polymorphism in *Leptographium longiclavatum*, indicating that these microsatellites will be useful in population genetic studies.

Approach:

- Simple sequence repeats (SSRs) were identified from expressed sequence tags (ESTs) and the draft genome sequence of *G. clavigera*.
- Primers were designed from sequences flanking the SSRs, and screened on fungal isolates using extracted haploid mycelium DNA.

Key Findings and Implications:

- Of 60 potential SSRs, eight were polymorphic and were used to genotype 53 isolates of *G. clavigera* from two locations. Fifty-one isolates yield amplicons, while two isolates failed to amplify at one locus each, suggesting a low frequency of null alleles.
- Allelic diversity varied between three to 10 per locus, and the 53 isolates yielded 45 unique genotypes.
- The populations sampled from the two sites were strongly differentiated.
- All markers also amplified a single amplicon in 21 isolates of *L. longiclavatum*, the second most common and pathogenic fungus associated with MPB.
- Two loci (SR45, SR47) demonstrated significant length polymorphisms and will be useful for population genetic studies of *L. longiclavatum*, while the eight markers will be useful for future population structure studies with *G. clavigera*.

J. KOOPMANS, H. -M. C. DE LA GIRODAY, B. S. LINDGREN, AND B. H. AUKEMA. 2009. TAKE ME TO YOUR LEADER: DOES EARLY SUCCESSIONAL VEGETATION SPATIALLY INHIBIT PISSODES STROBI (COLEOPTERA: CURCULIONIDAE)? ENVIRON. ENTOMOL. 38(4):1189-1196 [URL]

Document Type: Peer-Reviewed Publication

Core Messages:

- The white pine weevil (*Pissodes strobi)* is a herbivorous insect that reproduces in young spruce and pine trees.
- The influence of distribution and diversity of nonhost species on white pine weevil host-selection, distribution and abundance had not been studied.
- This study examined the spatial factors of host and nonhost trees that may influence white pine weevil host selection and distribution.
- Understanding weevil interactions with host and nonhost vegetation combined with existing knowledge of spruce defense mechanisms, could lead to new silviculture tools for dealing with white pine weevil in commercially important forests.

Approach:

- A population census (included the spatial location) was taken of an interior hybrid spruce stand with moderate levels of white pine weevil near Prince George, B. C. Two thirds of the stand were comprised of early-successional nonhost vegetation (i.e., alder, paper birch, black cottonwood, lodgepole pine, trembling aspen and Canadian buffaloberry).

Key Findings and Implications:

- The presence of Canadian buffalo berry, paper birch, black cottonwood and trembling aspen among spruce was correlated with reduced weevil colonization. Possible reasons for these patterns include, nonhost volatiles (defense chemicals), and changes in below-ground processes (e.g., nitrogen fixing and nutrient uptake), and overstory shade.
- Knowing the locations of nonhost trees can provide as much, or more, inference about the locations of weevil-attacked trees as knowing the locations of suitable or preferred host trees.
- There was no association between stem density (host or nonhost) and white pine weevil attacks.
- The study’s findings highlight areas for future research to help determine if nonhost vegetation in early successional stands can be used for MPB management in commercially important stands. For example:
    - Do present patterns of weevil colonization distribution in early stand development last into later forest successional stages.
    - Examining the alteration of host seeking behaviors by insects through exposure to nonhost chemical defenses.
    - Examining how competition in plant communities affects herbivore population dynamics by altering host plant defences.