UNIVERSITÉ DU QUÉBEC À MONTRÉAL

FREINER LA PROPAGATION DE RAVAGEURS DU BOIS PAR PHYTOSANITATION AUX MICRO-ONDES ET IDENTIFICATION D'ENVAHISSEURS POTENTIELS PAR TESTS DE PRÉFÉRENCE D'HÔTE

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MARTIN PAYETTE

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LISTE DES ABRÉVIATIONS

Français :

CIPV	Convention internationale pour la protection des végétaux
MEB	Matériaux d'emballage en bois
NIMP	Normes internationales pour les mesures phytosanitaires

Anglais :

DWV	Deadwood volume
EPPO	European and Mediterranean Plant Protection Organization
IPPC	International Plant Protection Convention
ISPM	International Standards for Phytosanitary measures
LDRTF	Lake Duparquet Research and Teaching Forest
MEA	Malt dextrose agar
WPM	Wood packing material

RÉSUMÉ

Les matériaux d'emballage en bois (MEB) sont des vecteurs importants pour l'introduction de larves d'insectes xylophages et de pathogènes forestiers. Des exemples célèbres d'espèces introduites par du bois importé, comme l'agrile du frêne, *Agrilus planipennis* (Fairmaire), et la graphiose de l'orme, *Ophiostoma ulmi* (Buisman) et *O. novo-ulmi* (Braiser), témoignent du danger que posent les pestes exotiques sur la biodiversité forestière et urbaine autant au niveau écosystémique qu'économique. Les normes internationales sur les matériaux d'emballage en bois No. 15 (NIMP No. 15) proposent soit la fumigation au bromure de méthyle ou le traitement thermique avec une combinaison température/temps de 56°C/30 min pour éliminer tout organisme à l'intérieur des MEB. Ce projet a deux volets. D'abord, avec le but de développer un traitement plus fiable et plus économique, j'ai testé l'efficacité de l'irradiaton utilisant des micro-ondes comme traitement phytosanitaire sur des larves de *Monochamus scutellatus* (Say) et sur quatre pathogènes fongiques d'arbres dans les MEB. Les résultats indiquent que la combinaison de 56°C/2 min a donné 100% de mortalité chez les larves et de 65°C/2 min a inhibé la régénération de la majorité des échantillons chez toutes les espèces sauf *Chondrostereum purpureum* (Pers.) Pouzar (1959). Le traitement peut efficacement tuer certains organismes dans le bois à de durées plus courtes (2 minutes ou moins) que les 30 minutes recommandées, mais de futurs expériences sont nécessaires pour évaluer les combinaisons température/temps optimales pour éliminer le plus d'espèces possible. Ensuite, j'ai tenté de déterminer quels insectes xylophages indigènes aux Québec avaient la capacité de sélectionner et de compléter leur cycle de vie dans une essence européenne, l'épinette de Norvège. Ceci avait pour but d'évaluer leur potentiel invasif en Europe ou dans des plantations déjà établies en Amérique du Nord et pour pouvoir les cibler dans de futures expériences avec les micro-ondes. J'ai identifié quatre coléoptères xylophages compatibles avec l'épinette de Norvège dont *M. scutellatus*. Cette approche a permis franchir une première étape pour déterminer le potentiel envahisseur de ces espèces. La capture de *M. scutellatus* supporte également la pertinence de l'utilisation de ses larves pour des études cherchant à optimiser le traitement d'irradiation aux micro-ondes ou à tester d'autres traitements phytosanitaires au Québec.

Mots clés :
Espèces envahissantes, insectes xylophages, pathogènes, traitement phytosanitaire, micro-ondes, irradiation, matériaux d'emballage en bois (MEB).

ABSTRACT

Wood packing materials (WPM) are an important vector for the introduction of larvae of xylophagous insects and pathogens. Famous examples such as the emerald ash borer, *Agrilus planipennis* (Fairmaire), and Dutch elm disease, *Ophiostoma ulmi* (Buisman) and *O. novo-ulmi* (Braiser), illustrate the enormous threat posed by nonindigenous pests on forest and urbain trees. The International Standards for Phytosanitary Measures No. 15 (ISPM No. 15) suggest either methyl bromide fumigation or heat treatment with a temperature/time combination of 56°C/30 min to eliminate all organisms inside the wood. This project has two sections. First, in an effort to develop a more reliable and economic treatment, I tested the reliability of microwave irradiation as a phytosanitary treatment on *Monochamus scutellatus* (Say) larvae and on four fungal tree pathogens in WPM. Results show that a combination of 56°C/2 min gave 100% mortality for the larvae and of 65°C/2 min inhibited most regeneration of cultures in all but *Chondrostereum purpureum* (Pers.) Pouzar (1959). The treatment is efficient at killing certain organisms at shorter time intervals (2 minutes or less) than the suggested 30 min, but future studies will be required to determine optimum temperature/time combinations to eliminate as many species as possible. Second, I determined the acceptability and survivability of xylophagous insects native to Québec with a European tree species, Norway spruce, to evaluate their potential as invaders in Europe or as pests for established plantations in North America and to target them specifically in future microwave trials. I identified four beetle species compatible with Norway spruce, including *M. scutellatus*. This approach was a first step in determining the invasive potential of these species. The capture of *M. scutellatus* during this study also supports the pertinence of using its larvae in studies for optimizing the microwave irradiation treatment or for testing other phytosanitary treatments in Québec.

Key words :
Invasive species, xylophagous insects, pathogenic fungi, phytosanitation, microwaves, irradiation, wood packing material (WPM).

INTRODUCTION

0.1 Les invasions biologiques et leurs conséquences écologiques et économiques

Les effets des invasions biologiques constituent une des principales menaces à la biodiversité et posent un risque important aux écosystèmes naturels, ruraux, et urbains (Allen et Humble, 2002 ; NRC, 2002 ; Poland et McCullough, 2006). La fréquence des invasions biologiques a augmenté énormément avec la multiplication des déplacements des humains, particulièrement en matière des échanges internationaux (Mack *et al.*, 2000). Les invasions biologiques liées aux activités humaines peuvent amener des conséquences négatives importantes autant au niveau écologique qu'économique (Holmes *et al.*, 2009 ; Mack *et al.*, 2000 ; NRC, 2002 ; Vitousek *et al.*, 1997). Selon Wilcove et al. (1998), la destruction d'habitat et les espèces envahissantes sont les deux causes majeures d'extinctions aux États-Unis, touchant respectivement 88% et 57% des plantes menacées. La croissance agressive des populations humaines, la fréquence et la rapidité grandissantes de ses déplacements, la perte d'habitats naturels au profit du développement agricole et urbain et la globalisation des marchés créent de plus en plus de chemins par lesquels des espèces opportunistes peuvent se rendre dans de nouvelles régions (Liebhold *et al.*, 1995 ; OTA, 1993 ; Pimentel *et al.*, 2000). Les déplacements plus rapides par voies marines et aériennes et sur de plus longues distances offrent un transport rapide et augmentent les chances de survie d'espèces exotiques (Mattson *et al.*, 1994 ; McCullough *et al.*, 2006 ; OTA, 1993 ; Work *et al.*, 2005).

Les invasions biologiques se déroulent en trois étapes : introduction, établissement, et propagation (Liebhold et Tobin, 2008 ; Mack *et al.*, 2000 ; NRC, 2002). L'introduction est le moyen par lequel une espèce non-indigène arrive dans un nouvel habitat. Lors de l'établissement, la nouvelle population devient autosuffisante et peut maintenir ses nombres constants dans le nouvel environnement. Dans la phase de

propagation, la population croît et étend sa présence sur une plus grande superficie. Cette dernière étape est souvent facilitée par des perturbations naturelles ou causées par l'humain. Il y a plusieurs mécanismes qui peuvent transformer une espèce non-indigène en espèce invasive. Une adaptation spécifique de l'espèce non-indigène peut lui donner un avantage compétitif contre les indigènes ou l'absence de prédateurs dans un nouvel habitat enlève un facteur important qui contraint normalement les populations. Par exemple, *Centaurea diffusa* Lam., une Asteraceae de l'Eurasie, est invasive en Amérique du Nord (Callaway et Aschehoug, 2000). Des exsudats phytotoxiques de ses racines lui donnent un net avantage dans la compétition pour des ressources contre les espèces nord américaines par rapport à des espèces en Eurasie qui, par coévolution avec elle, ont pu développer des adapataions aux composés toxiques.

Historiquement, le Canada et les États-Unis ont accueillis plus de 50,000 espèces invasives. Plusieurs ont été introduites intentionnellement à des fins d'agriculture, d'élevage, de restauration, de contrôle biologique et de loisir, mais la majorité ont été introduites accidentellement par voies de transport (Pimentel, Zuniga et Morrison, 2005). Bien que ce ne soit pas toutes les espèces non-indigènes qui sont envahissantes, le peu d'espèces qui le deviennent causent des dommages importants au niveau des écosystèmes et de l'économie. Il a été estimé que les espèces invasives amènes des pertes et des coûts d'environ 120 milliards $US par année aux États-Unis (Pimentel, Zuniga et Morrison, 2005). Cette étude couvrait les dommages et les coûts de contrôle causés par des espèces de plantes, de mammifères, d'oiseaux, de reptiles, d'amphibiens, de poissons, d'arthropodes, de mollusques, de microorganismes, d'animaux d'élevage et de pathogènes humains. En 2005, le budget fédéral inter-agences aux États-Unis pour la gestion de problèmes d'espèces envahissantes était de plus de 484 millions $US, une augmentation de 98 millions par rapport à l'année précédente (Simberloff, Parker et Windle, 2005). Ce mémoire traite de la problématique du contrôle des espèces insectes et fongiques qui s'attaquent au bois et

causent des dommages importants aux arbres urbains, forestiers et de plantations. Les dommages causées aux forêts par des insectes et pathogènes fongiques aux États-Unis amènent des coûts annuels de 4,2 milliards $US (Pimentel, Zuniga et Morrison, 2005). Encore aux États-Unis, les coûts annuels induits par les insectes xylophages, qui s'attaquent au bois des arbres et, lors de l'explosion d'une population envahissante, peuvent causer la mort à des individus en santé, sont estimés à 1,7 milliards $US en les dépenses gouvernementales et à 830 millions $US en pertes résidentielles (Aukema *et al.*, 2011).

0.2 Les matériaux d'emballage en bois comme vecteur de pestes et maladies forestières

0.2.1 Introduction aux matériaux d'emballage en bois

Des vecteurs importants des espèces envahissantes d'insectes xylophages et de champignons qui dégradent le bois sont les matériaux d'emballage en bois (MEB) (terme anglais : wood packing material [WPM]) (Brockerhoff *et al.*, 2006 ; Haack, 2001 ; McCullough *et al.*, 2006 ; Work *et al.*, 2005). Les MEB sont principalement représentés par les palettes de bois et jouent un rôle essentiel dans le transport et l'entreposage de biens et matériaux au niveau national ainsi qu'à l'échelle mondiale. Le bois de palettes est généralement de moins bonne qualité. En raison de leur facilité de construction et leur coût réduit, les palettes de bois sont retrouvées dans toutes les régions du monde et peuvent voyager vers de nombreuses localités différentes au cours de leur vie utile. Elles peuvent être construites selon divers modèles en fonction de l'utilisation désirée (AMPCQ, 2009 ; CRIQ, 2001). Elles peuvent être fabriquées de composantes brutes ou rabotées sur une ou deux faces et être vertes ou séchées (CRIQ, 2001). En général, le bois de conifères est utilisé pour construire de palettes moins coûteuses et jetables, de 9 mm à 15 mm d'épaisseur des planches, tandis que le bois de feuillus sert à construire des palettes réutilisables et recyclables, d'environ 20

mm d'épaisseur des planches (CRIQ, 2001). En 2000 au Canada, la Colombie-Britanique et l'Ontario étaient responsables de la croissance imporante des exportations du pays qui totalisent 63% des ventes à l'étranger (CRIQ, 2001). La contribution du Québec comptait pour 31% de la production et 9% des exportations de palettes au Canada, représentant une valeur de 87,7 millions $US et 16,4 millions $US respectivement. Il est estimé qu'entre 12,4 à 14 millions de palettes sont produites annuellement au Québec. La production et l'exportation des MEB forme alors une partie importante de l'économie québecoise et implique un grand nombre d'entreprises, petites ou grandes (CRIQ, 2001).

0.2.2 Les organismes qui s'y trouvent

Le bois non-traité qui compose les palettes de bois et autres MEB peut contenir les larves d'insectes xylophages et les pathogènes du bois qui étaient déjà présents avant l'abattage des arbres. Elles leur offrent un milieu propice et une protection lors du transport et constituent un vecteur important pour leur introduction dans des pays étrangers (Brockerhoff et al., 2006 ; Haack, 2001 ; McCullough et al., 2006 ; Work et al., 2005). Ces organismes peuvent menacer les populations d'arbres urbains, forestiers et de plantation dans leurs nouveaux habitats si les conditions sont propices à leur propagation (Allen et Humble, 2002).

0.2.2.1 Insectes

En 2001 aux États-Unis, Haack (2001) avait estimé que 400 insectes non-indigènes s'attaquant aux arbres et arbustes avaient établis des populations autosuffisances. Une étude plus récente a élevé ce nombre à 455 (Aukema *et al.*, 2011). Les familles de coléoptères de Cerambycidae, Curculionidae (plus spécifiquement dans la sous-famille des Scolytinae) et Buprestidae produisent plusieurs des parasites les plus

reconnues pour les dommages causés aux populations d'arbres étrangères et sont les insectes les plus souvent identifiés dans les MEB lors d'inspections aux sites d'entrées aux États-Unis et en Nouvelle-Zélande (Brockerhoff, 2009 ; Haack, 2006). Ce sont des espèces qui pondent leurs œufs sous l'écorce et dans le pré-cambium des arbres. Les larves sont alors transmises lorsque le bois est coupé et utilisé dans la construction de MEB. Pour la majorité des espèces problématiques de ces familles, la période immature se déroule dans les branches, le tronc ou dans les racines de l'arbre hôte (Arnett Jr et Thomas, 2001 ; Arnett Jr *et al.*, 2002). Elles sélectionnent parfois des arbres en santé, mais s'attaquent la plupart du temps aux arbres malades, moribonds ou récemments morts, puisque les défenses de celles-ci sont ainsi moins efficaces (Solomon, 1995). Plusieurs espèces n'ont qu'une génération et peut-être une seconde génération partielle à chaque année, tandis que d'autres produisent plusieurs générations qui se chevauchent (Craighead, 1923). En général, un cycle de vie dure environ un an, mais peut varier à 2 et même 5 ans chez certaines espèces (Craighead, 1923 ; Yanega, 1996). Le temps de génération peut aussi varier à l'intérieur d'une même espèce selon le climat et la photopériode de chaque région (Baier, Pennerstorfer et Schopf, 2007 ; Hansen, Bentz et Turner, 2001). Les adultes émergent du bois au printemps et pondent leurs œufs sous l'écorce de l'hôte à l'intérieur de quelques semaines. Ceux-ci éclosent de quelques jours à plusieurs semaines plus tard et, chez les Cerambycidae et Buprestidae, les larves passent à travers plusieurs phases de croissances, devenant plus larges et creusant dans le xylème vers le centre de l'arbre avant de remonter vers la surface et devenir nymphe (Craighead, 1923 ; Yanega, 1996). Les Scolytinae, plus connues en anglais sous le nom de « bark-beetle », restent dans le pré-cambium durant leur croissance larvaire (Arnett Jr *et al.*, 2002). Les tunnels et galeries créés par les adultes et les larves bloquent la circulation de substances nutritives de l'arbre, l'affaiblissant et, dans certains cas extrêmes, causant sa mort. Chez les Scolytinae, dont les larves ne sont pas capables de digérer la matière ligneuse, les adultes transportent des champignons symbiotiques qui dégradent le bois et peuvent à leur tour causer des dommages importants (Humble et

Allen, 2006 ; Klepzig *et al.*, 2001). Les larves n'ayant pas émergés avant le début de l'automne entrent en diapause jusqu'au printemps et émergent en tant qu'adultes au printemps. Plusieurs espèces peuvent être contraintes à attaquer une seule espèce ou genre d'arbre, mais d'autres peuvent sélectionner plusieurs genres différents, mais demeurent spécifiques soit aux conifères ou aux feuillus.

En Amérique du Nord, le longicorne asiatique, *Anoplaphora glabripennis* (Motschulsky), est un exemple d'espèce invasive ayant causée des dommages importants aux populations urbaines de feuillus. Il était déjà un parasite de feuillus urbains en Chine avant d'être repéré à New York en 1996 et à Chicago en 1998 (Fleming *et al.*, 2003). Des arbres attaqués à répétition sont morts à l'intérieur de quelques années après l'infestation initiale. En 2000, plus de 4 720 arbres à New York et 1 390 en Illinois ont dû être abattus, ce qui représente des coûts de 25 millions $US (Nowak *et al.*, 2001). Il a été repéré à Toronto en 2003 et après une longue campagne d'éradication semblait avoir été complètement éliminé au Canada selon une annonce récente du gourvenement (CFIA, 2013a). Il a par contre de nouveau été identifié près de l'aéroport international Pearson en périférie de Toronto (CFIA, 2013c). L'agrile du frêne, *Agrilus planipennis* (Fairmaire), est un parasite relativement récent en Amérique du Nord. Il a été identifié en 2002 aux alentours de Détroit, au Michigan, et de Windsor, en Ontario (Poland et McCullough, 2006). Il peut tuer des arbres de grande taille, trois ou quatre ans après infestation et de petits arbres après une année. Dans le sud-est du Michigan en 2004, environ 15 million de frênes ont été tués par l'agrile du frêne (Poland et McCullough, 2006). Il est encore aujourd'hui présent en Ontario et au Québec sur l'îles de Montréal et les région qui l'entourenté (CFIA, 2013b). Les régions à risques sont hautement régulées par des prohibitions de transport de matériaux dérivés du frêne à l'extérieur d'elles et par des campagnes d'élimination d'arbres infestés pour ralentir la prolifération de l'insecte. Il a été prédit que son territoire continuera à s'étendre dans le nord-est des États-Unis jusqu'à être présents dans plus de 25 états (Kovacs *et al.*, 2010).

Certains insectes peuvent causer des problèmes en étant vecteur de maladies forestières. Le nématode du pin, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, utilise les longicornes du genre *Monochamus* comme vecteur de transmission vers des arbres de pin (*Pinus* sp.) affaiblis ou mourants dans lesquels il se nourrit et se reproduit (Mamiya, 1976). Son origine présumée est l'Amérique du Nord, où il n'est vu que comme un parasite mineure, tandis que, depuis le début du 20^{e} siècle, il cause des dommages importants dans les forêts de pin du Japon (Bergdahl, 1988 ; Dropkin, 1989 ; Kosaka *et al.*, 2001). Il a été par la suite introduit en Chine et en Corée (Fielding et Evans, 1996 ; Kosaka *et al.*, 2001 ; Shi *et al.*, 2012 ; Suzuki, 2002 ; Zhao *et al.*, 2007) et est maintenant présent au Portugal et en Espagne (Abelleira *et al.*, 2012 ; Robertson *et al.*, 2011). Certains pays, ayant détectés la présence du nématode dans des MEB importés (Finlande) ou voulant en prévenir l'occurance (Suède, Norvège, Corée du Sud), ont émis des restrictions sévères sur l'importation de MEB venant de pays où il est établi (Dwinell, 1997). Le longicorne noir, *Monochamus scutellatus* (Say), une espèce indigène au Québec, est candidat pour être vecteur de la transmission du némade du pin vers l'extérieur de la province.

Une autre famille importante relativement aux MEB est celle des Siricidae (Hymenoptera). Leur cycle de vie est similaire à celui des familles de Cerambycidae et de Buprestidae. Ils pondent leurs œufs dans l'écorce et les larves creusent vers le centre de l'arbre avant d'émerger comme adultes après une à deux années de croissance (Morgan, 1968). Les larves passent par 5 à 11 phases larvaires. Puisqu'elles ne digèrent pas le bois, elles se nourrissent plutôt de pathogènes fongiques du bois qui utilisent les adultes comme vecteur d'arbre en arbre et ces champignons peuvent être une des causes de la mort des arbres en plus des tunnels créés par les larves. Les femelles sont partiellement parthénogénétiques puisqu'il n'est pas nécessaire pour elles d'avoir copulé pour pondre des œufs mâles haploïdes. Cette famille est principalement connue par l'espèce *Sirex noctilio* (Fabricius), une espèce originaire de l'Eurasie et du nord de l'Afrique (Carnegie *et al.*, 2006). Elle

s'est établit au début du 20ième siècle en Nouvelle-Zélande et a été introduite en Tasmanie en 1951 pour être détectée ensuite en Australie en 1961 où elle continue de causer des dommages importants aux plantations de *Pinus radiata* et à d'autres arbres du même genre (Carnegie, Eldridge et Waterson, 2005 ; Carnegie *et al.*, 2006 ; Morgan et Griffith, 1989). Carnegie et al. (2006) ont déterminé qu'advenant son introduction, *S. noctilio* a le potentiel de pouvoir persister dans plusieurs pays de l'Amérique du Nord et du Sud, dans le sud de l'Afrique et en Chine.

0.2.2.2 Champignons

Les pathogènes sont plus difficiles à détecter que les insectes xylophages, ce qui rend difficile leur contrôle (Allen et Humble, 2002). Ils sont tout de même souvent retrouvées dans le bois importé (Jacobs *et al.*, 2003 ; Kim *et al.*, 2005 ; Mireku et Simpson, 2003). Les champignons sont dangereux puisqu'ils peuvent à la fois être transportés dans le bois et utiliser les insectes xylophages comme vecteurs (Humble et Allen, 2006). Les pathogènes fongiques non-indigènes ont été responsables de plusieurs épidémies au courant des deux derniers siècles (Allen et Humble, 2002). Un exemple marquant est le chancre de l'écorce (anglais : chestnut blight), causé par le champignon *Cryphonectria parasitica* (Murr.), qui a décimé les populations de châtaigniers aux États-Unis et en Europe (Bissegger, Rigling et Heiniger, 1997). Un autre exemple est la graphiose de l'orme, ou maladie hollandaise de l'orme (anglais : Dutch elm disease), attribuée à deux espèces ascomycètes, *Ophiostoma ulmi* (Buisman) et *Ophiostoma novo-ulmi* (Braiser), qui ont toutes deux causées des dommages aux proportions pandémiques aux populations d'ormes de l'Amérique du Nord et de l'Europe au courant du 20ième siècle (Brasier, 2000 ; Mireku et Simpson, 2003). En Amérique du Nord, deux espèces de Scolytinae ont été confirmées comme étant vecteur de la maladie, le *Hylurgopinus rufipes*, qui est indigène, et le *Scolytus multistriatus* (Marsh.), qui a été introduit à partir de l'Europe (Jacobi, Koski

et Negron, 2013 ; Webber, 2000). Un autre exemple plus récent vient de certaines espèces du genre *Phytophthora* qui sont envahissantes dans certains coins du monde et causent des maladies des racines, l'apparition de chancres et la perte de feuilles (Broembsen, 1989 ; Hansen, 2008). *Phytophthora ramorum* (McPherson *et al.*, 2013 ; Rizzo *et al.*, 2002 ; Werres *et al.*, 2001) est la cause primaire de l'encre du chêne rouge, une maladie amenant la mort d'un grand nombre de chênes en Californie et en Oregon depuis 1993 et également présent en Europe (McPherson *et al.*, 2013 ; Rizzo *et al.*, 2002 ; Werres *et al.*, 2001). La maladie a atteint un niveau épidémique et ses effets à longtermes sont très inquiétants (Rizzo et Garbelotto, 2003). De plus, la présence de *P. ramorum* dans un arbre augmente sa vulnérabilité face aux attaques des insectes xylophages, particulièrement les Scolytidae (McPherson *et al.*, 2013).

Comme mentionné plus haut, le contrôle d'espèces envahissantes est complexe et coûteux (Aukema *et al.*, 2011 ; Lodge *et al.*, 2006) (Allen et Humble, 2002 ; NRC, 2002 ; Pimentel, Zuniga et Morrison, 2005). Une des meilleures façons de réduire de telles dépenses est de prévenir l'introduction des espèces. La section suivante décrit les accords internationaux et les traitements appliqués au MEB qui tentent de mitiger le problème.

0.3 Règlementations et traitements

La présence d'organismes potentiellement envahissants à l'intérieur des MEB et la nécessité de contrôler leur transmission constituent un problème d'une grande valeur économique pour les entreprises qui les produisent. La Convention internationale pour la protection des végétaux (CIPV) a établis les Normes internationales pour les mesures phytosanitaires (NIMP) afin d'étudier, prévenir et contrôler la dissémination d'organismes de quarantaine pour réduire l'occurrence de leur introduction et établissements (FAO, 2009). Les NIMP No. 15 proposent deux traitements à être appliqués sur les MEB pour éliminer les organismes qui se trouvent à l'intérieur : la

fumigaton au bromure de méthyle et le traitement thermique (FAO, 2009). La fumigation au bromure de méthyle doit respecter un programme permettant d'atteindre un produit de concentration/temps minimal pendant 24 h et ce pour une température et une concentration finale spécifique selon les combinaisons choisis. Le traitement thermique requiert que le centre du bois atteigne une température minimale de 56°C pour au moins 30 minutes (FAO, 2009). En autant que ces conditions sont respectées, le traitement peut prendre la forme de séchage à l'étuve, d'imprégnation chimique sous pression favorisée par la chaleur, d'irradiation aux micro-ondes, ou autres. Il est également nécessaire que tous MEB soient écorcés, indépendamment du traitement utilisé. Finalement, les MEB traités selon les méthodes approuvées par les NIMP No. 15 doivent être estampillés et doivent l'être de nouveau s'ils ont été recyclés.

Ces traitements ont cependant chacun leurs désavantages. L'écorçage peut être efficace pour enlever les organismes se trouvant dans l'écorce et réduire les chances d'attaque après traitement (Haack et Petrice, 2009 ; McCullough *et al.*, 2007), mais il peut arriver que quelques traces d'écorce, bien que minimes, soient encore présentes sur les MEB après traitement en quantités suffisantes pour les besoins des larves (Ray et Deomano, 2007). Le bromure de méthyle n'est adéquat pour tuer les organismes que jusqu'à une profondeur de 50 mm à l'intérieur du bois versus des épaisseurs pouvant excéder cette profondeur dans les MEB (Kunstadt, 1998). De plus, c'est un composé qui contribue à la dégradation de la couche d'ozone (WMO, 2006) et son utilisation est strictement réglementée par les signataires du Protocole de Montréal relatif aux substances qui appauvrissent la couche d'ozone (UNEP, 2000). Les signataires du protocole, dont le Canada, doivent réduire l'utilisation du bromure de méthyl et cesser complètement l'utilisation en 2015 (Madé, 2012).

Le traitement thermique conventionnel est préférable à la fumigation au niveau de son impact sur l'environnement. Il a été démontré qu'il est efficace à 56°C/30 minutes contre le *Tetropium cinnamopterum* Kirby (Mushrow *et al.*, 2004). Il a

cependant été démontré qu'il n'est pas toujours 100% efficace contre les pré-nymphes (la phase inactive entre les stades larvaires et le stade de nymphe pendant laquelle l'insecte passe l'hiver) d'agrile du frêne, qui ont survécus à des combinaison température/temps de 60°C/30 min et de 50°C/60 min dans une étude (Myers, Fraser et Mastro, 2009) et de 55°C/120 min dans une autre (Haack et Petrice, 2009 ; McCullough *et al.*, 2007). De plus, le traitement à 56°C/30 min n'est pas suffisant pour éliminer tous pathogènes fongiques dans le bois puisque la résistance à la chaleur des champignons varie d'une espèce à l'autre (Ramsfield *et al.*, 2010) et la combinaison reconnue pour tous les éliminer est de 67 °C/ 75 min (Kutz, 2005).

L'irradiation aux micro-ondes est un traitement qui est de plus en plus étudié comme alternative au séchage à étuve conventionnel (Fleming *et al.*, 2003 ; Fleming *et al.*, 2004 ; Fleming *et al.*, 2005 ; Henin *et al.*, 2008 ; Hoover *et al.*, 2010 ; Nzokou, Tourtellot et Kamdem, 2008). Les micro-ondes seraient en mesure de cibler l'humidité dans le bois, tels que celle présente chez les êtres vivants et donc d'offrir un traitement plus fiable que le séchage par étuve qui doit chauffer le substrat au complet à partir de l'extérieur. Ces études indiquent que ce traitement pourrait être aussi efficace, sinon plus, que les traitements présentement utilisés et demanderait une durée considérablement plus courte. Le tableau 0.1 cite des combinaisons température/temps déjà étudiés avec les micro-ondes dans le contexte des MEB et les parasites qui y sont reliées. Le traitement a démontré une capacité d'obtenir près de 100% de mortalité chez des larves de Cerambycidae avec des combinaisons températures/temps maximales de 60 °C/~5 min (*Hylotrupes bajulus*) (Fleming *et al.*, 2003 ; Fleming *et al.*, 2004 ; Fleming *et al.*, 2005 ; Henin *et al.*, 2008 ; Hoover *et al.*, 2010 ; Nzokou, Tourtellot et Kamdem, 2008), de 60 °C/5 min (*A. glabripennis*) (Fleming *et al.*, 2003 ; Fleming *et al.*, 2004 ; Fleming *et al.*, 2005 ; Henin *et al.*, 2008 ; Hoover *et al.*, 2010 ; Nzokou, Tourtellot et Kamdem, 2008) et de 62 °C/2,31 min (*Plectrodera scalator*) (Fleming *et al.*, 2003 ; Fleming *et al.*, 2004 ; Fleming *et al.*, 2005 ; Henin *et al.*, 2008 ; Hoover *et al.*, 2010 ; Nzokou, Tourtellot et Kamdem,

2008), chez des larves de nématode du pin avec des combinaisons de 62 °C/2 min (Fleming *et al.*, 2003 ; Fleming *et al.*, 2004 ; Fleming *et al.*, 2005 ; Henin *et al.*, 2008 ; Hoover *et al.*, 2010 ; Nzokou, Tourtellot et Kamdem, 2008) et de 60 °C/ 2,31 min (Fleming *et al.*, 2003 ; Fleming *et al.*, 2004 ; Fleming *et al.*, 2005 ; Henin *et al.*, 2008 ; Hoover *et al.*, 2010 ; Nzokou, Tourtellot et Kamdem, 2008). Seulement une étude a tenté de tester le traitement sur des larves de l'agrile du frêne (Fleming *et al.*, 2003 ; Fleming *et al.*, 2004 ; Fleming *et al.*, 2005 ; Henin *et al.*, 2008 ; Hoover *et al.*, 2010 ; Nzokou, Tourtellot et Kamdem, 2008). Celle-ci a obtenu 100% de mortalité à 65 °C pour 30 min. Cependant, les expériencs ont été faites sur des bûches non-écorcées, ce qui explique l'efficacité diminuée du traitement au niveau du temps nécessaire afin d'obtenir une mortalité totale. Ces résultats suggèrent que l'irradiation aux micro-ondes est une avenue de recherche prometteuse en vertue des hauts taux de mortalité à des durées de traitement très réduits par rapport aux 30 min recommandés par les NIMP No. 15. Il semble cependant n'y avoir aucune étude qui ait été conduite sur l'effet des micro-ondes sur les champignons dans le bois. Des expériences ont déjà eu lieu avec les micro-ondes sur certaines des espèces de bactéries, de champignons et de nématodes dans le sol avec des résultats prometteurs pour des durées plus courtes (Ferriss, 1984), mais rien de conclusif sur l'effet sur le bois peut en être tiré. Comme mentionné plus haut, dans le contexte des MEB, le traitement thermique conventionel par chauffage externe n'est pas suffisant pour causer la mortalité de toute les espèces fongiques dans le bois à 56°C/30 min (Ramsfield *et al.*, 2010). Une forme d'irradiation, fréquences-radio, a cependant déjà été testée pour stériliser le bois et a démontrer une capacité d'obtenir 98% à 100% de mortalité chez certaines espèces fongiques après 2 minutes de traitement (Tubajika *et al.*, 2007). La similarité des traitements pourrait indiquer le grand potentiel des micro-ondes.

Afin de rafiner les connaissances déjà acquises dans des études précédentes sur la prévention d'introductions d'espèces exotiques et dans l'optique de présenter le Québec en tant que partenaire économique fiable et sécure en ce qui converne ses

exportations de MEB, cette étude veut (1) déterminer l'efficacité de l'irradiation aux micro-ondes comme traitement phytosanitaire sur les larves d'une espèce insecte et quatre espèces fongiques présentes au Québec et d'une certaine importance économique et (2) étudier une méthode pour identifier des espèces insectes du Québec avec un haut potentiel d'exportation par les MEB afin de les cibler comme espèces à risque et de haute importance pour des traitements phytosanitaires. La portée de cette étude devrait dépasser la sphère locale du Québec vers l'international (1) en appuyant les études précédentes sur l'irradiation aux micro-ondes exécutées sur des essences d'arbres et espèces insectes et fongiques différentes et (2) explorant de nouvelles possibilité pour prédir des envahisseurs potentiels. Les deux sections suivantes décrivent les objectifs principaux de ce mémoire.

0.4 Objectif 1 : Évaluer l'efficacité de l'irradiation aux micro-ondes au Québec

Comme mentionné précédemment, des études portent déjà sur le potentiel de l'irradiation aux micro-ondes. Parmi les espèces ciblées dans ces études, on retrouve des espèces reconnus pour leurs activités envahissantes, tel le longicorne asiatique (Fleming *et al.*, 2003), l'agrile du frêne (Nzokou, Tourtellot et Kamdem, 2008) et le nématode du pin (Fleming *et al.*, 2005 ; Hoover *et al.*, 2010). Les études ont toutes testé des températures de traitement près de 56°C mais avec des durées très variables. Certaines études expérimentent avec des traitements thermiques sur des champignons dans le bois (Ramsfield *et al.*, 2010 ; Tubajika *et al.*, 2007), mais je n'ai trouvé aucune étude qui teste le potentiel de traitement phytosanitaire des micro-ondes sur les champignons. Le tableau 0.1 présente les combinaisons température/temps utilisées dans ces études. On peut également observer que les dimensions ainsi que le type de bois sur lequel le traitement est testé varient énormément. Considérant que l'objectif global de ces études est d'identifier une combinaison température/temps adéquate afin d'éliminer toutes catégories de vivants à l'intérieur des MEB, elles

offrent conjointement des résultats utiles mais non-conclusifs, vu les différences entre les doses létales.

Dans le cadre d'une éventuelle introduction du traitement à une échelle industrielle au Québec, il s'avère nécessaire de déterminer l'efficacité du traitement sur des pièces de bois de dimensions comparables à celles trouvées dans les MEB. Ces traitements seront réalisés en utilisant des parasites déjà présents sur le territoire et courant le risque de se trouver dans le bois utilisé pour construire des palettes. Des durées de traitements réduites par rapport au séchage conventionnel seront utilisées afin de déterminer si le traitement serait profitable pour les entreprises québécoises. L'objectif du premier chapitre de cette maîtrise sera de déterminer l'efficacité du traitement dans ces conditions. J'ai alors sélectionné le longicorne noir, *Monochamus scutellatus* (Say) une espèce de longicorne présente sur le territoire québécois qui s'attaque à la majorité des essences de conifères indigènes et qui est abondante et simple à capturer dans les forêts mixtes et de conifères. C'est un parasite mineur au Québec puisqu'il n'affecte principalement que la qualité de bois coupé, ce qui le rend par contre à risque d'être retrouvé dans les MEB. L'association nématode du pin aux espèces du genre *Monochamus* fait également de *M. scutellatus* un bon candidat pour être un parasite de quarantaine (EPPO, 1997, 2012). J'ai également sélectionné quatre espèces fongiques présentes au Québec qui s'attaquent au bois, soit *Gremmeniella abietina* (Lagerberg) Morelet and *Heterobasidion annosum* (Fr.) Bref., and *Chondrostereum purpureum* (Pers.) Pouzar and *Mycosphaerella populorum* G.E. Thompson. Ces quatre espèces sont distribuées à travers plusiseurs pays et peuvent être des parasites mineurs dans les forêts et plantations de certaines régions (EPPO, 1980, 2009 ; Lygis *et al.*, 2004) ou, comme dans le cas de *C. purpureum*, peuvent servir comme bioherbicide pour le contrôle de plantes ligneuses indésirables (Pitt *et al.*, 1999 ; Ramsfield *et al.*, 1996). Elles ne causent présentement pas les mêmes inquiétudes que des maladies ayant atteint des niveaux épidémiques comme l'encre du chêne rouge (*P. ramorum*), mais ont tout de même été sélectionées comme

espèces modèles pour tester le traitement. Les espèces seront traitées dans des blocs de dimensions similaires à ce qui est retrouvé dans plusieurs palettes de bois à partir de bois de peuplier faux-trembles (*Populus tremuloides* Michx.) et de pin gris (*Pinus banksiana* Lamb.), des essences couramment utilisées en construction de palettes.

0.5 Objectif 2 : Identifier et cibler des insectes xylophages spécifiques afin de valider le traitement phytosanitaire commercial par les micro-ondes

Puisque les traitements phytosanitaires sur les MEB constituent avant tout une approche préventive, le deuxième volet du projet explore ce thème plus loin. Les études discutées dans la section précédente tentent majoritairement de déterminer l'efficacité de traitements phytosanitaires préventifs sur des espèces déjà classifiées comme envahissantes. L'objectif premier du présent projet est de déterminer si l'irradiation aux micro-ondes serait une option avantageuse et durable pour les NIMP No. 15 et pour le Québec. Dans ce contexte, les espèces que nous voulons ciblées pour le traitement ne sont pas celles qui entrent dans la province, mais bien celles qui pourraient en sortir. Il serait donc intéressant de voir si l'on peut déterminer le potentiel invasif d'espèces indigènes du Québec dans des régions étrangères. Les traitements phytosanitaires pourraient alors être ciblées vers les espèces qui démontrent le plus grand risque.

Puisque les espèces indigènes deviennent rarement des parasites dans leur habitat d'origine, il est difficile d'évaluer si elles deviendraient envahissantes dans des conditions différentes. Dans le cas des insectes xylophages, l'un des critères les plus importants pour évaluer si une espèce introduite aura du succès dans une nouvelle région est la présence d'hôtes compatibles (Carnegie *et al.*, 2006 ; Hanks, Paine et Millar, 1993 ; Niemela et Mattson, 1996 ; Ødegaard, Diserud et Østbye, 2005 ; Roques, Auger-Rozenberg et Boivin, 2006). L'hôte est important puisqu'il doit d'abord être attirant pour l'adulte et ensuite, les larves doivent pouvoir compléter leur

croissance à l'intérieur (Niemela et Mattson, 1996 ; Ødegaard, Diserud et Østbye, 2005 ; Roques, Auger-Rozenberg et Boivin, 2006). Certaines espèces sont plus spécialisées que d'autres et les hôtes compatibles seront limités aux membres de la même espèce ou du même genre d'hôte. Les plus généralistes quand à eux pourront sauter d'une famille d'hôte à l'autre (Lindelöw et Björkman, 2001). L'Europe et l'Amérique du Nord au nord du Mexique sont très similaires au niveau du climat et de la composition de la faune et de la flore (Mattson *et al.*, 2007 ; Niemela et Mattson, 1996). De plus, il y a eu historiquement beaucoup de déplacements humains et de marchandises entre les deux continents. Pour ces raisons, ils partagent maintenant des espèces qui ont été introduites dans l'un ou l'autre des territoires et qui sont maintenant bien établies dans leur nouvel habitat (Mattson *et al.*, 2007 ; Niemela et Mattson, 1996). L'existence de plantations d'essences exotiques aux fins d'activités humaines dans l'un ou l'autre des continents pourrait également facilité l'établissement dans une nouvelle région d'espècs insectes introduites qui utilisent ces essences d'arbre comme hôtes dans leur habitat d'origine.

Dans cette deuxième partie de ce projet, des bûches d'une essence européenne, l'épinette de Norvège, *Picea Abies* Karst., ont été placées sur différents sites forestiers québécois afin de tenter d'attirer des insectes xylophages et les résultats ont été comparés avec les espèces qui se sont attaquées à deux essences indigènes, l'épinette noire (*Picea mariana* [Mill.]) et l'épinette blanche, (*Picea glauca* [Moench]). L'épinette de Norvège fut choisie pour son importance écologique et économique, en vue notamment de l'énorme étendue sur lequel on la retrouve en Europe et en Russie (Bucci et Vendramin, 2000). Elle est également présente en Amérique du Nord dans des plantations.

Tableau 0.1 Combinaisons température/temps de plusieurs études utilisées sur divers parasites dans le bois.

Espèce	*Traitement*	Températures	Temps	Matériaux	Étude
Agrilus planipennis (Buprestidae)	Micro-ondes	50, 55, 60, 65°C	30 min	Bûches non-écorcées: volumes variables	Nzokou et al., 2008
Anoplaphora glabripennis (Cerambycidae)	Micro-ondes	60°C	0,5, 1, 2, 3, 3,5 et 5 min	Blocs: 10,2 x 10,2 x 10,2 cm et 10,2 x 10,2 x 2,5 cm	Fleming et al., 2003
Hylotrupes bajulus (Cerambycidae)	Micro-ondes	Autour de 55°C	Entre 1 et 60 min	Blocs: 15 x 6 x 2,2 cm. 80 x 15 x 2,2 cm et 140 x 15 x 2,2 cm	Henin et al., 2008
Plectrodera scalator (Cerambycidae)	Micro-ondes	62°C	Non-spécifié	Blocs: 10,2 x 10,2 x 50,8 cm et 2,5 x 10,2 x 50,8 cm	Fleming et al., 2005
Bursaphelenchus xylophilus (Nematoda)	Micro-ondes	62°C	Non-spécifié	Blocs: 10,2 x 10,2 x 50,8 cm et 2,5 x 10,2 x 50,8 cm	Fleming et al., 2005
Bursaphelenchus xylophilus (Nematoda)	Micro-ondes	40, 48, 52, 54, 56, 58, 60, 62, 63, 65, 67, 70°C	1 min	Blocs: 2,5 x 3,8 x 0,64 cm et 10,2 x 10,2 x 25,4 cm	Hoover et al., 2010
Divers (Fungi)	Fréquences radio	65°C	2 min	Blocs: 15,5 x 10 x 10 cm	Tubajika et al., 2007
Divers (Fungi)	Submersion dans l'eau	41, 46, 51, 56, 61, 66, 71, 76°C	<1 ,30, 60, 120 min	Blocs: 0,30 x 0,1 x 0,05 cm	Ramsfield et al., 2010

CHAPITRE I

Efficacy of microwave irradiation for phytosanitation of wood packing material

Martin Payette[1,*], Timothy T. Work[1], Pascal Drouin[2], and Ahmed Koubaa[1]

[1]Forest research centre, NSERC UQAT-UQAM Chair in Sustainable Forest Management, Université du Québec à Montréal, Département of biological sciences
[2] LignoP consultant, Rouyn-Noranda

[*]Corresponding author

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1.1 Abstract

Wood packing materials (WPM) are important vectors of invasive xylophagous insects and pathogenic and decomposer wood fungi. The International Plant Protection Convention (IPPC) introduced the International Standards for Phytosanitary Measures No. 15 (ISPM No.15) to regulate the development of treatments to sanitize WPM and prevent the introduction and movement of forest pests. ISPM No.15 suggest that heat treatments of 56°C for 30 min be applied on all WPM. In this study, the efficacy of microwave irradiation was tested on *Monochamus scutellatus* larvae and on four different pathogenic fungi, *Gremmeniella abietina*, *Heterobasidion annosum*, *Chondrostereum purpureum*, and *Mycosphaerella populorum*, five species of economic significance in Québec, in both jack pine and trembling aspen,. We explored the possibility of shortening the duration of treatment as a possible means of reducing costs while assuring 100% mortality. We irradiated *M. scutellatus* larvae at 56, 61, and 66 °C for 1, 2, and 3 min and the four fungal species at 50, 55, 60, 65, 70, 75, and 90 °C for 0.5, 1, or 2 min. Fungi were tested at a wider range of temperatures to account for possible higher variation of resistance between species. We obtained 100% mortality in larvae at 56°C for 2 min. The fungi species were much more resistant to the treatment. *G. abietina* was eliminated at 75 °C/0.5 min, *H. annosum* at 90 °C/1 min, *M. populorum* at 90 °C/2 min, and *C. purpureum* was still present at the highest temperature/time combination used. We demonstrated the capacity of microwave irradiation to kill the larvae and fungi at short treatment times, though lethal temperatures for the fungi were very high. Future studies should test a wider range of treatment times and expand trials to include more insect and fungal species to determine which temperature/time combination will allow us to reduce time while assuring 100% efficiency.

1.2 Résumé

Les matériaux d'emballage en bois (MEB) sont des vecteurs importants d'espèces insectes xylophages envahissantes et de pathogènes fongiques décomposeurs du bois. La Convention internationale pour la protection des végétaux (CIPV) a introduit les normes internationales sur les matériaux d'emballage en bois No. 15 (NIMP No. 15) afin de réguler le développement de traitements pour stériliser les MEB et pour prévenir l'introduction et le transport de pestes forestières. Les NIMP No. 15 suggèrent des traitements de chaleur de 56°C pour 30 min pour les MEB. Dans cette étude, l'efficacité de l'irradiation aux micro-ondes a été testée sur des larves *Monochamus scutellatus* et sur quatre pathogènes fongiques des arbres, *Gremmeniella abietina*, *Heterobasidion annosum*, *Chondrostereum purpureum* et *Mycosphaerella populorum*, cinq espèces d'une importance économique au Québec, dans des bloques de pin gris et de peuplier faux-trembles. Nous avons exploré la possibilité de réduire la durée des traitements afin de réduire les coûts tout en s'assurant d'obtenir 100% de mortalité. Les larves de *M. scutellatus* ont été irradiées à 56, 61 et 66 °C pour 1, 2 et 3 min et les quatres espèces fongiques à 50, 55, 60, 65, 70, 75 et 90 °C pour 0.5, 1, or 2 min. Les champignons ont été testés avec un plus grand étendu de températures pour tenir compte de la possibilité d'une variation dans la résistance entre espèces. Nous avons obtenu 100% de mortalité chez les larves à 56°C pour 2 min. Les espèces fongiques étaient plus résistantes au traitement que les larves. *G. abietina* a été éliminé à 75°C pour 0.5 min, *H. annosum* à 90°C pour 1 min, *M. populorum* à 90°C pour 2 min et *C. purpureum* était encore présent à plus haute combinaison de température/temps utilisée. Nous avons démontré la capacité de l'irradiation aux micro-ondes pour éliminer les larves et espèces fongiques avec des courtes durées de traitements, bien que les espèces fongiques nécéssitaient des températures beaucoup plus élevées. Il serait nécessaire dans des études futures de tester un plus grand étendu de durées de traitement ainsi que de tester le traitement sur d'autres espèces insectes et fongiques pour déterminer quelle combinaison de tempréture/temps permettra de réduire la durée tout en gardant une efficacité de 100%.

1.3 Introduction

Invasive forest insect pests and plant pathogens cost an estimated $2.1 billion US each year in losses and damage within the United States (Pimentel, Zuniga et Morrison, 2005). Wood boring beetles from the family Cerambycidae are among the most frequent and problematic insect groups which are regularly introduced through wood packing material (WPM) (Brockerhoff, 2009 ; Haack, 2006). Many serious pathogens are transported through dunnage, insect vectors, wood chips, and WPM (Mireku et Simpson, 2003). Large-scale use of microwave irradiation to eliminate wood boring insects and fungal pathogens inside WPM, including wood pallets, may become an important part of the overall effort to minimize the introduction of invasive pests across borders. The International Plant Protection Convenion (IPPC) published the International Standards for Phytosanitary Measures No. 15 (ISPM No. 15) which set guidelines for the regulation of WPM in international trade in order to stem the accidental introduction of invasive pests (FAO, 2009). Signatory countries, such as Canada and the United-States, have adopted these guidelines and inserted them into their own regulations for the control of out- and in-bound WPM (CBSA, 2012 ; USDA, 2004). Currently, the ISPM No. 15 standard recognizes two methods for the treatment of wood packing material: methyl bromide fumigation and heat treatment. Methyl bromide fumigation is regulated according to specific temperature and concentration combinations for a treatment period of 24 hours, whereas heat treatments require that wood core temperature be held at 56 °C for 30 min, a temperature/time combination determined initially to kill the pine wood nematode (*Bursaphelenchus xylophilus* [Steiner & Buhrer] Nickle), the causative agent of pine wilt disease (Ramsfield *et al.*, 2010). Methyl bromide is however a short-lived gas which has been shown to contribute to the depletion of the ozone (WMO, 2006). The Montreal Protocol on Substances that deplete the ozone layer singled out methyl bromide and signatory countries are required to have completely phased out its use by 2013 (Madé, 2012 ; UNEP, 1994). Contracting parties to the IPPC are therefore

encouraged to search for alternatives to fumigation (FAO, 2009). Conventional heating in kilns can only heat the wood core by transfer of energy from the outside-in. Dielectric heating, such as occurs during microwave irradiation, affects water molecules more directly and can raise the inner temperature of wood much faster than conventional heating. Dielectric permittivity is a measure of how a medium is capable of being polarized by an electromagnetic field. The relative permittivity of water is very high whereas that of wood is much lower. Organisms within the wood are therefore affected more directly than they would be when treated with conventional heating. Several studies have tested the effects of microwave irradiation on the dielectric properties of wood to test the distribution of heat and the effect of moisture content, and have found that higher moisture contents lower the treatment's capacity to heat wood (Antti et Perré, 1999 ; Koubaa *et al.*, 2008). The consensus is that higher percentages of moisture absorb more heat and lower the capacity of the microwaves to heat the wood. Heating efficiency also varies according to structural factors, such as the direction of the grain and the heterogeneity of the wood's interior (Antti et Perré, 1999). This does not however cause changes in the structural integrity of the wood and it has been suggested that moving the applicator or rotating the wood could remedy the situation (Antti et Perré, 1999 ; Fleming *et al.*, 2005). Several studies have tested the efficacy of the treatment on insect pests within the wood for phytosanitation purposes and found it to be effective at temperatures ranging from 60 to 65 °C for much shorter time intervals (<5 min) than the 30 min suggested by the ISPM No. 15 (Fleming *et al.*, 2003 ; Fleming *et al.*, 2004 ; Fleming *et al.*, 2005 ; Henin *et al.*, 2008 ; Hoover *et al.*, 2010 ; Nzokou, Tourtellot et Kamdem, 2008 ; Ramsfield *et al.*, 2010). Many studies however did not focus on determining the optimal temperature/time combination and mostly tested temperatures for one treatment time or tested much higher times than are necessary to obtain 100% mortality with many wood pests.

To test microwave irradiation as a viable phytosanitary treatment northeastern North America, we selected larvae from a common species of Cerambycidae, *Monochamus scutellatus* (Say), which is native to North America, as well as four pathogenic fungi, *Gremmeniella abietina* (Lagerberg) Morelet, *Heterobasidion annosum* (Fr.) Bref., *Chondrostereum purpureum* (Pers.) Pouzar and *Mycosphaerella populorum* G.E. Thompson.

M. scutellatus lays its eggs beneath the bark of weak and dying conifers (Peddle, 2000). The larvae bore deep into the wood and are often the cause of economic losses in the lumber industry as they attack cut logs in fields and lumberyards, reducing the quality of the wood. Though *M. scutellatus* has not yet been shown to be so, many species in the *Monochamus* genus (*M. carolinensis* [Olivier], *M. altematus* Hope, and *M. salruarius* [Gebler]) are vectors of the pine wood nematode which has caused heavy damages in pine forests in Japan, China, Korea and Taiwan (Fielding et Evans, 1996 ; Kosaka *et al.*, 2001 ; Shi *et al.*, 2012 ; Suzuki, 2002 ; Zhao *et al.*, 2007) and in Portugal and Spain (Abelleira *et al.*, 2012 ; Robertson *et al.*, 2011), encouraging many countries to set strict restrictions on all import of WPM from countries where the pine wood nematode is present (Dwinell, 1997).

Pathogenic fungi capable of killing trees are of great economic significance. Epidemics of Dutch elm disease (Dedic et Zlatanovic) (*Ophiostoma ulmi*; *Ophiostoma novo-ulmi* [Brasier]) and sudden oak death (*Phytophthora ramorum* [Werres et al., 2001]) are examples of the risks posed by non-indigenous fungi. The four fungi species used in this study were selected based on their presence in the northeastern North America and on their likeliness of selecting hosts commonly used in wood pallet production in this region. In addition, they are minor pests in forests and plantations of certain regions and have all been reported as being found outside North America, meaning that their transport via imported and exported wood is a likely occurrence. *H. annosum* is an economically significant conifer root and butt rot fungus in the Northern Hemisphere (Asiegbu, Adomas et Stenlid, 2005 ; Lygis *et al.*,

2004). *C. purpureum* is the causal agent of silver leaf disease on many fruit trees and scrubs (Beever, 1970) and is used as bioherbicide against unwanted non-indigenous trees (Pitt *et al.*, 1999 ; Ramsfield *et al.*, 1996). *G. abietina* is the causal agent of scleroderris canker and attacks mainly pine in North America and sometimes spruce (EPPO, 2009 ; Wallace, 2012). Finally, *M. populorum* is the causal agent of septoria canker and attacks all species of *Populus* in North America as well as some exotic and hybrid poplars (EPPO, 1980).

This study's aim was to determine minimum microwave irradiation combinations of temperature and time required to eliminate cerambycid larvae (*M. scutellatus*) and four different fungal wood pathogens (*G. abietina*, *H. annosum*, *C. purpureum*, and *M. populorum*), all indigenous or established in northeastern North America, in industrial grade wood such as would be found in wood pallets and other types of wood packing materials, using time intervals considerably shorter than the ISPM No. 15 standard of 30 min for thermal treatment. Based on the results from previous studies on the heat treatment of wood-boring insect larvae (Fleming *et al.*, 2003 ; Fleming *et al.*, 2004 ; Fleming *et al.*, 2005 ; Henin *et al.*, 2008 ; Hoover *et al.*, 2010 ; Nzokou, Tourtellot et Kamdem, 2008 ; Ramsfield *et al.*, 2010) and of fungal pathogens (Ramsfield *et al.*, 2010 ; Tubajika *et al.*, 2007), we hypothesized that microwave 2 min treatments at temperatures of 56°C or higher would be sufficient to kill all larvae and at temperatures of 70°C or higher would be sufficient to provoke close to 100% (between 99% and 99.99%) mortality in all fungi species, given the generally higher resistance of fungi to harsh conditions. We also expect that the higher porosity of jack pine than trembling aspen will allow the microwaves to heat more effectively the organisms inside and therefore decrease the temperature level required to kill them in the softwood blocks versus in the hardwood blocks.

1.4 Materials and methods

1.4.1 Equipment

For all experiments, we used a 2.45 GHz BP-111 compact microwave processor with an oven chamber dimensions of 33 by 33 by 20.5 cm (Microwave Research and Applications, Inc., Laurel, MD) to irradiate wood blocks containing insects and fungi. The system included an integrated temperature probe and Cal Control® 9500 temperature/process controller. The system is designed to provide a steady rate of temperature increase up to a specified level to which it is then maintained, all the while delivering uniform heating within the chamber. We controlled the program settings and monitored the temperature readings with Cal Control® CALgrafix Controller Software.

The CALgrafix software was also used to record temperature readings for post-treatment evaluation of temperature variation. The readings were used to remove any data-points where the temperature during treatment varied by more than 1 °C over or under the desired value. In all treatments on larvae and several treatments during the trials on fungi species in trembling aspen blocks (see section 1.3.6), temperature readings recorded with the software were lost.

1.4.2 Cerambicid larvae

The *Monochamus scutellatus* larvae were collected from black spruce logs obtained in October 2012 at the Rivière-aux-rats sawmill near La Tuque, Québec. The logs came from a stand in the Mauricie region that had burned the previous spring. The logs were cut to approximately 30 cm. We split logs with an axe until we found sign of a larval gallery and then chiselled the wood carefully until the larvae could be safely extracted. Almost half of larvae found in the logs were already dead or lacking vigour (low mobility, consumption of wood, and/or frass production). Those with

obvious signs of low vigour were not kept. We verified both dead and live specimens to species using Craighead (1923). All specimens extracted were *M. scutellatus*. Live larvae were kept separately in indivdual Petri dishes to avoid antagonistic interactions among larvae. Each dish contained a moist towelet, wood shavings and small pieces of wood cut from the logs in which the larvae were found. They were kept at room temperature for no more than three days before treatment. A total of 66 larvae were extracted.

1.4.3 Wood Decomposing Fungi

We obtained four species of wood decomposing fungi from the Forest Pathology Laboratory at the Université de Laval: *Gremmeniella abietina* (strain CFL-87-0064) and *Heterobasidion annosum* (strain CFL-535), which are associated with conifers, and *Chondrostereum purpureum* (strain CFL-529) and *Mycosphaerella populorum* (strain CFL-1326), which are associated with deciduous trees. They were originally part of the collection at the Laurentian Forestry Centre and were isolated from trees in the province of Québec.

Cultures were received in June of 2011 and incubated at room temperature. In December 2011, we conducted preliminary mortality tests to establish a range of heat tolerances for each species. Two replicates for each species were subjected to temperatures of 60, 65, 70, 75 and 80 °C for a period of 2 and 5 min (Table 1.1). Based on these results, the temperatures used in the irradiation treatments were fixed at 24 (control), 55, 60, 65, 70, 75, and 90°C. We used 10 replicates per temperature/time combination. Instead of 1, 2, and 3 min for the duration of treatment like with the larvae, we opted for 0.5, 1, and 2 min to assure there would be enough samples that survived at shorter times to make an adequate regression analysis. *G. abietina* and *H. annosum* were inoculated and irradiated in jack pine blocks and *C. purpureum* and *M. populorum* were inoculated and irradiated in trembling aspen.

1.4.4 Block Preparation

Jack pine, *Pinus banksiana* Lamb., and aspen, *Populus tremuloides* Michx., wood blocks were provided by Tembec Inc., in La Sarre, Québec. All blocks were 3.8 by 6.4 by 20 cm. All blocks were kiln dried for 24 hours at 103°C and weighed. For the *M. scutellatus* larvae man made tunnel, we used a 1.3 cm (0.5 in) spade bit to drill a 3.8 cm deep hole at the center of one of the narrow longitudinal sides of the blocks. Another hole, approximately 0.6 cm wide and 1.9 cm deep, was drilled into the wide longitudinal surface of the blocks, at the center and approximately 1.3 cm to the side of the larva's tunnel to insert the temperature probe and measure the block's core temperature (Figure 1.1).

For fungal irradiation, we drilled four holes equidistant in the forward facing side of each block (same as in Figure 1.1). The holes were 1.6 cm wide and 3.8 cm deep. We made them wider than for the larvae to make extraction of the wood pellets easier. A hole, again 0.6 cm wide and 1.9 cm deep, was drilled for the thermal probe through the top of each block between holes 1 and 2, and between holes 3 and 4. Both the holes drilled for the larvae and those drilled for the wood pellets were prepared so as to be facing the microwave door and be perpendicular to the direction of the microwaves within the chamber.

To simulate natural accumulation of moisture in wood, blocks were soaked in water for a minimu of 12 hours before treatment. The blocks were weighed immediately before insertion of either larvae or pathogens. Moisture content was calculated by dividing weight difference between wet and dry blocks divided by their dry weight:

$$MC = (ww - dw) / dw \times 100$$

where MC = moisture content (%),

ww = wet weight,

dw = dry weight.

1.4.5 Treatment of Cerambycidae larvae

We placed the *M. scutellatus* larvae at the center of jack pine blocks and did tests at 24, 56, 61, 66°C for 1, 2 and 3 min. A single larva was placed in a block immediately prior to irradiation and the hole was plugged. The plug was made from the same type of wood using a 1.3 cm plug-cutter. There were six replicates per treatment at 56, 61, and 66°C and four replicates per treatment at room temperature (24°C, with the power turned off), which served as an experimental control.

Mortality was assessed immediately after treatment based on lack of movement, dehydration, and/or discoloration. In all cases, the larvae were placed back in their respective Petri dishes on a new moist towelet. A second verification was performed 24 hours after treatment to see if there was movement with prodding. This approach was based on that of Fleming et al. (2005) and of Fleming et al. (2003).

1.4.6 Treatment of pathogenic fungi

All laboratory equipment used to handle fungi was sterilized immediately prior to and following use.

The four fungi were freshly cultivated on malt extract agar incubated at 28 °C prior to starting the liquid culture. An agar pellet aseptically collected of each strain was

transfer to a 1 L Malt Extract broth. The bottles were incubated at room temperature under slow agitation (50 rpm) for two weeks prior to the microwave treatments. Hyphal and spores germ count was determined by plate counts on MEA following serial dilution in peptone water buffer. This accounted for 3.4 $\log_{10}$ germs ml^{-1} for *G. abietina*, 3.8 for *H. annosum*, 4.8 for *C. purpureum*, and 3.7 for *M. populorum*.

Wood pellets 1.3 cm wide and 1.3 cm high were prepared with a plug-cutter from the same stock of wood as the blocks. Pellets were heat sterlized, at 103°C for at least 9 hours. Pellets were submerged and kept in a solution of their assigned organism 30 min prior to treatment.

For each temperature/time combination, an inoculated pellet from each of the organisms associated with the same wood species (*G. abietina* and *H. annosum* in jack pine and *C. purpureum* and *M. populorum* in aspen) was placed in either hole no. 1 or no. 2 of the same block at the same time. The holes were shut with plugs made from the same stock of wood as the blocks. The average time to reach the desired core temperature was 60 sec. After treatment, the pellets were removed aseptically as quickly as possible with tweezers. We then cut three to six shavings off of each pellet with a scalpel and placed them on a malt extract agar surface. The block was weighed and then reused, this time placing the inoculated pellets in holes no. 3 or no. 4. Holes were never used more than once to prevent contamination and to to evaluate if the position in the wood block would affect survival.

Wood shavings were incubated on malt dextrose agar (MEA) medium at room temperature for four weeks and inspected visually for presence or absence of growth after 14 days and 28 days. Species isolates were identified based on morphology. The treatment was not considered lethal if the isolates grew on the medium from the wood shavings after irradiation.

In the pilot study examining, *G. abietina* and *H. annosum* in jack pine, we confirmed the presence of both species in control treatments (24°C with no power). However, in

addition to these species, we observed bacterial and/or fungal contamination as evidenced by the presence of different morphotypes in some controls, which competed for space with the study cultures. Furthermore, contamination was not observed in samples that were irradiated with microwaves.

During the second trial, using *C. purpureum* and *M. populorum* in aspen, antibacterial MEA medium (Bengal rose) was used to avoid contamination on either controls or irradiated samples. However, the use of the antibacterial agent inhibited growth of *M. populorum*. For this reason, we repeated the second trial on *C. purpureum* and *M. populorum* using the same medium as the first trial. There was again evidence of contamination in controls and none in irradiated samples. We therefore considered that the contaminants did not inhibit growth of the treated species and were mostly likely by temperatures equal to or higher than 50°C.

The presence of wood shavings in the hole for the temperature probe would occasionally result in faulty temperature readings. We excluded from the analysis all individual repetitions in which this occurred or where the process controller did not keep the temperature at ± 1°C around the desired value. As temperature readings weren't available for some of the treatments on *C. purpureum* and *M. populorum* (50°C/1 min, 55°C/2 min, 60°C/0.5 min, 60°C/2 min, 65°C/0.5 min, 70°C/0.5 min, 70°C/1 min, 75°C/1 min, 75°C/2 min, 90°C/0.5 min), a separate analysis was made with the original data for comparison.

1.4.7 Data Analysis

For the larvae trials, we used a binomial-response generalized linear model with bias reduction to account for quasi-complete separation of datapoints. This occurs when one or more predictor values are seperated by the outcome variable and the model therefore predicts some of the values perfectly, which in turn gives a predicted

probability of 1 or nearly 1. The brglm package in R version 3.0.0 (R Development Core Team 2013, Kosmidis, 2013) allowed us to create a generalized linear model and eliminate any small-sample bias by introducing a penalized likelihood (Firth, 1993). The package allows us to have a bias-reduced estimator that is smaller than the maximum likelihood estimator and gives estimates and standard errors that are finite as opposed to maximum likelihood estimates with complete or quasi-complete separation (R Development Core Team 2013, Kosmidis, 2013). The notation for creating a model is the same, except that the brglm notation is used instead of glm. We then separated models for each treatment time with only temperature as a fixed effect to determine lethal temperature at a probability of 99 % and 99.99 %.

For the fungi trials, we analyzed two separate datasets. The first contained all original data and the second was a final adjusted dataset with points removed due to post-assessement of temperature variation higher than 1°C < 0. All data were analysed with a binomial-response generalized linear model with a logit link function using the glmer function in the lme4 library with R version 3.0.0 (R Development Core Team 2013,Bates, Maechler et Bolker, 2013). Duration of irradiation, temperature and moisture content were used as fixed variables, and position within the block was used as a random factor. We then separated models for every treatment time using only temperature as a fixed variable in order to predict lethal temperature at 99% and 99.99% at each of these times.

We calculated confidence intervals for all predicted lethal temperatures to verify whether they encompassed the ISPM No. 15 standard of 56°C.

1.5 Results

1.5.1 Cerambycid larvae

All but two larvae irradiated with microwaves died during or within 24 hours of the treatment (Table 1.2). Both subjects that were still alive after 24 hours had been treated at 56 °C for 1 min. All the control treatments (24 °C) survived. The minimum temperature/time combinations at which all subjects were killed were 56 °C held for 2 min and 61 °C for 1 min.

A logit regression showed quasi-complete separation, in which a covariate predicts almost perfectly the outcome of the response variable. In this case, tested temperatures above the control of 24°C caused the death of all but two larvae whereas all larvae at the control temperature survived. The regression analysis gives a predicted probability close to or equal to 1 and an extremely high standard error. After adjustment through bias reduction of maximum likelihood estimates with the brglm package in R version 3.0.0 (Kosmidis, 2013), the probabilities and standard errors for the covariates were much lower and easier to interpret.

Treatment time was not significant. Larval weight, ranging from 0.1089 g to 0.9549 g and averaging 0.4714 g, showed no significant effect or relationship to mortality. Wood moisture content, which varied between 16.42 % and 73.54 % with an average of 36.24 %, was also not significant (Table 1.3).

Predicted lethal temperature (LT) and their confidence intervals are presented in Table 1.4. The LT values at a probability (P) of 99.99 % are higher by a difference of 35.5 °C at 1 min and of 28.6 °C at 2 and 3 min versus those at a probability of 99%. The predicted LT at P = 99 % are higher by a difference of 24.5 °C at 1 min and of 10.1 °C at 2 and 3 min versus the observed LT. The large discrepancy between the predicted LT at 99 % and 99.99 % probability as well as with observed LT is probably due to the small sample size. We can nevertheless note that 56 °C falls

within the confidence intervals of all predicted lethal temperatures except one (1 min; p = 99.99 %).

1.5.2 Pathogenic Fungi

Observable LT were different for each of the four fungal species in either the original datasets or in the final adjusted datasets where samples with temperature variability were removed (Figure 1.2). *H. annosum*, *C. purpureum* and *M. populorum* still showed signs of growth after being treated at 90 °C for 0.5, 1 and 1 min respectively, while for *G. abietina* there is no growth as of 75 °C/0.5 min in the original dataset and as of 70 °C/2 min in the final adjusted dataset. For *G. abietina*, *H. annosum* and *M. populorum*, however, there is a wide gap that separates the few cases of growth at higher temperatures from the cluster of growths at lower ones. All observed results are presented in Table 1.5

For both the original and final datasets, a generalized linear mixed model for binomial data revealed significant effects of treatment temperature and time for *G. abietina* and *H. annosum* (Table 1.6). Both variables had negative effects on post-treatment regeneration of each species. For *C. purpureum* and *M. populorum*, only temperature treatment had a significant negative effect on post-treatment regeneration (Table 1.6). For all four species, there was no significant effect of wood moisture content, which ranged from 14.21 % to 25.72 % and averaged 20.49 % in the blocks of jack pine and from 4.14 % to 78.43 % and averaged 30.09 % in the blocks of aspen. In the original data, variances and standard deviations for holes as a random effect were all 0 for *H. annosum* and *C. purpureum* and below 0.00001 for *M. populorum*. They were higher for *G. abietina* (variance = 2.82; SD = 1.68). In the final adjusted dataset, the variances and standard deviations for *H. annosum*, *C. purpureum* and *M. populorum* were all non-equal to 0 but below 0.00001. They were again higher for *G. abietina* (variance = 0.54; SD = 0.74). The variances and standard

deviations are small enough that we can interpret that there is little to no difference between holes and within the same hole, indicating that sample location in the oven and in the block did not overly affect estimates for fixed effects.

Predicted lethal temperatures with their confidence intervals at each treatment time are shown in Table 1.7. Differences in predicted LT between the original and final adjusted datasets vary in most cases between 0.2 °C and 5.5 °C, though in three instances the variation is greater than 23 °C (*G. abietina*/0.5 min/p = 99.99 %; *C. purpureum*/2 min/p = 99 % and p = 99.99 %). As with the larvae, there is a large discrepancy between the predicted temperatures at 99 % and 99.99 %. This is again probably due to the small sample size. None of the predicted LT/time combinations for any of the species had a predicted LT lower or equal to the ISPM standard of 56 °C. It was only the case for *G. abietina* where the confidence intervals included 56 °C, though this was only for one value in the original data (1 min/p = 99 %) and two values in the final adjusted data (1 min/p = 99.99 %).

1.6 Discussion

All *M. scutellatus* larvae were killed at 56 °C for 2 min and at 61 °C for 1 min or higher. Since we did not observe any abnormalities in the temperature readings for the two surviving larvae at 56 °C for 1 min, we must conclude that this is not a sufficient amount of time to guarantee 100 % mortality. It is however promising to note that results from this study indicate that the ISPM No. 15 standard for heat treatment of 56 °C for 30 min is more than adequate to eliminate the larvae, as the duration of treatment was shorter a factor of 15. Other studies that have tested microwave irradiation on xylophagous insect larvae showed varying results. In dry wood, it was necessary to irradiate Asian longhorn beetle larvae for 5 sec at 60 °C to obtain 100% mortality (Fleming *et al.*, 2003). In the same study, larvae treated in green wood blocks at 60 °C had 100 % mortality when the treatment time was three

min or over. A later study obtained 100 % mortality on cottonwood beetle (*Plectrodera scalator* [Fabricius]) larvae by heating wood up to at least 62 °C before removing them from the wood (Fleming *et al.*, 2005). Other researchers found that microwave irradiation for 30 min on wood infested with the emerald ash borer did not inhibit 100% of adult emergence at any temperature (Nzokou, Tourtellot et Kamdem, 2008). They proposed that uneven heat distribution might be the cause of the treatment's ineffectiveness. As they used recently cut log segments still covered in part with bark, we hypothesize that the heterogeneity of the wood due to the bark and the probably high moisture content may have affected the treatment's effectiveness versus cut wood which has had time to dry and contains no bark. Henin et al. (2008) irradiated larvae of the cerambycid *Hylotrupes bajulus* (L.) and found that a wood core temperature exceeding 55 °C and a surface temperature of 60 °C were enough to obtain 100 % mortality. In all studies, except that of Nzokou et al. (2008), larvae were placed in wood blocks. A summary of findings indicate that temperatures around 60 °C are enough to kill Cerambycidae larvae in wood blocks with microwave irradiation, though the present study and those mentioned above set various starting conditons and do not follow a single standard method. To accelerate the recognition of this treatment by the IPPC for the ISPM No. 15, a standard for wood dimensions and microwave and measuring equipment should be established to determine the optimal temperature/time combination for all commonly used wood species in WPM around the world and for the xylphagous insect species with the highest risk of being transported in them. The present study offers a set number of conditions, such as commonly used wood dimensions and an efficient microwave emitter, on which a standard method could be based on.

This study has shown that microwave irradiation can kill wood infecting fungi at time intervals much shorter than the 30 min (Table 4) suggested by ISPM No. 15 for conventional heat treatments, though the minimum temperature of 56 °C is not

sufficient at the short time intervals that we tested (FAO, 2009). We have also determined that different species vary in susceptibility to heat treatment (Figure 1).

This corroborates the results from a study that tested conventional heating using heated water on 11 wood-decay fungi species, with three strains per species, in wood blocks (Ramsfield *et al.*, 2010). They found that lethal temperatures varied between species of wood fungi. In all but one species, exposure to 61 °C for less than 1 min was sufficient to prevent regrowth. In the resistant species, *Schizophyllum commune*, 76 °C for less than 1 min was lethal. They suggested that *S. commune*'s resistance was due to its being able to form chlamydospores, a thick-walled structure produced by many fungi species which increases tolerance to heat.

H. annosum is a chlamydospore-forming fungus which may explain why its observed and predicted LT in this study showed higher heat resistance than *G. abietina*, which was treated in the same jack pine blocks. Inactivation of mycelia of *H. annosum* is reportedly 35 °C, while conidia and ascospores, more resistant structures, are inactivated at 45 °C for 60 min (Otrosina et Cobb Jr, 1989). Drier climates and warm weather favour its development (Kliejunas *et al.*, 2010) and adhesion to conifer tissues is enhanced at temperatures 30 to 70 °C, though germinability of the spores is not (Kliejunas *et al.*, 2010).

G. abietina development is optimal at high humidity and at temperatures of 10 – 15 °C, with the capacity to grow at or near 0 °C (Barklund et Unestam, 1988). It is intolerant to higher temperatures and manifests rapid suppression of growth from 20 to 25 °C (Dorworth et Krywienczyk, 1975). This may be due to its range being confined to parts of the northern temperate zone and points to its low resistance to heat relatively to the other three species. It is also notable that its growth was much slower than the other three and only presented small clusters of hypha at mid-level or at the bottom of the aqueous solutions before treatment. The development of spores in its case must take more time and may explain its low resistance to the treatment.

Results showed a high temperature tolerance from *C. purpureum* compared to *M. populorum*, which was treated in the same aspen blocks. A growth at the surface of the aqueous solution in which it was kept before treatment indicates that there may already have been presence of spores. *C. purpureum*, however, grows in temperate climates and does not seem to present a particularly high tolerance to heat. The optimal temperature for basidiospore release is reported to be 18 °C and sporophores have a high affinity for moisture (Spiers, 1985). Germtube development is optimal at 25 to 27 °C with an upper limit of 37 °C (Spiers et Hopcroft, 1988). In *M. populotum*, ascospores are realeased between 3 and 30 °C with the optimal temperature being 27 °C (Thompson, 1941).

All four species have been reported to be killed at temperatures much lower than those at which they were treated during this study (75 °C for *G. abietina* and 90 °C or more for *H. annosum*, *C. purpureum*, and *M. populorum*). This may be due to the short duration during which they were irradiated. As mentioned, Ramsfield et al. (2010) found that 61 °C for less than 1 min was sufficient to kill all but one of 11 species of fungi. Another study did the same on four species of pathogenic fungi with radio-frequency and found that temperatures between 60 and 70 °C for 2 min were enough to kill between 98 % and 100 % of all cultures (Tubajika *et al.*, 2007). These two studies had similar results to one another using two different modes of heating. As radio-frequency is a form of dielectric heating, we would have expected our results to be similar. We have found no mention in the literature pertaining to any particular tolerance to heat of the four species tested in the present study. All we can do is speculate as to the possibility of cross-contamination between samples during post-treatment manipulation of the cultures, causing sterilized mediums to be accidentally inoculated.

It is difficult to interpret variation in heat resistance between species that were irradiated in different types of wood since differences in the microstructure of wood cause differences in its dielectric properties (Duchow et Gerhardt, 1996). Generally,

softwoods have a much more ordered and open microstructure with longer and larger cells than hardwoods. This means that the volume porosity of softwoods is usually higher than hardwoods. The permittivity of air is 1, which means that higher porosity in wood decreases its permittivity. Irradiation treatments are therefore likely to be more efficient when the permittivity of the medium surrounding unwanted organisms is lower. In one study, it was indeed shown that microwave drying for fir blocks was much more intensive than for beech blocks (Dedic et Zlatanovic, 2001). We would therefore expect microwaves to have a stronger effect on fungal pathogens in the jack pine blocks than in aspen. However, there was no clear demarcation in heat resistance between species in aspen or in jack pine blocks. In both the observed and predicted LT, *C. purpureum* and *M. populorum* (both species in aspen blocks) showed more heat resistance than *G. abietina*, though *M. populorum* did not show higher resistance than *H. annosum*. This is hard to interpret, however, since predicted LT are much higher for *M. populorum* than *H. annosum* at a treatment time of 1 min but lower at a time of a 2 min.

It was expected that LT would descend with each increase in time. The species whose reaction to the treatment resembled this pattern most was *H. annosum* (Table 1.7, Figure 1.2). The predicted lethal dose for the final adjusted datasets dropped by 12.9 °C at $P = 99$ % and by 17.8 °C at $P = 99.99$ % from 0.5 to 1 min and then only varied by 1.4 °C and 2.0 °C from 1 to 2 min. The three other species reacted differently. There was a 6.4 °C drop at $P = 99$% and 11.0 °C drop at $P = 99.99$ % for the lethal dose from 0.5 to 1 min for *G. abietina*, but at 120 °C it increased to 5.4 °C and 17.7 °C higher than the lethal temperature for 0.5 min. For *C. purpureum*, LT increased considerably with every increase in time. Those for *M. populorum* varied very little for 0.5 and 2 min, but were almost 30 °C higher than both these times at 1 min. The unexpected increase of predicted lethal temperatures at longer treatment times may simply indicate that 2 min are not sufficient with the current temperatures

tested to insure that these three species are completely incactived and unable to regenerate from their more resistant structures and life stages.

There is a large discrepancy between observed LT and predicted LT as well as between predicted LT at $P = 99$ % and $P = 99.99$ %. Values at $P = 99.99$ % also seem very high when compared to other studies (Ramsfield *et al.*, 2010 ; Tubajika *et al.*, 2007). The gaps between predicted values at 99 % and 99.99 % probabilities in both larvae and fungi may be due to lack of precision caused by the small sample size. This also probably explains the large confidence intervals, as there is an inverse square root relationship between sample size and confidence intervals. For the difference between observed results and predicted LT, another study dealing with microwaves had a similar problem when testing microwaves on the pine wood nematode and suggested that it was due to variability in the data used for the analyses (Hoover *et al.*, 2010).

Moisture content in the jack pine blocks for the treatments on *H. annosum* and *G. abietina* only varied between 14.71 % and 25.72 while variation was much higher in the trembling aspen blocks (4.14 % – 78.43 %) for treatments on *C. purpureum* and *M. populorum* and in jack pine blocks (16.42 % – 73.54 %) for treatments on *M. scutellatus* larvae. Even in the cases of higher variation, moisture content did not significantly affect mortality. Microwaves penetrate deeper into wood when moisture content is lower, which should have inhibited their capacity to cause mortality in organisms in the wood (Zielonka *et al.*, 1997). However, the temperature probes were placed in such a fashion that they were never more than 1.5 cm away from the organisms in the wood, making it unlikely that high variations in temperature occurred between the probes and the organisms.

In the present study, despite the fact that placement of the inoculated wood samples in the blocks did not show any significant effects on mortality of fungi, the small dimensions of the wood blocks may have eliminated the problem of unbalanced

heating within the wood sample. Fleming et al. (2005) showed that trials on cerambycid larvae yielded nearly 100 % mortality when the samples were set on a turntable and rotated during treatment compared to when samples stayed in a fixed position. Sample blocks in these experiments were at least four times larger than those in the present study. It stands to reason that in larger industrial scale samples, homogeneity of heat distribution will be an important issue to consider. We have not found any other studies than Fleming et al. (2005) looking at wood sample rotation as a means of equalizing heat distribution in the context of phytosanitation. Rotation of the wood samples may reduce the required lethal temperature and time and circumvent the problem of wood heterogeneity such as was encountered in Nzokou et al. (2008).

1.7 Conclusion

We found that *M. scutellatus* larvae were eliminated from jack pine blocks at an observed temperature/time combination of 56 °C for 2 min and at a predicted value of 66 °C for 2 min at 99 % probability. In the experiments on pathogenic fungi, results were variable. The fungi species were much more resistant to the treatment. *G. abietina* was eliminated at a temperature/time combination of 75 °C/0.5 min, *H. annosum* at 90 °C/1 min, *M. populorum* at 90 °C/2 min, and *C. purpureum* was still present at the highest temperature/time combination used. These values are surprisingly high but seem nevertheless encouraging as they indicate that microwave irradiation were capable of eliminating Cerambycidae larvae and all tested species of pathogenic fungi but *C. purpureum* in WPM at much shorter treatment times than the current recommended time of 30 min for heat treatments. In the event that industrial-scale microwave equipment would entail more or less the same costs as conventional ovens, microwave irradiation could considerably reduce time and increase cost efficiency for phytosanitary treatments in Québec. Future experiments should take

into account longer treatment times and a wider range of temperatures to increase predictive power of analyses. It will also be necessary to evaluate and compare the resistance to temperature of additional pathogenic fungi species to give a better idea of how high the temperature would need to be to eliminate a much wider spectrum of species. To determine the optimal temperature/time combination from an economical standpoint, we suggest a future study to evaluate the cost and benefits of reducing treatment time and increasing temperature.

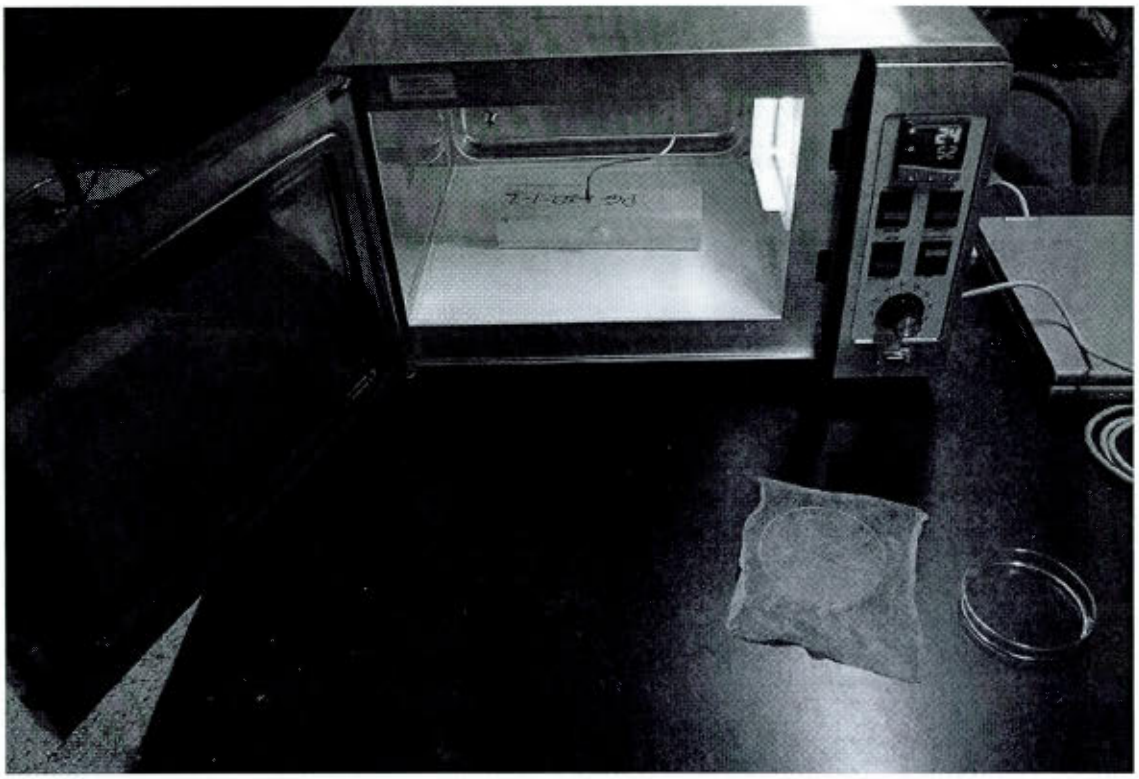

Figure 1.1 BP-111 compact microwave processor with temperature probe inserted in jack pine block. The hole containing the larva is plugged on the front-facing side.

Table 1.1 Preliminary survival results for fungal pathogens with temperatures and times.

	60 °C		65 °C		70 °C		75 °C		80 °C	
	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min
C. purpureum	2/2	1/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
G. abietina	0/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
H. annosum	2/2	1/2	2/2	0/2	1/2	0/2	1/2	0/2	1/2	0/2
M. populorum	1/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2

Table 1.2 Survival results of *M. scutellatus* larvae with treatment temperatures and times.

Time	Temperature (°C)	Survivors
1 min	24	4/4
	56	2/6
	61	0/6
	66	0/6
2 min	24	4/4
	56	0/6
	61	0/6
	66	0/6
3 min	24	4/4
	56	0/6
	61	0/6
	66	0/6

Table 1.3 Outcome estimates (including β estimate, standard error, Wald statistic [z value], P-value [P]) for binomial generalized linear model of *M. scutellatus* larvae mortality following microwave irradiation according to temperature, time, moisture content (MC), and larval weight (Stillwell).

	β Estimate	Standard Error	z value	P (>|z|)	
Intercept	12.358	4.983	2.480	0.013	*
Temperature	-0.240	0.072	-3.334	0.001	***
Time	-1.915	1.173	-1.633	0.103	
MC	0.070	0.045	1.562	0.118	
LW	-1.946	3.485	-0.558	0.577	

Note: Significant results at $P \leq 0.05$. $P < 0.1$, '.' ; $P < 0.05$, '•' ; $P < 0.01$, '••' ; $P < 0.001$; '•••'.

Table 1.4 Predicted lethal temperatures of *M. scutellatus* larvae with 99% and 99.99% probability for each treatment time with standard errors and confidence intervals (CI).

M. scutellatus	1 min		2 min		3 min	
	p = 99%	p = 99.99%	p = 99%	p = 99.99%	p = 99%	p = 99,99%
Lethal Temperature (°C)	80.5	116.0	66.1	94.7	66.1	94.7
Standard Error	12.6	28.2	11.1	21.1	11.1	21.1
CI	24.6	55.3	21.8	41.4	21.8	41.4
CI Lower Limit	55.9	60.7	44.2	53.3	44.2	53.3
CI Upper Limit	105.1	171.3	87.9	136.1	87.9	136.1

Table 1.5 Survival results for all four pathogenic fungi with temperatures and times after removal of errors due to measurement inaccuracy. In bold are results for the original datasets and in normal font are results for the datasets with datapoints removed because of high variation in temperature measurements. Results in italic were not corrected due to unavailability of temperature measurements.

		Jack Pine				Trembling Aspen			
Time (minutes)	Temperature (°C)	*G. abietina*		*H. annosum*		*C. purpureum*		*M. populorum*	
0.5	24	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10
	50	**8/10**	9/11	**10/10**	11/11	**7/10**	6/8	**7/10**	7/8
	55	**5/10**	3/7	**9/10**	6/7	**7/10**	6/7	**3/10**	2/7
	60	**3/10**	3/9	**5/10**	5/9	**1/10**	***1/10***	**1/10**	***1/10***
	65	**2/10**	0/5	**2/10**	0/5	**6/10**	***6/10***	**0/10**	***0/10***
	70	**0/10**	0/6	**0/10**	0/6	**0/10**	***0/10***	**0/10**	***0/10***
	75	**1/10**	0/7	**0/10**	0/7	**1/10**	1/8	**0/10**	0/8
	90	**0/10**	0/10	**1/10**	1/10	**0/10**	***0/10***	**0/10**	***0/10***
1	24	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10
	50	**8/10**	6/7	**10/10**	7/7	**5/10**	***5/10***	**7/10**	***7/10***
	55	**2/10**	2/5	**0/10**	0/5	**7/10**	7/9	**1/10**	1/9
	60	**0/10**	0/10	**0/10**	0/10	**4/10**	3/7	**6/10**	4/7
	65	**0/10**	0/10	**0/10**	0/10	**5/10**	5/10	**3/10**	3/10
	70	**0/10**	0/9	**0/10**	0/9	**1/10**	***1/10***	**0/10**	***0/10***
	75	**0/10**	0/9	**1/10**	1/9	**1/10**	***1/10***	**0/10**	***0/10***
	90	**0/10**	0/10	**0/10**	0/10	**2/10**	2/10	**1/10**	1/10
2	24	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10	**10/10**	10/10
	50	**5/10**	3/6	**10/10**	6/6	**6/10**	5/9	**7/10**	6/9
	55	**2/10**	2/9	**2/10**	2/9	**7/10**	***7/10***	**2/10**	***2/10***
	60	**1/10**	0/9	**2/10**	1/9	**3/10**	***3/10***	**1/10**	***1/10***
	65	**0/10**	0/10	**0/10**	0/10	**5/10**	5/10	**0/10**	0/10
	70	**1/10**	1/9	**1/10**	1/9	**5/10**	5/10	**0/10**	0/10
	75	**0/10**	0/9	**0/10**	0/9	**0/10**	***0/10***	**0/10**	***0/10***
	90	**0/10**	0/10	**0/10**	0/10	**6/10**	4/9	**0/10**	0/9

Table 1.6 Outcome estimates (including β estimate, standard error, Wald statistic [z value], P-value [P]) for binomial generalized linear mixed model of fungi mortality following microwave irradiation according to temperature, time, and moisture content (MC). In bold are results for the original datasets and in normal font are results for the datasets with datapoints removed because of high variation in temperature measurements.

	β Estimate		Standard Error		z value		P (>|z|)			
G. abietina										
Intercept	**-20.947**	-12.461	**5.225**	2.598	**-4.009**	-4.796	**< 0.001**	***	< 0.001	***
Temperature	**0.370**	0.219	**0.089**	0.043	**4.172**	5.112	**< 0.001**	***	< 0.001	***
Time	**0.021**	0.015	**0.010**	0.007	**2.165**	2.296	**0.030**	*	0.022	*
MC	**0.260**	0.190	**0.390**	0.255	**0.667**	0.742	**0.505**		0.458	
H. annosum										
Intercept	**-13.290**	-13.227	**2.553**	2.311	**-5.206**	-5.725	**< 0.001**	***	< 0.001	***
Temperature	**0.221**	0.221	**0.041**	0.037	**5.323**	5.896	**< 0.001**	***	< 0.001	***
Time	**0.016**	0.014	**0.007**	0.006	**2.300**	2.290	**0.021**	*	0.022	*
MC	**0.448**	0.381	**0.259**	0.231	**1.725**	1.651	**0.084**	.	0.099	.
C. purpureum										
Intercept	**-3.997**	-3.825	**0.806**	0.766	**-4.962**	-4.997	**< 0.001**	***	< 0.001	***
Temperature	**0.075**	0.074	**0.012**	0.012	**6.188**	6.297	**< 0.001**	***	< 0.001	***
Time	**-0.006**	-0.007	**0.004**	0.004	**-1.400**	-1.802	**0.162**		0.072	.
MC	**-0.113**	-0.190	**0.161**	0.155	**-0.704**	-1.223	**0.481**		0.221	
M. populorum										
Intercept	**-8.562**	-9.621	**1.633**	1.757	**-5.243**	-5.477	**< 0.001**	***	< 0.001	***
Temperature	**0.160**	0.179	**0.027**	0.030	**5.900**	6.017	**< 0.001**	***	< 0.001	***
Time	**0.004**	0.004	**0.006**	0.006	**0.768**	0.713	**0.443**		0.476	
MC	**-0.100**	-0.149	**0.200**	0.197	**-0.501**	-0.755	**0.616**		0.450	

Note: Significant results at P ≤ 0.05. P < 0.1, '.' ; P < 0.05, '•' ; P < 0.01, '••' ; P < 0.001; '•••'.

Table 1.7 Predicted lethal temperatures for the four pathogenic fungi with 99% and 99.99% probability for each treatment time with confidence interval value. In bold are results for the original datasets and in normal font are results for the datasets with datapoints removed because of high variation in temperature measurements.

	0.5 min		1 min		2 min	
G. abietina						
p = 99%	65.4 ± 8.7	**79.9 ± 11.7**	59.0 ± 5.0	**59.4 ± 4.8**	70.8 ± 12.1	**73.7 ± 12.1**
p = 99.99%	75.2 ± 14.3	**103.9 ± 23.2**	64.2 ± 8.5	**66.3 ± 9.1**	92.9 ± 26.6	**98.3 ± 26.6**
H. annosum						
p = 99%	81.4 ± 11.7	**79.9 ± 9.1**	68.5 ± 9.1	**65.9 ± 7.8**	69.9 ± 9.3	**70.1 ± 8.2**
p = 99.99%	102.0 ± 22.3	**99.0 ± 17.7**	84.2 ± 18.9	**79.3 ± 15.9**	86.2 ± 19.3	**85.7 ± 16.4**
C. purpureum						
p = 99%	88.3 ± 14.9	**89.4 ± 15.1**	115.8 ± 29.2	**114.8 ± 28.4**	146.5 ± 52.3	**170.9 ± 75.6**
p = 99.99%	118.1 ± 30.4	**121.6 ± 30.8**	173.6 ± 59.3	**172.0 ± 57.6**	232 ± 104.2	**278.2 ± 149.4**
M. populorum						
p = 99%	64.1 ± 6.6	**65.9 ± 7.8**	93.8 ± 19.0	**94.1 ± 18.4**	65.1 ± 8.1	**64.8 ± 7.7**
p = 99.99%	74.5 ± 12.9	**79.3 ± 15.9**	135.3 ± 40.0	**134.7 ± 38.5**	78.5 ± 16.9	**77.6 ± 15.8**

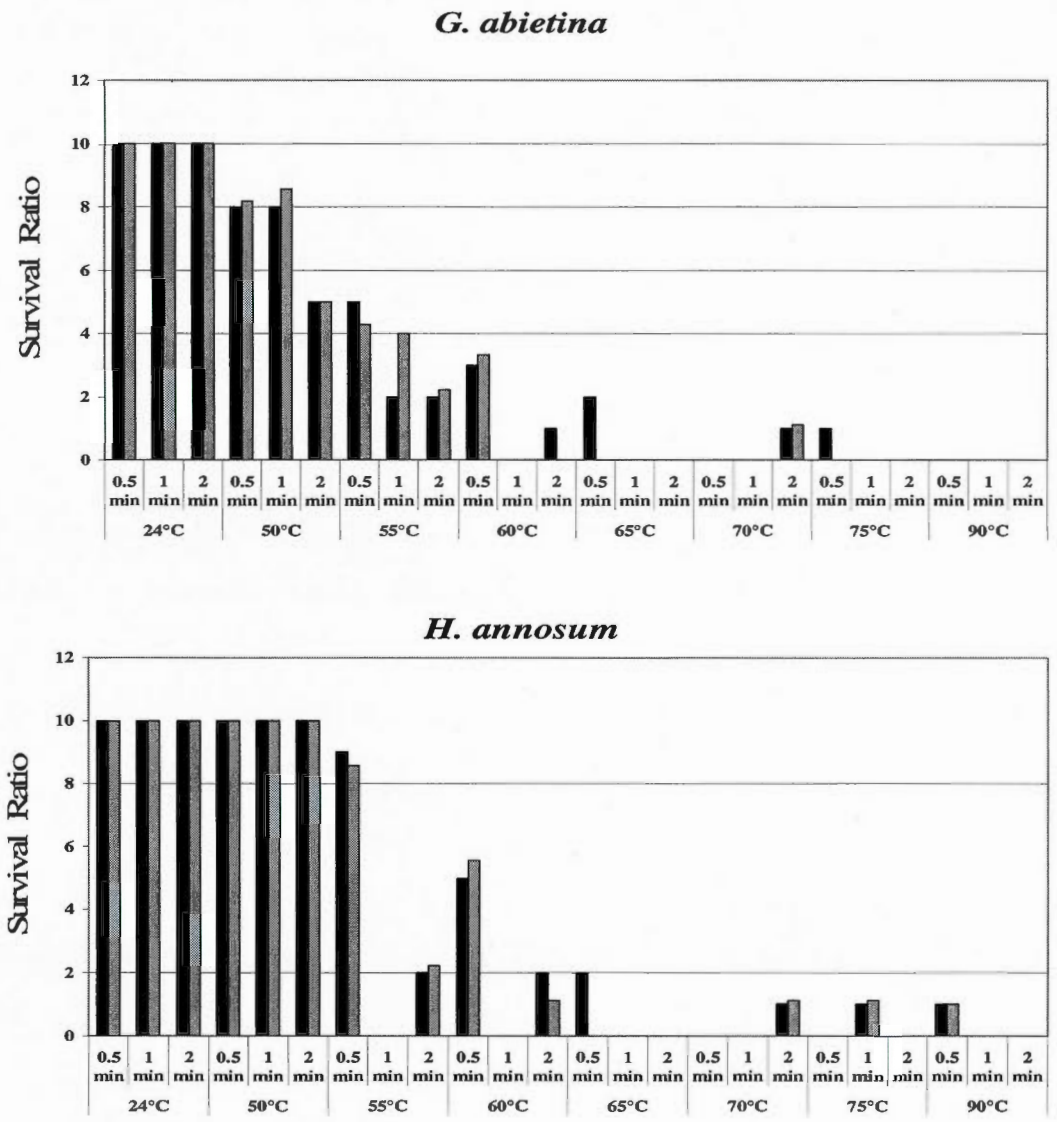

Figure 1.2 (1) Survival ratios for the four pathogenic fungi with temperature-time combinations. Ratios for the final dataset adjusted for temperature variations were all brought to an equivalent denominator (10). Bars in black represent data with datapoints from the original datasets, bars in grey represent data with the final adjusted data points, and bars in white represent series that could not be adjusted.

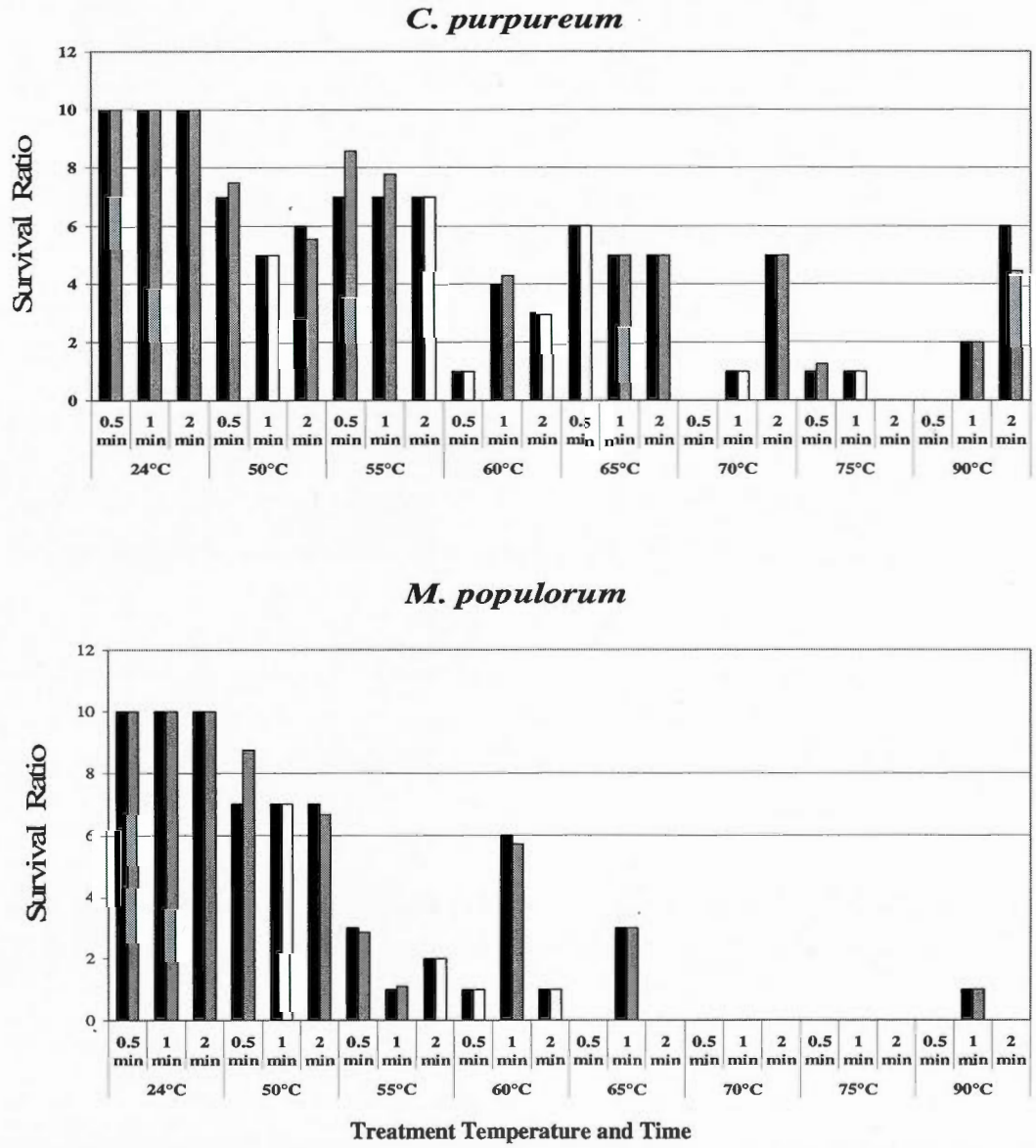

Figure 1.2 (2) Survival ratios for the four pathogenic fungi with temperature-time combinations. Ratios for the final dataset adjusted for temperature variations were all brought to an equivalent denominator (10). Bars in black represent data with datapoints from the original datasets, bars in grey represent data with the final adjusted data points, and bars in white represent series that could not be adjusted.

CHAPITRE II

Colonization of Norway spruce (*Picea Abies* [L.] H. Karst.) by xylophagous insects indigenous to Québec's boreal forest

Martin Payette'* and Timothy T. Work

Forest research centre, NSERC/UQAT/UQAM Chair in Sustainable Forest Management, Université du Québec à Montréal, Département des sciences biologiques

*Corresponding author

In preparation for submission

2.1 Abstract

One of the most important determining factors behind the successful establishment of invasive xylophagous insects is the presence of compatible hosts. The capacity of insects to complete development on novel wood species has significant implications for their transport through wood packing material (WPM) and establishment in new habitats as well as their interactions with plantations of non-indigenous species established within their native range. In this study, our objective was to identify indigenous North American species that can complete their larval development on freshly cut logs of Norway spruce, *Picea Abies* L. Karst, a species of Europeen origin, to compare their relative preference for a non-indigenous host relative to two native species, *Picea glauca* (Moench) Voss and *Picea mariana*, (Mill.) Britton, Sterns & Poggenburg. We placed 48 Norway spruce logs in uncut boreal forest stands in 2010 alongside white and black spruce logs and collected them after two months of exposure. We placed all logs in separate emergence cages and reared them for the following 19 months. Four xylophagous beetles were able to readily complete their development in Norway spruce: *Monochamus scutellatus* (Say), *Dryocoetes autographus* (Ratzeburg), *Polygraphus rufipennis* (Kirby), and *Hylobius congener* Dalla Torre. Debarking can reduce the transmission of bark beetles, though they can still be intersepted at ports of entry. *M. scutellatus* larvae, however, bore deep in the wood instead of staying in the bark, making its transmission through WPM a high risk. The capacity for these species to colonize Norway spruce deadwood could ease the process of establishment should they be introduced in the tree's native range, thus setting the stage for it potentially becoming a pest. It is also notable that species of the *Monochamus* genus are vectors of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner &Buhrer 1934) Nickle 1970, an important pest of *Pinus* spp. in many countries. Future studies should conduct host preference assays in laboratory conditions with the identified species to further determine optimal factors for Norway spruce colonization. This approach is interesting for the possibilities it offers in targeting specific species for phytosanitary treatments before they become pests.

2.2 Résumé

Un des facteurs déterminants de l'établissement d'espèces insectes xylophages envahissantes est la présence d'hôtes compatibles. La capacité d'insectes à compléter leur développement sur de nouvelles espèces d'arbres a des implications importantes au niveau de leur probabilité à être transporté dans des matériaux d'emballage en bois (MEB) et à pouvoir s'établir dans des régions étrangères ainsi que de pouvoir coloniser des plantations composées d'espèces d'arbres non-indigènes dans leur région d'origine. Dans cette étude, nous avions comme but d'identifier des espèces indigènes en Amérique du Nord pouvant compléter leur développement larvaire dans des bûches récemment coupées d'épinette de Norvège, Picea Abies L. Karst, une espèce d'origine européenne, pour comparer leurs préférences relativement à deux essences indigènes, Picea glauca (Moench) Voss and Picea mariana, (Mill.) Britton, Sterns & Poggenburg. Nous avons placé 48 bûches d'épinette de Norvège dans la forêt boréale en 2010 à côté de bûches d'épinette noire et d'épinette blanche et les avons récupérées après deux mois. Chaque bûche a été placée dans une chambre d'émergence séparée pour 19 mois. Quatre espèces coléoptères xylophages ont complété leur développement dans l'épinette de Norvège : Monochamus scutellatus (Say), Dryocoetes autographus (Ratzeburg), Polygraphus rufipennis (Kirby) et Hylobius congener Dalla Torre. L'écorçage peut réduire la transmission d'espèces qui sont plus souvent retrouvées dans l'écorce, bien qu'elles puissent tout de même être interceptées dans des ports d'entrée. Par contre, les larves de M. scutellatus creusent vers le centre du bois leur rendant plus à risque d'être transporté dans les MEB. La capacité de ces espèces à coloniser des bûches d'épinette de Norvège pourrait faciliter le processus d'établissement si elles sont introduites dans la région d'origine de l'arbre ou dans des plantations de celle-ci, ce qui mènerait potentiellement à leur classification en tant que pestes. Il est aussi d'intérêt que les espèces de genre *Monochamus* sont des vecteurs du némotde du pin, *Bursaphelenchus xylophilus* (Steiner &Buhrer 1934) Nickle 1970, une peste importante de *Pinus* spp. dans plusieurs pays. De futures études devraient entreprendre des tests de préférence d'hôtes dans des conditions contrôlées en laboratoire avec les espèces identifiées afin d'isoler plus de variables et ainsi déterminer les conditions optimales pour leur colonisation de l'épinette de Norvège. Cette approche est intéressante puisqu'elle pourrait éventuellement permettre de cibler des espèces spécifiques pour des traitements phytosanitaires avant qu'elles ne deviennent nocives.

2.3 Introduction

The successful establishment of non-indigenous xylophagous insects in a new habitat depends on the availability of suitable hosts, among other ecological factors (Niemela et Mattson, 1996). Though bark- and wood-boring insects that are introduced into new ranges through wood packing materials (WPM) can become important pests (Brockerhoff, 2009 ; Brockerhoff *et al.*, 2006 ; Haack, 2001 ; McCullough *et al.*, 2006 ; Work *et al.*, 2005), they must first establish a viable population. To determine which species are likely to become established, it is important to consider the many factors that can influence the susceptibility of a host tree to a new insect species. Phylogenetic distance affects host specificity for phytophagous insects and can help estimate arthropod richness at local and global levels (Ødegaard, Diserud et Østbye, 2005). However, this effect has been shown to vary, with generalized species being able to colonize a wide range of hosts in different families of conifiers and/or angiosperms, and other more specialized insects only being able to attack congeners (Roques, Auger-Rozenberg et Boivin, 2006). The success of non-indigenous insects therefore highly depends on how selective they are. As the density of competitors within the same tree can be a factor in the selection of a host by the adult or of the success of the larvae inside the wood (Hanks, Paine et Millar, 1993), more specialized insects will possess adaptations giving them a competitve advantage over the generalized insects. This can be due to bark thickness and wood properties such as specific nutritional or secondary substances and structural features that vary less between trees of the same genus or family (Smith, Bancroft et Tropp, 2002). In any case, it is likely that, for most species, the more closely related the tree species in the insect's new range are to those in its native habitat, the higher its success is likely to be.

Many studies have performed host preference trials with xylophages in varying contexts. The susceptibility of already established exotic plants to native bark-beetles (Coleoptera: Curculionidae: Scolytinae) has been studied (Bertheau *et al.*, 2009). It

was observed that non-indigenous trees were just as likely to be colonized as native trees but that the presence of the prefered host of a bark-beetle species in the area increased the likelihood of a non-indigenous tree being colonized. Other studies have looked at the preferences of non-indigenous insects with respect to native plants (Eager *et al.*, 2004 ; Siegert et McCullough, 2003 ; Smith, Bancroft et Tropp, 2002). In light of the likely eventual migration or introduction of the pine shoot beetle, *Tomicus piniperda* (L.), in the southern and western United-States, a study observed the suitability of several pines from these regions for the beetle in laboratory conditions (Eager et al., 2004). They determined that *T. piniperda* was indeed capable of colonizing many species with varying brood success. Based on the high abundance of *Pinus taeda* L., one of the trees tested and selected by *T. piniperda*, they concluded that it would be a prime target in the eventuality of the beetle's introduction in the tree's range. Such studies may help plan proper control and prevention methods by increasing understanding of the preferences of existing pests among potential hosts or of the susceptibility of non-indigenous trees (suce as plantation trees) to native insects.

Since invasive species can have an important economic impact due to the immense costs of damages and control (Pimentel, Zuniga et Morrison, 2005), a preventative and more proactive alternative to the approaches mentionned above would be to determine in advance what xylophagous species from a specific region, not yet considered pests, could colonize wood from abundantly present trees in a foreign habitat and thus establish a viable population that could eventually become invasive. Identifying species with a potential for establishment in the tree's native range will allow us to plan adequate prevention methods specific to these insects. To our knowledge, none have attempted a pre-emptive study to observe the suitability of tree species to insects not yet established in its native habitat.

Tree species from Europe could serve to test the above-mentioned approach and determine what xylophagous species from the North American boreal forest should

be targeted for prevention. Trade between North America and Europe has greatly increased the frequency of species introductions from one continent to the other. This, added to their similar climates as well as to ancient land bridges that created chances for migrations, have resulted in both continents having very similar flora and fauna (Mattson *et al.*, 2007 ; Niemela et Mattson, 1996). The presence of related trees may offer an increased chance of finding suitable hosts for species introduced from one to the other. One of the most abundant and widely distributed tree species in Europe is Norway spruce (*Picea abies* L. Karst). Its distribution extends from the Ural mountains to the western Alps, and from northern Scandinavia to the Balkan mountains and it is one of the most important European trees from an ecological and economical standpoint (Bucci et Vendramin, 2000). As spruce species such as white spruce (*Picea glauca* [Moench] Voss) and black spruce (*Picea mariana*, [Mill.] Britton, Sterns & Poggenburg) are widely distributed in the boreal forests of North America (Farrar, 1995), the Norway spruce may offer a perfect opportunity for North American xylophages adapted to white or black spruce to successfully establish in Europe. Its continuous distribution and tolerance of more southern climates compared to other conifers also suggests that insects ill-adapted to the shorter photoperiods and longer winters of northern Europe may have less difficulty attacking Norway spruce at lower latitudes and in a context of global climate change.

In this study, we assess the acceptability (whether or not an insect will feed on a chosen host) and suitability (the capacity of a tree species to host an insect from hatchling to reproducing adult) of deadwood from an non-indigenous spruce (Norway spruce) relative to two native spruces (white spruce and black spruce) for bark- and wood-boring insects native to the northern Québec boreal forest. We expect that the shared genus of all three species will make Norway spruce compatible for many native insects. We measured colonization of logs from all three species placed in the boreal forest over a two month period during the summer of 2010, when bark- and wood-boring insects reproduce. Though this study cannot determine which species

will become tree killers in a new habitat, it may provide insight into how a non-indigenous species can quietly integrate into a native ecosystem or, *vice versa*, how a native insect species can readily attack deadwood from a non-indigenous tree.

2.4 Materials and methods

2.4.1 Tree species and study area

Bait-logs of two native North American spruce species (white spruce, *P. glauca*, and black spruce, *P. mariana*) and one non-indigenous European spruce species (Norway spruce, *Picea abies*) were used to analyze preferences among Québec-native bark- and wood-boring insects within congeneric trees with different origins. We cut a total of 54 bait-logs 70 cm long and averaging 16.6 cm in diameter (min = 9 cm; max = 28 cm) from Norway spruce, white spruce, and black spruce. We were careful to select healthy trees so as to reduce the chance of them already being colonized. The three Norway spruce trees from which the logs were cut were found near Hertel Lake at the Gault Research Preserve on Mont St-Hilaire in the Montérégie region of Québec on June 25th, 2010. The logs were then frozen for 12 hours at -70°C at the Université de Québec à Montréal to kill as many of the organisms as possible already in the wood. Two white spruce trees and one black spruce tree were cut at the Lake Duparquet Research and Teaching Forest (LDRTF) on July 5th, 2010. The logs were frozen for 12 hours at only -10°C in the freezers that were available on location. Space and time constraints prevented us from freezing them for a longer period. As it was the middle of summer and the insects inside were most likely at their lowest level of cold-hardiness, the sharp temperature drop may have been enough to kill most athropods inside. Larvae of *Monochamus alternatus* Hope (Coleoptera: Cerambycidae), a Japanese relative of *M. scutellatus* (Say), a common wood-borer in North American boreal forest, have been shown to have a supercooling point – the temperature below which an animal's chemical protection is unable to keep it from freezing – that varies

with the seasons and is at its highest during the summer (-6.2°C) (Ma *et al.*, 2006). However, larvae of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae), can reportedly have a much lower supercooling point, even during the summer (as low as -20.7°C in June) (Bentz et Mullins, 1999). In any case, we placed two logs of each species in emergence chambers immediately after freezing to determine if any arthropods were left over to bias results. No bark- or wood-borers emerged from them after 12 months.

We placed the other 48 bait-logs in the LDRTF (approx. 48°30' N, 79°22' W) in the northwestern portion of Québec. The LDRTF is situated in the southern part of the boreal forest and in the northern part of the Clay Belt. The nearby city of La Sarre (approx. 42 km northeast of the LDRTF) has a daily average temperature of 0.7°C and annual average precipitation of 889.8 mm (Canada, 2012). Forest fires are the main disturbance type in the area (Bergeron, Grondin et Blouin, 1999). The LDRTF is in the balsam fir (*Abies balsamea* [L.] Mill.) and white birch (*Betula papyrifera* Marsh.) mixedwood forest (Grandtner, 1966). Mesic sites are composed of white birch, trembling aspen (*Populus tremuloides* Michx.), and white spruce (*Picea glauca*) (Bergeron et Bouchard, 1984), while hydric sites are characterized by tamarack (*Larix laricina* [DuRoi] K. Koch), black spruce (*Picea mariana*), eastern white cedar (*Thuja occidentalis* L.), and black ash (*Fraxinus nigra* Marsh.) (Bergeron *et al.*, 1982).

2.4.2 Experimental design

We divided the 48 bait-logs among four stands, each separated by at least 1 km. Log diameter and length were measured before deployment and the extremities of all logs were coated with paraffin to reduce desiccation. We placed one bait-log of each species at the same spot on the ground in a three-pointed star pattern (Figure 1.1) to offer a choice for ovipositing females. We set up four of these stations with a

minimum 15 m buffer from each other in each stand. All logs were deployed on July 10th, 2010, and collected on September 14th, 2010.

To assess its possible influence on captures, we estimated deadwood volume surrounding each station using the line intersect method (Van Wagner, 1969). This is done by measuring the diameter of all deadwood that crosses three 10 m transects that spread out from the center of a station following the path of one of the three logs:

$$V = (\pi^2/8L) \Sigma d$$

where: V= volume per unit area (m^3/ha),

d = piece diameter at intersection,

L = length of transect.

2.3.3 Emergence chambers and identification

Once collected, we placed the logs in emergence chambers. These were made of 25.4 to 30.5 cm wide Sonotubes cut to 1 m in length. The ends were sealed with a black fabric with 1 mm wide holes to let air in while minimizing the passage of light. At the center of one end, a hole leading to a collection cup filled with propylene glycol was cut in the middle of the fabric to let light in and attract emerged arthropods (Figure 1.2). We replaced the collection cups regularly to identify emerged adults. We opened the chambers in April 2012, after 19 months, and collected all arthropod carcasses which had emerged but hadn't reached the collection cups.

The emergence chambers were kept at the LDRTF Research Station. We placed the logs from two stations in each stand at room temperature (Mean T° = 24.0°C) and left the other half in an unheated shelter (Mean T° = -5.1°C; Max T° = 19.2°C; Min T° =

-36.5°C) to see whether exposure to seasonal temperatures would affect adult emergence. They were transferred to room temperature as of May 5th, 2011.

Bark- and wood-boring insects such as longhorn beetles (Coleoptera: Cerambycidae), bark-beetles and weevils (Coleoptera: Curculionidae), and woodwasps (Hymenoptera: Siricidae) were identified to the species level (Bright, Canada. Dept. of Foreign et International, 1976 ; Papp, 1984 ; Schiff *et al.*, 2006). We identified other beetles and Hymenoptera to family (Arnett Jr et Thomas, 2001 ; Arnett Jr *et al.*, 2002), all other insects to order or sub-order (Diptera) (Borror, Triplehorn et Johnson, 1989), and remaining arthropods to class.

2.4.4 Statistical Analysis

We chose four beetle species that were captured in sufficiently high numbers for separate analysis using a backward stepwise regression with a generalized linear mixed model (GLMM) and Poisson distribution in the lme4 package with R version 3.0.0 (Bates, Maechler et Bolker, 2013). We assessed the influence of the following fixed effects on the abundance of each of the four most abundant species of xylophageous coleoptera (*Dryocoetes autographus* [Ratzeburg], *Polygraphus rufipennis* [Kirby], *Hylobius congener* [Dalla Torre et al.], and *Monochamus scutellatus* [Say]): sampling site, trap, tree species, log diameter, surrounding deadwood volume (DWV) and average emergence chamber wintering temperature. We only took into account data from logs having wintered at room temperature for *H. congener*, as there were no emergent adults from logs having wintered freezing temperatures. Interactions between fixed effects were tested but were not significant and so were not included.

2.5 Results

A total of 13440 emerged adult arthropods were captured from the logs after 19 months in emergence chambers. The most abundant orders were Coleoptera (42.1%, mainly Cerambycidae and Curculionidae), Diptera (55.8%), and Hymenoptera (1.0%). Overall abundances of adult arthropods according to log species are shown in Table 2.1. We identified 16 families of Coleoptera. We only identified to species the insects that were in the Cerambycidae (2 species) and in the Curculionidae (2 weevil and 2 bark beetle species) families. The four most commonly collected among these species were Cerambycidae: *Monochamus scutellatus* (Say), Curculionidae: Molytinae: *Hylobius congener* Dalla Torre, Curculionidae: Scolytinae: *Dryocoetes autographus* (Ratzeburg), and Curculionidae: Scolytinae: *Polygraphus rufipennis* (Kirby). We also identified two woodwasps (Hymenoptera: Siricidae) to species: *Urocerus albicornis* (Fabricius) and *Urocerus cressoni* (Northon). No xylophagous beetle species were captured from the six control logs.

Fixed effects and model parameters are presented in Table 2.2. The importance of fixed effects varied between the four species analysed in Poisson regression models. All four species showed a capacity to use Norway spruce logs and complete their larval development within them, though they varied in their host preferences with respect to all three log types. *M. scutellatus*, *D. autographus* and *P. rufipennis* were significantly affected by tree species. Table 2.2 shows the abundances of the four beetle species in each tree. *M. scutellatus* abundance was significantly higher in white spruce than in the two others. *D. autographus* abundance in black spruce was significantly lower than in Norway spruce and white spruce. *P. rufipennis* showed a higher preference for white spruce than for Norway spruce. Though total abundance of *P. rufipennis* was the lowest in black spruce, the model showed no significant difference between its mean abundance in black spruce logs versus either Norway spruce or white spruce. This may be due to the uneven distribution of *P. rufipennis*

among traps and stands (Figure 2.3). In any case, it still demonstrated the capacity to use Norway spruce deadwood.

There was no significant effect of DWV on abundance for any of the species. *M. scutellatus* responded positively to log diameter (β = 1.228; P = 0.028) and was the only beetle to be significantly affected by it. Emergence was significantly lower for *P. rufipennis* in logs having spent the winter at freezing temperatures (686 inside versus 51 outside; P = 0.001). For *H. congener*, wintering at freezing temperatures completely inhibited emergence. Neither *M. scutellatus* nor *D. autographus* was affected by wintering temperature.

Distribution of each species among stands, traps, and tree species are represented in Figure 2.3. The coefficient of variation for *H. congener*, calculated with the standard deviation and mean only of logs having wintered at room temperature, was high (353.0%), showing much variation in its distribution. The coefficient of variation for the three other species was calculated with the standard deviation and mean for all logs. *M. scutellatus* and *P. rufipennis* had similar and high coefficients of variation (234.8% and 254.0%), which can be explained by the fact that they were clumped and absent from many trap locations. *D. autographus* had the lowest coefficient (158.3%), showing the least amount of variation in its distribution, and was the most widespread and abundant of the four (Figure 2.3).

2.6 Discussion

The study revealed eight species that stood out as possible candidates for introduction via wood packing material. There were two Cerambycidae, *M. scutellatus* and *Tetropium cinnamopterum* Kirby, two weevils (Curculionidae: *H. congener* and *Pissodes rotundatus* LeConte), two bark-beetles (Curculionidae: *D. autographus* and *P. rufipennis*), and two woodwasps (Siricidae: *U. albicornis* and *U. cressoni*).

The four analyzed species of beetles, *M. scutellatus*, *D. autographus*, *P. rufipennis* and *H. congener*, all used Norway spruce as a host and were able to emerge as adults. They attacked Norway spruce in similar proportions to white spruce, an endemic species which all four beetles sometimes use as host. *M. scutellatus*, *P. rufipennis* and *H. congener* had a mean abundance that was highest in white spruce and second highest in Norway spruce, though *H. congener* was not significantly correlated with any one species. For *D. autographus*, mean abundance was most correlated with Norway spruce and white spruce and was generally more common and abundant in all three.

The selection of Norway spruce by *M. scutellatus* is unsurprising. The beetle is found throughout North America and.its preferred host is eastern white pine (*Pinus strobus* L.), but it also attacks white spruce, black spruce, red spruce (*Picea rubens* Sarg.), jack pine (*Pinus banksiana* Lamb.), red pine (*Pinus resinosa* Ait.), balsam fir (*Abies balsamea* [L.] Mill.) and occasionally tamarack (*Larix laricina* [DuRoi] K. Koch) (Wilson, 1962). It is a fire-associated xylophage and attacks recently killed, dying and weakened trees (Saint-Germain, Drapeau et Hébert, 2004a, 2004b), and is a minor economic pest in Québec as it decreases the quality of freshly cut wood. Its wide range of hosts from different families, including congeners of Norway spruce, may explain its capacity to colonize it. Another interesting detail is that *M. scutellatus* was positively affected by log diameter. Other studies indicate that *M. scutellatus* selects mostly trees with larger diameters (Hughes et Hughes, 1982, 1987 ; Saint-Germain, Drapeau et Hébert, 2004a, 2004b). In smaller trees with thinner bark and a larger surface per unit of volume, heat from exposure to the sun or to fire can lead to desiccation and large variations in temperature and moisture content, possibly degrading the subcortical region and rendering the tree less attractive and the general environment too unstable for developing larvae (Furniss, 1965 ; Saint-Germain, Drapeau et Hébert, 2004b). Norway spruce generally grows to be slightly wider (90 cm) (Petrides, 1973) than white (60 cm) and black spruce (30 cm) (Farrar, 1995).

This would suggest that the generally wider diameter of Norway spruce may facilitate its selection by *M. scutellatus* should it be introduced into regions where Norway spruce is present. The overall emergence of *M. scutellatus* was not affected by overwintering conditons, suggesting that the larvae are well adapted to variations in temperature and moisture in the wood. As Cerambycidae larvae bore deep into the heartwood, they are most likely well insulated and moisture loss is slower.

The capture of *M. scutellatus* in Norway spruce logs is also significant because of the association that *Monochamus* species have with the pine wood nematode (*Bursaphelenchus xylophilus* [Steiner and Buhrer] Nickle) as vectors between trees. All *Monochamus* spp. are included in the European and Mediterranean Plant Protection Organization's (EPPO) list for species absent from the EPPO region but to be treated as quarantine pests (EPPO, 2012).

D. autographus had a high affinity for Norway spruce. This is explained by the fact that it is already present all through North America and Eurasia and is a generalist that attacks all confiers in its range, including Norway spruce (Bright, Canada. Dept. of Foreign et International, 1976). It is established in Europe as far as northern Norway (70° N) and is well adapted to subarctic regions, having a flexible life-cycle, while important pests such as *Tomicus piniperda* L. and *Ips typographus* (Linnaeus) with obligate one-year life-cycles aren't as successful (Johansson, Nilssen et Andersen, 1994). Its success is due to its versatility in habitat requirements, its dispersal capability, its capacity to reproduce without being synchronized with other members of its species (i.e. without swarming), to reproduce at least twice successively in one piece of bark, and to develop without quiescence or diapause when temperatures are favourable (Johansson, Nilssen et Andersen, 1994). These factors have allowed it to become well established across the Northern Hemisphere. Its adaptability in high latitdudes may explain why overwintering at freezing temperatures did not affect total emergence. However, its already ubiquitous

distribution without it being a severe pest makes its eventual classification as a quarantine pest unlikely and its accidental transport via WPM less of a concern.

P. rufipennis is present throughout Canada and the United States and, like *D. autographus*, attacks all conifers in its range (Bright, Canada. Dept. of Foreign et International, 1976). In northern regions, *P. rufipennis* has one generation per year while it can have up to three further south. It has been known to kill small, stressed trees of up to 25.4 dbh. (Schmid et Frye, 1977). Its capacity to live throughout a large North-South distribution, affinity for a wide range of possible hosts and flexible life-cycle may give it an advantage in the case of a possible introduction in Europe. This is added to its ability to colonize Norway spruce deadwood, which would facilitate its establishment. It was not significantly affected by diameter, though this is contrary to results from previous studies which have found that thicker phloem and bark is preffered by bark-beetles in general (Amman, 1972 ; Bowers, Borden et Raske, 1996 ; Haack et Slansky Jr, 1987 ; Reid et Glubish, 2001). The lack of significant differences in colonization of larger diameters may point to too little variation in log diameters. Adult *P. rufipennis* emerged much less from logs having overwintered at freezing temperatures. Though this species is found at high latitudes (as high as Alaska), our results suggest that cold has a strong influence on the survival of its larvae. Snow cover is an important factor in protecting overwintering larvae from the cold and desiccation (Lombardero *et al.*, 2000) and the logs exposed to freezing temperatures during this study were sheltered from the snow, which may have affected their survival. An alternate option for overwintering of the logs in future studies would be to keep the logs outside at ground level and covered wih a tarp in order to protect them from animals while simulating natural conditions. Regardless of the effect of the storage conditons of the logs on the larvae, *D. autographus* did not show the same susceptibility to cold, indicating that *P. rufipennis* is much less suitably adapted to temperatures as low as -36.5°C which occurred during the winter of 2010-2011.

H. congener used all three tree species equally. It is a bark weevil which occurs from the northeastern coast of Canada to Alaska and its larvae feed mainly on the inner bark of logs and stumps of red pine, Scots pine (*Pinus sylvestris* L.) and eastern white pine, but will also attack larch and spruce (Drooz, 1985). In the eastern provinces and in Maine, it is an important pest of newly planted conifer seedlings which the adults search for in clearcut and burned forests (Eidt *et al.*, 1995 ; Welty et Houseweart, 1985). The fact that it can attack a diverse range of conifers, including Scots pine, another tree native to Europe and Asia, means that it may be capable of colonizing deadwood in Europe. It showed no specifc preference for variation in diameter. However, a relative of *H. congener*, *Hylobius warreni* Wood, is a common pest of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm. ex S. Wats.) in British Columbia and has shown a preference for root collars with a larger diameter (Duke et Lindgren, 2006). As mentioned above, larger tree diameters provide a better environment for the development of wood and bark-boring larvae so that Norway spruce deadwood may be a convenient breeding ground for newly arrived adults of *H. congener*. Another important detail on their emergence was that no individuals of *H. congener* were captured from logs that overwintered at freezing temperatures and so may be susceptible to high temperature variation to an even larger degree than *P. rufipennis*.

The volume of deadwood surrounding the trap-logs had no significant effect on recruitment for any species. The current study focused on the effect of DWV in the immediate area surrounding each trap location. Other studies indicate that DWV does not have an important effect on saproxylic and xylophagous communities at a local level, but that placement and quality of deadwood and regional supply have a much greater influence (Franc *et al.*, 2007 ; Vodka, Konvicka et Cizek, 2009). Xylophagous beetles and wasps are generally very mobile and most likely much less affected by DWV on a small scale. Vodka et al. (2009) showed that exposure to sunlight and vertical position of wood had a far greater influence on abundance and diversity at

this level. Also, substrate type (log, snags and tops, and scortched *vs* unscortched) and forest management (clear-cut, mature managed and forest reserves) tend to attract different assemblages of saproxylic and xylophagous communities (Hjältén *et al.*, 2007). Future studies on the attractiveness of non-indigenous tree species to indigenous xylophages should look into these factors as well as regional distribution and supply of deadwood to better understand what variables affect insect loading outside of the trees' specific characteristics.

Attractive kairomones play a large role in selection of a host (Allison *et al.*, 2001 ; Allison, Borden et Seybold, 2004 ; Francardi *et al.*, 2009 ; Haberkern et Raffa, 2003 ; Ibeas *et al.*, 2007 ; Lindelöw, Eidmann et Nordenhem, 1993 ; Schiebe, 2011 ; Silk *et al.*, 2007 ; Sweeney *et al.*, 2010). The three log species were set beside one another at each station. Attractants from anyone of the logs or from beetles that were already on scene may have played a role in attracting other adults to the general area, but then the selection of a specific species may have simply been dependant on insect loading in each log. Thus, though the results show that many xylophagous species are capable of selecting Norway spruce at a local level, we cannot infer whether they can actually be attracted to them from longer distances. This ambiguity could be resolved in future research by keeping log species separate, placing a small group of logs of each species in the same stand but distanced by a reasonable buffer between them such as was done by Siegert and McCullough (2003). We can still infer however that, in mixed plantations composed of closely related native and non-native trees, native insects could be attracted by the indigenous trees and then be able to attack the non-indegenous trees, as has been demonstrated in a previous study (Bertheau *et al.*, 2009).

Probably the most important vector for the introduction of non-indigenous bark- and wood-boring insects are WPM (Brockerhoff, 2009 ; Haack, 2006). Regulations for the construction of WPM now require that wood be debarked before use (FAO, 2009). Though in many cases WPM may still have traces of bark on them after

debarking (Ray et Deomano, 2007), we suspect that any insects present so close to the surface would not benefit from much protection from phytosanitary treatments such as kiln drying or microwave irradiation. This greatly reduces the risk of bark-beetles and bark-boring weevils being introduced through WPM. It would be more likely for the one Cerambycidae of the four analyzed species in this study, *M. scutellatus*, to be found in WPM, given that its larvae bore deeper into the wood than the three others, which only dig a few millimetres into the phloem.

Very few adults of *T. cinnamopterum* (6), *P. rotundatus* (6), *U. albicornis* (6), or *U. cressoni* (12) were captured and none of them emerged from Norway spruce. Nevertheless, some of these species possess characteristics that give them the potential to become pests if introduced in the right conditions. *T. cinnamopterum* are capable of attacking several conifer species (Drooz, 1985). They are related to *T. fuscum* (F.), a non-indigenous species which was first found in Halifax, Nova Scotia, in 1999 (Smith et Hurley, 2000). It is originally from Europe, where it attacks primarily Norway spruce. In Canada, it attacks many species also attacked by *T. cinnamopterum* such as red spruce, white spruce, and black spruce (Saint-Germain, Drapeau et Hébert, 2004b ; Smith et Humble, 2000). Both species produce and are attracted to aggregation pheromones with similar compounds (Silk *et al.*, 2007 ; Sweeney *et al.*, 2010). Such similarities (and others) may offer them an advantage in each other's native habitats. The small pine weevil, *P. rotundatus*, is associated with conifers and lays its eggs under the bark (Drooz, 1985). There is very little documentation on them, suggesting that they are not of any particular economic importance. Their relative, the white pine weevil, *P. strobi* Peck, is an important pest of many conifers in Canada, including Norway spruce (Alfaro, 1995). Although *P. strobi* attacks the terminals of young pine and spruce, other *Pissodes* species generally only attack the bole and root collar of dying trees, which means they aren't likely to be found in wood used in the construction of WPM (Furniss et Carolin, 1977). There is also very little literature dealing specifically with either *U. albicornis*

or *U. cressoni*. *U. albicornis* is reported to have been introduced into Great Britain in the past, but without successfully establishing itself (Morgan, 1968). Members of the *Urocerus* genus are widespread in the forests of Canada, except *U. cressoni*, which is only reported in eastern Canada (Goulet, 1992). Siricidae, like wood-boring beetles, are subcortical insects that can cause a great amount of damage through the creation of larval galleries and, in most cases, the introduction of symbiotic fungi that break down wood (Coyle et Gandhi, 2012). *Sirex noctilio* F. is a species with a biology similar to that of species in the *Urocerus* genus and well known because of its invasiveness (Morgan, 1968). It originated from Eurasia and has been introduced into many countries of the Southern Hemisphere, where Siricidae are rare, as well as in North America (Long, Williams et Hajek, 2009). In its introduced ranges, *S. noctilio* attacks live trees and contributes to tree mortality, attacking mainly pines but also larch, fir, and spruce (Coyle et Gandhi, 2012 ; Gandhi *et al.*, 2010). *Amylostereum areolatum* (Fr.) Boidin is the main fungal symbiont of S. *noctilio* and a close relative of A. chailletii (Pers. : Fr.) Boidin, a symbiont associated to some Sirex spp. and *Urocerus* spp., including *U. albicornis*, though no mention is made of *U. cressoni* (Morgan, 1968 ; Stillwell, 1966 ; Thomsen et Koch, 1999). Though litterature concerning *Urocerus* spp. is not as extensive as that on *S. noctilio*, we can infer that similarities in their biology could make *Urocerus* spp. have comparable reactions to those of *S. noctilio* where conditions are analogous to those that allowed *S. noctilio* to become an important pest.

2.7 Conclusion

This study determined that at least four xylophagous insect species of the northeastern North American boreal forest, *M. scutellatus*, *P. rufipennis*, *D. autographus* and *H. congener*, are capable of selecting Norway spruce in the form of deadwood as host in more or less equivalent proportions to the native white and black

spruce. *D. autographus* is capable of adapting to a wide range of latitudes, though it is already present throughout the range of Norway spruce in Eurasia and does not appear to pose a serious threat as a pest. Since survival of larvae of *P. rufipennis* and *H. congener* was highly affected by cold temperatures, they would seem to be more vulnerable to variations in temperature and moisture in the wood could have an impact on their success against more competitive native species in Europe. Larvae of bark-beetles and bark-boring weevils such as these three species are also less likely to survive or be found in WPM, the main vectors for xylophagous insect introductions, as phytosanitary treatments and debarking eliminate many viable organisms found at the surface of the wood. Larvae of *M. scutellatus* bore deep into the heartwood of boles and are better protected from variations in temperature and humidity in the environment, giving them an advantage during travel in WPM. We confirmed its preference for larger diameters and this would make Norway spruce an appealing and abundant host. As *Monochamus* spp. are known to be the main insect hosts of the pine wood nematode and are already on the EPPO list, the potential for *M. scutellatus* to be able to cross host barriers in Europe should be of great concern.

All four species are generalists in there native ranges. As Norway spruce is not native to the boreal forest of northern Québec, it is to be expected that it would not have attracted specialist insects. By extension, introduced generalist xylophages in Europe from North America will be less likely to compete successfully with natural enemies of Norway spruce who are better adapted to its defenses. Nevertheless, the objective of the present study was to determine acceptability and suitability of Norway spruce logs for xylophagous insects from the North American boreal forest, and future studies may use this data to evaluate the capacity to compete of the four captured beetle species against European specialists.

Since host barriers are one of the most important factors for the successful establishment of non-indigenous xylophagous insects, the approach tested in this study is a stepping stone towards pre-emptive identification of potential pests.

Though it has its limits, as the many factors that are specific to the environment of European forests (photoperiod, competitors, natural enemis such as predators and parasites, choice of hosts and landscape heterogeneity) cannot be replicated in field conditions in North America, it is too risky to test exotic insects in European forests. A next step would be to remove the constraints posed by North American field conditions by using the information aquired in this study in controlled laboratory experiments where the Norway spruce could be isolated from the native trees and the insects reared specifically for the purpose of choice assays. We could then determine the traits that the insects possess allowing them to successfully attack the foreign tree and those that the tree possesses which attract the insects.

In more general terms, this could help better understand the characteristics and life-history traits behind the success of invasive pests so that a set list of criteria could eventually be applied towards identifying potential invasive insects not yet established in foreign habitats. Such lists would allow for the development of phytosanitary treatments tailored to the potential pests from each country. Another application would be to evaluate the threat of local insects to plantations of non-indegenous trees and to develop control programs specific to these insects.

Figure 2.1 Picture of log trap with Norway spruce, white spruce, black spruce.

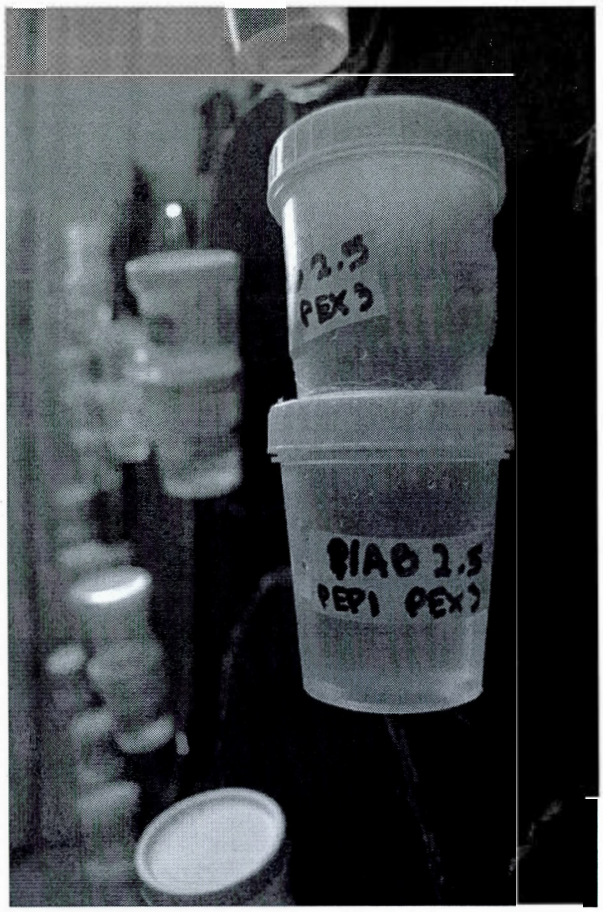

Figure 2.2 Collection cup on the emergence chambers containing propylene glycol. The bottom cup can be unscrewed and replaced.

Table 2.1 Insect emergence from 48 logs collected in the summer of 2010 by order and family and by tree species and placement of the emergence chambers during the fall of 2010 and the winter of 2011.

Taxon	Norway spruce		White spruce		Black spruce		Total
	Out	In	Out	In	Out	In	
Coleoptera							
Cerambycidae							
Monochamus scutellatus (Say)	0	10	1	15	0	12	38
Tetropium cinnamopterum Kirby	0	0	1	0	5	0	6
Total	0	10	2	15	5	12	44
Curculionidae							
Hylobius congener Dalla Torre	0	33	0	50	0	7	90
Pissodes rotundatus LeConte	0	0	0	4	0	2	6
Total	0	33	0	54	0	9	96
Scolytinae							
Dryocoetes autographus (Ratzeburg)	1086	1511	1216	749	25	149	4736
Polygraphus rufipennis (Kirby)	26	146	18	465	7	75	737
Total	1112	1657	1234	1214	32	224	5473
Total	1112	1690	1234	1268	32	233	5613
Other Coleoptera	7	11	7	9	2	13	49
Total Coleoptera	1119	1711	1243	1292	39	258	5662
Hymenoptera							
Siricidae *Urocerus albicornis* (Fabricius)	0	0	0	3	1	2	6
Urocerus cressoni (Northon)	0	0	1	0	0	11	12
Urocerus (Unknown)	0	0	0	0	1	0	1
Total	0	0	1	3	2	13	19
Parasitoid Wasps	36	7	2	3	55	12	115
Formicidae	1	0	0	1	0	0	2
Unkown	0	0	0	0	2	0	2
Total Hymenoptera	37	7	3	7	59	25	138
Araneae	0	1	0	14	0	18	33
Chilopoda	1	1	0	1	0	2	5
Diptera	6715	332	25	46	299	78	7495
Hemiptera	0	0	0	3	0	1	4
Lepidoptera	2	1	1	0	5	6	15
Neuroptera	2	0	5	3	2	3	15
Psocoptera	27	4	10	3	26	3	73
Total	7903	2057	1287	1369	430	394	13440

Table 2.2 Outcome estimates (including β estimate, standard error, Wald statistic [z value], P-value [P]) for poisson generalized linear mixed model for the four most abundant Coleoptera species according to tree species, tree diameter, deadwood volume (DWV), and wintering temperature (Inside vs Outside).

	β Estimate	Standard Error	z value	P (>|z|)	
M. scutellatus					
Intercept	-6.086	2.842	-2.142	0.032	*
N vs. W	-1.858	0.773	2.402	0.032	*
N vs. B	-0.693	0.486	-1.424	0.154	
W vs. B	1.165	0.588	1.981	0.048	*
Diameter	1.228	0.560	2.193	0.028	*
DWV	1.212	1.250	0.970	0.332	
In vs Out	4.966	3.189	1.557	0.119	
D. autographus					
Intercept	3.435	0.804	4.272	< 0.001	
N vs. W	-0.050	0.739	-0.068	0.946	
N vs. B	3.911	0.734	-5.328	< 0.001	***
W vs. B	3.961	0.733	-5.406	< 0.001	***
Diameter	0.742	0.480	1.544	0.123	
DWV	0.247	0.512	0.481	0.630	
In vs Out	0.597	1.005	0.593	0.553	
P. rufipennis					
Intercept	-2.311	0.989	-2.336	0.019	*
N vs. W	2.020	0.989	2.042	0.041	*
N vs. B	0.456	0.948	2.042	0.631	
W vs. B	-1.563	0.907	1.725	0.085	.
Diameter	0.822	0.448	1.835	0.066	.
DWV	0.407	0.600	0.678	0.498	
In vs Out	2.930	0.890	3.292	< 0.001	***
H. congener					
Intercept	0.739	0.935	0.791	0.429	
N vs. W	1.189	1.229	-0.967	0.334	
N vs. B	2.245	1.268	-1.771	0.077	.
W vs. B	1.056	1.221	-0.864	0.387	
Diameter	-0.529	0.573	-0.924	0.355	
DWV	-0.218	0.943	-0.232	0.817	
In vs Out	-	-	-	-	

Note: Significant results at $P \leq 0.05$. $P < 0.1$, '.' ; $P < 0.05$, '•' ; $P < 0.01$, '••' ; $P < 0.001$; '•••'. N = Norway spruce; W = white spruce; B = black spruce.

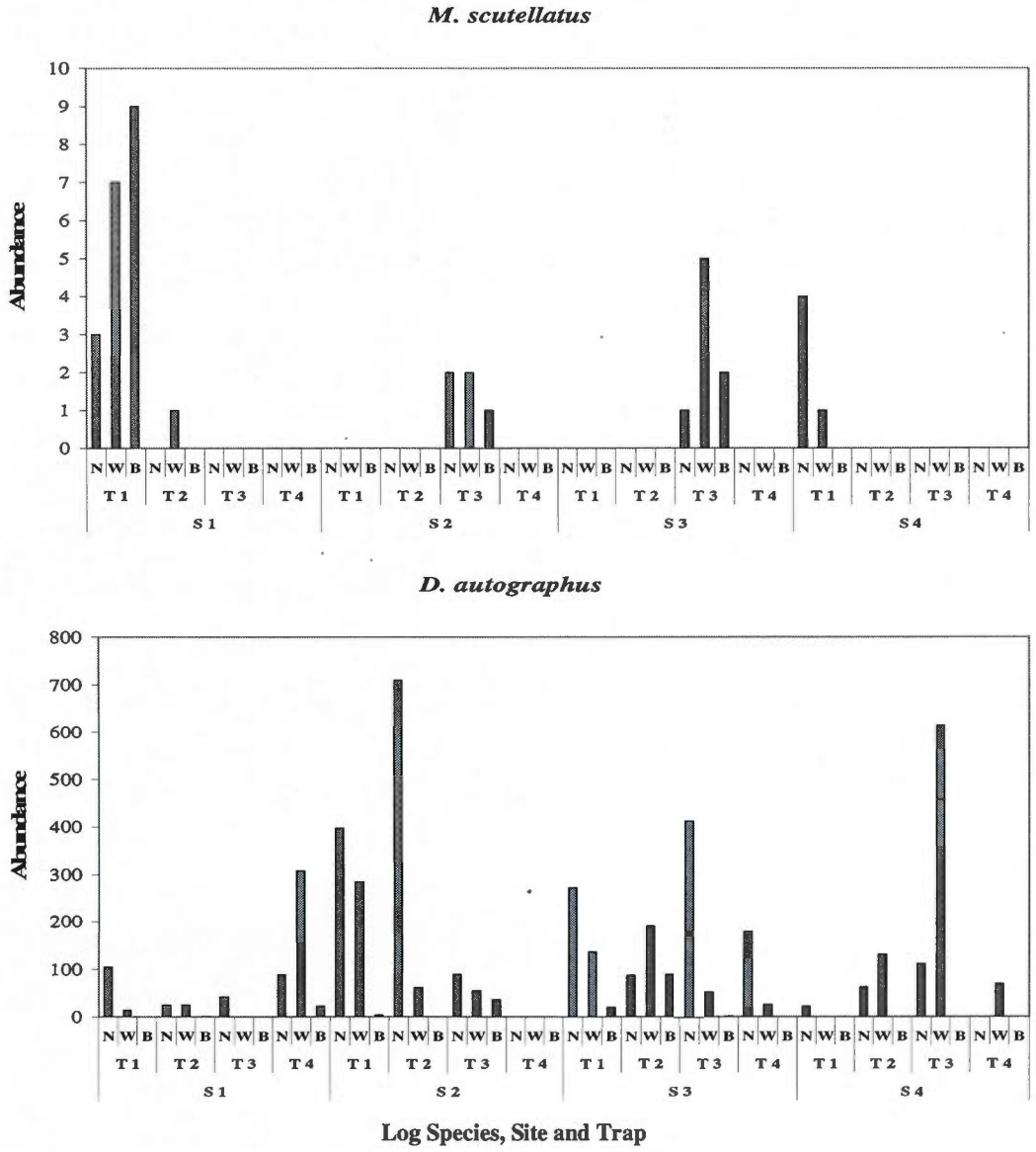

Figure 2.3 (1) Distribution of abundance in log species at different site (S) and trap (T) locations. N = Norway spruce; W = White spruce; B = Black spruce.

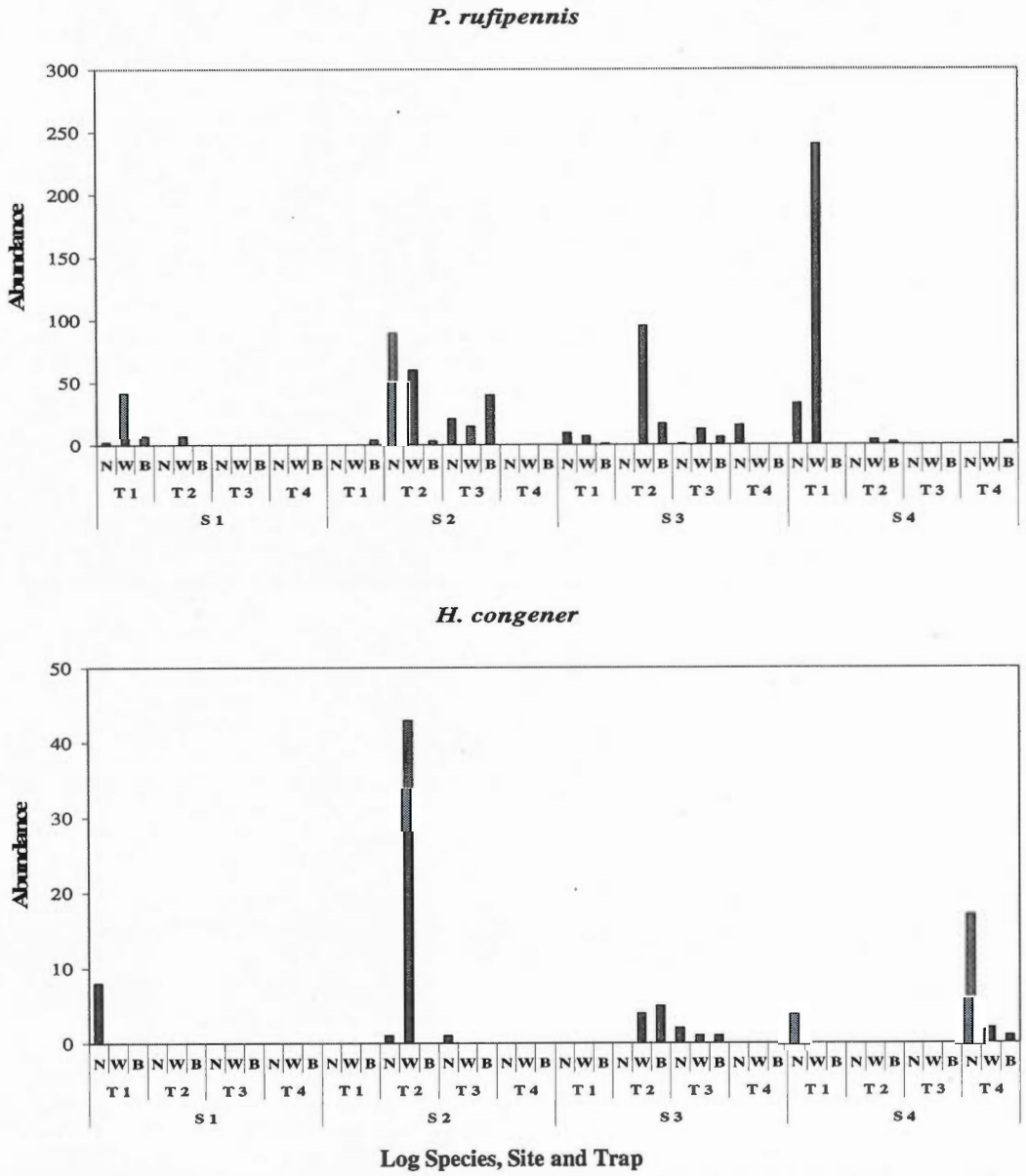

Figure 2.3 (2) Distribution of abundance in log species at different site (S) and trap (T) locations. N = Norway spruce; W = White spruce; B = Black spruce.

CONCLUSION

Le but premier de mon projet de maîtrise était de tester l'efficacité de l'irradiation aux micro-ondes comme traitement phytosanitaire sur les organismes se trouvant dans les MEB. Il s'inscrivait dans un projet plus vaste en collaboration avec le laboratoire d'Ahmed Koubba, chercheur se spécialisant en biomatériaux à l'Université du Québec en Abitibi-Témiscamingue (UQAT), et la Chaire de recherche du Canada sur la valorisation, caractérisation et transformation du bois de l'UQAT. L'objectif général de cette collaboration vise l'établissement éventuel de l'irradiation aux micro-ondes comme traitement phytosanitaire au Québec en tenant compte des aspects économiques, physiques et biologiques. Mon volet portait sur l'aspect biologique et pour atteindre ce but, j'ai sélectionné les paramètres des expériences en fonction du contexte québécois. Pour tester le traitement aux micro-ondes, j'ai sélectionné cinq espèces présentes sur le territoire de la province qui s'attaquent au bois mort, moribond ou malade et qui seraient donc susceptibles d'être transmises par voit de MEB : les larves de longicorne noir, *M. scutellatus* et les champignons *G. abietina*, *H. annosum*, *C. purpureum*, et *M. populorum,* qui dégradent le bois. Ces espèces sont la cause de dommages à des degrés variables au niveau d'arbres vivants et du bois de coupe et semblent *a priori* être des candidats susceptibles de devenir envahissants sous les bonnes conditions dans un nouveau territoire. Ensuite, me basant sur les suggestions des standards NIMP No. 15 pour les traitements de chaleur (chauffer le bois à 56°C et le tenir à cette température pendant 30 min), j'ai testé des températures autour de la norme établis de 56°C. Puisque l'utilisation du traitement par l'industrie forestière dépend essentiellement de la possibilité de réduire les coûts, j'ai testé des temps considérablement plus courts (0,5 à 3 min) que la suggestion de 30 min. J'ai aussi tenté de faire concorder le type de bois dans lequel les organismes ont été traités à celui régulièrement utilisé dans la construction de MEB en utilisant des blocs fait à partir d'essences et avec des dimensions similaires à ce dernier.

Selon mes résultats, le traitement semble pouvoir effectivement éliminer plusieurs organismes se trouvant à l'intérieur du bois et ce avec des durées beaucoup plus courtes. Les larves de longicorne noir étaient hautement susceptibles au traitement. Une température de 56°C tenue pendant deux minutes était suffisante pour obtenir 100% de mortalité dans les échantillons. Ceci concorde avec les observations d'autres études ayant testées le traitement par micro-ondes sur des larves d'autres espèces de Cerambycidae et sur le parasite de longicornes du genre *Monochamus*, le nématode du pin, dans des blocs de bois (Fleming *et al.*, 2003 ; Fleming *et al.*, 2005 ; Hoover *et al.*, 2010). Dans ces études, 62°C pendant une minute était la combinaison minimale pour obtenir 100% de mortalité.

Par contre, les pathogènes fongiques testés étaient beaucoup plus résistants. Pour *G. abietina*, *H. annosum* et *M. populorum*, les premiers cas à atteindre 100% de mortalité dans les 10 réplicats et pour une combinaison de température/temps étaient de 60°C/1 min, 55°C/1 min et 65°C/0.5 min respectivement. Cependant, dans tous les cas, de la regénération a été observée à des températures beaucoup plus hautes, allant jusqu'à 90°C chez *H. annosum* et *M. populorum*. Pour les échantillons de *C. purpureum*, il y a eu des survivants dans presque toutes les combinaisons. Ceci témoigne de la difficulté à évaluer une véritable température létale chez des organismes avec des formes et cycles de vie aussi complexes que ceux des champignons. La production de structures protectrices comme les ascospores, basidiospores et chlamydospores chez plusieurs espèces est difficile à prédire et est très variable d'une espèce à l'autre. De plus, la précision des résultats est plus diffile à évaluer étant donné les précautions additionnelles (environnement et outils stériles) à entreprendre lors des manipulations expérimentales.

Un autre obstacle à l'interprétation des données a été la mesure des variations de température lors des traitements. Certaines mesures montraient que la température variait parfois de plus de 1°C au-dessus ou en-dessous de la température désirée. Celles-ci ont été éliminées des analyses. Il y a cependant certaines combinaisons de

tempréatures/temps pour lesquelles les données de mesures ont été perdues ou n'ont pas été enregistrées. C'est le cas pour toutes les données de longicornes et pour certaines de celles de *C. purpureum* et de *M. populorum* (indiquées dans la section 1.3.1). J'ai tout de même décidé de réaliser une analyze sur les données de larves puisque je n'avais pas observé de hautes variations lors des manipulations. Pour les champignons par contre, il était important de pouvoir faire les mêmes corrections sur les données de températures pour *C. purpureum* et *M. populorum* et celles qui ont été faites sur *G. abietina* et *M. populorum* afin de pouvoir comparer les résultats. Comme compromis, j'ai inclu une analyse avec les données corrigées pour les variations de températures et une autre pour les données originales. Dans le cas de *C. purpureum* et *M. populorum*, pour lesquels des mesures de température étaient manquantes, j'ai inclu une analyze avec les données partiellement corrigées pour obtenir une idée de la variation de la température létale.

Il est évident selon les résultats – autant après l'analyse des données originales qu'avec l'analyse des données corrigées – que le traitement à irradiation aux micro-ondes n'est pas aussi efficace pour les champignons que pour les larves de longicornes. Je propose que le point le plus important du traitement devrait être qu'il soit capable d'éliminer les larves d'insectes comme les Cerambycidae, les Buprestidae et les Siricidae qui creusent à l'intérieur du bois. La mobilité des adultes émergeant leur accorde un niveau de risque plus grand que les champignons qui, eux, doivent souvent s'associer justement à des insectes pour être transportés d'un arbre à l'autre. Dans la même mesure où les conditions proposées par le standard NIMP No. 15 pour le traitement de chaleur à convection (56°C pour 30 min) ont été développées principalement pour le nématode du pin et ses insectes hôtes (Ramsfield *et al.*, 2010), des combinaisons températures/temps adaptées à l'irradiation aux micro-ondes pourraient tenir compte principalement de la menace que représente les larves d'insectes xylophages et le nématode du pin. D'un autre côté, les micro-ondes ne semblent pas nécessiter des temps de traitement aussi longs que le traitement de

chaleur à convection. Sous cet angle, il est probable que la combinaision de 56°C/30 min soit beaucoup plus efficace avec les micro-ondes qu'avec le traitement à convection et que, sans changement dans les normes par rapport au traitements de chaleur, l'irradiaiton aux micro-ondes réussirait à éliminer une proportion beaucoup plus grande de champignons pathogènes du bois. Il serait donc important d'envisager des expériences qui établiraient un rapport entre l'efficacité du traitement aux micro-ondes et celui par chaleur de convection aux mêmes combinaisons de température et temps. Avec ces données, il sera donc possible de déterminer le meilleur compromis au niveau de l'utilisation d'énergie, du temps et du niveau d'efficacité de strérilisation.

Ceci se rapporte à la deuxième partie du projet qui était plus exploratoire. Les combinaisons de température/temps létales peuvent varier d'une espèce à l'autre. Afin de déterminer les paramètres optimaux pour le traitement (température/temps), il est d'abord nécessaire d'identifier et de cibler les organismes qui courent le plus grand risque de devenir envahissants. J'ai donc tenté de le faire pour des insectes xylophages de la forêt boréale québécoise en sélectionnant un des facteurs les plus importants dans la détermination du succès d'une espèce introduite, la présence d'hôtes compatibles. J'ai placé des bûches d'épinette de Norvège dans la forêt boréale québécoise avec des bûches d'épinette blanche et d'épinette noire dans le but d'attirer et de capturer des insectes xylophages indigènes capables de sélectionner et de compléter leur cycle de vie à l'intérieur d'une essence européenne. Quatre espèces de coléoptères ont été capturées dans l'épinette de Norvège en abondances comparables à leurs abondances dans aux moins des deux essence indigènes. *M. scutellatus* a été capturé en plus grande abondance dans l'épinette blanche, mais plusieurs adultes ont tout de même émergés de l'épinette de Norvège. Le choix de cette espèce pour les expériences du chapitre 1 a donc été en quelque sorte justifié, puisqu'elle semble avoir un minimum de compatibilité avec l'essence européenne. Le Scolytinae *D. autographus*, qui est déjà présent en Eurasie, est sans surprise l'espèce qui a

démontré le plus d'affinité pour l'épinette de Norvège. Cependant, sa présence en Eurasie fait en sorte que sa réintroduction ne risquerait pas de créer plus de risques d'invasion. Le Scolytinae *P. rufipennis* et le charançon *H. congener*, qui peuvent être des pestes mineures au Canada, ont été retrouvés en proportions presque équivalentes dans les trois essences. Ils ont par contre réagi moins bien que les deux autres au froid et possiblement à la dessiccation des bûches en raison de leur entreposage à température sous le point de congélation à l'air libre plutôt que sous la neige comme en milieu naturel. Leur faible résistance aux variations environnementales pourrait être un facteur qui diminuerait leurs chances de s'établir en Europe. Le fait que *D. autographus*, *P. rufipennis* et *H. congener* sont des insectes qui se retrouvent rarement à une profondeur de plus de quelques millimètres dans le phloème diminu la possibilité qu'ils se retrouvent dans les MEB et qu'ils survivent aux traitements phytosanitaires.

Cette approche semble prometteuse parce qu'elle a confirmé le choix initial de l'espèce de longicorne à étudier. Parce contre, puisque l'expérience a été faite dans une forêt au Québec, il n'est pas possible d'identifier tous les facteurs pouvant influencer les choix des insectes indigènes pour l'essence non-indigène. Pour avoir une meilleure idée des préférences d'espèces capturées par cette méthode, il serait nécessaire de répéter l'expérience en testant les préférences en laboratoire de chaque espèce individuellement. Une autre possibilité serait de répéter l'expérience dans une forêt européenne et de comparer les traits des insectes européens capturés avec ceux des insectes nord américains.

Nous pouvons également noter que cette approche pourrait être utile pour tester la viabilité d'une plantation d'arbres non-indigènes. Plusieurs traitements différents pourraient être testés en faisant varier la composition en espèces indigènes ou exotiques, la moyenne d'âge ou l'état de santé dans différents groupes expérimentaux.

Une fois raffinée, l'approche générale proposée par la deuxième partie de ce mémoire nous permettra de dresser une liste d'espèces xylophages avec un potentiel invasif. Il sera ensuite possible de rassembler les charactéristiques leur conférant un avantage qui sont déjà connues dans la littérature ou à étudiées. L'objectif à long terme sera d'utiliser cette matrice de charactères pour identifier à l'avance le potentiel d'établissement d'insectes xylophages chez des essences non-indigènes et dans des régions étrangères. Ceci aurait des retombés au niveau des stratégies de plantations d'arbres non-indigènes ainsi que celles de contrôle et de prévention de l'introduction d'insectes non-indigènes. La recherche sur les traitements phytosanitaires, comme l'irradiation aux micro-ondes, pourrait alors cibler des espèces qui ne sont pas nécessairement encore envahissantes, mais dont nous connaissons le potentiel d'établissement.

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