Biology and Management of the Dutch Elm Disease Vector, *Hylurgopinus rufipes* Eichhoff (Coleoptera: Curculionidae) in Manitoba

by

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Abstract

Hylurgopinus rufipes, the native elm bark beetle (NEBB), is the major vector of Dutch elm disease (DED) in Manitoba. Dissections of American elms (*Ulmus americana*), in the same year as DED symptoms appeared in them, showed that NEBB constructed brood galleries in which a generation completed development, and adult NEBB carrying DED spores would probably leave the newly-symptomatic trees. Rapid removal of freshly diseased trees, completed by mid-August, will prevent spore-bearing NEBB emergence, and is recommended. The relationship between presence of NEBB in stained branch sections and the total number of NEEB per tree could be the basis for methods to prioritize trees for rapid removal.

Numbers and densities of overwintering NEBB in elm trees decreased with increasing height, with >70% of the population overwintering above ground doing so in the basal 15 cm. Substantial numbers of NEBB overwinter below the soil surface, and could be unaffected by basal spraying. Mark-recapture studies showed that frequency of spore bearing by overwintering beetles averaged 45% for the wild population and 2% for marked NEBB released from disease-free logs. Most NEBB overwintered close to their emergence site, but some traveled ≥4.8 km before wintering.

Studies comparing efficacy of insecticides showed that chlorpyrifos gave 100% control of overwintering NEBB for two years as did bifenthrin: however, permethrin and carbaryl provided transient efficacy. NEBB showed a gradual increase in development rate with increasing constant temperature. Lipid content of overwintering NEBB was higher in late fall than in mid-winter, which might show that depletion of fat reserves could jeopardize survival, but could be a result of conversion to cryoprotectants.

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Chapter 1: Introduction

Dutch elm disease (DED) is one of the most destructive diseases of most elm species throughout their range (Karnosky, 1979; Gibbs et al., 1994). Elm trees are native in many areas of the Northern Hemisphere and have been extensively planted to improve the aesthetics of streets, parks and boulevards. Prior to DED, they were the dominant ornamental tree in many cities throughout North America, Europe and China. Elms also provide wood for fuel and furniture, livestock feed, medicine and shelter, and give support for vines (Ghelardini and Santini, 2009). Regrettably, beginning early in the 20th century in western Europe, two devastating pandemics of DED afflicted the elms in Europe and North America (Brasier, 2000; Guries, 2001). The first epidemic of DED, which ended in the 1940s, was caused by *Ophiostoma ulmi* (Buisman) Nannf., while the aggressive *O. novo-ulmi* Brasier is responsible for the current epidemic (Brasier, 1991).

Dutch elm disease was first observed in the United States in 1930 in Ohio (Schlarbaum et al., 1997) where symptomatic American elms, *Ulmus americana* L., were observed with the disease (May, 1930). In Canada, the first diseased trees were found in Quebec in 1944 (Pomerleau, 1945, 1961). In western Canada, DED was first identified in Manitoba in 1975 (Ives and Petty, 1976; Hildahl, 1977) and in Saskatchewan in 1981 (Sterner and Davidson, 1982). In Alberta, one diseased elm tree has been reported (Tewari et al., 2001).

The Dutch elm disease fungus is transmitted from tree to tree by beetle vectors and occasionally through root grafts (Rioux, 2003). In Manitoba, the native elm bark beetle, *Hylurgopinus rufipes* Eichhoff. (Coleoptera: Curculionidae: Scolytinae) is the

major vector of *O. ulmi* and *O. novo-ulmi* (Hildahl, 1977), although a small number of the smaller European elm bark beetle, *Scolytus multistriatus* Marsham (Coleoptera: Curculionidae: Scolytinae) have been caught in pheromone traps in Manitoba (Westwood, 1991a; Pines, 2009). Recently, there has been concern about the potential of the invasive alien species *Scolytus schevyrewi* (Semenov) to vector DED (Jacobi et al., 2007); however, in Manitoba and Saskatchewan this species avoids American elm and prefers Siberian elm (*Ulmus pumila* L.), which does not become infected with DED (Veilleux, 2012) . Information on the life history of *H. rufipes* in Manitoba is summarized in Fig. 1, and the major life stages are pictured in Figs. 2−4. In Manitoba, *H. rufipes* overwinters as an adult (Anderson, 1996) (Fig. 2) at the base of healthy elm trees (Anderson and Holliday, 2003). In spring, adults move to elm crowns where their feeding on twigs allows any pathogen spores they carry to infect the trees (Hildahl, 1977; Takai et al., 1979). After crown feeding, adults construct brood galleries in dead or dying elm trees and females lay eggs in niches at the side of the galleries (Swedenborg et al., 1988) (Fig. 3). Larvae (Fig. 4) tunnel outwards from the parental gallery and after completion of feeding they pupate. The new generation adults emerge and move to healthy elms to feed and overwinter.

In Manitoba and Saskatchewan, although naturally-occurring elm has a restricted distribution along lakes and in river valleys, elm is an important shade tree and has been planted extensively in urban areas (Davidson et al., 1964). Since DED was first detected in 1975 in Manitoba (Hildahl, 1977), provincial and municipal governments have been actively involved in DED management programs (Westwood, 1991a) which focus on elms in and around urban areas. As part of this program more than 270,000 diseased elms

have been removed since 1975 (Westwood, 1991a; City of Winnipeg, 2008a). Currently, about 5,000 diseased trees are removed annually by the Urban Forestry Branch of the City of Winnipeg (City of Winnipeg, 2008b). The main challenge is to find sustainable and cost effective ways to reduce the rate of loss of the remaining elm trees in Manitoba in order to preserve them as an important urban resource for future generations. Despite the vigor of the management program, elm trees continue to be killed at a rate of 2−3% per annum, and improved performance of the management program is desirable in order to maintain annual losses at 2% or less (Barwinsky and Domke, 2012).

A major component of management programs for DED is the management of the vector beetles. Vector management includes removal and destruction of dead or diseased trees to prevent them from becoming sources of beetles transporting pathogen spores. Another aspect of vector management is insecticidal control of beetles in overwintering sites at the base of healthy elm trees. Improvements in the efficacy of both these management options would help lower annual elm loss rates further.

The tree removal program is carried out by identifying and tagging infected elms in summer and then removing them before the following spring. For logistic reasons, most trees are removed after the onset of winter. Removals on this schedule eliminate sources of inoculum in years following disease symptom detection but would not prevent disease transmission if spore-carrying beetles emerged from infected trees in the same year as symptom detection. It has generally been assumed that, in Manitoba, *H. rufipes* seldom breed and produce offspring in an infected tree in the same year that symptoms are detected. However, the City of Winnipeg conducted a small study of trees that were identified as >50% dead and were removed in winter. Of these trees, about 67% were

infected with DED, and 38% (up to 55% in some sites) also contained considerable numbers of brood galleries, some of which had exit holes (Robbie-Draward, 1995). The study raised the question of whether breeding in recently infected elms is significant enough to contribute substantially to the population of overwintering *H. rufipes*, and whether emerging beetles carry the pathogen. If newly-diagnosed trees are a major source of *H. rufipes*, and particularly if many of these beetles are spore-bearing, a program of "rapid removal", in which diagnosed trees are removed immediately, may be warranted. "Rapid removal" is regarded as the most efficient DED management technique (Stipes, 2000), but the associated logistical challenges of removing large numbers of trees in a timely manner are considerable. An evaluation is needed of whether, under Manitoba conditions, rapid removal would provide benefits outweighing these difficulties.

Insecticide treatments to the base of elm trees are effective in controlling overwintering *H. rufipes* (Gardiner, 1976a; Gardiner and Webb, 1980; Lanier et al., 1984). The control of overwintering populations of *H. rufipes* is a major component of DED management in the prairies (Westwood, 1991a). Originally, insecticidal applications to control overwintering *H. rufipes* were made to the basal 2–3 m of elms (Gardiner and Webb, 1980). However, Anderson and Holliday (2003) found that the number of beetles found at heights above 55 cm was negligible and so insecticide applications above this height are unnecessary. Consequently, Anderson and Holliday (2003) recommended that applications of insecticides should be restricted to the bottom 55 cm of each tree to focus on the target beetle to maximize the effect of the treatment. Reducing the amount of bark area treated with insecticides would significantly reduce cost and environmental impact (Anderson and Holliday, 2003).

Chlorpyrifos is the only active ingredient registered for basal applications for beetle control in Canada (Health Canada, 2010) and its persistence has been demonstrated (Jin et al., 1996; Oghiakhe and Holliday, 2011). However, concern about the toxicity and deleterious environmental effects of organophosphate pesticides has affected their use especially among home owners. Chlorpyrifos is one of the organophosphate pesticides under re-evaluation in Canada, as announced by the Pest Management Regulatory Agency (PMRA) in June 1999 in the Re-evaluation Note REV99-01, *Re-evaluation of Organophosphate Pesticides* (Health Canada, 2000a). Identification of a less acutely toxic alternative to chlorpyrifos for basal spraying would make the public more accepting of this management tool and increase its usage. Since the appearance in the media of adverse publicity on the use of chlorpyrifos, public apprehension about basal application has continued to intensify (City of Winnipeg, 2011, 2013; Manitoba Government, 2012). Such anxiety adds to the difficulty of using even registered products to spray elms on privately owned property. Restricted access to conduct basal applications to elms on private property impairs the effectiveness of the overall management program.

It is important to identify potential areas of improvement in Manitoba's existing DED management program, which has remained largely unchanged since DED was first reported in the Province 38 years ago. Improving the program and reducing the rate of tree loss are safeguards against political decision makers deciding that the program is not cost-effective and terminating it.

There were three major objectives of my thesis research:

1. To determine whether *H. rufipes* can complete development in newly-diagnosed elm trees in the same growing season as symptoms become detectable, and can emerge carrying spores; and if this occurs, to investigate ways of predicting which symptomatic trees are likely to contribute most to the population of spore-bearing beetles.
2. To investigate fine-scale vertical distribution and other characteristics of the overwintering biology of *H. rufipes* in Manitoba.
3. To examine alternatives to chlorpyrifos and to determine their suitability for control operations, particularly with respect to their persistence of efficacy against native elm bark beetles.

Each objective is the subject of a research chapter, respectively chapters 3−5, and chapter 5 has already been published. In addition chapter 2 is a review of relevant literature, chapter 6 is the general discussion and chapter 7 contains two appendices documenting research results that are not considered publishable, but could be useful to future DED researchers, particularly in the Prairie Provinces.

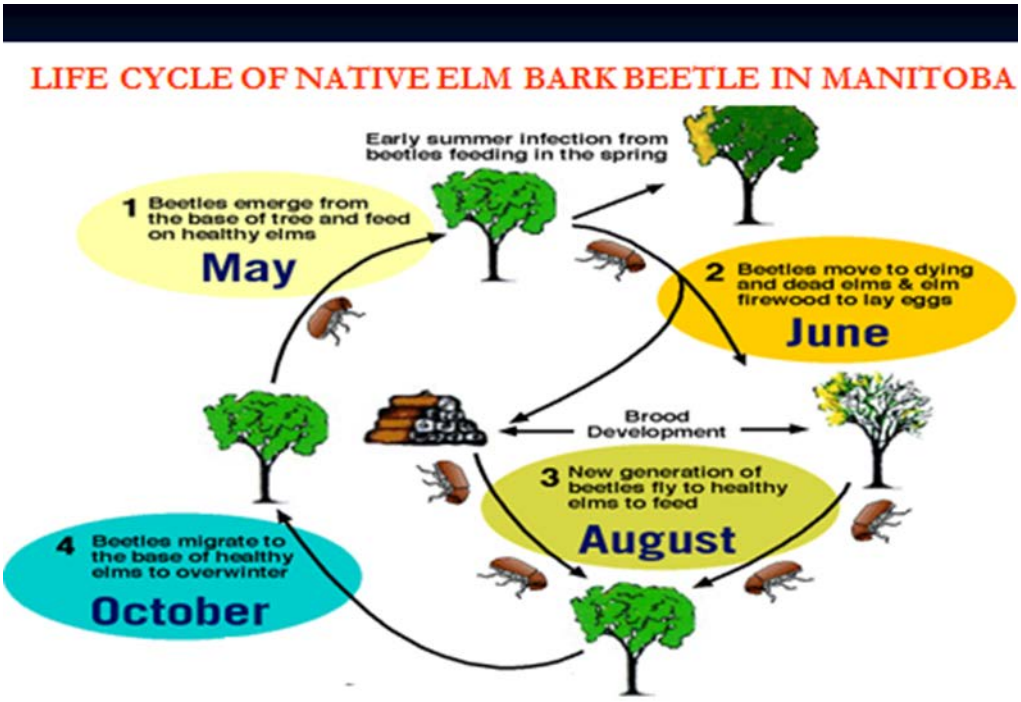

Figure 1. Life cycle of *Hylurgopinus rufipes* in Manitoba. Image credit: Jon Leferink, Manitoba Conservation and Water Stewardship, Winnipeg. Used with permission.

Figure 2. Lateral view of adult *Hylurgopinus rufipes*. Photo credit: Baker and Bambara, North Carolina State University, Bugwood.org (Creative Commons Licensing).

Figure 3. Female *Hylurgopinus rufipes* surrounded by newly laid eggs in niches. Photo credit: S. Oghiakhe.

Figure 4. Larvae of *Hylurgopinus rufipes* in their galleries in the laboratory. Photo credit: S. Oghiakhe.

Chapter 2: Literature review

Introduction and review objectives

Dutch elm disease (DED) is a fatal disease of most species of elm trees. The arrival of the disease in North America near the beginning of the 20th century and the practice of growing elms in monocultures along city streets have caused a rapid spread of the disease, destroying millions of elms in forests, shelterbelts and cities around the world (Dunn, 2000). In Canada, DED was first detected in Quebec in 1944 and spread to Ontario in 1946 (Pomerleau, 1961, 1965a), New Brunswick in 1957 (Davidson and Newell, 1957), Manitoba in 1975 (Ives and Petty, 1976; Hildahl, 1977) and Saskatchewan in 1981 (Sterner and Davidson, 1982; Schacherl, 1996). In Alberta, one diseased elm tree was found in 1998 and was promptly removed (Tewari et al., 2001).

The first report of DED in St. Paul, Minnesota was in 1961 and within the next two decades, Minnesota had lost between 10 and 20 percent of its 140 million elms to the disease (French et al., 1980). In Minnesota, annual losses to DED have varied between 1% and 3% in the past 20 years in communities that have effective control programs, but it is estimated that 1 million elms remain with a resource value exceeding 1 billion dollars (Minnesota Department of Agriculture, 2013). Since the disease was first detected in 1975 at Brandon, Selkirk and along the Red River in Wildwood Park in Winnipeg (Hildahl, 1977), provincial and local authorities have been proactively involved in a DED management program (Jeffrey, 1982; Westwood, 1991a). In 1975, the year that the disease was detected, the Province of Manitoba introduced the Dutch elm disease management program designed to curtail the spread of the disease and help communities

rebuild their urban forests. Later, the Dutch elm disease Act was passed by the Manitoba legislature on 29 June, 1998 (Manitoba Government, 1998) providing the regulatory and enforcement framework to implement the management strategies for DED.

The fungi that cause DED, *Ophiostoma ulmi* (Buisman) Nannf. and *O novo-ulmi* (Brasier), enter the xylem vessels of small branches when scolytine vectors feed. In North America, these vectors are the smaller European elm bark beetle, *Scolytus multistriatus* (Marsham), and the native elm bark beetle, *Hylurgopinus rufipes* (Eichhoff). Initial symptoms of DED are wilting and yellowing of leaves, which is termed flagging. Later, DED results in death of the branches and ultimately the entire tree (USDA, 1975; Gibbs and Smith, 1978).

Methods for managing DED in the prairies are similar in different communities. However, there have been varying levels of success in different jurisdictions, perhaps because of differences in budgetary allocations, program priority and available expertise. In general, the main elements of the DED control program in the Prairie Provinces include: surveillance, sampling, pruning, sanitation, basal trunk treatment, beetle trapping, legislation, public awareness and education, cost-share agreements with communities, buffer zones, integrated management, reforestation and research (Westwood, 1991a).

In 2008, total program expenditure for DED control in Manitoba (outside of the City of Winnipeg) was approximately CAD $2.4 million and operated in 38 cost sharing communities and in buffer zones surrounding the Cities of Winnipeg and Brandon, both of which have their own management programs (Pines, 2009). In 2008, the City of Winnipeg allocated about $3 million annually to control DED (Domke, 2005; City of

Winnipeg, 2008a). The costs of the management programs are small compared to the value of the elm resource they are designed to protect.

My thesis research was intended to facilitate improvements to the current DED management program, which has remained effectively unchanged since the disease was first reported in Manitoba. Innovative, environmentally sound and cost effective solutions are imperative due to increasing budgetary constraints for urban forestry, among other competing needs for funding. Without improvements to the program, there is a danger that the costs of maintaining an acceptable rate of tree loss may become unsustainable.

There is a vast amount of literature published on DED. Studies on DED vectors over a broad range of geographical locations underscore their importance in disease transmission especially in Europe and North America: Pertinent examples are Finnegan (1957), Sinclair and Campana (1978), Gibbs (1978, 1979), Anderbrant and Schlyter (1987), Webber (1990, 2000), Basset et al. (1992), Brasier (2000, 2001), McLeod et al. (2005), and Lefévre et al. (2006). This review will focus on hosts, vectors and pathogens that are most relevant to the current situation in Manitoba.

Biology of the organisms involved in Dutch elm disease

Biology of American elm, *Ulmus americana* L. (Ulmaceae)

American elm is a tall, graceful tree that occurs naturally in a variety of habitats across much of the eastern United States and Canada (Little, 1971; Bey, 1990). Varieties include American elm or white elm (*U. americana* var. *americana*) found throughout eastern North America (Little, 1971; Bey, 1990), and Florida elm (*U. americana* var. *floridana*

Chapman), which occurs in coastal areas from North Carolina to central Florida (Duncan and Duncan, 1988; Godfrey, 1988,).

Shattuck (1905) provides important early information on the biology of American elm through his microscopic study of morphology during the period of fertilization and embryo growth. Bey (1990) provides an account of the properties of *U. americana* from which much of the following is derived. Throughout its range, flower production, seed maturity and seed fall of *U. americana* occur in the spring. Flowers are wind-pollinated, and seed fall is mostly finished by the middle of March in the South and by the middle of June in the North. *Ulmus americana* trees are largely self-sterile and only about 1.5 % of self-pollinated flowers produce viable seed in Canada (Bey, 1990). Pollination is reduced when spring is wet because anthers do not open in humid air. (Lee and Lester, 1974)

Ulmus americana trees may begin to produce seed by 15 years of age; and seed production is prolific from about 40 years of age and may continue until trees are 300 years old (Bey, 1990). Seeds are winged, lightweight and easily dispersed by the wind. Seeds germinate on the soil surface, usually within 6 to 12 days of dispersal, but germination may occur as late as 60 days after dispersal. In a few seeds germination may be delayed to the next spring. Optimum germination occurs when night and day temperatures are 20 °C and 30 °C, respectively. Mineral soil presents the best medium for establishment of American elm seedlings (Bey, 1990).

On good sites, trees may grow to a height of 30–38 m and reach a diameter at breast height (1.5 m above ground level) of 122 –152 cm; trees frequently reach 24 m on medium sites. In open or scattered stands, most *U americana* bifurcate close to the

ground and produce broad canopies. American elms frequently live 175 –200 years, and trees more than 300 years old have been reported (Bey, 1990).

Research on the genetics of *Ulmus* has focused on breeding trees with DED and phloem necrosis resistance, and integrating the resistance with attractive plant qualities (Bey, 1990). Despite high selection intensity, DED resistance of *U. americana* cultivars is of lower quality compared to resistant trees obtained from Asian or European species (Bey, 1990). It is difficult to hybridize *U. americana* because the species has a chromosome number which is double that of all the other elms, therefore most crosses exclude *U. americana* (Bey, 1990). Ager and Guries (1982) studied barriers to interspecific hybridization between *U. americana*, which is tetraploid, and five elm species (diploid) and report self-incompatibility in one *U. americana* in which there was inhibition of germination of pollen and of pollen tube on the stigma. Efforts to hybridize *U. americana* with *U. pumila* have not been successful (Santamour, 1970; Townsend and Santamour Jr, 1993). Whittemore and Olsen (2011) used flow cytometry to evaluate 81 wild *U. americana* from throughout the range of the species and four cultivated trees from the northeastern United States and the central Atlantic coastal plain: most specimens were tetraploid, as previously found, but 21% of the wild trees sampled were diploid, a ploidy level not previously confirmed for the species. Whittemore and Olsen (2011) conclude that *U. americana* is genetically heterogeneous, and recommend further study of the origin and relations of the different ploidy levels as a promising approach to developing DED resistant *U. americana*.

Methods involving genetic engineering and biotechnology are currently being evaluated to determine whether DED-resistant elms can be obtained by introducing

desirable genes into elms of interest (Shukla et al., 2012; Khoshraftar et al., 2013), and it is possible these techniques could be used to produce DED resistant *U. americana*. Bolyard and Sticklen (1993) report on the regeneration of American elms from leaf explants. Cerato-ulmin, a toxin from *O. ulmi*, has been produced in an expression vector in *E. coli* and has been shown to have biological activity. Altered forms of this toxin could be used as non-toxic competitive inhibitors of cerato-ulmin and DNA-encoding inhibitors could then be engineered into elms for expression of the inhibitors (Bolyard and Sticklen, 1993). The ß-glucuronidase reporter gene has been used to establish a transformation system for producing transgenic American elms, and promising preliminary results were obtained (Bolyard and Sticklen, 1993).

A technique has been developed for conserving germplasm from old American elm trees that have withstood DED epidemics and have the potential to produce disease resistant trees (Shukla et al., 2012). The method uses *in vitro* propagation of buds from old trees for cloning (Shukla et al., 2012). Resulting rooted plantlets grown in the greenhouse environment have a 90% survival rate. The technique developed for American elm will aid in increasing resistant clones, facilitate long-term conservation of elite genotypes, and could also be used in conservation of tree species threatened with extinction (Shukla et al., 2012).

Hubbes (2004) has shown that elm seedlings and 15–20 year old trees acquire resistance to *O. novo-ulmi* when injected with non-aggressive *O. ulmi*. The effectiveness of the acquired resistance depends on the tree's genetic composition and health, and on environmental factors. Bernier and Hubbes (1994) tested the lethal and mutagenic effects of ultraviolet irradiation on *O. ulmi sensu lato*. Ultraviolet treatment raised the prevalence

of mutants in five natural progenies representing *O. ulmi* and *O. novo-ulmi*; thus, ultraviolet treatment might yield fungal mutants of value in programs for developing induced or constitutive resistance (Bernier and Hubbes, 1994).

The 31.5 Mb genome of *Ophiostoma ulmi* has been sequenced and estimates show about 8,639 putative genes (Khoshraftar et al., 2013). Khoshraftar et al. (2013) were able to screen for genes linked to virulence and hypothesize metabolic pathways related to virulence enzymes. Sequencing of the genome will facilitate understanding of the genetic basis of pathogenicity by *O. ulmi* and is in line with the view of Brasier (1983a) that effective breeding for resistance requires an understanding of the genetic basis of pathogenicity and the range of pathogenic variation. Currently, the geographical centre of origin of DED is unknown, although is thought to be in eastern Asia (Heybroek, 1976). The centre of origin may well be the centre of fungal diversity, and hence the source of new strains and races of the pathogen which could attack European and American elms, including disease resistant ones, in future (Brasier, 1983a). With the progress being made to produce elms that are resistant to DED using genetic engineering techniques, I expect in the next decade there will be disease resistant American elm cultivars with suitable agronomic characteristics for planting.

Biology of *Hylurgopinus rufipes* (Eichhoff)

According to Poole and Gentili (1996) and Arnett et al. (2002), *Hylurgopinus rufipes*, belongs to Superfamily Curculionoidea, family Curculionidae, and is in the subfamily Scolytinae. Previously, Scolytinae was termed Scolytidae as it was considered to be a family, but now it is recognized that bark beetles, although highly specialized, are within the family of true weevils, Curculionidae. Further information on the taxonomy of *H.*

rufipes has been provided by Eichhoff (1869), Wood (1979) and Rabaglia and Lanier (1981).

Hylurgopinus rufipes is the major vector of DED in Manitoba (Hildahl and Wong, 1965; Hildahl, 1977) and Saskatchewan (Schacherl, 1996). Information on the life history and habits of *H. rufipes* in Manitoba has been published by Hildahl (1977), Anderson (1996) and Anderson and Holliday (2003); in Ontario, by Finnegan (1957); in Minnesota by Landwehr et al. (1981) and Swedenborg et al. (1988); in New York by Martin (1938) and Thompson and Matthysse (1972) and in Connecticut by Kaston (1939).

A thorough description of the life stages of *H. rufipes* has been completed by Kaston (1936) in Connecticut. *Hylurgopinus rufipes* adults excavate galleries that are oriented across the grain of the wood (Kaston, 1939; Lanier, 1978). Eggs may be laid closely packed on both sides of the gallery (Kaston, 1939). Eggs are pearly white and shiny (Kaston, 1936). They are oblong to oval and measure about 0.66 mm by 0.38 mm (Kaston, 1936). Larvae of *H. rufipes* develop through either five or six instars (Kaston, 1939). Larvae are legless, white grubs with amber-coloured head capsules (Kaston, 1936; Lanier, 1978). The larval body is curved and is capable of contracting and expanding substantially (Kaston, 1936). Full grown larvae can reach 3.5 to 4.0 mm in length with a head capsule measuring 0.8 to 0.9 mm wide (Kaston, 1936). The width of the head capsule is approximately 3/4 that of the body and can be differentiated from the larvae of the *Scolytus multistriatus*, where the head capsule is 1/2 the width of the body (Lanier, 1978). Mean duration of larval development varies from a minimum of 29 days at 24.5 $^{\circ}$C under laboratory conditions, to about 40 or 50 days under ambient conditions, in the field during the summer months (Kaston, 1939).

The appearance of the pupal stage of *H. rufipes* varies depending on size of the pupal chamber and stage of pupal development (Kaston, 1936). The total length of the pupa, including caudal spines, is about 3.3 mm and the pupa is about 1.5 mm at the widest point (Kaston, 1936). The body bears a variable number of setae, which are as pronounced at the end of the pupal period as at the beginning (Kaston, 1936). The colour of the head region changes gradually from white to brownish red as the pupa ages, but the pupal body remains white until adult eclosion (Kaston, 1939). Pupae can be sexed by comparing the seventh and eighth abdominal tergites (Kaston, 1936). In females, tergite seven is enlarged and a posterior portion of tergite eight is exposed. In males, tergites seven and eight are equal in length. The mean duration of the pupal period at 24.5 $^{\circ}$C and 65% RH, is 7.26 days (Kaston, 1939).

Length of adult *H. rufipes* varies depending on sex: in males, mean length of body from vertex to posterior border of elytra is about 2.5 ± 0.09 mm, while in females, mean length is about 2.6 ± 0.10 mm (Kaston, 1936). The adult is cylindrical, brownish-black and thinly clothed with short stiff yellow hairs; the club of the antennae is sub-ovate, nearly twice as long as wide, and more shiny towards the base (Kaston, 1936). Newly emerged adults are more evenly brown with the head alone darker than the rest of the body (Kaston, 1936). Abdominal characteristics of male and female *H. rufipes* show distinct differences. In males, the seventh tergite features processes that serve as stridulatory plectra, whereas the seventh tergite of females is rounded (Kaston, 1936). A series of parallel ridges on the underside of the apex of the left elytron, found in both males and females, serves as the stridulatory *pars stridens* (Kaston, 1936). Stridulation can be used to determine sex in new adults with more than 99% accuracy (Lyons, 1982).

Stridulation does not occur in female beetles (Lyons, 1982; Swedenborg et al., 1988; 1989). According to Lanier (1978), adult *H. rufipes* may be distinguished from *S. multistriatus* by the shape of the abdomen; the former has a rounded convex shape and the latter a concave posterior.

Overwintering

Kaston (1939) showed that overwintering *H. rufipes* larvae can survive exposure to −28 °C; however, none of the larvae that have already begun spring feeding are able to survive freezing conditions. Overwintering larvae start to develop as temperature permits, complete development, and emerge as adults in June and July (Finnegan, 1957; Thompson and Matthysse, 1972; Lanier, 1978). These adults produce offspring that may either overwinter as larvae or adults depending on the rate of development (Landwehr et al., 1982). Localities where *H. rufipes* larval overwintering occurs include southwestern Ontario (Finnegan, 1957) and New York (Thompson and Matthysse, 1972).

Becker (1939) noticed that adult *H. rufipes* overwinter in healthy elm trees. In Connecticut, based on dissection of a healthy tree in winter, overwintering occurs at various heights above ground, but the density and percentage of survival of adults is greatest in the lower part of the tree where the bark is thick and lowest in the upper part where bark is thin (Kaston, 1939). In Massachusetts, the greatest numbers of overwintering tunnels are found near the ground on the trunk and on the exposed roots of elm trees (Becker, 1935). In Manitoba, *H. rufipes* overwinters as an adult in the basal 55 cm of healthy elm trees (Anderson and Holliday, 2003). Other authors have found a greater number of overwintering beetles near the ground; unfortunately, quantitative data were not reported (Thompson and Matthysse, 1972; Gardiner and Webb, 1980; Strobel

and Lanier, 1981). Adult overwintering has been reported in Manitoba (Anderson, 1996), Saskatchewan (Schacherl, 1996), southwestern Ontario (Finnegan, 1957), New Brunswick (Magasi et al., 1993), central Minnesota (Swedenborg et al., 1988), and Connecticut (Kaston, 1939).

The proportion of *H. rufipes* overwintering in the adult or larval stage varies among regions in North America (Finnegan, 1957; Thompson and Matthysse, 1972; Takai et al., 1979; Swedenborg et al., 1988). In southwestern Ontario, *H. rufipes* has one generation and an incomplete second generation per year (Finnegan, 1957); in Connecticut, it has one and a half generations per year (Kaston, 1939). In Minnesota, there is generally a single generation with winter spent in the adult stage, although in some circumstances and locations larval overwintering occurs (Landwehr et al., 1982; Swedenborg et al., 1988). In Manitoba, there is one generation per year and *H. rufipes* overwinters only as an adult (Anderson, 1996).

Life cycle following adult overwintering

Overwintering *Hylurgopinus rufipes* adults are active in the spring and burrow in their overwintering tunnels before emerging (Kaston, 1939; Thompson and Matthysse, 1972; Lanier, 1978). At about 20 $^{\circ}$C, beetles leave their tunnels and begin to fly (Lanier, 1978), usually in late April or early May. In Manitoba, adult emergence occurs from mid-April to early June (Hildahl, 1977).

Following emergence, adults move to the crowns of healthy American elm to feed (Kaston, 1939; Lanier, 1978). More beetles are attracted to trees with pruning wounds than to those without wounds (Landwehr et al., 1981; 1982). Feeding grooves are usually formed in branches measuring 2 to 10 cm diameter (Thompson and Matthysse, 1972;

Lanier, 1978). The feeding tunnels usually penetrate to the phloem layer and score the wood (Lanier, 1978). During feeding, adults carrying *O. novo-ulmi* pathogens on their body transfer the spores into the xylem of healthy elms (Hildahl, 1977; Takai et al., 1979; Gardiner, 1981).

After feeding, adults move into larger diameter branches or stems of dead or dying elm trees to construct brood galleries and lay eggs in niches at the side of the galleries (Kaston, 1939; Thompson and Matthysse, 1972; Lanier, 1982**;** Swedenborg et al., 1988). Egg galleries may also be constructed in recently cut elm wood (Hildahl, 1977). In many areas, gallery construction and oviposition occurs in May and June. *Hylurgopinus rufipes* is a poor disperser and prefers forest habitat and shaded areas such as woodlots, riverbanks, and shelterbelts (Martin, 1938; Kaston, 1939; Lanier, 1978). Hosts that are stressed or moribund or cut elms are mostly suitable for breeding, and healthy elms are not used for oviposition (Lanier, 1982; Millar et al., 1986). The attractiveness of elm logs is closely related to their moisture content, and the underside of logs suffers more attack than the upper because of the heat from the sun (Kaston, 1939).

Bark beetles of species that mass attack to overcome healthy trees have highly-developed communications systems but, in contrast, beetles such as *H. rufipes* that construct brood galleries in moribund trees have simple systems of chemical and acoustical communication (Rudinsky and Ryker, 1977; Swedenborg et al., 1989). Attraction of *H. rufipes* to elm trees is mediated by host volatiles (Gardiner, 1979; Peacock, 1979; Millar et al., 1986; Swedenborg et al., 1988), and *O. novo-ulmi* metabolically exploits diseased elms to raise output of four volatile semiochemicals, thereby increasing their attractiveness to *H. rufipes* (McLeod et al., 2005). There may

also be a short-range pheromone used by the male beetle to find the female gallery (Lanier, 1982, 1983; Swedenborg et al., 1988), although this has not been identified. Swedenborg et al. (1989) report three different types of stridulation by male *H. rufipes*, one that occurs in response to stress, a second prior to copulation at the entrance to the gallery, and a third in response to rival males. Swedenborg et al. (1988) outlined a sequence of chemical and acoustic behavior in brood wood colonization by *H. rufipes*: female *H. rufipes* are attracted by tree volatiles and initiate galleries, males are also attracted to tree volatiles and use a pheromone to find the gallery entrance, at the gallery entrance, male beetles stridulate and touch the female beetles which signifies their presence and willingness to copulate. Other males arriving after the first may challenge the residing male beetle with rivalry stridulation. The gallery ceases to be attractive to male beetles after a while and the resident pair of beetles continues with gallery construction.

Hylurgopinus rufipes egg galleries are formed in the inner bark of the host and generally have an entrance hole near the centre of the gallery with two tunnels extending across the grain of the wood (Becker, 1935; Kaston, 1939). Eggs are laid in niches along both sides of the gallery (Becker, 1935; Kaston, 1939). The first egg is laid about a week after the entrance tunnel is started and as many as six eggs may be laid in one day (Kaston, 1939). Female beetles lay a single set of eggs in a season and the parent beetles die that season (Kaston, 1939). Incubation depends on temperature, but eggs usually hatch after about a week (Kaston, 1939).

Larvae of *H. rufipes* feed along the wood grain, perpendicular to the egg gallery (Kaston, 1939). At completion, larval tunnels are usually between 50 and 65 mm in

length, although there are variations (Kaston, 1939). Larval development is completed by late June or early July in Connecticut, but may extend into August in the northern parts of the beetle's range (Kaston, 1939). The pupal stage lasts about one to two weeks, depending on temperature (Kaston, 1939). Emergence of new adults occurs over several months, usually peaking in late July and early August in Connecticut; adult emergence may be earlier if temperatures have been high, or may be later in colder climates (Kaston, 1939). Early-emerging adults may construct brood galleries and give rise to a generation that overwinters in the larval stage (Kaston, 1939), but there is no evidence that this occurs in Manitoba (Anderson, 1996). *Hylurgopinus rufipes* adults emerging from pupal chambers in late summer fly to healthy elms and excavate feeding tunnels (Becker, 1935; Kaston, 1939). These feeding tunnels exist in every part of the tree, but are less frequent in the crown than in the lower trunk and large limbs (Becker, 1939). Feeding persists into September, when the beetles look for sites to overwinter (Finnegan, 1957; Lanier, 1983). As the temperature drops, *H. rufipes* adults move from the branches to the base of the tree to overwinter (Lanier, 1983; Anderson and Holliday, 2003). In Manitoba, adults may construct new tunnels near the base of trees as late as the end of October (Anderson, 1996).

Biology of *Ophiostoma novo-ulmi*

Taxonomy and nomenclature of Ophiostoma novo-ulmi

Because of the successive discovery of its various developmental stages, the nomenclature of the fungus that causes DED has undergone many changes over the years (Buchel and Cornelissen, 2000). After the discovery of DED in 1919 by Spierenburg (1921), the fungus was isolated from a symptomatic tree and named *Graphium ulmi*

Schwarz (Schwarz, 1922); subsequently Wollenweber (1927) and Buisman (1928) showed that this fungus is the causal agent of DED. Buisman (1932) identified the sexual stage of *G. ulmi* and renamed the fungus *Ceratostomella ulmi* (Schwarz) Buisman. In 1934, Nannfeldt further revised it from *Ceratostomella ulmi* to *Ophiostoma ulmi* (Buism.) Nannf. (Melin and Nannfeldt, 1934). Moreau (1952), Hunt (1956) and Upadhyay (1981) suggested the name *Ceratocystis ulmi* (Buisman) C. Moreau. However, based on the morphology of the asexual states (anamorphs) of the fungus, the name *Ophiostoma ulmi* (Buism.) Nannfeld is preferred (Weijman and De Hoog, 1975; De Hoog and Scheffer, 1984), as it embraces all developmental stages of the fungus (Sinclair and Campana, 1978).

The latest examination of the classification of the genus *Ophiostoma* within the Ascomycota division has been provided by Agrios (1997). The genus *Ophiostoma*, which comprises more than a hundred species (Hausner et al., 1993; Okada et al., 1998), belongs to the class Pyrenomycetes, order Ophiostomales. Van Wyk and Wingfield (1990) introduced Ophiostomales to refer to the related genera *Ophiostoma, Ceratocystis sensu stricto,* and *Ceratocystiopsis.* Species in the genus *Ophiostoma* are unusual within the Ascomycetes because of the presence of chitin and cellulose, rather than only chitin, in the cell wall (Cherif et al., 1993). Besides the ascospores (sexual spores), *Ophiostoma* species produce three different asexual (anamorph) spore types: *Pesotum*-type spores, *Sporothrix* (Cephalosporium)-type spores, and yeast-like spores (Van Wyk and Wingfield, 1990; Agrios, 1997).

Three species of *Ophiostoma* have been found to cause DED: *O. ulmi* (Buism.) Nannfeld, *O. novo-ulmi* Brasier and *O. himal-ulmi* Brasier (Brasier, 1991; Brasier and

Mehrotra, 1995). *Ophiostoma ulmi sensu lato (s.l.)* applies to the first two species (Dewar and Bernier, 1993). *Ophiostoma novo-ulmi* occurs as two separate races, the Eurasian and the North American (Houston, 1985; Brasier, 1991, 2001), which are now designated as subspecies (Brasier and Kirk, 2001).

Life cycle of Ophiostoma ulmi sensu lato

Detailed accounts of the life cycle of *Ophiostoma ulmi s.l.* have been presented by Gibbs and Smith (1978), Lea and Brasier (1983), Webber and Brasier (1984) and Webber et al. (1987). The following is a summary of this information. Except when existing as spores on the surface of a beetle vector, for its entire life cycle (Fig. 5), the fungus remains associated with the elm tree. During that association, *Ophiostoma ulmi s.l.* occurs in either pathogenic or saprophytic stages.

The pathogenic stage involves invasion and spread of *O. ulmi s.l.* in the xylem of infected elms (Gibbs and Smith, 1978) (Fig. 5). The pathogenic phase normally begins when a spore-bearing vector beetle feeds in the crown of the host tree, and produces a feeding groove (Webber and Brasier, 1984). There are variations in structure, size and location of feeding grooves (Webber and Brasier, 1984). Although the feeding groove may provide a good environment for growth and sporulation of *O. ulmi s.l.*, the proportion of xylem infections is low relative to the proportion of feeding grooves with detectable spores of *O. ulmi s.l.* (Webber and Brasier, 1984). The frequency of xylem infection may depend on whether spores from the beetle surface can transfer directly to xylem vessels exposed by beetle feeding. Such direct transfer appears to be infrequent; it appears that more usually there is a transitional mycelial stage, in which *O. ulmi s.l.* initially colonize the feeding groove and, using this nutrient base, mycelia develop and

penetrate into the xylem (Webber and Brasier, 1984). The infection process may be inhibited by mycophagous mites and antagonistic microorganisms in the feeding groove, and so the higher the spore load delivered by the vector beetle, the higher the probability of successful xylem infection (Webber and Brasier, 1984). Competition among genetically diverse *O. ulmi s.l.* ascospores may affect the success of infection, but if beetles are carrying spores from asexual mycelial conidia or synnemata these are probably of the same genotype and unlikely to be antagonistic (Webber and Brasier, 1984). During the initial stages of xylem infection, competition determines which variety occupies the larger part of the tree (Webber and Brasier, 1984) (Fig. 5). All the above procedures may be hindered by the host tree's chemical or physical barriers like growth suppressing substances, thick bark, narrow xylem vessels, and tylose formation (Elgersma, 1982). Successful infection by the pathogenic phase results in severe wilt symptoms because the xylem is obstructed; tree death is the usual result (Swinton and Gilligan, 2000). Xylem blockage is believed to result from the effects of cerato-ulmin and possibly other toxins (Scheffer et al., 1987).

In the saprophytic stage, the fungus colonizes the phloem or inner bark of moribund or dead elms (Gibbs and Smith, 1978) (Fig 5) and may make contact with immature or newly-emerged adult bark beetles that are also inhabiting the bark (Gibbs and Smith, 1978). Successful completion of the life cycle of DED is contingent on the ability of the pathogen to impart spores to newly produced beetles (Webber et al., 1987) (Fig. 5). Spores carried by breeding beetles into the bark can be responsible for the fungal colonization of the bark around the brood galleries (Clinton and McCormick, 1936; Brasier and Gibbs, 1975), and subsequently these fungi may be the source of spores

carried by beetles leaving the tree (Webber and Brasier, 1984). Alternatively, inoculum present in the pathogenic stage in xylem vessels can move into the bark and convert to the saprophytic stage; this is influenced by hyphal extension rates, the amount of *O. ulmi s.l.* carried into the bark by beetles and the result of intraspecific competition between different genotypes of *O. ulmi s.l.* (Webber et al., 1987). The recycling into the pathogenic stage from the saprophytic stage is important for the preservation of pathogenic vigor, as continuous cycling and transmission of the saprophytic stage provides no opportunity for selection for virulence (Webber et al., 1987). Spores carried by individual beetles are usually genetically heterogeneous (Webber et al., 1987).

During the saprophytic stage, brood galleries produced by the beetles become occupied by mycelium and the fruiting bodies of *O. ulmi s.l.* (Webber et al., 1987). The type of spores acquired by beetles is based on the fruiting structures of *O. ulmi s.l.* present (Webber and Brasier, 1984). Spore stages formed in brood galleries do not directly contribute spores to emerging beetles as, during the final larval instar, each larva digs a pupal cavity that is isolated from the gallery by a frass plug (Webber and Brasier, 1984). During the moult to the pupa, the exoskeleton, intestinal lining and attached spores from the larval stage are shed (Webber and Brasier, 1984). Adults emerge by boring directly outwards from the pupal chamber and most of the *O. ulmi s.l.* spores they carry come from inoculum in the pupal chambers (Webber and Brasier, 1984). That inoculum is either carried into the chambers by the larva or by mites and nematodes (Jacot, 1936; Brasier, 1978), or is from fungal hyphae germinating in the pupal chamber from the neighbouring bark (Webber and Brasier, 1984). The suitability of the pupal cavity for production of more *O. ulmi s.l.* spores is dependent on moisture and nutrients, which are

more available in the outer bark (Webber and Brasier, 1984). Hence, pupation position, which differs among beetle species, affects the ability of *O. ulmi s.l.* to produce spores in the pupal chamber and thus the quantity of spores available (Webber and Brasier, 1984).

Transmission of Ophiostoma ulmi sensu lato

In addition to being vectored by beetles, *O. ulmi s.l.* may infect healthy trees through root to root transmission by suckers (Peace, 1960) or natural root grafts (Verrall and Graham, 1935), and transport of infected logs. However, beetles are responsible for short- and medium-range dispersal (Webber and Brasier, 1984), both into the pathogenic stage through feeding grooves and into the saprophytic stage through establishment of new breeding galleries (Swinton and Gilligan, 2000). Medium to long-range transport is of major importance in the epidemiology of DED within infested regions, taking place over distances of the order of several to tens of kilometres, although little is known about the frequency distribution of flight distance from diseased to healthy trees (Swinton and Gilligan, 2000).

Frequency of bearing spores is greatly dependent upon vector species. For example, in Spain, Webber (1990) isolated *O. ulmi* spores from only 6% of *Scolytus kirschii* Skalitzky, in comparison to ≥64% of *S. multistriatus* and ≥98% of *S. scolytus* (Fabricius) from Rubena; in Guadalajara, the proportions were 100% for *S. scolytus*, 35% for *S. multistriatus* and <8% for *S. kirschii*. Numbers of spores carried by beetles differ between and within the species. Emerging *Scolytus scolytus* adults in Rubena carried between 1 and 350,000 *O. ulmi* spores, with only one beetle spore free. Spore loads on *S. multistriatus* from Guadalajara were 1−57,000, and the few *S. kirschii* beetles carrying *O. ulmi* spores had loads of ≤300 (Webber, 1990).

In Europe, ten species of phoretic mites have been found on the exterior of *S. pygmaeus, S. scolytus* and *S. multistriatus*, and on mites of four species *O. novo-ulmi* spores were detected (Moser et al., 2010). Two mites, *Trichouropoda* n. sp. and an undescribed parasitoid mite, *Pyemotes* n. sp., have been found in association with *H. rufipes* collected from Winnipeg (J. C. Moser personal communication, September, 2009). The significance of these mites and other mites as possible carriers of DED has been discussed by Jacot (1934, 1936) and Moser et al. (2010).

Antagonistic interactions

Grazing by mites, nematodes, Collembola and dipteran larvae reduces the amount of *O. ulmi s.l.* spores in elm bark, although the negative effect may be compensated for by the arthropods in spreading *O. ulmi s.l.* spores on galleries and bark fissures (Lea and Brasier, 1983). Besides *O. ulmi s.l.* the bark is occupied by many other pathogens, a factor likely to affect the amount of available spores is competition of these pathogens with *O. ulmi s.l.* (Webber and Brasier, 1984). Webber and Brasier (1984) have shown that four fungal species living on the bark are as good or better colonizers as *O. ulmi s.l.* These fungal species are likely tough competitors of *O. ulmi s. l.* during bark colonization (Gibbs and Smith, 1978) and capable of reducing the bark area occupied by *O. ulmi s. l.* (Webber and Brasier, 1984). There is evidence that competition and disruption of *O. ulmi s.l.* in bark may originate from *O. ulmi s.l* itself (Webber and Brasier, 1984). Both hyphal fusion of *O. ulmi s.l.* controlled by vegetative compatibility genes (Webber and Brasier, 1984) and a cytoplasmically transmitted disease, possibly associated with double stranded RNA, can affect the ability of distinct genotypes of *O. ulmi s.l.* to survive the saprophytic stage and contribute spores on vector beetles (Brasier, 1983b).

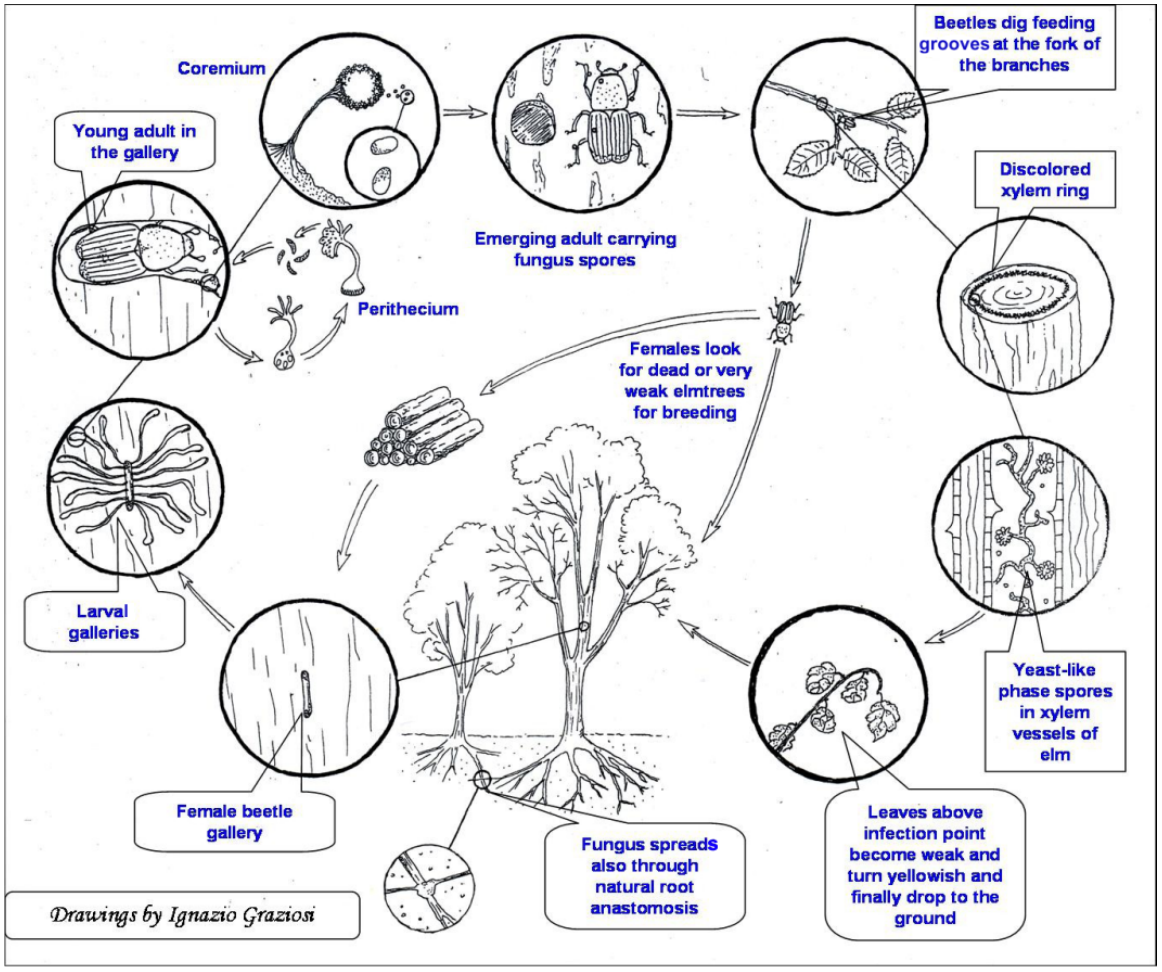

Figure 5. Life cycle of Dutch elm disease. Image credit: Ghelardini and Santini (2009). Used under copyright conditions of Società Italiana di Selvicoltura ed Ecologia Forestale [SISEF].

Interactions involving *Hylurgopinus rufipes*

Hylurgopinus rufipes is the primary vector of DED in the northern parts of the range of American elms, including Canada (Lyons, 1982; Landwehr et al., 1982). Despite the presence of *S. multistriatus* in Manitoba (Buth and Ellis, 1981; 1982), no breeding population has been identified (Westwood, 1996). *Scolytus multistriatus* does not survive in places where temperatures of −20 °C occur, due the vulnerability of the overwintering larvae (Strobel and Lanier, 1981; Lanier and Peacock, 1981; Lanier et al., 1984).

In Connecticut, New Jersey and New York, Parker et al. (1947) report that it is unusual for >10% of *S. multistriatus* to carry spores of *O. ulmi*; the frequency of spore-bearing was slightly higher for *H. rufipes* (Parker et al., 1948). In Ontario, Gardiner (1976b) found that 31−35% of overwintering adults of *H. rufipes* in fall and 26.1−27% in spring carry spores, and in southern Ontario, fungal spores retrieved from adult *H. rufipes* in the spring averages 45% (Takai et al., 1979). Spores are carried on the exoskeleton surface and not in a protective mycangial cavity and so, after a period of flight, beetles may lose all or part of their original spores (Webber and Brasier, 1984; Webber, 2000).

Not all feeding by spore-bearing beetles results in an infection. Based on studies with *S. multistriatus*, only 3 to 5% of inoculations result in xylem infection (Webber and Brasier, 1984). Takai et al. (1979) report that 0 to 74% of feeding attempts by *H. rufipes* result in inoculation. Adult *H. rufipes* feed in the larger branches, where the probability of xylem contact is less, and potentially decreasing disease transmission. However, elm trees are more likely to become infected with DED if inoculation takes place in large branches; when inoculation takes place in smaller twigs trees are sometimes able to isolate the infection (Thompson and Matthysse, 1972). *Hylurgopinus rufipes* pupates

mostly in the inner bark (Kaston, 1939), a position that favours increased spore production, and hence more inoculum on emerging beetles (Webber and Brasier, 1984). In Quebec, adults emerging from brood wood in August are more likely to carry spores than those emerging earlier or later; in this area, a maximum of 50% of the beetles are infective (Pomerleau, 1965b). In Sault Ste. Marie, Ontario, overwintering adult beetles carry a high spore load, which persists for an extended period of time; in contrast, adults from overwintering larvae carry few fungal spores (Gardiner and Roden, 1977).

The preference for different elm species differs depending on beetle species, and these preferences influence the chances of successful pathogen transmission (Webber, 2000). In Manitoba, the main alternative *Ulmus* species to *U. americana* is Siberian elm, *Ulmus pumila*. In *U. pumila* trap logs at two locations in Manitoba over three years, no brood galleries of *H. rufipes* were found, but an average of 49% of the 90 *U. americana* logs set out in the same locations contained brood galleries (Veilleux, 2012). In Manitoba, the density of entrance holes of overwintering *H. rufipes* in *U. pumila*, was between 31and 51% of that in *U. americana* in the same location, and densities in *U. pumila* declined with distance to the closest *U. americana* (Anderson and Holliday, 2000). Overwintering *H. rufipes* also exhibit preference for height and diameter of trees. Between 0 and 190 cm above ground, the densities of entry holes, wintering tunnels, and beetles show a logistic relationship with the diameter of the trunk: densities are close to zero at diameters less than 10 cm and reach a location-specific asymptote at diameters greater than 20 cm (Anderson and Holliday, 2003).

In central Minnesota, adult *H. rufipes* emerging in spring are attracted to dying or dead elms, but adult beetles emerging in the summer are attracted to healthy elm trees

(Swedenborg et al., 1988). The spring-emergent adults fly primarily in the evening; once landed on a tree, females initiate brood galleries and short range interactions among beetles occur, possibly mediated by pheromones and stridulation (Swedenborg et al., 1988). Long-range attraction, resulting in tree colonization, can be accounted for by attraction to host volatiles alone (Swedenborg et al., 1988). Attraction to colonizing beetles, presumably through induced host volatile production in stressed or moribund trees, has been demonstrated by application of herbicides such as monosodium methyl arsonate (MSMA) or cacodylic acid to cuts made in the bark (Lanier, 1989; Pines and Westwood, 1996).

McLeod et al. (2005) report that *O. novo-ulmi* synergistically manipulates infected elms to increase production of four volatile semiochemicals, thus enhancing the trees' attractiveness to *H. rufipes* and increasing inoculum dispersal which benefits the fungus. The vector is also benefitted by the synergism. The pathogenic phase produces a supply of dead trees that provides additional brood wood resources that allow population increases of the beetle vector (Webber et al., 1987).

The history of the two epidemics of DED in the northern hemisphere (Brasier, 1990; Brasier and Mehrotra, 1995; Brasier and Buck, 2001) can provide insight into the interactions between the fungal pathogens, insect vectors and the host tree (Brasier, 1983a). Before the arrival of *O. novo-ulmi* in Europe, the elm population was subject to periodic flare-ups of the less aggressive pathogen, *O. ulmi* (Brasier, 1983a). It is likely that the first tree killed by the aggressive pathogen was used as breeding material by local beetles already carrying the less aggressive pathogen. A large number of beetles leaving this tree carry the less aggressive pathogen, and some of the aggressive pathogen. This

process results in an increase in frequency of infections caused by the less aggressive pathogen to well above its previous level, and a gradual rise in the number of elms succumbing to the aggressive pathogen (Brasier, 1983a). Vectoring the pathogen in elm populations that were for the most part susceptible to *O. novo-ulmi* has been highly beneficial to the elm bark beetles (Webber, 2000). Through killing additional elms and providing more brood trees for the beetle, infections initiated by the aggressive pathogen begin to increase, rapidly overtaking those initiated by the less aggressive pathogen. Consequently, the less aggressive pathogen goes into decline (Brasier, 1983a). Most of the accessible large elms are killed, resulting in a collapse of the beetle population and that of the aggressive pathogen. The less aggressive pathogen is now virtually eliminated in the main epidemic areas, and survives in a few isolated pockets of elm untouched by the aggressive pathogen (Brasier, 1983a). Elm seedlings and root suckers regenerate in large numbers. When large enough to support beetle breeding, they are attacked by the aggressive pathogen. In regions with several vector species, the reduced size of available breeding material can affect species composition of the vector guild. In Europe, the result is that the primary beetle vector, *S. scolytus* goes into decline, and smaller beetles such as *S. multistriatus* and *S. ensifer* Eichhoff become of major importance in disease transmission (Brasier, 1983a). Field elms are largely reduced to understory populations. Brasier and Mehrotra (1995) report the endemic presence of *O. himal-ulmi*, which causes DED in the western Himalayas. There appears to be a balance between the local elm hosts, fungus and the bark beetles in this region.

The appearance of *O. novo-ulmi* in Manitoba in 1975 (Hintz et al., 1993; Temple et al., 2006) and in 1981 in Saskatchewan (Hintz et al., 1993), the presence of a

population of *H. rufipes*, and the highly susceptible *U. americana* led to the killing of thousands of trees. In Western Canada, the fungus is genetically uniform and consists entirely of *O. novo-ulmi* (Hintz et al., 1991, 1993). The appearance of DED in the prairies resulted in an increased population of vectors because of increased supply of brood wood. The larger population of beetles increased the efficiency of transmission, thus causing an outbreak of the disease which radiated out from the initial infection sites.

Dutch elm disease in Manitoba

Dutch elm disease was identified in 1975 in Manitoba (Ives and Petty, 1976) and in Saskatchewan in 1981 (Sterner and Davidson, 1982). Although naturally-occurring elm is restricted to wetter locations in southern areas of Manitoba and Saskatchewan, elm is among the commonest shade tree planted in urban areas (Davidson et al., 1964).

The major DED vectors in North America, *H. rufipes* and *S. multistriatus* (Finnegan, 1957; Davidson et al., 1964), show a gradient of relative importance in Manitoba and to the south. *Scolytus multistriatus* is the major vector in southern Minnesota, including the area of Minneapolis-St. Paul, and *H. rufipes* tends to be found in parks, woods, and along rivers, and less frequently on boulevard trees; in northern Minnesota, *H. rufipes* is the major vector and is found on boulevard trees as well as in wooded areas (French et al., 1980). Manitoba and Saskatchewan are both located at the northern limits of the range of *S. multistriatus*, and although a few have been caught (Buth and Ellis, 1981; Westwood, 1991b; Knowles, 2002), the beetle is not considered a major vector in these provinces because it generally does not survive winter (Lanier, 1982; Lanier and Peacock, 1981; Lanier et al., 1984; Schacherl, 1996). *Hylurgopinus rufipes* is the major vector in Manitoba and Saskatchewan (Hildahl and Wong, 1965;

Brasier, 1991; Westwood, 1991a; Schacherl, 1996) and occurs throughout the natural range of *U. americana* in Manitoba and Saskatchewan (Hildahl and Wong, 1965).

In genetic surveys, *O. novo-ulmi* aggressive subgroup is the predominant causal agent in Manitoba and Saskatchewan (Hintz et al., 1993; Temple et al., 2006). The DED pathogen in this region is remarkably and persistently genetically uniform (Temple et al., 2006).

In Manitoba, the only tree host of DED is *U. americana*. Dutch elm disease now occurs in most of the range of *U. americana* in Manitoba, having spread beyond the southern and central parts of the province from the Manitoba-Ontario boundary into Saskatchewan and north to the Saskatchewan River (Knowles, 2002). Before DED was discovered in the province, there were several millions of elms mainly in wild stands in southern Manitoba and in boulevards and parks in the City of Winnipeg (Manitoba Environment, 1993). There is a significant population of wild elms growing along riverbanks in Manitoba, where many of the trees are quickly infected because of the presence of beetles carrying spores. Most of Winnipeg's annual elm loss since 2000 has been made up of wild elms (Benson, 2006). Because of DED, although elms continue to be valued in urban forests, the total population of urban elms in Winnipeg has been significantly reduced. Winnipeg had approximately 275,000 elms in 1975 (Domke, 2005). In 2008, there were approximately 160,000 elms left in the urban forest, and elms grown in boulevards in Winnipeg represented approximately 40% of all boulevard trees (City of Winnipeg, 2008a). The mean number of elms destroyed by DED in the 10 years up to 2008 was 5,000 trees annually (City of Winnipeg, 2008a). The main challenge is to

find sustainable and cost effective ways to reduce the loss of remaining elm trees in Manitoba to preserve them as an important urban resource for future generations.

Economics of Dutch elm disease in Manitoba

Before DED was discovered in Manitoba, the population of *Ulmus* species in south and central parts of the province was about 20 million trees (Jeffrey, 1982), worth millions of dollars. From the beginning of the 20th century more than five million elm trees were planted in rural shelter belts, towns and cities (Westwood, 1991a). The greatest economic effect of DED is the destruction of valuable elms along city streets or near important or historical buildings. In Winnipeg, about 35% of trees on boulevards and in parks are elms (Barwinsky and Domke, 2012).

A cost benefit analysis of the integrated DED management program in Manitoba between 1975 and 1990 was performed by Westwood (1991a). In 1990, the estimated value of the urban elm forests within communities and the City of Winnipeg in Manitoba exceeded $1.1 billion (Westwood, 1991a). Expenditures of approximately $10 million from 1981 to 1990 had conserved $276,204,000 worth of elm trees in 35 rural communities. Westwood (1991a) concluded that the integrated DED management program has been a worthwhile investment, and that about $5,010,000 had been saved since 1981 by managing DED.

Domke (2005) estimated based on similar experiences in U.S. cities that, without the management program, Winnipeg would have lost approximately 18% of its elm trees per year (Domke, 2005). Over 37 years, the average annual loss rate caused by DED in Winnipeg had been 1.38%; however individual annual loss rates have varied significantly over this period. Disease incidence had progressively increased over the 5 years before

2011, when the loss rate increased to the highest yet at 3.46% (Domke, 2012). The target is to reduce annual elm losses to DED to no more than 2% (Barwinsky and Domke, 2012).

A continued annual loss rate >2% may lead to disease pressure where the City of Winnipeg is unable to successfully control DED (Domke, 2012), and so this prompted an economic analysis for Winnipeg in 2012. At that time, the estimated number of elms on boulevards and in parks was 84,000, and with an estimated value for an average boulevard elm tree of $9,600, the appraised value of elm asset on boulevards and in parks alone was estimated to be $806 million (Barwinsky and Domke, 2012). If Winnipeg ceases to control DED, the estimated cumulative removal costs may be as high as $119 million by 2028 and it will cost $50.4 million to replace the 84,000 boulevard and park elms (Barwinsky and Domke, 2012). In addition, there would be a loss of environmental, economic, social benefits and quality of life (Barwinsky and Domke, 2012). By 2012, the City of Winnipeg had spent about $69 million to manage DED and preserve Winnipeg's elm population (Domke, 2012). The most recent estimate of annual cost for DED management in Winnipeg is $3.7 million, of which the Province of Manitoba contributes $1 million (Barwinsky and Domke, 2012). The average annual cost over the 37 years for DED management in Winnipeg has been $1.91 million; this amount was spent on surveillance, tree removal, elm bark beetle control, fungicide injection, tree pruning, tree replacement, public education and research (Barwinsky and Domke, 2012).

Control of Dutch elm disease, with emphasis on the prairie region

The management of DED has been described by Campana and Stipes (1981), Strobel and Lanier (1981), Lanier (1982) and Stipes (2000). Dutch elm disease must be effectively

controlled in order to conserve urban and rural elm trees. Disease management is based on lowering the chance for new infection of healthy elms (prophylaxis), or increasing the probabilities for recovery of elms after infection with pathogens (therapy) (Stipes, 2000). Prophylactic control methods include vector control, prevention of root grafts, pruning or removal of diseased elms and development of resistant species. Therapeutic control measures include surgical techniques and chemical injection (Campana and Stipes, 1981).

Dutch elm disease surveys

Surveillance is the efficient and methodical assessment of all the elm trees in a designated area to discover both symptomatic and hazard elms in addition to sites where elm firewood is stored (Trees Winnipeg, 2013a). In Winnipeg, surveillance is carried out by the Forestry Branch, City of Winnipeg. In most places in the City of Winnipeg, DED surveillance is done twice during the summer. Since 1996, the City's Forestry Branch has also conducted weekly surveys of some areas in the city with higher incidences of DED (Trees Winnipeg, 2013a). In Winnipeg, there are a number of groups of citizens who have been trained to detect DED and who monitor their neighbourhood trees. The data collected in the field is entered into a database and made available to removal operations staff (Trees Winnipeg, 2013a).

In participating rural communities, the DED program is managed by Manitoba Conservation and Water Stewardship on a cost-shared basis (Manitoba Conservation and Water Stewardship, 2013). Starting in mid-May each year, provincially appointed inspectors and officers are sent to search for elm firewood in each of the participating communities. After this, usually starting in mid-June, the inspectors begin to survey for trees that are either ≥40% dead or that are infected with DED (Manitoba Conservation

and Water Stewardship, 2013). Inspections are repeated throughout the summer, and each community is surveyed up to three times by provincial inspectors (Manitoba Conservation and Water Stewardship, 2013). These surveys require that inspectors, who usually work in pairs, visually survey every property in the community. Under *The Forest Health Protection Act (May 2009)***,** inspectors and officers may enter private property for the purpose of inspection without permission from the property owner (Manitoba Conservation and Water Stewardship, 2013). In the process of determining if a tree has DED, the inspectors may take a sample from the tree using pole pruners to look for a characteristic stain under the bark. If elm wood, hazard trees or DED infected trees are found on the property, inspectors record the detection, mark the material with orange spray paint, and leave a letter at the property or mail a letter to the property owner to let them know what was found and who to contact with any questions (Manitoba Conservation and Water Stewardship, 2013). As DED is a designated forest threat, an officer or inspector can order a property owner to remove the designated material themselves or can bring a removal crew on to the property to have the material removed at the owner's expense (Manitoba Conservation and Water Stewardship, 2013).

Sanitation

The removal of diseased trees is vital for reducing the transmission of DED fungal spores. Sanitation has been described as the cornerstone of disease suppression (Lanier and Epstein, 1978; Campana and Stipes, 1981). Complete sanitation demands that all diseased trees with beetles be removed. Sanitation involves detection, isolation, removal and disposal of diseased trees and is the key to successful management of DED (French et al., 1980). It also important to excavate, treat and remove tree stumps promptly.

Prompt sanitation is the best single method of containing DED, but an effective, rigidly implemented integrated system by which the fungal inoculum levels and vector densities are reduced is the ultimate control system (Stipes, 2000). However, due to resource constraints, total sanitation is not feasible where the main reservoir for vectors and pathogens is large numbers of elm trees growing in the wild (Campana and Stipes, 1981).

The annual tree removal and disposal program starts with the summer season surveys described above, during which infected elms are tagged on the basis of crown discolouration and die-back. These trees are then removed before the following spring. Most trees are removed during the winter season when other urban forestry activities are less pressing and when the ground is frozen and so less easily damaged by heavy equipment. Tree removal using this schedule can be effective in removing sources of inoculum in years following disease symptom detection. However, winter removal would not provide optimum suppression of disease transmission if, in the same year as symptom detection, a generation of beetles developed and emerged from infected trees carrying spores. Such a developing generation could contribute significantly to the vector population, and could transmit pathogen spores from the diseased tree to healthy trees. The first evidence that this may occur came from an exploratory survey carried out by the City of Winnipeg, in which 38% of symptomatic trees that were scheduled for winter removal had *H. rufipes* brood galleries, and some had exit holes (Robbie-Draward, 1995). From 2004 to 2010 in Manitoba, Veilleux et al. (2012) assessed the annual prevalence of DED in communities with winter removal of symptomatic trees, and in comparable communities where symptomatic trees were removed within a few weeks of symptom detection (rapid removal). In rapid removal communities the annual prevalence of elm

infection (1.5 ± 0.2%) was significantly lower than in communities with autumn/winter removal (3.1 ± 0.4%).

Basal insecticide application

In the prairies, insecticidal applications to the base of elm trees are effective in killing overwintering beetles (Jin et al., 1996). It has been shown that the application of 0.5% a.i. of chlorpyrifos to the lower trunk of elm trees is effective in prevention of overwintering by adult *H. rufipes* and in reducing the incidence of DED pathogen (Gardiner, 1976a; Gardiner and Webb, 1980). Chlorpyrifos applications to a height of 2.5 m control between 83% and 100% overwintering *H. rufipes* (Gardiner and Webb, 1980). In Manitoba conditions, chlorpyrifos is effective for two years (Jin et al., 1996). Similar applications in Minnesota reduce numbers of *H. rufipes* emerging in spring by 93% (Landwehr et al., 1982).

Basal applications may kill beetles at emergence, but mostly it is expected that *H. rufipes* mortality will occur when beetles enter the base of a tree. At that point, typically, insecticide residues will be higher than in spring, and beetles entering by chewing tunnels have more contact with residues than those exiting the tunnel. Gardiner and Webb (1980) report a progressive decrease of chlorpyrifos residues on branches of trees sprayed with 0.5% mixture in June (0.030 mg/cm^2 bark surface), September (0.015 mg/cm^2 bark surface) and December (0.008 mg/cm^2 bark surface).

According to the recommendation by Anderson and Holliday (2003), basal insecticide sprays should be limited to the basal 55 cm of each elm from an original height of about 2 m, because the number of live overwintering *H. rufipes* found above 55

cm was not significant. They recommended more research to determine whether further decreases in height of applications are advisable.

Spray applications are conducted in rural municipalities in the province if the municipality takes part in the DED cost share agreement administered by Manitoba (Golinoski, 2012). The City of Winnipeg carries out basal insecticide applications between August and October, and applies insecticides to all boulevard and park elm trees. A new recommendation has been made that all American elm trees in the City of Winnipeg be included in the basal spray program to improve its integrity (Barwinsky and Domke, 2012). Homeowners in Winnipeg do not pay for basal insecticide application (Golinoski, 2012). In the last two years, the City of Winnipeg has refocused the basal insecticide application to treat elms growing in riverbanks around the City (Golinoski, 2012). Riverbanks harbour high populations of *H. rufipes* and DED pathogens. Making these areas major priorities by directing limited resources is considered the most prudent way to manage DED in the City of Winnipeg (Golinoski, 2012).

Biological control

Populations of vectors of DED are affected by natural biological control agents, which could therefore affect frequency of disease transmission (Lanier and Epstein, 1978). Possible predators include mites, nematodes, insects and birds, while fungi and bacteria are parasites of one or more stages of *H. rufipes.* In Connecticut, the most common parasite of *H. rufipes* is the braconid wasp, *Spathius canadensis* Ashmead (Kaston, 1939). Beetle larvae of the penultimate and ante-penultimate instars are attacked by *S. canadensis*, but by far the largest numbers are attacked in the last instar (Kaston, 1939). Mites are often found in the galleries of *H. rufipes* and a number of species are borne on

the exoskeleton of the beetles (Kaston, 1939; Moser et al., 2010) Specimens of the mite, *Pediculoides dryas* Vitzthum, are often found attached to the intersegmental membrane behind the prosternum (Kaston, 1939). Sometimes the mites are seen eating the eggs, and occasionally the larvae of the beetle (Kaston, 1939). Some clerid beetles are predators of bark beetles: Hopkins (1893) reported *Thanasimus dubius* Fabricius as a predator of *H. rufipes*. In Connecticut, the most common clerid predator is *Enoclerus nigripes* Say (*quadriguttatus* auct.), which is a voracious feeder: in the laboratory, one *E. nigripes* can consume five adult *H. rufipes* in succession (Kaston, 1939). Another predator is the fly, *Lonchaea polita* Say, which is sometimes found in large numbers. It pupates in the egg galleries of *H. rufipes* (Kaston, 1939). It is not known what influence these natural enemies of *H. rufipes* have on vector populations in Manitoba.

The potential of using nematodes for biological control of elm bark beetles has been investigated (Kaston, 1939; Tomalak and Welch, 1982; Jones and Welch, 1982). Tomalak and Welch (1982) report that sphaerulid nematodes infect third instar larvae of elm bark beetles and emerge as juveniles of the next generation. Presence of parasites in the haemocoel during beetle development causes numerous abnormalities in insect organogenesis, especially of the gonads, and beetle mortality is due to histological degradation (Tomalak and Welch, 1982). *Hylurgopinus rufipes* can be infected and killed in the laboratory by the nematode *Neoaplectana carpocapsae* (DD-136) (Jones and Welch, 1982). The successful use of DD-136 in biological control depends upon the creation of a moist microenvironment capable of sustaining live nematodes until they can enter the insect host. A hydrophilic colloid, Tenogum® is found to be non-toxic to DD-

136, and potentially useful against *H. rufipes* when sprayed with DD-136 in suspension (Jones and Welch, 1982).

Root graft severance

Dutch elm disease may spread through grafted roots (Verrall and Graham, 1935; Peace, 1960; Epstein, 1978). Direct DED transmission from diseased to adjoining healthy elms through root grafts can be blocked by soil injection with sodium methyl dithiocarbamate (SMDC) (vapam®) (Epstein, 1978) or by physically detaching grafts; both treatments require excavating to a depth of about 75 cm. To be efficacious, these measures must be deployed before the pathogen in the symptomatic elm is passed via the graft to a healthy elm.

Preventive pruning

Preventive pruning involves elimination of moribund branches from non-symptomatic trees to remove brood material. Well established young trees should be pruned to improve their form, and to eventually control the height and extent of branches as they mature (Allen, 2009). It is important to remove rubbing and broken branches, and any branches or multiple stems that are too crowded (Allen, 2009). The optimum time to prune is from September to mid-December and from February to early April (Allen, 2009) which avoids the spring period for movement of emerging infected beetles to new trees. In Manitoba it is illegal to prune elm trees from 1st April to 31st July inclusive, as pruning elms during these periods makes them attractive to *H. rufipes* (Allen, 2009). In May and June more overwintering adults of *H. rufipes* are caught on American elms that have been pruned than on those that are not pruned; on pruned elms, fewer beetles are caught when pruning wounds are treated with dressing compound (Landwehr et al.,

1981). After mid-July, there is no appreciable difference in the number of summer-emerging *H. rufipes* caught on pruned elms compared with that on unpruned elms (Landwehr et al., 1981).

Regulatory control

In 1981, *The Dutch Elm Disease Act* was enacted to establish procedures for DED management in Manitoba (Manitoba Government, 1998). The major elements of the Act and Regulation involve: a ban on pruning of elm trees from 1st April to 31st July; a ban on storage or unauthorized transport of elm wood; regulations for the appropriate destruction of diseased wood by burial, burning or chipping; and the authority of inspectors to enter premises and conduct control operations (Trees Winnipeg, 2013b). *The Dutch elm Disease Act* has been repealed and the same measures are now included in *The Forest Health Protection Act*, in force since 2009 (Manitoba Government, 2013).

Fungicide injection

Fungicide injection may be used to protect elm trees from DED. The fungicide is injected into the water conducting vessels (Kondo, 1972) by root flare injection or trunk injection, of which the former is more common (Trees Winnipeg, 2013a). For effective protection, fungicide injection should be carried out when elms are fully leafed out, but before July (Trees Winnipeg, 2013a). The fungicides Propiconozole (Alamo®) and the broad-spectrum benzimidazole (Eertavas®) are registered for application in Canada (Trees Winnipeg, 2013a). In Fredericton, annual repetition of the treatment is considered necessary (Magasi et al., 1993). In Winnipeg, it is recommended that the fungicide be applied every two to three years as part of integrated DED prevention (Barwinsky and Domke, 2012). Injection costs on average between $200 and $300 per treatment for a

mature elm (Barwinsky and Domke, 2012), and because of the cost and difficulty of the method, only high value elms should be treated. Therapeutic fungicidal treatment of symptomatic elms should be not carried out unless <10% of the crown has wilted; prompt removal of diseased branches must be done as well (Trees Winnipeg, 2013a).

In Winnipeg, the use of Dutch Trig® as a bio-control vaccine for American elms is being evaluated by the City of Winnipeg (Wiebe, 2009; Dutch Trig, 2013). A special injector is used to introduce a suspension of *Verticillium albo-atrum* Reinke & Berth (Ascomycota: Hypocreales) spores into the tree, with the intent of activating the trees defences against fungal infection (Dutch Trig, 2013).

Trap trees

The trap tree technique is an effective and economical method for managing DED. Beetles are attracted to treated elms but produce fewer new breeding adults than in untreated elm trees (Lanier, 1989). Trees killed with herbicides attract the beetles (Lanier, 1989), and could be sprayed with an insecticide to control landing beetles. In Manitoba, monosodium methane arsenate (MSMA), was applied to elm trunks to investigate whether the treated elms would become potent trap trees for the native elm bark beetle (Pines and Westwood, 1996). Within 18 days of herbicide application all treated elms were dead, and significantly more *H. rufipes* were attracted to these trees than to control trees. However, MSMA is no longer available or registered for use along with many arsenical pesticides. In 2009, the Environmental Protection Agency decided to phase out MSMA, cacodylic acid and its sodium salt and all organic arsenicals as part of the transition to less toxic herbicides (Environmental Protection Agency, 2012).

Integrated disease control

Integrated pest (disease) control is the use and integration of multiple systems and methods to manage/control a pest. An effective integrated system by which the fungal pathogens levels and vector densities are lowered is the ultimate regimen. Dutch elm disease management must be tailored for individual needs where the disease is either starting or where it has become established (Stipes, 2000). Integrated control of DED in Manitoba is used to protect urban elms from the disease. Major components of an integrated disease control program include: legislation, site-specific inventory of trees within control areas, elm tree sanitation by pruning and removal, basal spraying with an insecticide to kill overwintering elm bark beetles, preventative and curative tree injections with fungicides, replacement of elms with alternative species, surveillance, research, education and public information and the community Elm Guard Program (Westwood, 1991a). An integral part of the management program is to establish a shared responsibility between governments and communities through the development of the Cost Sharing Agreements. These partnerships are designed to share costs of management activities such as basal spraying, tree replacement and pruning, between Manitoba Conservation and communities (Westwood, 1991a). Implementation of the integrated disease control components is conducted in different jurisdictions of Manitoba by Manitoba Conservation, City of Winnipeg and Trees Winnipeg. Buffer zones, managed by Manitoba Conservation, surround Brandon and Winnipeg, and serve to reduce vector and pathogen movement from wild elms into these cities. To maintain the integrity of the buffer zones that obstruct disease entry through the LaSalle, Red and Seine River corridors, the City of Winnipeg recently sent a formal recommendation to the Province to

reinstate the Ritchot and Springfield buffer zones and reinstate basal spray treatments to control overwintering *H. rufipes* (Barwinsky and Domke, 2012). With the exception of those in buffer zones, protection for wild elms has not been carried out in any jurisdiction because of financial limitations (Campana and Stipes, 1981).

In Saskatchewan, DED management is supervised by the Forest Service Branch of Saskatchewan Ministry of Environment (McIntosh et al., 2005). Saskatchewan Agriculture, Food and Rural Revitalization and the Saskatchewan Dutch Elm Disease Association work cooperatively with Saskatchewan Environment to facilitate the management of DED (McIntosh et al., 2005). In previous years, Saskatchewan Ministry of Environment conducted all the DED surveillance; however, from 2001 to 2010, DED surveillance and removal services were under contract (McIntosh et al., 2005). The major components of the provincial DED management program included: legislation, public awareness and education, cost-share communities, surveillance, sampling, diagnostics, pruning, buffer zones, sanitation, beetle trapping, basal chlorpyrifos applications, diversification of urban forests and research (McIntosh et al., 2005). Dutch elm disease management activities are administered and enforced under *The Forest Resources Management Act* (Saskatchewan Government, 2005), which has been subject to some amendments by the Statues of Saskatchewan since it was passed.

Saskatchewan Ministry of Environment partnered with 43 communities to share the costs of program delivery (McIntosh et al., 2009), as part of which two surveys (late June or early July and August) were conducted annually to detect DED symptoms. Cities with >15,000 residents conduct their own surveillance ((McIntosh et al., 2005). Buffer zones 2 km wide have been established around high risk communities with large urban

elm populations. Removal of diseased and hazard elms in communities and buffer zones were carried by Saskatchewan Ministry of Environment under contract (McIntosh et al., 2005). In 2010, when DED had been reported in about 25 communities in Saskatchewan, the provincial government resolved to move to a model that emphasizes shared responsibility for DED management activities and reduced its DED control program, saving the province $400,000 annually (ForestTalk, 2010). While larger communities may continue with management activities, smaller jurisdictions lose the disease monitoring programs the province had been implementing (ForestTalk, 2010) but are encouraged to implement their own monitoring activities. However by 2012, thirteen communities had continued to implement their own DED surveillance and removals programs (McIntosh, 2012). The Ministry of Environment is committed to continuing regulatory controls, scientific and technical support, diagnostic services, and monitoring and removals in buffer zones, but municipalities will be responsible for the elm trees that are within their own boundaries (Saskatchewan Legislature, 2010).

Potential improvements to disease management in Manitoba

The aims of the thesis research were to develop biological knowledge to improve vector and disease management in Manitoba, and to provide practical information to urban forest managers so that they can enhance the efficacy of the integrated DED control programs they operate.

The first objective of this study was to determine whether *H. rufipes* can complete development in newly-diagnosed elm trees in the same growing season as symptoms become detectable, and can emerge carrying spores; and if this happens, to investigate ways of predicting which symptomatic trees are likely to contribute most to the

population of spore-bearing beetles. This objective addresses concerns raised by the study of Robbie-Draward (1995) and may show the mechanisms underlying the phenomena reported by Veilleux et al. (2012).

The second objective was to investigate the fine-scale vertical distribution and other characteristics of overwintering *H. rufipes* in Manitoba. This objective arises from the recommendation (Anderson and Holliday, 2003) that basal insecticide sprays should be limited to the bottom 55 cm of each elm because few overwintering *H. rufipes* survive above this height, and the further recommendation for research to determine whether height of basal insecticide applications could safely be reduced even more.

The third objective was to study the efficacy of alternatives to chlorpyrifos for basal applications for control of overwintering *H. rufipes*. This objective arose because of concerns about the future availability of chlorpyrifos as a result of reviews in both the United States (Environmental Protection Agency, 2010) and Canada (Health Canada, 2007).

Chapter 3: Brood development of *Hylurgopinus rufipes* Eichhoff (Coleoptera: Curculionidae) in American elm trees newly diagnosed with Dutch elm disease in Manitoba

Introduction

Dutch elm disease was initially observed in the Netherlands and France in 1919 and 1921, respectively (Guyot, 1921; Spierenburg, 1921, 1922;). Schwarz (1922) isolated and named the causative fungus as *Graphium ulmi*, which was later classified by Melin and Nannfeld (1934) as *Ophiostoma ulmi* (Buisman) Nannf. Brasier (1991) reclassified the "aggressive strain" of *O. ulmi* as *O. novo-ulmi*, and in 2001, Brasier and Kirk (2001) assigned the two subpopulations of *O. novo-ulmi*, recognized as the Eurasian (EAN) and North American (NAN) races, to the *novo-ulmi* and *americana* subspecies respectively.

The spread of DED in Europe and North America has been reviewed by Gibbs (1978). Following the discovery of DED in 1975 in Manitoba at Selkirk, Brandon and in Winnipeg (Hildahl, 1977), it has killed enormous numbers of American elm trees in the Province. A genetic survey conducted in Winnipeg, Manitoba and in Western Canada shows that *O. novo-ulmi* aggressive subspecies is the predominant causal agent (Hintz et al., 1993; Temple et al., 2006). *Hylurgopinus rufipes* (Eichhoff) is the dominant vector of *O. novo-ulmi* in Manitoba (Hildahl and Wong, 1965; Westwood, 1991a), although *Scolytus multistriatus* (Marsham) has also been reported (Buth and Ellis, 1981). Manitoba Conservation monitored for *S. multistriatus* with pheromone traps throughout southern Manitoba and only eight were caught between 1982 and 2006 (Pines, 2009). These numbers are insufficient for *S. multistriatus* to be an important vector of DED in Manitoba.

The life cycle of *H. rufipes* varies among regions. Overwintering adults of *H. rufipes* emerge in spring from the base of elm trees and move to healthy elms to feed (Kaston, 1939; Landwehr et al., 1981). Disease transmission occurs during the feeding

process when spores of the fungus, attached to the beetles' cuticle are transferred to the water-conducting vessels of the tree (Hildahl, 1977; Takai et al., 1979). This results in early summer (June/July) infection of elm trees by the pathogen. After spring feeding, beetles move to dying and/or diseased elm trees or elm logs where they construct brood galleries, and females lay eggs (Thompson and Matthysse, 1972; Swedenborg et al., 1988). The eggs hatch into larvae that feed in galleries under the bark. In *H. rufipes*, females excavate horizontal tunnels that run transverse to the wood grain, and lay eggs in niches adjacent to the tunnel. The smaller larval tunnels that radiate from the major tunnel run perpendicular to the wood grain (Kaston, 1939; Bright, 1976). Gallery excavation for *H. rufipes* differs strikingly from *Scolytus multistriatus*; in *S. multistriatus* the main tunnels for egg laying are vertical and along the wood grain while the smaller larval tunnels align transversely with the wood grain (Kaston, 1939). In August, following brood development, adult *H. rufipes* of the new generation emerge and move to healthy elms to feed. In fall, adult beetles move to the base of healthy elm trunks to overwinter in the bark (Strobel and Lanier, 1981; Anderson and Holliday, 2003). Elsewhere *H. rufipes* may overwinter in the adult or larval stage (Finnegan, 1957; Thompson and Matthysse, 1972; Takai et al., 1979; Swedenborg et al., 1988) but, in Manitoba, there is one generation per year and *H. rufipes* overwinters only as an adult (Anderson, 1996).

Sapstain, also known as blue stain, is a grey, black or bluish discolouration of sapwood caused by the presence of pigmented fungal hyphae (Seifert, 1993) that use fatty acids, simple carbohydrates, and triglycerides and other constituents of the xylem layer (sapwood) (Wang et al., 1995). Most of these fungi belong to two genera of ascomycetes,

Ophiostoma and *Ceratocystis* (Kirisits et al., 2012), and they include the DED fungus (Holmes, 1981). The symbiotic relationships involving bark beetles and ophiostomatoid fungi benefit the fungi, which gain from transport to a new host, and the bark beetles, which gain suitable brood wood through fungi inhibiting tree defenses or killing the tree (Six and Wingfield, 2011).

Since DED was initially reported in 1975 in Manitoba (Hildahl, 1977), provincial and local authorities have been proactively involved in integrated disease management programs involving techniques such as pruning, spraying insecticide, injecting fungicide, legislative controls (pruning bans and restricted movement of elm wood) and sanitation. Sanitation is a preventative action involving detection, isolation, removal and disposal of diseased elm trees (French et al., 1980). The major goal of sanitation is to prevent transmission of spores from infected to uninfected trees. In Manitoba, surveillance of elm trees for DED is carried out in the spring and summer by inspectors from the City of Winnipeg, Urban Forestry Branch and Manitoba Conservation, Forestry Branch. Trees showing characteristic disease symptoms are identified and marked with red paint for removal. Symptoms that inspectors use in DED detection include yellow flagging of foliage, and whether there is blue staining beneath the bark.

It has generally been assumed that, in Manitoba, delay of removal of newly-symptomatic trees until the following winter did not compromise the objectives of sanitation. However, the City of Winnipeg (Robbie-Draward, 1995) conducted a small study of elm trees that were identified as more than 50% dead and were removed early. Some of these trees were infected with DED, and also contained brood galleries. The study raised the question of whether breeding in newly infected elms is successful and

whether beetles carrying the pathogen emerge from these trees before winter. If newly-diagnosed trees are a major source of *H. rufipes*, and particularly if many of these beetles are spore-bearing, a program of "rapid removal", in which newly diagnosed trees are removed immediately, may be appropriate. Therefore, the objectives of this study were to determine whether *H. rufipes* can complete development in newly-diagnosed elm trees in the same growing season as symptoms become detectable and emerge carrying spores, and to investigate ways of predicting which symptomatic trees are likely to contribute most to the population of spore-bearing beetles.

Material and Methods

In each of June 2006 and 2007, 30 American elm trees exhibiting symptoms of new DED infection were tagged on the south bank of the La Salle River in the Camp Amisk (49° 43′ 21″ N; 97° 10′ 14″ W) portion of La Barriere Park, located 5 km south of Winnipeg (Fig. 6). Dutch elm disease symptoms were identified visually by the presence of either green or brown wilted leaves on elm trees, or by the presence of both symptoms on the same tree, depending on the stage and progression of the disease. As all previously-tagged trees at this site had been removed, it was known that the trees tagged were newly symptomatic. At the time of tagging, and at one month intervals until leaf fall, each tagged tree was photographed from at least two angles to allow charting of the location and progression of disease symptoms. In 2006, four randomly selected tagged trees were felled on each of the following dates: 26 June, 24 July, 21 August and 4 December, and one tagged tree was felled on 2 October. In 2007, four tagged trees were felled on 25 June, 23 July, 13 August and 3 December, and one tagged tree was felled on 17 September. On each date, the trunk and branches > 2.5 cm diameter of one complete

felled tree were cut into labelled sections, removed from the site, and taken to the laboratory for debarking. When four trees were felled, from each of the remaining three trees, one symptomatic branch from the site of initial infection and another branch from the opposite side of the tree to the site of initial infection were sectioned and removed for debarking. Tagging was carried out with the aid of crews from Manitoba Conservation and the City of Winnipeg. Tree felling was carried out with the assistance of a felling crew from Manitoba Conservation, Forestry Branch. Labelled sections of trees were taken to the University of Manitoba where they were stored at 5 °C in a controlled environment cooler until dissection. Labels allowed the location of branches to be related to previously taken photographs of the trees, so that proximity and relationships of the sections could be depicted (Fig. 7). For each felled tree, tree height and compass direction of symptoms was determined at the time of felling using a Silva Type 15 compass equipped with a clinometer, and the diameter at breast height (DBH) was determined using a diameter tape.

In the laboratory, each log was measured to calculate the bark area, and then the bark was carefully removed using a hammer and a chisel. Bark pieces were examined under a binocular microscope for the presence and location of brood galleries and the stage of insects in galleries. Staining symptoms of DED were charted when observed during bark peeling.

Detection of *Ophiostoma novo-ulmi* in samples

For the assessment of all types of samples taken from sampled trees, malt extract agar (2%) was prepared by mixing 20 g malt extract (Difco, Franklin Lakes, NJ) supplemented with 1 g yeast extract, (Gibco, Paisley, UK) and 20 g bacteriological agar

(Gibco), in 1 L deionized water (Molnar, 1965). The mixture was sterilized by autoclaving for 25 min at 121 °C and 414 kPa in an AMSCO (Eagle series) autoclave. After cooling, the medium was dispensed into 10 cm Petri dishes in a micro flow hood and left to solidify at room temperature.

During dissection of tree and branch sections, larvae, pupae and adult beetles were picked from galleries using sterile forceps, and placed individually in 1.5 ml microcentrifuge tubes and stored at 4 °C. A bio incinerator (Monoject Scientific, Sherwood Medical, St. Louis, MO) was used to sterilize forceps between picking insects from different galleries. Insects of each stage were plated singly in Petri dishes within 48 h of collection from the tree section. Cultures were incubated at ambient laboratory temperature and relative humidity for up to 10 days in alternating light and dark cycles. All plates were labeled and wrapped with parafilm to maintain moisture and to prevent the accidental escape of fungal spores. Plates were examined every second day with a dissecting microscope for the presence of the characteristic mycelial or synnemal state of *O. novo ulmi*. Detailed identification techniques and colony morphology are provided in Stipes and Campana (1981).

Pieces of xylem tissue 4 to 6 cm long and about 3 cm wide were removed from randomly selected sections of each dissected tree; one piece was removed from each selected section using a sterile chisel and hammer. Pieces were cut through the cambium into the active xylem to enhance detection of any viable *O. novo-ulmi* fungi that were present. Each piece was then picked up with a sterile pair of forceps and individually kept in 16.8 x 14.9 cm sandwich bags and stored at 4 °C. Xylem samples were plated singly within 48 h of tree dissection. Plates were incubated and assessed, as described above.

To determine whether *H. rufipes* adults carry *O. novo-ulmi* spores in their gut, the adults were surface sterilized using a modification of the method of Burges et al. (1979); Lam and Pedigo (2000) and Watson et al. (2000). Before each beetle was sterilized, elytra and hindwings were removed with forceps to prevent contamination by fungal spores trapped between wings. Each beetle was immersed in a solution of Tween 20 and 50% sodium hypochlorite for 15 minutes, and then rinsed once in 70% ethanol and five times in sterile distilled water. Efficiency of surface sterilization was tested by plating out the washings and a few beetles. Dissection of beetles to remove the gut was carried out as described by Burges et al. (1979) with slight modifications. The dissections were carried out on beetles from trees felled between 26 June and 2 October, 2006. Dissections were performed on a sterile glass slide and the excised gut was smeared individually on medium in a Petri dish. Cultures were incubated and assessed as previously described.

Frass of emerged adults from random samples of brood galleries on trunks and branches was collected from the gallery with sterile forceps and stored in 1.5 ml micro centrifuge tubes at 4 °C before plating. Frass samples from individual beetles were plated singly in a Petri dish within 48 hours of removal from the tree section. Plates were incubated and assessed as described above.

Hylurgopinus rufipes adults used in experiments to determine spore loads were collected from sections of the symptomatic elm trees felled at Camp Amisk in 2006 and from beetles collected from trap logs kept in rearing cages in a controlled environment chamber at 5 °C in the Department of Entomology, University of Manitoba. Beetles were picked from galleries using sterile forceps, and individually kept in microcentrifuge tubes and stored at 4 °C until needed. Determination of spore loads on single beetles was

carried out as described by Wainhouse et al. (1998). Single beetles were homogenized with a sterile disposable pestle in 0.25 ml sterile water, and the homogenate was spread over a Petri dish containing 2% malt extract agar. Plates were incubated in alternating light and dark cycles in the laboratory as previously described. 'Colony forming units' (CFU) on each plate were counted after 4 days. In sample plates with fungal colonies, sterile toothpicks were used to pick and streak out the fungus for five single colonies on separate plates to confirm the presence of *O. novo-ulmi*.

Statistical analysis

Summary statistics of distributions of diameters and of height above ground of dissected sections of trunk and branches were determined for whole trees felled in 2006 and 2007. The relationship of presence or absence of *H. rufipes* to attributes of sample sections and trees was studied using log-linear analysis of contingency tables (Bishop et al., 2007) and regression analysis. All analyses were carried out using Systat® 13 (Systat, 2009) and the alpha level for significance was 0.05.

Results

Tables 1 and 2 show the characteristics of the symptomatic American elm trees felled at the Camp Amisk site in 2006 and 2007. In 2006, diameter at breast height (DBH) ranged from 10.0 cm to 44.0 cm, while height ranged from 9.3 m to 17.5 m. Dutch elm disease symptoms at the time of felling ranged from trees with no leaves and completely bare crown to trees with brown wilted leaves; in June wilting was localized in some trees. In 2007, DBH ranged from 10.0 cm to 59.0 cm, while height ranged from 8.7 m to 18.0 m.

Dutch elm disease symptoms at the time of removal ranged from trees with no leaves and completely bare crown to trees with brown and green wilted leaves throughout the crown.

A progressive transformation of *H. rufipes* from one developmental stage of its life cycle to another was observed on trees felled on each of five dates in 2006 and 2007 (Table 3). In both years, *H. rufipes* adults were present in brood galleries in newly-symptomatic trees in June and there were small larvae. In later samples, larvae were larger. Larval development occurred from June to August or September. Pupae and adults were seldom found in July and August, but were evident in September and October. Although counts of eggs were not made because of their small size, those found in June and July were usually embedded in galleries and covered with frass.

The 2007 data in particular show the enormous level of variation among individual trees in the numbers of *H. rufipes* present (Table 3). Part of this variation may be attributed to differences in bark area sampled, and so to manage this source of variation and to incorporate this information from trees where only two branches were dissected, data were expressed as numbers per m^2 of bark area (Table 4). The number of insects per gallery tended to be higher in July and in August and to be lower in fall and winter samples in 2006. With the exception of August, the same trend was evident in 2007. The single adult found in August 2007 was not in a gallery.

Data in Table 4 are for all trees including branch samples. In both years adults and larvae were relatively abundant in June samples. In 2006, larvae were the dominant stage in July and August, and adults were present in the sample from early October; a few beetles remained in trees over winter, and these beetles appeared to be dead. In 2007, July and August samples yielded few *H. rufipes* and galleries. Larvae, pupae and adults were

present in the September/October tree which was felled on 17 September. The insects in the December 2007 samples appeared to be dead, although this was not confirmed for pupae, which are not mobile.

In 2006 and 2007, the proportion of *H. rufipes* from tree dissections carrying spores of *Ophiostoma novo-ulmi* ranged from 50% to 77% for larvae, 67% to 100% for pupae, and 0% to 100% for adults (Table 5). In branch sections, the proportion ranged from 61% to 88% for larvae, and all the 5 pupae collected from the 24 July branch sections carried spores. There were no adults found in branch samples in 2006. In 2007, the proportion of *H. rufipes* carrying spores of *Ophiostoma novo-ulmi* ranged from 88–100% for larvae, 100% for pupae and 91–100% for adults in tree and branch sections. In order to determine whether spores of *O. novo-ulmi* occurred in the gut of adult *H. rufipes*, cultures were made from beetles collected from elm tree dissections in 2006. All the beetles tested from branches (N = 17) had spores in their gut while 86% of the beetles from the trunk (N = 22) had spores in the gut. Beetles used in the experiment were offspring adults collected from galleries in symptomatic logs.

In 2006, the proportions of xylem and frass carrying spores of *Ophiostoma novo-ulmi* in tree and branch sections ranged from 100% for xylem to 90%–100% for frass. In 2007, all xylem and frass samples that were tested carried spores of *Ophiostoma novo-ulmi* (Table 6).

Table 7 shows the frequency with which *H. rufipes* carried spores of *Ophiostoma novo-ulmi* shown by the number of colony-forming units (CFU) associated with beetles emerging from trap logs set out at Glenlea Research Station in May 2006 and from dissected sections of symptomatic elm trees felled at Camp Amisk in 2006. *O. novo-ulmi*

spores were detected on 18 of 21 beetles collected from dissected sections of diseased elm trees, and from 20 of 21 beetles collected from a random sample of trap logs. There was no significant difference between the percentage of beetles bearing spores emerging from trap logs and dissected sections of diseased trees. On dissected sections of diseased trees, Colony Forming Units (CFU) per beetle ranged from 0 to 234. On trap logs, CFU per beetle ranged from 0 to 1200. Overall, 62% of beetles emerging from dissected sections and 57% of beetles emerging from trap logs had greater than 100 CFU.

Tables 8, 9 and 10 show summary statistics of distributions of above ground height and diameters of dissected sections of trunk and branches of whole symptomatic American elm trees felled at Camp Amisk in 2006 and 2007. Distributions of height above ground showed that except for the September 2007 tree there were no *H. rufipes* or galleries in the lowest available sections of the trunk. *Hylurgopinus rufipes* and galleries tended to be higher on the trunk than if distributed according to availability of section heights: in many trees they utilized the highest section as maxima were identical, regardless of weighting. Also, *H. rufipes* tended to be most frequent in the higher sections as 10 and 25 percentiles for beetles were usually higher than for available sections in individual trees and when pooled or averaged over all trees (Table 8). Distributions of galleries and beetles found on the trunk did not show great preference for any particular diameter (Table 9).

In branches, *H. rufipes* and galleries were not distributed through all available diameters (Table 10). Minimum section diameters for distributions weighted by *H. rufipes* and galleries were greater than for unweighted data. Also, maximum section diameters were often smaller for distributions weighted by *H. rufipes* and galleries than

for unweighted data. Except for June 2007, standard deviations for distributions weighted by number of *H. rufipes* and galleries were smaller than for unweighted data, and the percentiles also showed that *H. rufipes* and galleries tended not to be in the smallest available branches or the largest available branches. For *H. rufipes* it appeared that 65% of the population in branches in the June 2006 tree was in branches of diameter 8−11 cm in diameter, even though 10% of branch segments were within this range of diameters. In general, the bottom of trunks and branches of small diameter were underutilized by *H. rufipes*; in many trees, the largest diameter branches were also not favoured. Based upon presence and absence of *H. rufipes* in sections using pooled data for 2006 and 2007 (Table 11), the frequency of infestation of branch sections was significantly higher ($LR\chi^2$ = 20.5, df = 1, $P < 0.001$) than the frequency of infestation of trunk sections. No doubt part of the lower frequency of infestation of trunk sections is attributable to the avoidance by *H. rufipes* of the lowest sections of the trunk that was demonstrated in Table 8.

When data for whole symptomatic trees sampled in 2006 and 2007 were pooled, there were highly significant relationships between xylem staining and the presence of *H. rufipes* in dissected sections (Table 12) ($LR\chi^2$ = 18.4, df = 1, $P < 0.001$). The relationship between xylem staining and *H. rufipes* presence did not differ significantly between trunk sections and branch sections ($LR\chi^2$ = 3.6, df = 1, $P = 0.056$). The relationship between the percentage of stained branch sections with *H. rufipes* present and the log transformed total number of *H. rufipes* per elm tree (Fig. 8) was significant (F = 16.2, df = 1, 7, P = 0.005). The relationship was $Log_{10}y = 1.15 + 0.02885x$, where y = Total *H. rufipes* in the tree and x = % stained branch sections with *H. rufipes*

Discussion

The 2007 removal data at Camp Amisk illustrate the enormous level of variation among individual trees in the numbers of *H. rufipes* present. Part of this variation may be attributable to differences in tree size, and so to manage this source of variation, data were expressed as numbers per m^2 of bark area. However, even when expressed as densities, beetle numbers in 2007 were still quite variable. Characteristics that make trees attractive to *H. rufipes* are unknown, so it is not easy to determine which trees will contain more beetles. There does not seem to be any correlation between a tree characteristic and high numbers of beetles, and dissecting newly-symptomatic elm trees is a costly and extremely labour-intensive process.

The data obtained in the 2006 and 2007 field seasons showed that newly symptomatic trees were suitable for construction of brood galleries. It appeared that most of the tiny and small-sized larval galleries were newly constructed from recently hatched larvae. Larval development occurred from June until August or September. Adults found in September/October samples are assumed to be of the new generation and about to leave their brood tree. Adult emergence may also be inferred from the presence of pupae. According to a temperature dependent model of pupal development (Kaston, 1939), under late summer temperature conditions in Manitoba, adults are expected to emerge from pupae within about 10 days of pupation. In the October 2006 tree, the low number of insects per gallery was an indicator that most galleries were empty and that the majority of beetles had already left the tree. In the December samples of both years, there were few insects, and all of them were dead. The results showed that native elm bark

beetles were able to complete their life cycle in newly-symptomatic trees in the same year as DED detection.

Although data from 2007 illustrate the extreme variability in infestation levels on individual trees, the same general pattern as in 2006 was evident. However, the number of larvae in the September 2007 sample, and the number of dead insects in the December 2007 samples suggest that not all insects successfully completed development in 2007. In Manitoba, *H. rufipes* does not overwinter in the larval stage.

It has generally been assumed that, in Manitoba, *H. rufipes* seldom breed and produce offspring in an infected tree in the same year that symptoms are detected. However, the City of Winnipeg (Robbie-Draward, 1995) conducted a small study of trees that were identified as >50% dead and were removed in winter. Of these trees, about 67% were infected with DED, and 38% (up to 55% in some sites) also contained considerable numbers of brood galleries, some of which had exit holes. It is not known whether the infections of the trees in this study were new, or from a previous year. However, the study raised the question of whether breeding in recently infected elms contributes substantially to the population of overwintering *H. rufipes*, and whether emerging beetles carry the pathogen. My study has demonstrated that, at least in some years, adult beetles can emerge from trees that were diagnosed earlier in the same year.

Perhaps the assumption that development is not normally completed in newly-symptomatic trees arises from the observation that visual symptoms of DED often appear after the time when *H. rufipes* brood are developing in brood galleries. However, it is now known that adult *H. rufipes* are not dependent upon visual cues to detect diseased trees. Infected elm trees emit semiochemicals that attract vector beetles (McLeod et al.,

2005). Visual symptoms, called ‘flagging’ may appear after beetles have already detected symptomatic host material using chemical cues.

Temperature greatly influences development of bark beetles (Wermelinger and Seifert, 1998), including *H. rufipes* (Kaston, 1939). Higher temperatures that are below the species’ upper lethal limit reduce the time to complete development (Heliövaara and Peltonen, 1999). Winnipeg had one of its warmest summers in 2006, with a mean of 19.8 °C (Environment Canada, 2012a) between June and August, higher than the 30 year average for this period of 18.3 °C (Environment Canada, 2007). Thus, although development was completed in 2006, in years with temperatures closer to the long term average, completion of development might not occur for *H. rufipes*. In 2007, summer temperatures were consistent with the long-term average in Manitoba and some of the beetles did not complete development and died in brood galleries.

The intergovernmental committee on climate change (IPCC, 2007) predicts that future storm frequency and average temperature will increase and severe phenomena including flooding, dry spells and heat spells will intensify (Cubasch et al., 2001). Such changes may influence the life cycle of *H. rufipes* in Manitoba. More frequent years with higher temperatures during the growing season would increase the number of years during which development is successfully completed in symptomatic trees. Temperature also regulates the onset of flight in spring (Heliövaara and Peltonen, 1999) and, as with other bark beetles (Forsse, 1989), higher spring temperatures could allow earlier onset of flight and reproduction in *H. rufipes*. Increased temperatures and longer growing seasons may result in decreased winter mortality (Whittaker and Tribe, 1996; Virtanen et al., 1996), and a shift to both larval and adult overwintering of *H. rufipes* in Manitoba, as

currently occurs in central (Gardiner, 1981) and southern (Finnegan, 1957) Ontario. In addition to effects on insect vectors, climate change could affect other aspects of host-pathogen interactions: warmer temperatures could increase pathogen development and transmission rates, increase the number of cycles per year, increase overwintering survival of pathogens, and change host susceptibility to infection (Harvell et al., 2002). Increased storm frequencies with wind-felled elm trees would provide more breeding material for *H. rufipes*. In a warmer climate, higher evaporation will lead to water shortage and increased host susceptibility, especially later during the summer.

The methodology I used to assess spore distribution on and in beetles and tree components is standard and adequate. A CFU is a viable individual cell which is capable of growing by binary fission into a colony of similar cells. The range of CFU on adult beetles was essentially the same for those dissected from sections of diseased trees and those that emerged from infected trap logs. This suggests that the percentages of spore-bearing new generation adults in dissections are a good guide to the frequency of spore-bearing beetles leaving a tree. Most new-generation beetles carried spores on the exoskeleton in my studies, but I also showed that a high proportion of these beetles carry viable spores in their gut, thereby increasing the total spore load available to the beetle to cause infection during feeding. From studies mainly with *Scolytus* species, factors affecting acquisition of *O. novo-ulmi* and *O. ulmi* spores by bark beetle vectors include beetle behaviour and location of pupal cells within the bark of diseased trees, and the favourability for pathogen development of environmental conditions in the pupal chambers (Webber, 2004). High temperatures may prevent spore formation in pupal cavities (Faccoli and Battisti, 1997), but mean air temperatures in Manitoba between June

and August were 19.8 °C in 2006 and 18.7 °C in 2007 (Environment Canada, 2012a, 2012b), quite close to the growth temperature optimum of 20−22 °C of *O. novo-ulmi* (Brasier et al., 1981). The high proportion of *H. rufipes* carrying *O. novo-ulmi* spores in 2006 and 2007 in my study might have been due to favourable temperatures for sporulation.

Parker et al. (1948) have shown that between 12.3% and 78.9% of adult *H. rufipes* carried spores as they emerged in July and August from trees infested with DED in New York State. Parker et al. (1948) also showed that 36.7% to 56.6% of *H. rufipes* emerging from diseased wood between October and early November carried spores. However, in New York State, *H. rufipes* is very much less effective as a vector than *S. multistriatus*, possibly because during feeding on twigs, *H. rufipes* is much less likely than *S. multistriatus* to make injuries that penetrate the wood of healthy trees and branches (Parker et al., 1948).

Unlike some bark beetles in which spores are carried in mycangia (Paine et al., 1997), in elm bark beetles, spores are carried on the exoskeleton (Webber, 2000). The absence of a mycangium may contribute to spore loss during flight, which may amount to 50% of the original spore load (Webber and Brasier, 1984; Webber, 2000). The high spore load I observed on the exterior of adult *H. rufipes* dissected from trees could be considerably reduced after a period of flight. However, if spores in the gut of these beetles survive winter and are transmitted during feeding in the crown in the spring, loss from the exoskeleton may be unimportant in the transmission process. Compared to the less aggressive *O. ulmi*, *O. novo-ulmi* requires fewer spores to successfully infect an elm tree through feeding grooves (Webber, 2004). In Manitoba, the combination of the

presence of the highly susceptible *U. americana*, the more aggressive *O. novo-ulmi* and favourable temperatures for its sporulation, and the presence of spores in the gut of *H. rufipes*, may contribute to effective transmission by *H. rufipes*.

Beetles that emerge with spores in late summer do not immediately transmit the spores to a new tree; transmission usually occurs when beetles are feeding on twig crotches in the crown in spring after they have over-wintered (Takai et al., 1979). Beetles over-winter in the base of healthy elm trees, but do not transmit spores at that time (Andersen and Holliday, 2003). Many beetles start feeding in August and continue into October or November before finally becoming dormant (Anderson, 1996). Over-wintering beetles probably rely on fat reserves that they accumulate through feeding after late summer emergence from brood galleries. Consequently, some proportion of the beetles emerging from symptomatic trees in September or later might have insufficient time to accumulate fat before winter, and might not survive. If such beetles have high levels of winter mortality, their contribution to transmission of *O. novo-ulmi* in Manitoba could be quite small. Studies of the fate of beetles emerging from newly-symptomatic trees would be very useful. However, in the field, beetles move from their brood tree to a healthy tree to feed and overwinter, and then, if they survive, move to the crown of a tree the following spring, where they feed and possibly transmit spores: following beetles through these two dispersal phases, involving two or more trees would be logistically challenging.

Despite the absence of studies of the fate of spore-bearing beetles that emerge from newly-symptomatic trees, it is necessary to determine how my results should be utilized in DED management. If these beetles die during winter, no transmission would

occur, and the practice of removing newly-symptomatic trees in winter is adequate. However, if these beetles survive and contribute considerably to spore transmission, “rapid removal”, in which diagnosed trees are removed immediately after symptom detection, may be warranted. The technique of “rapid removal” is regarded as the most efficient DED management technique (Barger, 1977; Stipes, 2000), but the associated logistical difficulties are great in jurisdictions with thousands of trees to remove rapidly, and an assessment of its cost effectiveness in operational conditions is desirable. Such an assessment was carried out in Manitoba by Veilleux et al. (2012), and showed that new infection rates were significantly less in communities using rapid removal than in those where removal of infected trees occurred in fall or winter. Some of the benefits identified in Veilleux et al. (2012) might arise because rapid removal could reduce root-graft transmission or transportation of diseased wood. However, the combination of the results of that study and the work reported here strongly suggest that, at least in some years, spore-bearing beetles emerging from newly-symptomatic trees contribute appreciably to transmission of DED, and it would be prudent to use rapid removal to prevent the possibility of this mechanism of transmission.

In large jurisdictions, such as Winnipeg, there is insufficient capacity to remove all newly symptomatic trees before adult emergence, and prioritization for rapid removal must be done. In my study, the total number of *H. rufipes* in newly-symptomatic trees ranged, during the period before adults were expected to have started leaving the tree, from 1 to 41,213. Clearly, symptomatic trees with the largest numbers of *H. rufipes* pose the greatest threat as a source of future infections and should have the highest priority for removal. My finding that the frequency of presence of *H. rufipes* in a dissected tree

section is associated with the presence of blue stain, and is related to total numbers of beetles in the tree could form the basis for a prioritization system. By scoring a small number of stained branch sections for the presence or absence of *H. rufipes*, a relationship such as this could be used to identify trees with the highest number of beetles.

There are many reports of the symbiosis between bark beetles and blue-stain fungi (Six and Wingfield, 2011 and references therein). Almost all of these reports deal with coniferous trees, and none deal with *H. rufipes*. Also, studies of association of spatial distributions of fungi and beetles have been at the among-tree scale or at larger scales, rather than within tree (e.g. Rumbold, 1931, 1936; Roe et al., 2011). Small-scale within-tree associations of distribution of blue-staining and bark beetle brood galleries might be expected because the fungus pre-conditions the tree for the normal development of bark beetle broods (Craighead, 1928). Possibly the release of attractive volatiles that *O. novo-ulmi* induces in infected elm trees (McLeod et al., 2005) occurs at small scale, and is responsible for the attraction of *H. rufipes* to fungal stained sections of tree, but other mechanisms could be involved.

In addition to preference for blue-stained sections, my research also showed within-tree preferences in siting of brood galleries: branch sections were preferred to trunk sections, higher trunk sections were preferred to lower trunk sections and branches of intermediate diameter were preferred to smaller or larger branches. Larger branches have thicker bark, and so the smallest branches may have insufficient bark to retain moisture to support larval development. *Scolytus kirschii* Skalitzky, which is a DED vector in Europe, utilizes larger diameter branches for brood rearing in more arid regions

than in moister regions (Six et al., 2005). Other bark beetles also exhibit preferences for specific portions of host trees. For example, in California, *S. multistriatus* prefers attacking the basal to mid-bole section of Chinese elm (*Ulmus parvifolia* Marsham) (Švihra, 1998) and avoids constructing brood galleries in the smallest branches of *Ulmus procera* (Hajek and Dahlsten, 1985). In bark beetles attacking conifers, thickness of the bark is a determinant of attack site of *Tomicus destruens* (Wollaston) (Gahdab, 2007) and *Ips pini* Say (Kolb et al., 2006) and the reproductive success of *I. pini* is greater in larger branches (Steed and Wagner, 2004). Through the effect on humidity in galleries, larger diameter branches with thicker bark would tend to favour spore acquisition by beetles, as sporulation by the pathogen is inhibited under dry conditions (Webber, 1990).

Implications for management of Dutch elm disease

My finding that spore-bearing *H. rufipes* adults can emerge from newly-symptomatic trees has important implications for DED transmission. Delaying tree removal until winter provides an opportunity for many spore-carrying beetles to leave newly-symptomatic trees. Whether these beetles are able to overwinter successfully and transmit the spores to susceptible spring wood the following year is unknown, but the demonstration by Veilleux et al. (2012) of the benefits of rapid removal supports the view that this type of transmission happens and contributes appreciably to the new infections. In jurisdictions where *H. rufipes* and *S. multistriatus* coexist, emergence of spore-bearing beetles can take place at most times during the growing season, and rapid removal has been shown to be extremely advantageous (Barger, 1977). As shown in this study, timing should be made an important part of sanitation removals in all jurisdictions with DED, and must be synchronised with vector biology to prevent emergence and dispersal of

beetles carrying spores. Therefore, I recommend that newly-symptomatic trees be removed as soon as possible after symptom detection, ideally before the end of August when, in warm years, new generation beetles can emerge.

The relationship between presence of *H. rufipes* in stained branch sections and the total number of beetles per tree could be the basis for a diagnostic tool to help prioritize trees for prompt removal and destruction. Further work on this relationship, with a focus on trees during the period of rapid tree removal, is advisable. The relationship could lead to more effective and efficient DED surveillance and removal of diseased elms. If this approach is to be implemented, it is desirable to dissect new trees from the June−August period when prioritization would need to be done, and to standardize the definition of a branch section.

Table 1. Characteristics of symptomatic American elm trees removed at Camp Amisk in 2006.

Date of removal and tree I. D.	Diameter at breast height (DBH[1])(cm)	Height (m)	Type of sample collected	[2]Description and extent and direction of centre of symptoms at time of sampling
26 June A	19.0	13.2	branches	Brown wilted leaves 148° SE around crown
26 June B	10.0	9.3	branches	Brown wilted leaves 170° SE around crown
26 June C	17.0	11.2	branches	Brown wilted leaves 90° SE around crown
26 June D	44.0	16.0	whole tree	Brown wilted leaves 360° around crown
24 July A	27.5	15.5	branches	Brown wilted leaves 360° around crown
24 July B	22.0	17.0	branches	Brown wilted leaves 360° around crown
24 July C	19.5	13.5	branches	Brown wilted leaves 360° around crown
24 July D	25.4	16.6	whole tree	Brown wilted leaves 360° around crown
21 August A	31.3	11.9	branches	Brown wilted leaves 360° around crown
21 August B	25.6	13.1	branches	Brown wilted leaves 360° around crown
21 August C	29.4	12.5	branches	Brown wilted leaves 360° around crown
21 August D	32.0	17.5	whole tree	Brown wilted leaves 360° around crown
2 October	22.0	13.1	whole tree	No leaves, crown completely bare
4 December A	11.1	12.3	branches	Brown wilted leaves 360° around crown
4 December B	14.0	10.5	branches	Brown wilted leaves 360° around crown
4 December C	19.1	12.8	branches	Brown wilted leaves 360° around crown
4 December D	26.4	16.0	whole tree	Brown wilted leaves 360° around crown

[1]Tree diameter at breast height was measured at 1.3 m above ground with a diameter measuring tape and calculated using the formula $D = C/\pi$, where D = diameter, C = circumference and $\pi = 3.14$.

[2]Symptoms direction was measured with a Silva Type 15 compass at a distance of 2 m from trees.

Table 2. Characteristics of symptomatic American elm trees removed at Camp Amisk in 2007

Date of removal and tree I. D.	Diameter at breast height (DBH[1])(cm)	Height (m)	Type of sample collected	[2]Description and extent and direction of centre of symptoms at time of sampling
25 June A	14.0	12.5	branches	Brown and green wilted leaves 360^{o} around crown
25 June B	22.3	10.2	branches	Brown and green wilted leaves 360^{o} around crown
25 June C	10.0	8.7	branches	Brown and green wilted leaves 360^{o} around crown
25 June D	32.0	12.0	whole tree	Brown wilted leaves 360^{o} around crown
23 July A	59.0	10.4	branches	Brown and green wilted leaves 360^{o} around crown
23 July B	57.0	11.6	branches	Brown and green wilted leaves 360^{o} around crown
23 July C	50.0	12.3	branches	Brown and green wilted leaves 360^{o} around crown
23 July D	41.0	18.0	whole tree	Almost bare with very few brown wilted leaves 360^{o} around crown
13 August A	20.4	13.6	branches	Brown and green wilted leaves 360^{o} around crown
13 August B	16.5	12.0	branches	Brown and green wilted leaves 360^{o} around crown
13 August C	12.1	9.5	branches	Brown and green wilted leaves 360^{o} around crown
13 August D	39.8	10.0	whole tree	Brown and green wilted leaves 360^{o} around crown
17 September	54.1	18.0	whole tree	A few brown and green wilted leaves 360^{o} around crown
3 December A	22.9	10.2	branches	No leaves; crown completely bare
3 December B	21.3	12.0	branches	No leaves; crown completely bare
3 December C	21.0	10.8	branches	No leaves; crown completely bare
3 December D	24.8	11.0	whole tree	No leaves; crown completely bare

[1]Tree diameter at breast height was measured at 1.3 m above ground with a diameter measuring tape and calculated using the formula $D = C/\pi$, where D = diameter, C = circumference and $\pi = 3.14$.

[2]Symptoms direction was measured with a Silva Type 15 compass at a distance of 2 m from trees.

Table 3. Numbers and percentage stage composition of *Hylurgopinus rufipes* collected from newly symptomatic American elm trees on five dates in 2006 and 2007. Data represent four trees per date except for October 2006 and September 2007 when one tree was sampled.

Year	Month	Total number of beetles in samples					Percentage stage composition		
		Sample type				Total for date			
		Whole	Branch	Branch	Branch		Larvae	Pupae	Adults
2006	June	927	0	0	0	927	93.5	0.3	6.1
	July	951	2961	1167	23	5102	99.8	0.3	0.0
	August	457	835	55	294	1641	98.8	1.1	0.0
	October	42	–	–	–	42	0.0	21.4	78.6
	December	6	0	0	0	6	83.3	0.0	16.7
2007	June	41213	0	0	0	41213	78.8	0.3	21.2
	July	30	0	0	0	30	100.0	0.0	0.0
	August	1	0	0	0	1	0.0	0.0	100.0
	September	1217	–	–	–	1217	35.9	13.3	50.8
	December	0	577	8	0	585	7.7	57.1	35.2

Table 4. Density of *Hylurgopinus rufipes* and brood galleries in dissected trees and branches in 2006 and 2007.

Date of felling	2006					2007				
	Numbers per m^2 (Mean±SE)[1]				Insects per gallery (Mean±SE)[1]	Numbers per m^2 (Mean±SE)[1]				Insects per gallery (Mean±SE)[1]
	Larvae	Pupae	Adults	Brood galleries		Larvae	Pupae	Adults	Brood galleries	
June	16.1±8.5	0.1±0.1	1.1±0.5	1.6±0.4	4.8±1.8	1487.0±388.3	0.0±0.0	357.0±42.1	580.0±72.0	2.8±0.2
July	153.4±35.0	0.4±0.3	0.1±0.1	21.4±4.1	6.5±0.9	1.0±0.7	0.0±0.0	0.0±0.0	0.06±0.05	15.0±0.0
August	98.0±25.0	1.0±0.6	0.0±0.0	16.1±3.2	6.5±1.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Sept/Oct	0.0±0.0	1.0±0.5	2.0±0.7	25.4±5.4	0.1±0.0	12.1±2.0	4.4±0.8	17.3±2.6	14.3±1.9	2.9±0.4
December	0.3±0.2	0.0±0.0	0.1±0.1	0.4±0.2	1.1±0.6	5.1±2.7	44.9±22.7	20.0±8.4	66.6±26.2	0.3±0.1

[1] Densities and insects per gallery were calculated for each dissected section, and these data were used to calculate means and standard errors.

Table 5. Percentage of *Hylurgopinus rufipes* from American elm tree dissections carrying spores of *Ophiostoma novo-ulmi*, and numbers of beetles tested in 2006 and 2007.

Year	Month	Percent of *H. rufipes* carrying spores (number of *H. rufipes* tested)					
		Whole-tree sections			Branch sections		
		Larvae	Pupae	Adults	Larvae	Pupae	Adults
2006	26 June	77 (819)	67 (3)	100 (31)	–	–	–
	24 July	58 (316)	–	0 (1)	61 (267)	100 (5)	–
	21 August	50 (106)	100 (2)	100 (1)	88 (362)	–	–
	2 October	–	100 (9)	91 (33)	–	–	–
	4 December	100 (5)	–	100 (1)	–	–	–
2007	25 June	88 (440)	–	92 (506)	–	–	–
	23 July	100 (20)	–	100 (1)	–	–	–
	13 August	–	–	100 (1)	–	–	–
	17 September	–	100 (9)	91 (33)	–	–	–
	3 December	–	–	–	88 (17)	100 (26)	86 (14)

Table 6. Percentage of xylem and frass from American elm tree dissections carrying spores of *Ophiostoma novo-ulmi* and number of samples tested in 2006 and 2007.

Year	Month	Percentage of xylem and frass carrying spores (number of samples tested)			
		Whole-tree sections		Branch sections	
		Xylem	Frass	Xylem	Frass
2006	26 June	100 (19)	90 (77)	100 (6)	—
	24 July	100 (23)	96 (69)	100 (8)	96 (67)
	21 August	100 (17)	97 (29)	100 (7)	100 (105)
	2 October	100 (32)	100 (106)	—	—
	4 December	100 (9)	100 (8)	100 (6)	—
2007	25 June	100 (12)	100 (58)	100 (4)	—
	23 July	100 (25)	100 (3)	100 (11)	—
	13 August	100 (19)	—	100 (11)	—
	17 September	100 (22)	100 (74)	—	—
	3 December	100 (7)	—	100 (8)	100 (12)

Table 7. Frequency with which *Hylurgopinus rufipes* carried spores of *Ophiostoma novo-ulmi* shown by the number of colony-forming units (CFU) (ranged from 0 to 1200) associated with beetles removed from dissected sections of symptomatic elm trees felled at Camp Amisk in 2006 and beetles that emerged from infected trap logs set out at Glenlea Research Station in May 2006.

Samples	No. of single beetle units	Percentage of beetles with spores	Mean CFU ±SD	Number of CFU from homogenate in CFU ranges		
				0−10	11−100	101−1200
Dissected sections	21	86	119.2 ± 72.5	3	5	13
Trap logs	21	95	159.1 ± 245.3	3	6	12

Table 8. Summary statistics of distributions of height above ground of dissected sections of trunks of whole symptomatic American elm trees* felled at Camp Amisk in 2006 and 2007. Height distributions are unweighted, to show distributions of samples; weighted by the numbers of *Hylurgopinus rufipes* to show height distributions of beetles; and weighted by number of galleries to show height distribution of galleries.

Date	Weighting	N	Height above ground (cm)								
			Min	Max	Mean	S.D.	Percentiles				
							10%	25%	50%	75%	90%
26 Jun 06											
	None	28	3	561	268	174	30	119	262	419	510
	H. rufipes	8	67	561	314	228	67	67	314	561	561
	Galleries	6	67	561	260	192	71	108	222	381	543
23 Jul 06											
	None	29	15	617	333	180	80	183	341	488	569
	H. rufipes	28	70	595	145	131	70	70	119	119	361
	Galleries	17	70	595	141	149	70	70	70	119	395
21 Aug 06											
	None	34	16	961	460	272	109	235	443	671	857
	H. rufipes	17	812	853	840	19	812	812	853	853	853
	Galleries	3	812	853	839	23	812	822	853	853	853
02 Oct 06											
	None	10	19	355	179	114	35	83	172	274	335
	H. rufipes	8	192	315	253	43	192	213	274	274	302
	Galleries	55	83	315	240	73	116	192	274	315	315
04 Dec 06											
	None	28	7	666	321	204	51	141	314	496	603
	H. rufipes	5	206	351	259	73	206	206	206	333	351
	Galleries	5	206	351	312	60	206	297	327	351	351
25 June 07											
	None	9	16	307	165	102	31	79	163	256	295
	H. rufipes	8310	124	307	248	49	163	208	278	278	307
	Galleries	2010	124	307	235	50	163	208	249	278	307
17 Sep 07											
	None	18	10	395	206	122	40	96	211	312	368
	H. rufipes	31	10	395	111	120	10	10	76	200	337
	Galleries	16	10	395	186	121	37	86	189	278	370
All trees (Pooled)											
	None	215	2	1268	360	262	56	153	310	524	732
	H. rufipes	8477	10	892	248	59	163	208	278	278	307
	Galleries	2112	10	892	236	61	163	208	249	278	307
All trees (Averaged)											
	None	10	—	—	323	202	59	152	313	491	606
	H. rufipes	10	—	—	217	93	154	160	215	265	322
	Galleries	10	—	—	226	75	149	180	220	260	360

*Trees felled on 23 July, 13 August and 3 December 2007 were not included in the table due to insufficient data, but are included in the pooled and averaged analysis

Table 9. Summary statistics of distributions of diameters of dissected sections of trunks of whole symptomatic American elm *trees felled at Camp Amisk in 2006 and 2007. Data are either unweighted, weighted by the numbers of beetles, or weighted by the number of galleries to show the distribution of samples, beetles and galleries respectively.

Date	Weighting	N	Diameter of trunk sections (cm)								
			Min	Max	Mean	S.D.	Percentiles				
							10%	25%	50%	75%	90%
26 Jun 06											
	None	28	30.0	52.5	39.9	5.3	35.1	36.7	38.0	42.0	48.5
	H. rufipes	8	36.5	50.0	44.0	5.7	36.5	39.8	43.0	50.0	50.0
	Galleries	6	34.0	50.0	40.8	5.6	34.3	36.5	40.7	43.0	49.3
23 Jul 06											
	None	29	18.0	30.0	21.7	2.8	18.4	19.9	21.0	23.0	25.3
	H. rufipes	28	19.0	26.0	24.1	1.9	21.2	24.0	24.0	26.0	26.0
	Galleries	17	19.0	26.0	24.5	2.1	20.8	24.0	26.0	26.0	26.0
21 Aug 06											
	None	34	14.0	28.5	19.1	3.6	15.0	16.5	18.0	21.6	23.0
	H. rufipes	17	14.0	27.0	15.1	3.1	14.0	14.0	14.0	15.0	15.0
	Galleries	3	14.0	15.0	14.3	0.6	14.0	14.0	14.0	14.7	15.0
02 Oct 06											
	None	10	19.0	34.5	22.4	5.1	19.0	20.0	20.0	21.8	31.7
	H. rufipes	8	19.0	20.0	19.4	0.5	19.0	19.0	19.0	20.0	20.0
	Galleries	55	19.0	21.8	19.6	0.8	19.0	19.0	19.0	20.0	20.0
04 Dec 06											
	None	28	12.0	45.0	31.6	6.2	27.7	29.2	30.7	33.0	39.6
	H. rufipes	5	30.4	32.5	31.8	1.0	30.4	30.8	32.5	32.5	32.5
	Galleries	5	30.4	32.5	31.8	0.9	30.4	30.4	31.0	31.4	32.5
25 Jun 07											
	None	9	28.0	35.0	29.8	2.3	28.0	28.4	29.0	30.4	33.7
	H. rufipes	8380	28.0	31.7	28.9	1.2	28.0	28.0	28.5	29.0	31.8
	Galleries	2010	28.0	31.7	29.1	1.2	28.0	28.5	29.0	29.0	31.8
17 Sep 07											
	None	18	6.5	67.0	43.5	14.8	20.2	40.5	48.3	50.0	59.1
	H. rufipes	31	6.5	67.0	52.9	11.7	43.5	48.5	52.5	62.0	62.0
	Galleries	16	6.5	67.0	45.2	15.0	19.4	45.3	48.5	51.0	61.1
All trees (Pooled)											
	None	215	6.5	67.0	28.3	10.2	18.0	21.0	26.0	34.0	43.0
	H. rufipes	8477	6.5	67.0	28.9	2.2	28.0	28.0	28.5	29.0	31.8
	Galleries	2112	6.5	67.0	29.0	2.9	28.0	28.5	29.0	29.0	31.8
All trees (Averaged)											
	None	10	—	—	28.7	5.3	22.5	26.4	28.3	30.5	36.1
	H. rufipes	10	—	—	21.6	2.5	19.3	20.4	21.3	23.5	23.7
	Galleries	10	—	—	20.5	2.6	16.6	19.8	20.8	21.5	26.6

*Trees felled on 23 July, 13 August and 3 December 2007 were not included in the table due to insufficient data, but are included in the pooled and averaged analysis

Table 10. Summary statistics of distributions of diameters of dissected sections of branches of the symptomatic whole American elm [*]trees felled at Camp Amisk in 2006 and 2007. Data are either unweighted, weighted by the numbers of beetles, or weighted by the number of galleries.

Date of felling	Weighting	N	Branch section diameter (cm)								
			Min	Max	Mean	S.D.	Percentiles				
							10%	25%	50%	75%	90%
26 Jun 06											
	None	159	2.6	35.0	10.2	8.3	3.5	4.3	6.5	13.9	27.0
	H. rufipes	919	4.0	23.0	10.3	2.4	6.5	8.5	11.0	11.5	11.5
	Galleries	76	3.5	23.0	9.8	4.1	5.0	6.5	11.0	11.5	15.9
23 Jul 06											
	None	23	4.0	17.0	9.1	3.9	4.3	6.1	8.0	13.0	15.0
	H. rufipes	923	4.5	15.0	7.1	2.1	4.5	5.8	7.0	8.0	8.5
	Galleries	146	4.5	15.0	7.4	2.2	4.5	5.8	7.0	8.0	9.8
21 Aug 06											
	None	28	3.0	12.5	6.4	2.5	4.0	4.5	5.8	7.5	9.9
	H. rufipes	440	4.5	8.5	5.0	0.9	4.5	4.5	4.5	5.0	6.5
	Galleries	41	4.5	8.5	6.1	1.7	4.5	4.5	5.0	8.0	8.5
02 Oct 06											
	None	67	1.7	45.0	6.9	6.4	2.1	3.0	5.0	8.8	14.9
	H. rufipes	34	7.0	18.0	12.6	4.4	7.0	8.0	16.0	16.0	18.0
	Galleries	323	4.5	45.0	13.8	4.1	8.0	10.0	15.0	16.0	18.0
04 Dec 06											
	None	39	3.5	26.5	13.5	5.5	7.4	9.6	12.5	17.9	20.8
	H. rufipes	1	10.0	10.0	10.0	—	—	—	—	—	—
	Galleries	2	10.0	10.0	10.0	0.0	10.0	10.0	10.0	10.0	10.0
25 Jun 07											
	None	57	3.0	22.0	11.9	5.9	5.5	6.5	10.0	17.6	20.9
	H. rufipes	32833	4.0	22.0	15.6	5.6	6.0	11.0	16.5	21.0	21.0
	Galleries	11795	4.0	22.0	15.2	5.3	7.0	10.0	16.0	20.0	21.0
17 Sep 07											
	None	72	8.0	42.0	22.4	6.7	14.9	18.0	21.8	26.0	32.0
	H. rufipes	1186	13.0	33.0	21.9	3.8	17.0	19.0	21.5	25.0	26.0
	Galleries	487	13.0	42.0	22.5	3.9	18.0	19.5	22.0	25.0	26.0
All trees (Pooled)											
	None	563	1.7	45.0	11.8	8.0	4.0	5.5	9.5	16.9	23.1
	H. rufipes	36367	4.0	33.0	15.3	5.9	6.0	10.0	16.5	21.0	21.3
	Galleries	12874	3.5	45.0	15.3	5.5	7.0	10.0	16.0	20.0	21.0
All trees (Averaged)											
	None	10	—	—	11.3	5.1	6.2	7.5	9.8	14.5	18.7
	H. rufipes	10	—	—	9.6	2.6	6.3	7.6	10.3	11.8	12.4
	Galleries	10	—	—	9.1	2.4	6.2	7.0	9.2	10.6	11.7

[*]Trees felled on 23 July, 13 August and 3 December 2007 were not included in the table due to insufficient data, but are included in the pooled and averaged analysis

Table 11. Contingency table showing the relationship between presence of *Hylurgopinus rufipes* beetles and whether sections are branches or trunks in symptomatic American elm trees pooled over all sections dissected in 2006 and 2007.

	Section		
	Branch	Trunk	N
Beetles absent	70.4 %	85.6 %	576
Beetles present	29.6 %	14.4 %	196
N	557	215	772

Table 12. Contingency table showing sections with xylem staining in relation to percentage of sections with *Hylurgopinus rufipes* present or absent pooled over trunks and branches in symptomatic American elm trees dissected in 2006 and 2007.

	Not stained	Stained	Total	N
Beetles absent	93.2 %	72.7 %	74.6 %	576
Beetles present	6.8 %	27.3 %	25.4 %	196
N	73	699		772

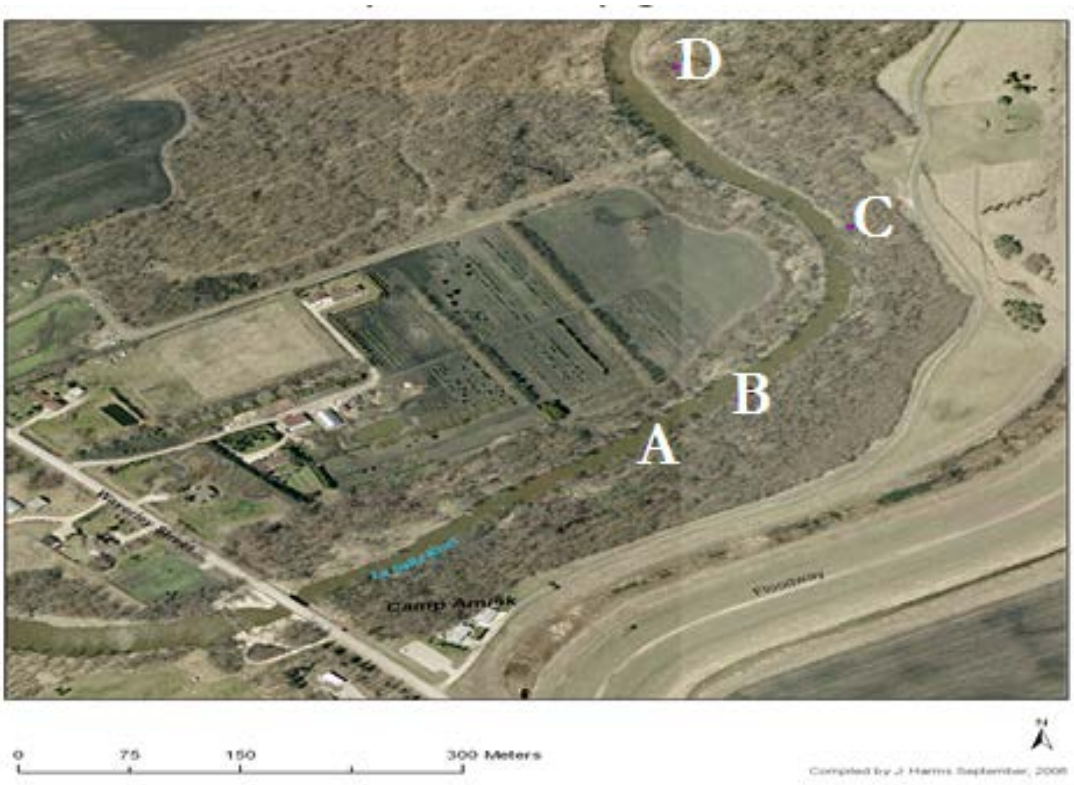

Figure 6. GPS locations of four newly-symptomatic American elm trees (A-D) felled at Camp Amisk, Winnipeg on June 26, 2006. Selected trees were felled and dissected at intervals from late June to December. Image credit: Jenny Harms, Protected Areas Specialist, Parks and Protected Spaces, Manitoba Conservation and Water Stewardship, Winnipeg. Used with permission

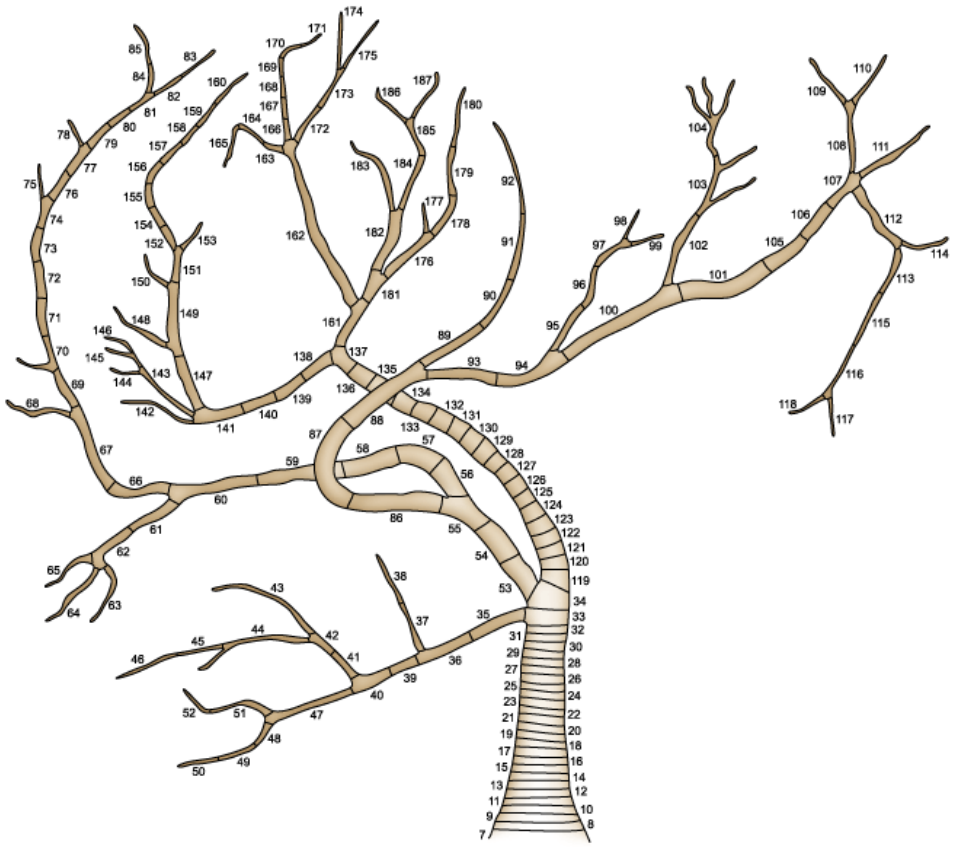

Figure 7. Diagrammatic representation of the symptomatic American elm tree felled at Camp Amisk on 26 June 2006 showing the labelling scheme for trunk and branch sections. Image credit: Jonathan Veilleux. Used with permission.

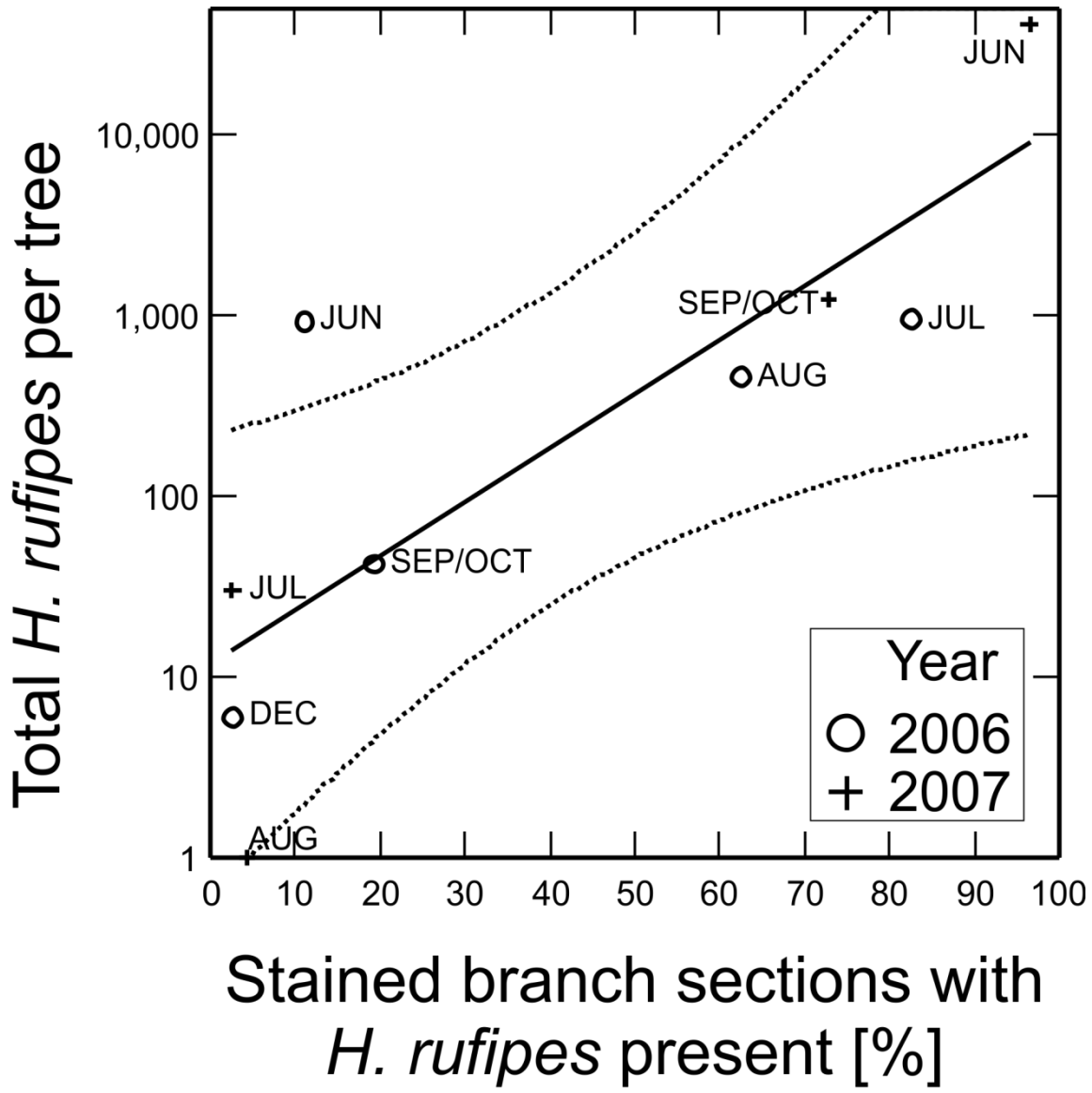

Figure 8. Relationship between stained branch sections with *Hylurgopinus rufipes* present (%) and total number of *H. rufipes* per American elm tree in 2006 and 2007 in Winnipeg. Dotted lines are the 95% confidence limits for the regression line, which is the solid line.

Chapter 4: Overwintering of the native elm bark beetle, *Hylurgopinus rufipes* Eichhoff (Coleoptera: Curculionidae), in American elm trees, *Ulmus americana* L. (Ulmaceae) in Manitoba

Introduction

The two main vectors of Dutch elm disease (DED) fungal pathogens (*Ophiostoma* (*Ceratocystis*) *ulmi* (Buismann) Nannfeldt and O. *novo*-ulmi Brasier) in North America are the native elm bark beetle, *Hylurgopinus rufipes* Eichhoff, and the smaller European elm bark beetle, *Scolytus multistriatus* Marsh (Collins, 1941; Parker et al., 1948). In Manitoba, Saskatchewan and North Dakota, *H. rufipes* (Coleoptera: Curculionidae), is the major vector of DED pathogens (Hildahl and Wong, 1965; Schacherl, 1996; Anderson and Holliday, 2003).

In northern regions, such as Minnesota and Manitoba, *H. rufipes* overwinter as adults (Landwehr et al., 1981; Swedenborg et al., 1988; Anderson and Holliday, 2003). *Hylurgopinus rufipes* adults appearing in late spring from overwintering sites at the base of elms proceed to the canopies of healthy elm trees to feed (Kaston, 1939; Landwehr et al., 1981); during feeding, transmission of spores may occur (Takai et al., 1979). Successful fungus transmission to elm trees occurs when feeding beetles penetrate to the xylem tissues and the tree's physiology is susceptible to infection (Takai et al., 1979). In Quebec, Pomerleau (1965b) found that American elm trees (*Ulmus americana* L.) are highly susceptible to *O. ulmi* infection from the end of May to late June, although this time is weather-dependent. According to Takai et al. (1979), in addition to the level of beetle activity in xylem, transmission efficiency depends on the level of contamination of beetles with fungal spores, which is at its peak in early June in central Ontario. After feeding, beetles move to dying and recently killed elms and fresh elm firewood and construct brood galleries and lay eggs (Thompson and Matthysse, 1972; Swedenborg et al., 1988). The eggs hatch into larvae that feed in galleries under bark. Beginning in

August, adult beetles of the new generation emerge and move to healthy elms to feed. The attractiveness of elm trees to *H. rufipes* is enhanced by *Ophiostoma novo-ulmi* infection (McLeod et al., 2005), which induces release of semiochemicals by diseased trees. In fall, adult beetles move to the base of healthy elm trunks and overwinter in the bark (Strobel and Lanier, 1981; Anderson and Holliday, 2003). In Manitoba, there is no evidence of successful overwintering in the larval stage, and there is one generation per year.

The control of overwintering *H. rufipes* by applications of insecticides to the basal part of the trunk of elm trees is important in control programs for DED (Gardiner, 1976a; Gardiner and Webb, 1980; Lanier et al., 1984; Oghiakhe and Holliday, 2011). In a previous study Anderson and Holliday (2003) reported that insecticide applications should be limited to the basal 55 cm of each elm tree — a marked reduction from an original recommended application height of about 2 m, because the number of overwintering *H. rufipes* alive above 55 cm was insignificant. They recommended more research to determine whether it is advisable to make further decreases in the height to which insecticide applications are made. The aim of this experiment was to investigate fine-scale vertical distribution and other characteristics of overwintering *H. rufipes* in Manitoba. During winter, healthy American elm trees were felled and their bases sectioned and removed to the laboratory for assessment of overwintering beetles.

Materials and Methods

Study sites

Camp Amisk site

Camp Amisk is part of the La Barriere Park system and is operated by The Manitoba Council of Scouts Canada. It is situated on the south bank of the La Salle River about 5 kilometers south of the Perimeter Highway, Winnipeg. The total area of Camp Amisk is 52 ha, of which the major area is mown grassland used for camping and recreation. The northern fringe is river bottom forest (Shay, 1984), and includes a large population of mature American elm trees growing beside the bank of the La Salle River. For the purpose of this study, the "Camp Amisk site" refers to the section of river bottom forest within Camp Amisk, which is from 50−200 m east of the Waverley Street Bridge and is on the south bank of La Salle River at latitude 49° 43′ 21″ N and longitude 97° 10′ 14″ W (Fig. 6). At this site, a section of river-bank forest 120 m long (East−West) was used. At the beginning of the study, the Camp Amisk site had many healthy and more than 15 diseased elms. Most of the trees were between 4 and 15 m from the river with diameter at breast height (DBH) ranging from 10 to 22 cm.

La Salle site

The La Salle site is located at 49° 42′ 03″ N, 97° 13′ 39″ W and is approximately 8 km south of Winnipeg's Perimeter Highway on the north bank of the La Salle River east of the town of La Salle (Fig. 9). The site is river bottom forest fringed on the north side by agricultural land and on the south side by the La Salle River. It measures 105 m along the river bank and 75 m in the North−South direction (from river bank to field) and at the

time of the study contained a natural population of more than 150 mature healthy American elm trees. At the site, a section of river-bank forest 68 m (East–West) long was used and there were no diseased elms in this section at the beginning of the experiment. Most of the trees that were used in the study were between 2 and 10 m from the river and were of DBH ranging from 21 cm to 48 cm.

Vertical distribution of over-wintering *Hylurgopinus rufipes*

Vertical distribution of overwintering *H. rufipes* in both sites was studied in winter 2007−8 and 2008−9 by felling selected healthy *U. americana* trees, sectioning their basal portions and, in the laboratory, dissecting them to reveal the overwintering insects. In late August 2007, healthy elms were randomly selected. At the Camp Amisk site, five trees were selected; 14 elms were selected at the La Salle site. The bark of each tree was scored with a double edged Japanese Style Pull Saw™ to provide markings at heights 5, 10, 15, 25, 35, 45 and 55 cm above the ground, thus delineating height zones between these cuts. On 4 February, 2008, the selected trees from each site were felled by cutting at ground level, and slices produced by cutting each stump at the score marks. Stumps of nine of the 14 selected trees at the La Salle site were excavated (Fig. 10) to a depth of 15 cm below ground level and roots cut at that depth to produce a section of trunk base and root flares from ground level to 15 cm below it. All slices were removed to the laboratory on the day of felling and stored in a cold room at 5 °C.

The study was repeated in 2008−9 at both the La Salle and the Camp Amisk sites using the same methods. On 1 August 2008, 24 healthy American elm trees were selected and tagged. At the La Salle site, there were no symptomatic elm trees at the time of tagging. On each of 17 November 2008 and on 17 February 2009, seven randomly

selected trees were felled at the La Salle site, the roots excavated to a depth of 15 cm and the basal 55 cm taken to the laboratory. Out of the 10 trees tagged at Camp Amisk, five were felled on 9 February and the basal 55 cm removed to the laboratory.

In the laboratory, sections were removed one at a time from cold storage and the bark on the trunk and root sections was removed, dissected and examined under a binocular microscope to detect tunnels and/or beetles (Figs. 11 and 12). The number and density of tunnels and beetles, and whether beetles were alive or dead, was recorded for each section. Beetles were picked from galleries using sterile forceps, and individually kept in microcentrifuge tubes at 4 °C for assessment of whether they were carrying *O. novo-ulmi* spores. A bio incinerator (Monoject Scientific, Sherwood Medical, St. Louis, MO) was used to sterilize forceps before picking beetles from another gallery. To allow beetle density to be estimated, surface area of each cylindrically shaped stump section was estimated by using the formula $2\pi rh$, where r is the radius of the section and h is its height. Surface area of sections consisting of irregularly shaped root flares was estimated by covering them with pieces of plastic sheet of geometric shapes; thus dividing the irregular area into triangles, rectangles, trapezoids, and squares for which areas can be calculated. Then, areas of these individual shapes were calculated and added together. Using a hammer and a sterile chisel, xylem tissue samples measuring 4 to 6 cm long and about 3 cm wide, were removed from parts of sections from three different heights where there were no *H. rufipes.* Pieces were cut through the cambium into the active xylem to increase the likelihood of detecting any viable *Ophiostoma* species fungi. Each piece was then picked up with a sterile pair of forceps from random samples of debarked trunk and branches and individually kept in 16.8 x 14.9 cm sandwich bags and stored at 4 °C. Frass

samples from randomly selected tunnels at each height were collected with sterile forceps and stored individually in 1.5 ml micro-centrifuge tubes and stored at 4 °C before plating.

Detection of *Ophiostoma novo-ulmi* spores

Preparation of malt extract agar

Two percent malt extract agar was prepared by mixing 20 g malt extract (Difco, Franklin Lakes, NJ), supplemented with 1 g yeast extract, and 20 g bacteriological agar (Gibco, Paisley, UK), in 1 L of distilled water. The mixture was sterilized by autoclaving for 25 min at 121 °C and 414 kPa. After cooling, the agar was dispensed into 10 cm Petri dishes in a micro flow hood and left to solidify at room temperature.

Culturing of O. novo-ulmi from H. rufipes and associated material.

Hylurgopinus rufipes adults that were picked from tunnels in stumps were plated singly in Petri dishes containing 2% malt extract agar within 48 hours of collection from stump sections. Cultures were incubated at ambient laboratory temperature and relative humidity for up to 7 days in alternating light and dark cycles. Xylem and frass samples were plated singly in Petri dishes containing 2% malt extract agar immediately after sampling or within 48 hours of collection. All plates were labeled and wrapped with parafilm to maintain moisture and to prevent contamination. Using a dissecting microscope, plates were examined every second day for the presence of the characteristic mycelial or synnematal stages of *O. novo-ulmi*. Identification techniques and assessment were based on Stipes and Campana (1981).

Mark-recapture studies on *H. rufipes*

In conjunction with the study of vertical distribution, a mark-recapture study was performed in which marked beetles were released from trap logs in August of 2007 and 2008, and "recaptured" in the overwintering beetle samples described above. On 6 August 2007, 17 trap logs were set out at the La Salle site along a 68 m length of the river bank. The logs in the river bank site were dusted with Blaze Orange coloured DayGlo® fluorescent powder (Fig. 13) (A. R. Monteith Limited, Mississauga, ON) using a universal duster so that all beetles that emerged would be marked with the powder (Pines and Westwood, 2008). On 19 August 2008, 15 trap logs from which adult beetles are expected to emerge were set out at the La Salle site along a 157 m length of the river bank and dusted with DayGlo® fluorescent powder (Fig. 14). All beetles recovered from the vertical distribution study were examined under an ultra violet light (Blacklight Blue by Osram Sylvania, Danvers, MA) to identify those marked with fluorescent powder on their body surface.

In addition to assessing marked beetles in elm trees, sticky traps were placed in both sites to monitor the population of *H. rufipes* and detect whether any of them were marked. Traps were 45.7 cm x 63.5 cm Elm Bark Beetle Sticky Traps (Great Lakes IPM Inc., Vestaburg, MI) and had no lure. They were stapled to healthy American elm trees at a height of 2.5 m. Traps were replaced with new traps every two weeks throughout the summer and fall of 2007 and 2008. At the time of collection in the field, each sticky trap was covered with wax paper for easy handling and storage. Traps were kept in the laboratory at 4 °C and then examined under the microscope to count the number of native elm bark beetles caught within the period. Beetles caught on traps were picked off the

trap surface and viewed under ultra violet light as described above. Then, beetles were positively identified after soaking in Histo-Clear™ II, a histological clearing agent (National Diagnostics, Atlanta, GA), to remove the trap adhesive from specimens.

In 2006, a pilot release was performed with 12 logs with DayGlo® fluorescent powder, placed in the La Salle site on 20 July 2006. Sticky traps were used to determine levels of recapture.

Statistical analysis

Likelihood ratio chi-square statistics were calculated for contingency tables relating the vertical height of stump sections and whether *H. rufipes* were alive or dead. Where a significant effect of height was found, binary logistic regression was performed to test for a relationship between height and *H. rufipes* survival. All statistical analyses were performed using Systat 13 (Systat 2009), and the alpha level for significance was 0.05.

Results

Tables 13−18 present results of dissections of bark of healthy trees from the La Salle site (Tables 13, 14, 16 and 18) and from Camp Amisk (Tables 15 and 17) in winters 2007−8 (Tables 13−15) and 2008−9 (Tables 16−18). Data for trees that were excavated to 15 cm below ground level are presented in Tables 13, 16 and 18; the remaining tables are for trees sampled down to the soil surface.

Numbers of overwintering *Hylurgopinus rufipes* adults are the raw data collected for each height range averaged over the stumps from the site on the sample date (Tables 13−18). These data are particularly valuable for assessing what proportion of the population is present at or below a particular height (Figs. 15–20). In all six tables, there

is a tendency for numbers of *H. rufipes* per height section to diminish with increasing height above the soil surface, even though the height interval of the upper four sections was twice that of the lower three sections. Within the height range of the samples, and considering only that portion of the *H. rufipes* population that overwintered above ground, the percentage of population in the basal 15 cm ranged from 71% to 93% (Figs. 16, 17 and 19). When considering only samples for those stumps that were excavated below ground, the percentage of population from 15 cm above ground down to below ground level averaged 80%; in these samples 25% of *H. rufipes* were below ground on February 4, 2008, and for both November 17, 2008 and February 17, 2009, the percentage below ground was 9% (Figs. 15, 18 and 20).

As the interval of height zones ranges from 5–15 cm and the surface areas of tree sections vary, total numbers of beetles are not a very good indicator of height preferences of insects or the intensity of interactions between them. For this purpose, density is more useful (Tables 13–18). Density of *H. rufipes* was always highest in the 0–5 cm height range and declined rapidly in height intervals above this zone up to a height of at least 35 cm. Above 35 cm, there was no consistent trend in densities with increasing height. Densities in samples taken below ground were less than those in the 0–5 cm and 5–10 cm zones immediately above (Tables 13, 16 and 18).

Density of tunnels decreased with increasing height across sites and dates (Tables 13–18), and in most cases, tunnel density was highest in the 0–5 cm height interval. Density of tunnels was considerable in stump sections removed from 0–15 cm below ground level (Tables 13, 16 and 18). The number of tunnels per *H. rufipes* is a measure of

tunnel occupancy and tended to be higher at the upper height intervals than at the lower ones with the exception of Camp Amisk in February 2008 (Table 15).

There was considerable variation in the proportion of live *H. rufipes* at the different sites and dates (Table 13−18). Comparisons among sites and dates of the frequency of living and dead overwintering *H. rufipes* for above-ground portions of stumps were almost all significant (Table 19). Overall, the percentages of beetles alive at the La Salle site and Camp Amisk on 4 February 2008 were 77% and 63% respectively. At the La Salle site on 17 November 2008, Camp Amisk on 9 February 2009 and La Salle site on 17 February 2009, 63%, 65% and 79% respectively were alive. Height significantly affected the proportion of *H. rufipes* alive for all sites and dates (Table 20). By calculating logistic regressions, it would be possible to observe any general trend of the distribution of living and dead *H. rufipes* with height. All but one of the regressions were significant but the values of McFadden's ρ^2 were low and the regressions did not account for a large proportion of the total Likelihood Ratio (LR) χ^2 (Table 20). Estimates of the 10^{th}, 50^{th} and 90^{th} percentiles show that there was no consistent trend with height for the dates and sites sampled (Table 20).

For unmarked beetles, there were no significant differences in frequency of spore bearing between sites in February 2008 ($LR\chi^2$ = 3.28, df = 1, P = 0.07) or between February and November 2008 for La Salle ($LR\chi^2$ = 0.30, df = 1, P = 0.6) (Table 21) or between sites for February 2009 ($LR\chi^2$ = 0.002, df = 1, P = 0.9) (Table 22). Overall, for both sites, the frequency of spore bearing in unmarked beetles was significantly lower in February 2009 than in 2008 samples ($LR\chi^2$ = 26.90, df = 1, P < 0.001). There is some

suggestion that frequency of spore bearing sometimes varied with height, but this was inconsistent (Tables 21 and 22).

Percentages of DayGlo®-marked *H. rufipes* at each height are given in Tables 13−18. The percentage of beetles marked, averaged over all heights, was 42.7±11.4% for trees sampled to 15 cm below ground (data in Table 13) and 40.4±10.3% for trees sampled to ground level (Table 14) at the La Salle site on 4 February 2008. The average percentage with marks was 53.4±9.9% at the La Salle site on 17 November 2008 (Table 16), when samples were taken to 15 cm below ground. Sixteen marked *H. rufipes* were found on five stumps excavated at Camp Amisk on 4 February 2008 (Table 15); 37% of the beetles had spores and were not marked. No marked *H. rufipes* were found on any of the five stumps excavated at Camp Amisk on 9 February 2009 (Table 17) or on the seven stumps excavated at the La Salle site on 17 February 2009 (Table 18).

Pooled over the three samples in 2008 (Table 21), 2% of marked *H. rufipes* that were plated had spores of *O. novo- ulmi*, which differed significantly ($LR\chi^2 = 150.98$, df = 1, $P < 0.001$) from the 45% of unmarked beetles in the same samples that carried spores. When each of the three samples were considered separately (Table 21), the frequency of spore bearing was also significantly lower for marked beetles, except in La Salle in November 2008, where beetle numbers were relatively small and two of the marked beetles carried spores. In samples in February 2009, no marked beetles were recovered, so comparisons of spore rates could not be done (Table 22). Relationships of frequency of spore carrying with height were variable and inconsistent (Tables 21 and 22).

Of the adult *H. rufipes* caught on sticky traps at the La Salle and Camp Amisk sites from 2006 to 2008 (Tables 23−25), marked *H. rufipes* were trapped only in 2007 (Table 24).

Discussion

This research and the previous studies by Anderson and Holliday (2000, 2003) were intended to define the location of overwintering *H. rufipes* in elm tree trunks. This would allow precise targeting for insecticidal management of DED to achieve optimum control.

In general, the methodology appeared effective. However, a major ice storm took place in Winnipeg on 9 February 2009 in which over 26 mm of freezing rain fell within 24 hours (Environment Canada, 2012c). The storm appeared to affect the number of *H. rufipes* that were found in stumps that were excavated at Camp Amisk (Table 17). Excavations done on that day were carried out in treacherous weather conditions. In addition although many beetles had DayGlo® marks in previous samples in winter 2008−9, beetles with markings were not found in samples taken on and after the storm. The lowest numbers of *H. rufipes* found at Camp Amisk during the study were in samples taken on the day of the storm.

Vertical distribution of numbers and density of overwintering *H. rufipes* showed basically the same above-ground pattern reported by Anderson and Holliday (2003): increasing with decreasing height. Overwintering *H. rufipes* showed a definite preference for the lower portion of the elm trunk. In Minnesota, *H. rufipes* overwinters as adults in the bottom 30 cm of elm trunks (Landwehr et al., 1982). Anderson and Holliday (2003) assessed beetles at 0−25 cm, 55–80 cm, 110–135 and 165–190 cm, and found few *H. rufipes* overwintering in or above the 55–80 cm zone. Anderson and Holliday (2003)

reported that above 55 cm, the number of living beetles was negligible and that insecticide application above this height was not necessary. My results showed that the majority of the *H. rufipes* population overwintered less than 15 cm above ground, with the basal 5 cm having the highest number of beetles. As shown, *H. rufipes* density was highest in the 0–5 cm height range, declining progressively up to a height range of 25−35 cm.

Density of tunnels of overwintering *H. rufipes* followed a similar trend to that for the beetles on the elm trunk with the highest densities at 0–5 cm height range. In Minnesota, Landwehr et al. (1982) reported that between 97% and 89% of dust piles produced by overwintering *H. rufipes* were within 30 cm and 15 cm from the ground, respectively. However, Anderson and Holliday (2003) observed that dust piles are not a reliable technique to estimate the numbers of overwintering *H. rufipes* because dust piles are cleared away by rain and wind, and because single dust piles may result from several *H. rufipes* excavating in close proximity. Using a 15 metre tall elm tree, Kaston and Riggs (1938) found that in Connecticut, where winters are milder than Manitoba, the density of tunnels was greatest between 4 and 10 m from the ground and that the total number of bark tunnels in this height range was 87% of the numbers in the whole trunk. The highest level of tunnel occupancy was at lower levels (76−122 cm above ground). My finding is similar to Anderson and Holliday (2003) who found that densities of tunnels are considerably higher in the bottom 25 cm than at 55–80 cm or above.

According to Gardiner (1981) and Anderson and Holliday (2003), as fall progresses *H. rufipes* adults forsake higher tunnels to construct new tunnels at the lower part of the trunk where most beetles overwinter. Anderson and Holliday (2003) suggested

that tunnels in the higher part of the trunk were most probably feeding tunnels. Kaston and Riggs (1938) and Kaston (1939) found that the ratio of unoccupied tunnels in the smaller branches, where the bark is thin, increases during October and early November; they believed that this indicated that some of the beetles had left to seek thicker bark in which to hibernate. The higher number of tunnels per *H. rufipes* at the upper height intervals than at the lower ones in my study is a pattern that is consistent with this hypothesis: many higher feeding tunnels have been abandoned and beetles have moved to hibernate in the lowest tunnel they construct, usually just above or below the soil surface.

I provide the first evidence that considerable numbers of *H. rufipes* overwinter below the soil surface where they may be unaffected by basal spraying. No previous published research results have shown that *H. rufipes* overwinter below ground, and finding beetles overwintering on root flares is of considerable interest. Gardiner (1981) refers to *H. rufipes* moving down the trunk from feeding to overwintering tunnels and penetrating the xylem area of the root flares to overwinter, but provides no data to support this statement.

In Manitoba in fall, adult *H. rufipes* move to the base of healthy elm trunks to overwinter in the bark (Anderson and Holliday, 2003). Different species of bark beetles are thought to overwinter at tree bases for various reasons. In Connecticut, Keen and Furniss (1937) have shown that severe winter temperatures are a primary mortality agent for overwintering western pine beetles (*Dendroctonus brevicomis* LeConte). In Newfoundland, Langor and Raske (1987) reported that average densities of overwintering adults/100cm^2 of the eastern larch beetle, *Dendroctonus simplex* Le Conte are greater 0−20 cm above ground than at 40−60 cm and 80−100 cm, and suggested that

beetles overwintering at the base of tree trunks are protected by snow from cold and woodpecker predation. Kaston (1939) dissected various sections of a tree in Connecticut, USA to determine whether adult *H. rufipes* were alive or dead and found that the density and percentage of survival are greatest in the lowest part of the tree where the bark is thick and lowest in the upper part where it is thin. Thick bark may provide protection for bark beetles from natural enemies, dehydration and cold and may enhance protection against freezing by providing a warmer microclimate. My results agree with Anderson and Holliday (2003) who report that most *H. rufipes* overwinter at height range 0−25 cm, and that the percentage of living beetles is greatest at this height range. The percentage alive is affected by geographic location and, in smaller trees (DBH ≤15 cm), is considerably lower in spring samples than autumn samples (Anderson and Holliday, 2003). Logistic regression of proportion alive on site and sample height is highly significant (Anderson and Holliday, 2003). As shown in my study, snow cover and greater bark thickness probably protect the overwintering beetles at the base of the tree from mortality from the cold and dry Manitoba winter.

In my study, the mean proportion of unmarked overwintering *H. rufipes* that carried spores of *Ophiostoma novo-ulmi* varied between 18% and 47% with the lowest values in the sparse samples from February 2009. These results are within the same range as those reported by Thompson and Matthysse (1972) who isolated spores from 6.9% to 57.1% overwintering *H. rufipes* adults in Connecticut, but higher than those reported by Parker et al. (1948) who found 1−2% of beetles in New York State carry spores in December–February. Spores of *O. ulmi* and *O. novo-ulmi* attach to elm bark beetles during adult development in pupal chambers in elm bark (Webber, 2004). The spore load

picked up by beetles is dependent on the level of spore colonisation and sporulation on the walls of the pupal chamber, and is determined by the location of the chamber in the bark, how long the beetle stays in the chamber, and the environment in the chamber (Webber, 2004). Numbers of spores of *O. novo-ulmi* vectored by individual beetles depend on whether pupation chambers are in the inner or outer bark. *O. novo-ulmi* produces most spores in chambers in the inner bark but few or none at all in chambers in the outer bark. Therefore, beetles that pupate in the outer bark have a lower probability of contacting the spores and emerging as vectors of DED (Webber, 2004). Although individual beetles can carry several thousands of spores on their body surfaces, some factors can lead to the loss of spore inoculum by a vector before successful disease transmission occurs (Webber, 2000). Spores can be lost through desiccation, ultra violet radiation and also after a period of flight the proportion of beetles still carrying a viable load of spores can be more than halved (Webber, 2000). In Ontario, Gardiner (1976b) reported that 31−35% of overwintering adults in fall and 26−27% in spring carry spores.

A number of studies have used fluorescent powder and marks and recapture techniques to study the movement of bark beetles (Safranyik et al., 1992; Turchin and Thoeny, 1993; Pines and Westwood, 2008). Beetle flight dispersal was monitored in Manitoba by Pines and Westwood (2008), who reported that in spring marked *H. rufipes* leaving overwintering sites were caught up to 750 m from their source, and that in summer marked beetles were caught up to 1 km from release sites. In both cases these represented the maximum distance at which traps were deployed. In 2007, a beetle was caught on a sticky trap at Camp Amisk before beetles were marked and released at the La Salle site in that year and so must have travelled to the Camp Amisk site either in fall

2006 or spring 2007. The 16 marked beetles found on the five stumps excavated at the Camp Amisk on 4 February 2008, must have come from the La Salle site where they were marked and released in the previous summer and arrived at Camp Amisk before winter. The straight-line distance between the two sites is 4.8 km. If the beetles were to fly along the river bank forest where most of the elms occur, the beetles would have travelled about 7.5 km. The considerable distance that adult *H. rufipes* are capable of flying has important implications for epidemiology and disease management. Beetles' ability to travel fairly long distances especially when many of them are carrying spores, may compromise control measures and contribute to DED transmission to areas that were previously free from the disease.

Despite evidence that some beetles disperse long distances, most beetles probably do not move far from emergence sites. With relatively few logs releasing beetles at the La Salle site, over 40% of overwintering beetles in the site in winter 2007−8 were marked, and in early winter of 2008−9 over 50% were marked. The La Salle site had no trees with DED during these two winters. In summer 2007, the nearest symptomatic tree (J. Leferink personal communication, 2013) to the site was 310 m to the west along the river bank and in the opposite direction, the nearest tree with symptoms was 870 m away in a straight line (1,360 m along the river bank). In summer 2008, there were two symptomatic trees about 90 m west of the site, and one to the east at a distance of 135 m. McLeod et al. (2005) found evidence that volatiles from *O. novo-ulmi*-infected trees are attractive to *H. rufipes* in late summer, as well as in spring. However attraction appears to have resulted in very few marked spore-free beetles emerging from my logs acquiring spores from nearby symptomatic trees before winter and then returning to overwinter in

the site. Most marked beetles appear to have moved relatively directly to overwintering trees. In the overwintering samples, 47% of unmarked beetles in February 2008 and 44% of unmarked beetles in November 2008 in the La Salle site were spore-bearing and must have come from outside of the site. Although the nearest detected source of spores was more than three times as far in 2007 than in 2008, the proportions of spore-bearing unmarked beetles were similar in both winters. Contribution of spore-bearing beetles to the population in the site would not only be a function of the distance to symptomatic tree sources, but would also depend on the highly variable number of beetles (refer to brood development studies in Chapter 3) that emerge from such trees.

Implications for Dutch elm disease management

The control of overwintering *H. rufipes* by applications of insecticides to the bottom of trunks of elm trees is an important part of DED management (Gardiner, 1976a; Gardiner and Webb, 1980; Lanier et al., 1984; Oghiakhe and Holliday, 2011). Experiments carried out in different parts of Manitoba, Ontario and New Brunswick in 1976 showed that spraying of 0.5% a.i. of chlorpyrifos to the basal 2.5 m of the trunks of elm trees controlled between 83% and 100% of overwintering *H. rufipes* (Gardiner and Webb, 1980). Similar applications in Minnesota reduced numbers of *H. rufipes* emerging in spring by 93% (Landwehr et al., 1982). Jin et al. (1996) and Oghiakhe and Holliday (2011) showed that, with appropriate insecticides, approximately 100% mortality of *H. rufipes* is attainable for up to two years after insecticide applications in Manitoba. Anderson and Holliday (2003) have shown that maximum effect per unit control of *H. rufipes* in Manitoba can be attained by focusing spray application between soil level and height 55 cm of the trunks of the biggest trees, and recommended research to determine

whether the upper limit of basal insecticide application can be further reduced while still effectively controlling overwintering *H. rufipes*. My current research shows that the majority of beetles overwinter very close to the soil surface. However, *H. rufipes* do appear to overwinter in substantial numbers at heights up to 55 cm and so, it is prudent to continue to target insecticide applications from ground level to a height of 55 cm. In my thesis I record, for the first time, that considerable numbers of *H. rufipes* overwinter below the soil surface. These probably do not come directly in contact with the insecticide applied in basal spraying operations, and that may explain why, despite aggressive integrated disease management efforts, Domke (2005) reports annual prevalence of about 2% over the first 30 years since DED was detected in Manitoba.

Basal spraying may have some effect on the numbers of surviving overwintering beetles below the ground surface. Anderson and Holliday (2003) suggested that *H. rufipes* move down the tree, constructing feeding tunnels at various heights, until they ultimately reach the lowest level at which they overwinter. During this process, they could encounter lethal doses of insecticide and be killed if they construct feeding tunnels in, or walk on, bark in the zone treated with insecticide. If so, reducing the height of insecticide treatment to <55 cm would reduce the probability of beetles encountering insecticide and could increase survival of beetles overwintering below ground. Dutch elm disease management would be greatly improved if an effective and environmentally friendly insecticide that can penetrate the soil and kill *H. rufipes* overwintering below soil surface is found; however this is an improbable combination of insecticide characteristics. Therefore, given the available means of insecticidal control, maintaining the current practice of basal applications to 55 cm above ground is probably wise.

My results showed that marked overwintering beetles can travel at least 4.8 km. We have also showed that 47% of overwintering beetles in stumps carried *O. novo-ulmi* spores when the nearest detected source of spores was >300 m away. The ability of spore carrying beetles to move considerable distances can aid in disease transmission. In communities using buffer zones in DED management to prevent beetles from entering urban forests, I recommend 4.8 km as the minimum width, contrary to the recommendation by Pines and Westwood (2008) that 1 km be used as the minimum width of buffer zones. In recommending 4.8 km as the minimum width, I recognize the following issues: that it is likely that the majority of beetles fly much less than 4.8 km as I know of only 17 beetles that flew this distance; that a 1 km zone probably is sufficient for all but a few percent of dispersers, but Pines and Westwood (2008) did not trap beyond 1 km, so we cannot be sure of the distance distribution and; that a 4.8 km buffer would be very expensive to maintain with perhaps little additional benefit. However managers, in using 1 km buffer zones should recognize that they are not preventing all beetles from traversing the zone.

Table 13. Mean (±SE) number of overwintering *Hylurgopinus rufipes* adults, *H. rufipes* density, tunnel density, tunnels per *H. rufipes*, percentage of *H. rufipes* alive and percentage of *H. rufipes* marked with DayGlo® powder at heights between 55 cm above and 15 cm below ground on nine stumps of American elm trees (DBH range 7–20.1 cm) at the La Salle site on 4 February 2008.

Height range (cm)	Number of *H. rufipes*	*H. rufipes* density/m^2	Tunnel density/ m^2	N (sections with *H. rufipes*)	Tunnels per *H. rufipes*	Percent of *H. rufipes*	
						Alive	Marked
45–55	6.1±1.6	116±26	1527±353	9	23.4±8.8	58±14	57±15
35–45	5.7±1.7	82±23	1329±279	9	27.3±9.5	45±13	41±15
25–35	4.0±0.9	98±25	1767±364	8	22.4±7.1	72±10	41±14
15–25	7.8±2.9	141±50	2760±658	8	25.7±8.2	50±7	47±14
10–15	10.8±3.8	377±129	5236±1773	9	38.3±17.3	59±11	38±14
5–10.	38.8±13.3	1649±752	8819±2153	9	9.7±2.5	59±5	40±12
0–5	56.9±10.7	2179±425	13214±3052	9	7.8±1.8	55±5	46±11
15 cm below 0	65.9±22.8	1195±458	4609±1497	7	5.5±1.4	49±7	50±12

Table 14. Mean (±SE) number of overwintering *Hylurgopinus rufipes* adults, *H. rufipes* density, tunnel density, tunnels per *H. rufipes*, percentage of *H. rufipes* alive and percentage of *H. rufipes* marked with DayGlo® powder found at heights between 0 and 55 cm above ground on five stumps of American elm trees (DBH range 9.2–12.7 cm) at the La Salle site on 4 February 2008.

Height range (cm)	Number of *H. rufipes*	*H. rufipes* density/m^2	Tunnel density/m^2	N (sections with *H. rufipes*)	Tunnels per *H. rufipes*	Percent of *H. rufipes*	
						Alive	Marked
45–55	0.4±0.4	7±7	3505±649	1	95.5±0.0	100±0	100±0
35–45	0.0±0.0	0±0	4713±832	0	—	—	—
25–35	1.4±0.9	28±18	4664±1165	3	160.0±68.3	20±20	33±33
15–25	6.0±1.9	119±43	6172±1704	5	79.7±23.1	49±13	86±18
10–15	45.0±22.2	1805±895	9188±2307	5	17.1±12.9	84±5	37±23
5–10	215.6±44.4	8531±2154	15634±3331	5	2.3±0.8	88±3	20±13
0–5	467.2±109.0	13717±3211	27645±9714	5	1.8±0.3	80±1	22±14

Table 15. Mean (±SE) number of overwintering *Hylurgopinus rufipes* adults, *H. rufipes* density, tunnel density, tunnels per *H. rufipes*, percentage of *H. rufipes* alive and percentage of *H. rufipes* marked with DayGlo® powder at heights between 0 and 55 cm above ground on five stumps of American elm trees (DBH range 12–22.4 cm) at Camp Amisk on 4 February 2008.

Height range (cm)	Number of *H. rufipes*	*H. rufipes* density/m^2	Tunnel density/m^2	N (sections with *H. rufipes*)	Tunnels per *H. rufipes*	Percent of *H. rufipes*	
						Alive	Marked
45–55	5.8±2.2	92±37	265±125	4	3.3±0.9	100±0	0±0
35–45	6.2±2.7	95±45	326±170	5	3.4±1.1	90±10	26±19
25–35	3.0±1.3	46±22	150±66	4	3.5±0.5	92±8	3±3
15–25	3.0±0.5	46±11	358±172	5	7.0±2.4	52±17	7±7
10–15	5.4±2.7	127±45	1015±403	5	13.2±7.0	92±5	0±0
5–10	19.0±9.9	454±171	2339±692	5	7.3±2.8	59±19	3±2
0–5	43.2±8.4	990±268	3379±934	5	3.8±1.1	65±10	4±2

Table 16. Mean (±SE) number of overwintering *Hylurgopinus rufipes* adults, *H. rufipes* density, tunnel density, tunnels per *H. rufipes*, percentage of *H. rufipes* alive and percentage of *H. rufipes* marked with DayGlo® powder at heights between 55 cm above and 15 cm below ground on seven stumps of American elm trees (DBH range 14.5–47.6 cm) at the La Salle site on 17 November 2008.

Height range (cm)	Number of *H. rufipes*	*H. rufipes* density/ m^2	Tunnel density/m^2	N (sections with *H. rufipes*)	Tunnels per *H. rufipes*	Percent of *H. rufipes*	
						Alive	Marked
45–55	7.3±5.4	52±33	1506±209	6	70.5±22.5	25±12	0±0
35–45	3.9±1.9	38±20	1994±351	4	38.9±5.1	14±8	52±28
25–35	6.1±2.3	68±24	2932±576	6	53.5±12.0	27±8	58±20
15–25	10.4±2.8	112±22	3765±502	7	43.6±10.3	43±15	54±15
10–15	35.4±8.4	921±303	7872±1146	6	9.9±1.8	54±17	58±15
5–10	103.9±33.6	2802±1067	13828±2034	7	12.2±4.8	68±11	60±11
0–5	157.3±85.8	4160±2387	12556±3487	7	8.5±3.2	63±12	76±9
15 cm below 0	64.1±42.9	1044±924	3136±1881	6	12.5±3.8	46±16	51±18

Table 17. Mean (±SE) number of overwintering *Hylurgopinus rufipes* adults, *H. rufipes* density, tunnel density, tunnels per *H. rufipes* and percentage of *H. rufipes* alive found at heights between 0 and 55 cm above ground on five stumps[*] of American elm trees (DBH range 5.4–7.6 cm) at Camp Amisk on 9 February 2009.

Height range (cm)	Number of *H. rufipes*	*H. rufipes* density/m^2	Tunnel density/m^2	N (sections with *H. rufipes*)	Tunnels per *H. rufipes*	Percent of *H. rufipes* alive
45–55	0.4±0.2	6±4	489±188	2	14.5±8.5	0±0
35–45	1.0±0.4	17±7	499±185	3	15.2±5.0	33±33
25–35	0.8±0.4	12±6	532±220	3	31.7±21.3	0±0
15–25	1.8±0.7	30±12	607±218	4	16.9±8.3	58±25
10–15	1.0±0.6	34±22	482±146	2	6.9±0.4	83±17
5–10	7.2±4.4	245±157	1136±310	4	6.6±3.2	74±11
0–5	35.4±23.3	1207±838	2639±836	5	11.6±8.0	44±14

[*]No marked *H. rufipes* were found on any of the five stumps.

Table 18. Mean (±SE) number of overwintering *Hylurgopinus rufipes* adults, *H. rufipes* density, tunnel density, tunnels per *H. rufipes* and percentage of *H. rufipes* alive at heights between 55 cm above and 15 cm below ground on seven stumps* of American elm trees (DBH range 4.8 – 8.3 cm) at the La Salle site on 17 February 2009.

Height range (cm)	Number of *H. rufipes*	*H. rufipes* density/m^2	Tunnel density/m^2	N (sections with *H. rufipes*)	Tunnels per *H. rufipes*	Percent of *H. rufipes* alive
45–55	0.6±0.4	11±8	703±278	2	12.9±2.9	83±17
35–45	0.4±0.3	7±5	765±268	2	33.0±3.0	25±25
25–35	0.6±0.4	9±7	1171±443	2	31.7±3.7	0±0
15–25	9.1±6.6	130±83	2081±860	5	48.0±25.7	58±13
10–15	16.4±10.5	460±265	3703±1297	5	14.6±6.2	78±7
5–10	42.6±19.8	1454±737	4996±1491	7	6.1±1.4	72±13
0–5	48.0±25.6	1711±952	8923±3184	7	11.7±3.2	83±5
15 cm below 0	16.9±14.9	94±62	1413±414	3	17.9±9.5	54±27

*No marked *H. rufipes* were found on any of the seven stumps.

Table 19. Comparisons among sites and dates of the frequency of living and dead *Hylurgopinus rufipes* for above-ground portions of stumps.

Source	Likelihood Ratio χ^2	df	*P*
Overall	463.6	5	< 0.001
Within Feb 08	275.8	2	< 0.001
Within La Salle Feb 08	262.9	1	< 0.001
La Salle vs Amisk Feb 08	12.9	1	< 0.001
La Salle vs Amisk Feb 09	20.4	1	< 0.001
Total among sites within dates	296.2	3	< 0.001
Among dates (All sites)	167.3	2	< 0.001
Among La Salle samples	451.0	3	< 0.001
Among dates at La Salle	188.1	2	< 0.001
Among dates at Amisk	2.5	1	0.112

Table 20. Chi-square tests, binary logistic regression and estimates of 10%, 50% and 90% percentiles from the regression for the effect of section height on the frequency of overwintering *Hylurgopinus rufipes* adults that were alive in stumps of American elm trees in Winnipeg in 2008 and 2009.

Date	Height range sampled (cm)	Site	Overall effect of height			Logistic regression on height						
			Likelihood ratio χ^2	df	*P*	Likelihood ratio χ^2	df	*P*	McFadden's ρ^2	Percentiles of *H. rufipes* alive (height [cm])		
										10%	50%	90%
04 Feb 08	-15 to 55	La Salle	15.6	7	0.029	9.1	1	0.003	0.004	-216	-17	1804
04 Feb 08	0 to 55	La Salle	34.6	6	<0.001	1.0	1	0.309	0.000	-312	-126	60
04 Feb 08	0 to 55	Amisk	57.8	6	<0.001	38.9	1	<0.001	0.072	-41	-4	34
17 Nov 08	-15 to 55	La Salle	317.6	7	<0.001	44.2	1	<0.001	0.012	-84	-2	80
09 Feb 09	0 to 55	Amisk	17.5	6	0.008	4.2	1	0.040	0.013	98	23	-51
17 Feb 09	-15 to 55	La Salle	25.1	7	0.001	12.1	1	0.000	0.012	112	45	-23

Table 21. Percentage of overwintering *Hylurgopinus rufipes* at each height of healthy American elm stumps that carried spores of *Ophiostoma novo-ulmi* at the La Salle site and at Camp Amisk in 2008.

Height range (cm)	% with spores (number tested)					
	4 Feb 2008 La Salle		4 Feb 2008 Camp Amisk		17 Nov 2008 La Salle	
	Unmarked	Marked	Unmarked	Marked	Unmarked	Marked
<0	56.0 (25)	0.0 (28)	—	—	33.3 (6)	— (0)
0–5	55.4 (101)	0.0 (33)	73.3 (15)	— (0)	50.0 (28)	25.0 (8)
5–10	45.8 (96)	0.0 (28)	86.7 (15)	— (0)	50.0 (16)	0.0 (4)
10–15	41.2 (34)	0.0 (19)	50.0 (16)	— (0)	35.3 (17)	— (0)
15–25	38.2 (34)	10.0 (20)	33.3 (18)	— (0)	66.7 (12)	— (0)
25–35	30.8 (26)	0.0 (12)	20.0 (15)	— (0)	0.0 (4)	— (0)
35–45	27.8 (18)	0.0 (14)	0.0 (15)	0.0 (5)	— (0)	— (0)
45–55	72.2 (18)	0.0 (17)	0.0 (15)	— (0)	0.0 (3)	— (0)
Pooled over height						
	47.4 (352)	1.2 (171)	37.6 (109)	0 (5)	44.2 (86)	16.7 (12)
Test of effect of marking on frequency of spore carrying						
LR χ^2	149.33		4.59		3.66	
df	1		1		1	
P	< 0.001		0.032		0.056	
Comparison of frequency of spore carrying at different heights (unmarked beetles only)						
Effect of height ranges on frequency						
LR χ^2	15.59		55.06		12.07	
df	7		6		6	
P	0.029		< 0.001		0.060	
Logistic regression on mid-point height						
LR χ^2	1.09		49.81		1.75	
df	1		1		1	
P	0.297		< 0.001		0.186	
McFadden's ρ^2	0.002		0.345		0.015	

Table 22. Percentage of unmarked overwintering *Hylurgopinus rufipes* at each height of healthy American elm stumps that carried spores of *Ophiostoma novo-ulmi* at the La Salle site and at Camp Amisk in February 2009. No marked beetles were recovered in these samples and, because of the small numbers of beetles, no assessments of spore-carrying were made for heights above 25 cm.

Height range (cm)	% with spores (number tested)	
	9 Feb 2009	17 Feb 2009
	Camp Amisk	La Salle
<0	—	28.6 (7)
0–5	37.5 (8)	25.0 (16)
5–10	0 (8)	8.3 (24)
10–15	0 (3)	20.0 (15)
15–25	33.3 (3)	18.2 (11)
Pooled over height		
	18.2 (22)	17.8 (73)
Comparison of frequency of spore carrying at different heights		
Effect of height ranges on frequency		
LR χ^2	6.46	2.82
df	3	4
P	0.091	0.589
Logistic regression on mid-point height		
LR χ^2	0.22	0.37
df	1	1
P	0.638	0.542
McFadden's ρ^2	0.011	0.005

Table 23. Total numbers of *Hylurgopinus rufipes* caught on sticky traps at Camp Amisk and the La Salle sites from 29 May to 27 October 2006. No marked beetles were caught.

Trapping period	Camp Amisk	La Salle	Total
29 May–12 June	0	0	0
12 June–23 June	6	6	12
23 June–30 June	0	1	1
30 June–14 July	10	1	11
14 July–31 July	3	1	4
31 July–14 July	1	1	2
14 July–30 August	1	1	2
31 August – 14 Sept	0	0	0
14 Sept–30 Sept	0	0	0
30 Sept - 27 Oct	0	0	0

Data from the Camp Amisk and the La Salle sites are totals from two traps.

Table 24. Numbers of *Hylurgopinus rufipes* including number of marked beetles caught on sticky traps at the La Salle and Camp Amisk sites in 2007.

Date	Total trapped at La Salle	Mean/ trap at La Salle	Total trapped at Camp Amisk	Mean/trap at Camp Amisk
20 April–04 May	1	0.5	1	0.5
4 May–18 May	26	4.3	1	0.5
18 May–1 June	9	1.5	0	0.0
1 June–15 June	30	5.0	5 **(1 marked)**	2.5
15 June–29 June	3	0.5	2	1.0
29 June–13 July	3	0.5	2	1.0
13 July–27 July	7	1.2	0	0.0
27 July–10 August	39 **(5 marked**)	6.5	2	1.0
10 August–24 August	19 **(6 marked**)	3.2	0	0.0
24 August–7 Sept	10	1.7	0	0.0
7 Sept–21 Sept	9	1.5	0	0.0
21 Sept–5 Oct	6	1.0	0	0.0
5 Oct –19 Oct	6	1.0	0	0.0

At La Salle, 20 April–4 May there were two traps; data from all other dates at La Salle are from six traps. Data from Camp Amisk are from two traps.

Table 25. Mean number of *Hylurgopinus rufipes* caught on sticky traps at the La Salle and Camp Amisk sites in 2008. No marked beetles were caught.

Date	Mean/ trap at La Salle	Mean/ trap at Camp Amisk
8 May–21 May	0.33	20.3
21 May–3 June	0	14.3
3 June–16 June	59.8	551.8
16 June–29 June	208.7	197.3
29 June–12 July	9.3	26.8
12 July–25 July	0.8	3.0
25 July–7 Aug	0.5	1.3
7 Aug–20 Aug	0.2	1.0
20 Aug–2 Sept	0.7	0.5
2 Sept–15 Sept	1.0	0.0
15 Sept–28 Sept	0.0	0.0
28 Sept–11 Oct	0.2	0.0

Data from the La Salle site are from six traps, at Camp Amisk data are from four traps.

Figure 9. Aerial view of the La Salle site. Copyright Google, DigitalGlobe. Used in accordance with the conditions of http://www.google.com/permissions/geoguidelines/attr-guide.html.

Figure 10. *Ulmus americana* stump cut 15 cm below the ground using a chain saw by a field technician from Manitoba Conservation at the La Salle site. Photo credit: Sunday Oghiakhe

Figure 11. *Ulmus americana* stumps debarked sequentially at different heights in the laboratory to count the number of overwintering adult *Hylurgopinus rufipes* and tunnels. Photo credit: Sunday Oghiakhe.

Figure 12. Tunnels containing frass made by overwintering adult *Hylurgopinus rufipes* on inner bark/phloem of elm. Photo credit: Sunday Oghiakhe.

Figure 13. Application of DayGlo® powder on elm logs at the La Salle site. Photo credit: Sunday Oghiakhe.

Figure 14. Elm logs covered with DayGlo® powder set out at the La Salle site. Photo credit: Sunday Oghiakhe.

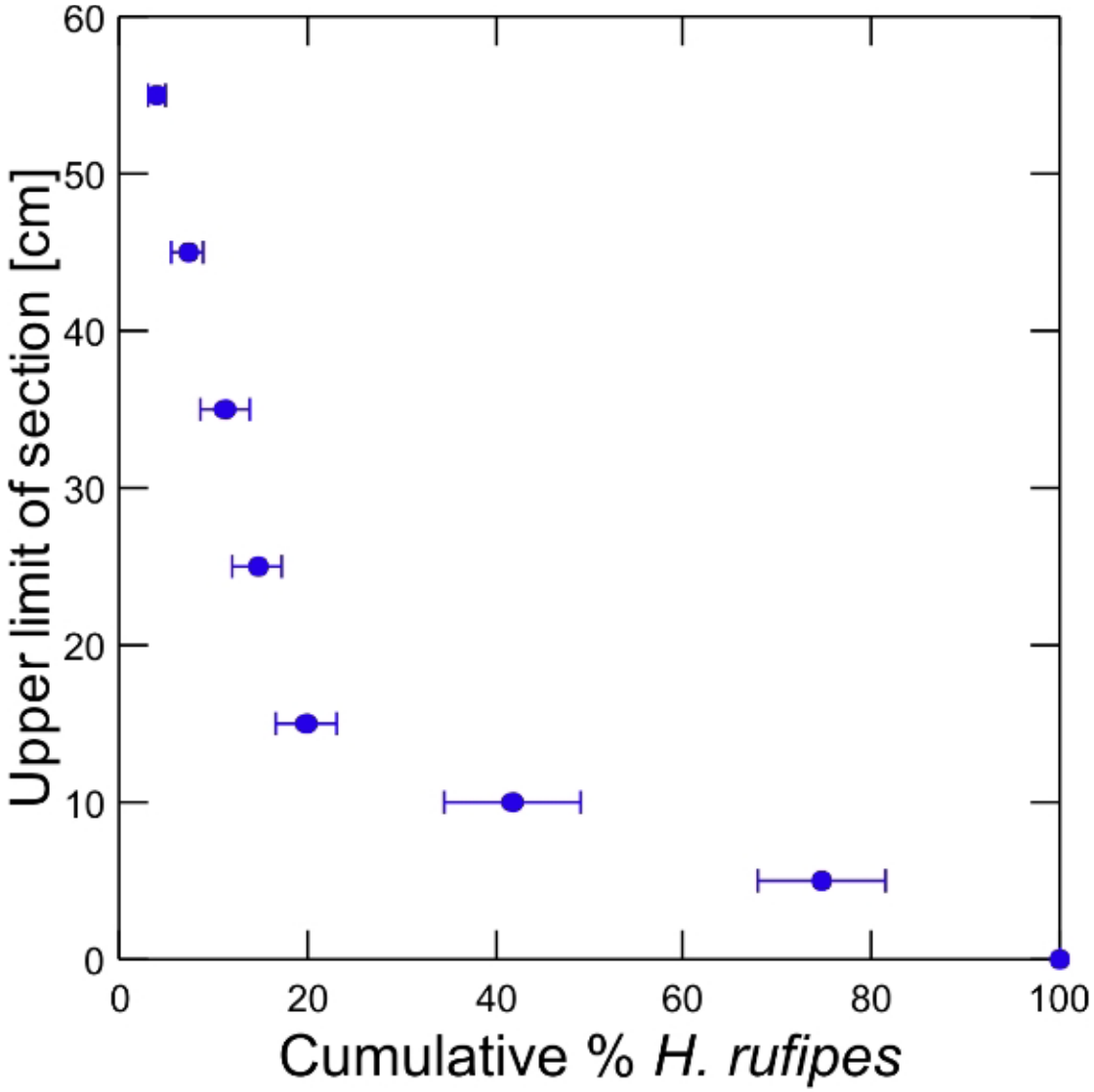

Figure 15. Relationship between cumulative percentage of overwintering *Hylurgopinus rufipes* and section height at the La Salle site on 4 February 2008. Height range sampled was from 55 cm to 15 cm below ground.

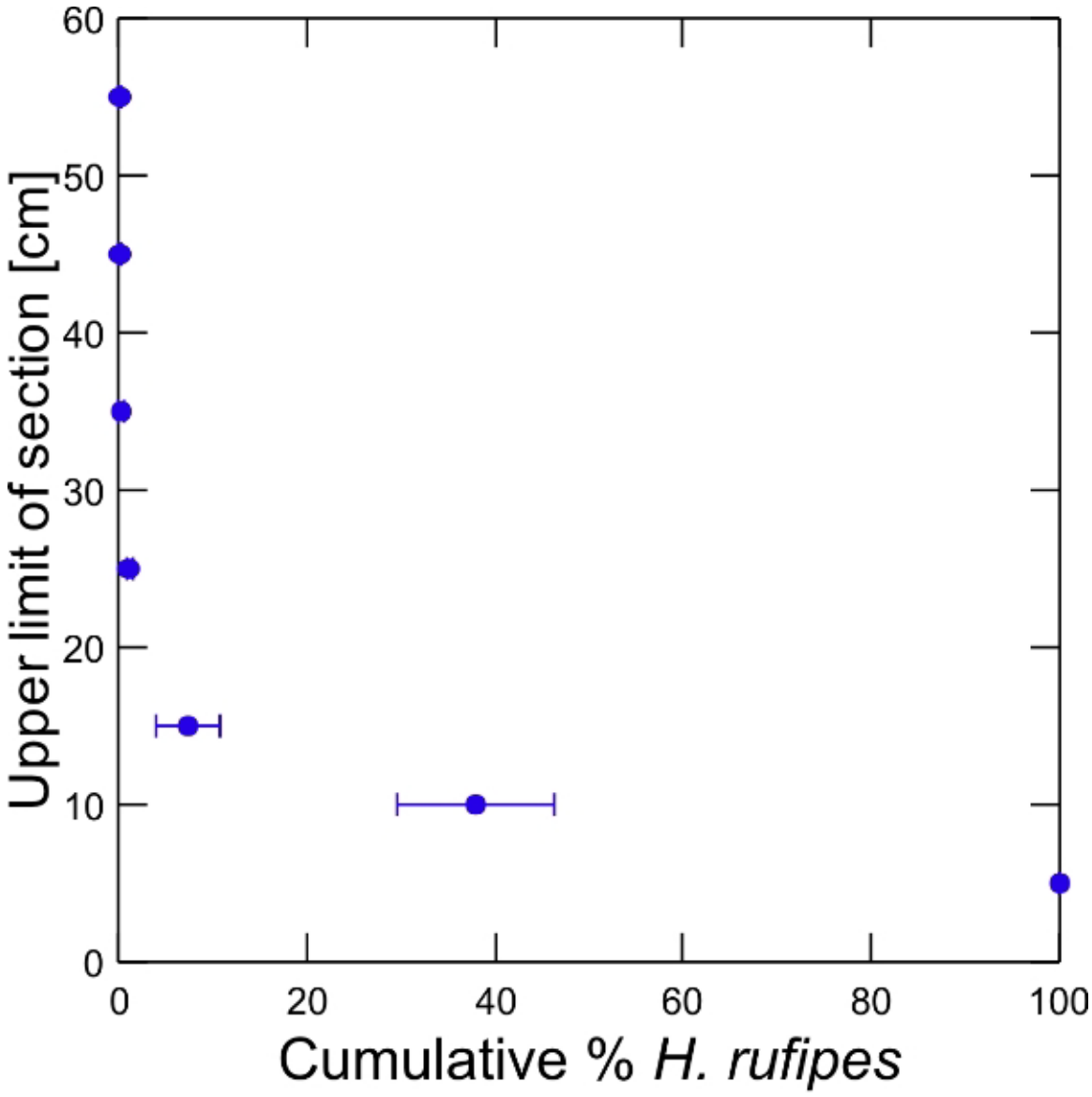

Figure 16. Relationship between cumulative percentage of overwintering *Hylurgopinus rufipes* and section height at the La Salle site on 4 February 2008. Height range sampled was 0–55 cm.

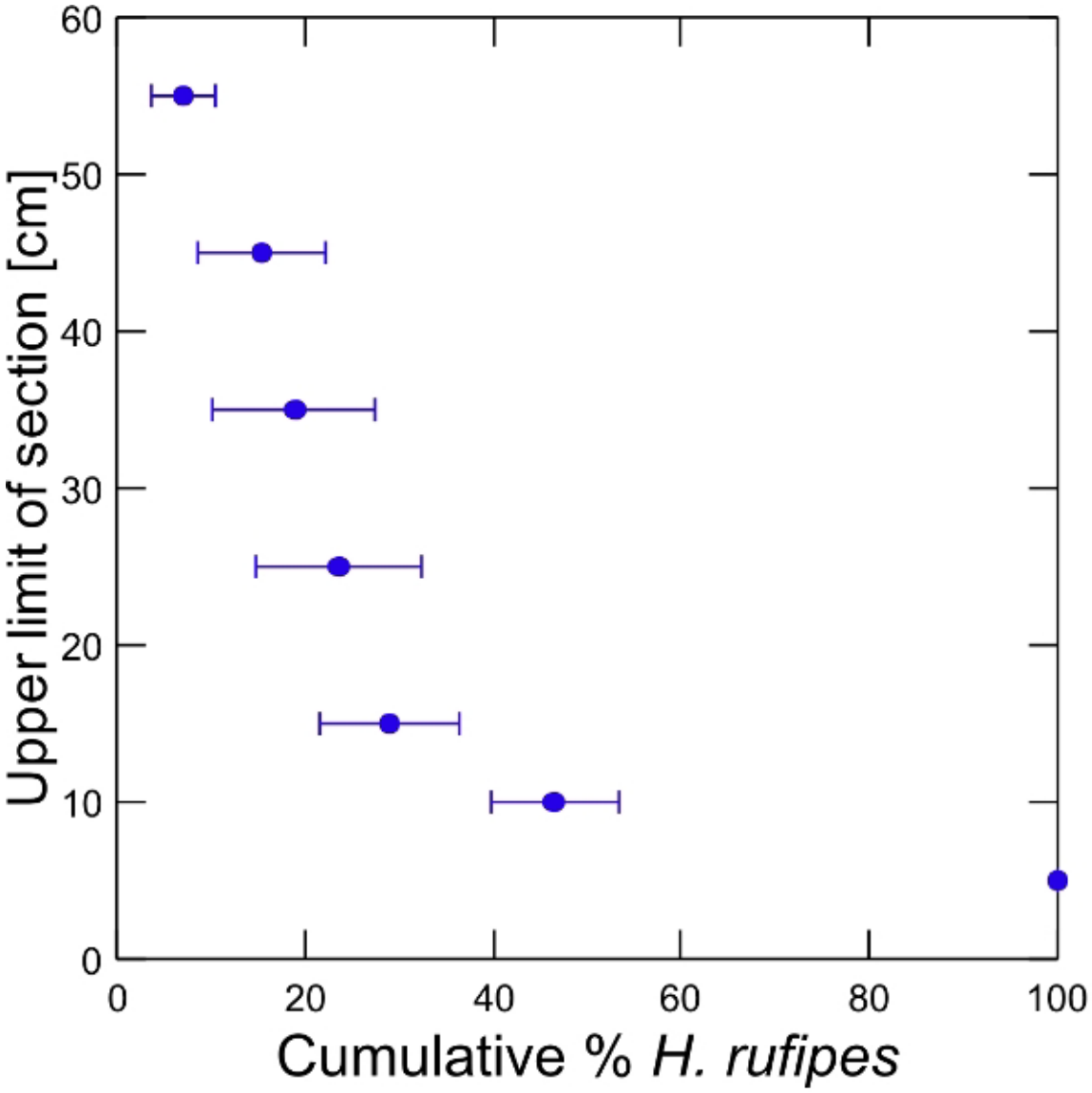

Figure 17. Relationship between cumulative percentage of overwintering *Hylurgopinus rufipes* and section height at the Camp Amisk site on 4 February 2008. Height range sampled was 0–55 cm.

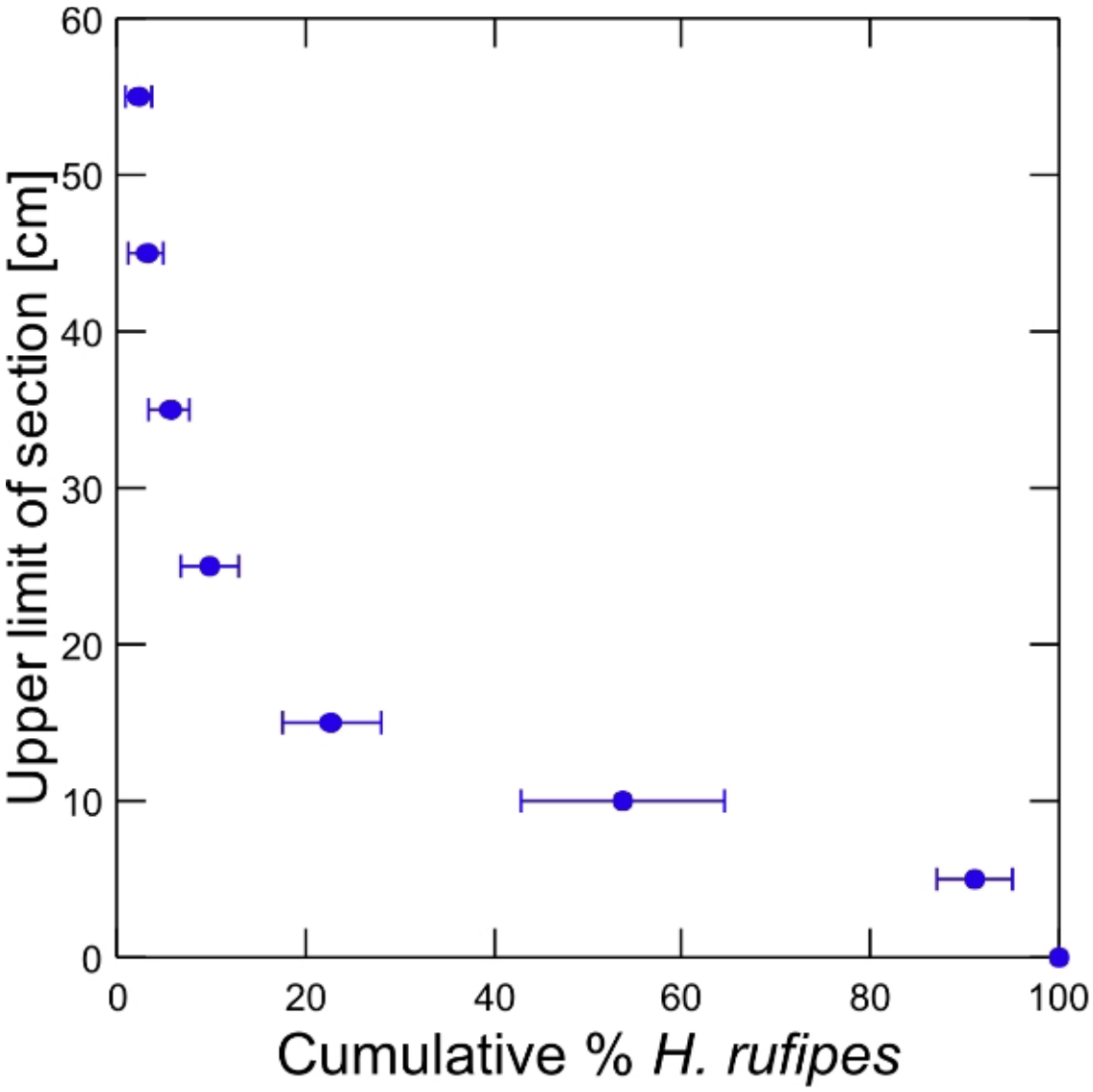

Figure 18. Relationship between cumulative percentage of overwintering *Hylurgopinus rufipes* and section height at the La Salle site on 17 November 2008. Height range sampled was from 55 cm to 15 cm below ground.

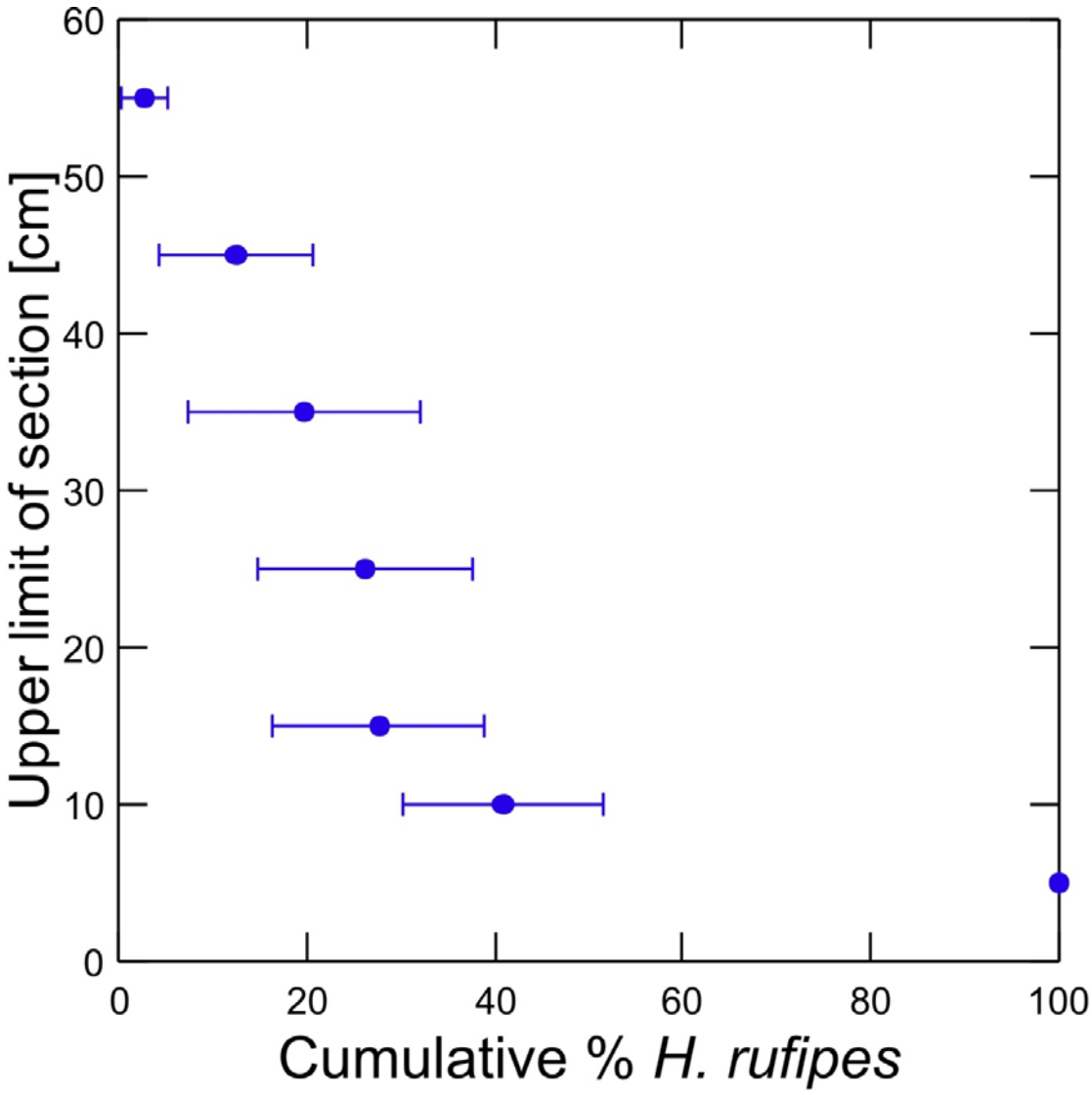

Figure 19. Relationship between cumulative percentage of overwintering *Hylurgopinus rufipes* and section height at the Camp Amisk site on 9 February 2009. Height range sampled was 0–55 cm.

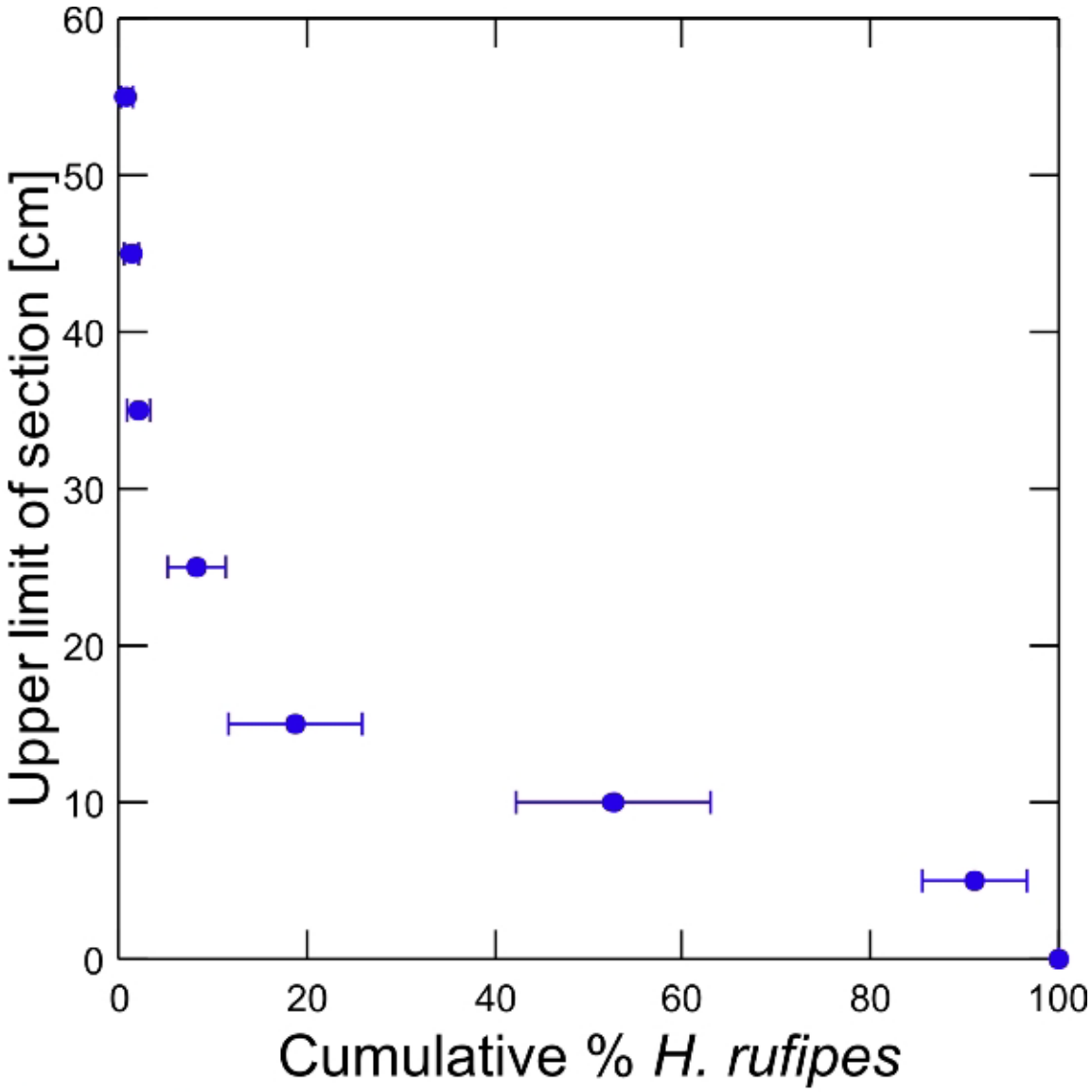

Figure 20. Relationship between cumulative percentage of overwintering *Hylurgopinus rufipes* and section height at the La Salle site on 17 February 2009. Height range sampled was from 55 cm to 15 cm below ground.

Chapter 5: Evaluation of Insecticides for Control of Overwintering *Hylurgopinus rufipes* (Coleoptera: Curculionidae)

Introduction

Dutch elm disease is a wilt disease of many *Ulmus* spp., and results from fungal infection. In Manitoba and Saskatchewan, the pathogen primarily attacks American elm trees (*Ulmus americana* L.), and is of an aggressive subgroup (Hintz et al., 1993), considered by Brasier (1991) to be a different species, *Ophiostoma novo-ulmi* Brasier. Dutch elm disease was first detected in Winnipeg and elsewhere in Manitoba in 1975 (Hildahl, 1977). In North American cities that do not have a DED control program, the estimated annual loss of American elm trees, in the years following the arrival of the disease, averages 18% and few American elms remain after 10 years (Domke, 2005). In Winnipeg, the annual rate of loss of American elms trees has been 2–3% and the inventory of American elms, which was 275,000 in 1975 is currently approximately 160,000 (Domke, 2005; City of Winnipeg, 2008a). The retention of the majority of the City's urban elms 35 years after the original infection is attributed to the effectiveness of an integrated disease management program (Domke, 2005), a portion of which involves management of the insect vector populations (Westwood, 1991a).

The native elm bark beetle, *Hylurgopinus rufipes* (Eichhoff) is the primary vector of DED pathogens in Manitoba, Saskatchewan and North Dakota (Hildahl and Wong, 1965; Stack et al., 1996). *Hylurgopinus rufipes* adults overwinter in the bark of healthy elm trees without contacting the conductive tissues of the tree; in Manitoba, most beetles overwinter within 55 cm of the ground surface in relatively large American elm trees (Anderson and Holliday, 2003). Insecticide treatments to the base of American elm trees control over-wintering native elm bark beetles, *H. rufipes* (Gardiner, 1976a; Gardiner and Webb, 1980; Lanier et al., 1984) and are expected to reduce both beetle populations and

the incidence of DED (Gardiner, 1976a). Currently the organophosphorus insecticide chlorpyrifos is the only active ingredient registered under a Restricted Use provision for this purpose in Canada (Health Canada, 2010). The persistence of efficacy of chlorpyrifos (Jin et al., 1996) allows basal applications to be made in alternating years. Chlorpyrifos has been subject to reviews in both the United States (Environmental Protection Agency, 2010) and Canada (Health Canada, 2007), and these have resulted in a reduced range of uses, leading to concerns about future availability of chlorpyrifos. The aim of this study was to study alternatives to chlorpyrifos to determine their suitability for basal applications, particularly with respect to their persistence of efficacy against native elm bark beetles. Alternatives were selected on the basis of registration status and evidence that they might be suited for basal applications against *H. rufipes*. Permethrin and carbaryl are registered in Canada for the prevention of entry of bark beetles into trees (Health Canada, 2010); bifenthrin is registered for prevention of elm bark beetle infestations in the United States (Environmental Protection Agency, 2009). The candidate insecticides were applied to elm trees in the field, and at intervals up to 3 years after application bark samples were removed for laboratory bioassays with adult *H. rufipes*.

Materials and Methods

Fall 2005 field experiment

At a site **(**49° 44' N, 97° 7' W) approximately 14 km south of Winnipeg, 15 healthy American elm trees, diameter at breast height (DBH) 19–27 cm, were selected and grouped on the basis of proximity and similarity of size into five blocks of three trees each. Trees were ≥3 m apart to avoid spray drift from one to another. On 19 September

2005 between 10 and 11 a.m., the lower 1 m of trunk of one tree in each block was sprayed (Fig. 21) to run-off (approximately 350 ml for a tree of 20 cm DBH) with a water-based spray of permethrin (Prelude 240^{TM} EC: 0.5% a.i. in water) or chlorpyrifos (Pro Dursban Turf Insecticide® EC: 0.48% a.i. in water), or water as a control. All applications were made with a backpack sprayer using the standard procedures for basal spraying, except that applications were made to a height of 1 m above ground to provide adequate bark surface for sampling. Weather records for the time of application were acquired from Environment Canada (2008) for Winnipeg International Airport (49° 55' N 97° 14' W), the nearest station with hourly records. During the time of applications temperatures rose from 16.1 ° to 17.2 °C and relative humidity declined from 68% to 63%; wind direction was approximately WSW with a speed of 9–11 km/h.

Bark disk samples (Fig. 22) were taken from randomly-selected positions within the treated area of each tree, using a 5.1 cm diameter hole saw driven by a battery-powered reversible electric drill. Bark disks were individually placed in self-sealing plastic bags and transported to the laboratory in a cooler containing freezer packs, and then stored at -20 °C in a freezer until bioassays were conducted. Bark disk samples were taken 1 h before the spray treatment, immediately after treatment, 1 h after treatment, and then at 4, 11, 35, 70, 248, 283, 640 and 725 d after treatment.

Fall 2006 field experiment

In August 2006, 42 healthy American elm trees (DBH 12–26 cm) in a stand of river bank forest with no DED were selected at a site **(**49° 42' N, 97° 15' W) about 100 m north of the La Salle River and east of the town of La Salle, Manitoba. The trees were blocked on the basis of proximity into six blocks of seven trees that were separated by ≥3 m to avoid

spray drift between trees. Within each block, five trees were randomly allocated to one of five treatments. Basal insecticide treatments were applied between 10 and 11 a.m. on 15 September 2006 as described for the previous experiment, and were: water-based sprays of permethrin (Prelude 240™ EC: 0.5% AI in water), carbaryl (Sevin XLR Plus® 2% AI in water), chlorpyrifos (Pro Dursban Turf Insecticide EC®: 0.48% AI in water), or bifenthrin (Onyx™: 0.06% AI in water), or a water control. During the applications, temperatures rose from 22.1 °C to 25.4 °C, relative humidity decreased from 62 to 51%, and wind direction was approximately SSE. Although wind speed at the airport weather station ranged from 19–24 km/h, that within the forested study site was less. Bark disk samples, as described above, were taken 1 hour before treatment, immediately following treatment, 1 hour following treatment and 4, 11, 35, 70, 140, 248, 283, 640, 725 and 1,163 days after treatment.

Bioassays

Adult *H. rufipes* were obtained from elm trap logs that had been exposed in spring in elm stands south of Winnipeg. In July or August, trap logs were removed from the field and stored at 5 °C. Adult beetles were collected by placing the logs in sealed plywood emergence boxes (Fig. 23), 183 x 56 x 56 cm, which were in a controlled environment room maintained at 22 °C with a light regime of 16:8 (L:D) hours. Two holes at each end of each box led to 3.8 liter clear glass jars to which the positively phototactic adult *H. rufipes* moved. Paper towels were placed in the jars to minimize beetle mortality in the condensed moisture that collected. Beetles were collected daily and used for bioassay within 24 hours of collection.

Bioassays were conducted following the method of Jin et al. (1996) with slight modifications. The bioassay chamber consisted of an ABS plastic cylindrical plumbing fitting, 5 cm internal diameter, and 3.9 cm high, closed at top and bottom with lids from 5 cm diameter Petri dishes (Fig. 24). Before the bioassay, the bark disk to be tested was placed on the bottom Petri dish lid, and the disk and lid sealed to the cylinder with paraffin wax so that the floor of the chamber was the bark disk. For the bioassay, 20 recently-emerged active adult *H. rufipes* were introduced to the chamber which was then closed at the top with the second Petri dish lid. The bioassay chamber was covered so that the beetles were in darkness, and maintained at room temperature (approximately 22 °C) for 48 hours, after which mortality of beetles was assessed. For each field experiment, bioassays were conducted simultaneously on all treatments in a replicate block for a particular time since treatment: 60 beetles were needed for each replicate for the 2005 fall experiment and 100 beetles were needed for each replicate of the 2006 fall experiment.

Analyses

Mortality data from bioassays of disks removed before insecticide treatment were compared to determine if there were pre-existing differences among treated trees. Frequencies of dead and living insects from bioassays of disks removed after treatment were subjected to binary logistic regression (Hosmer and Lemeshow, 2000). Initially, a complete logistic regression model was fitted that included time since application, main effects of spray treatment (effects on the location parameter of the logistic regressions), and their interaction with time since application (effects on the slope of the regression). Where a significant overall treatment effect was detected, partitioning of the likelihood ratio (LR) χ^2 was used to compare the water control with insecticide treatments, and to

compare mortality in the chlorpyrifos treatment with that in potential alternative insecticides. As different spray treatments affected both overall mortality and the pattern of mortality over time since application, treatments were considered to affect both the location parameter and the slope of the regression, and so 2 df were associated with each partitioned treatment effect. For some treatment × time combinations, all frequencies were 0, so loss of degrees of freedom was avoided by adding $\Delta = 0.5$ to all frequencies (Bishop et al., 2007). Analyses were performed in Systat (2009), and the experiment-wise α level for significance was set at 0.05. The α level for partitioning treatment effects was adjusted for the number of tests using the Bonferroni correction (Abdi, 2007). For graphical presentation of the results, mortality in each treatment was corrected for control mortality using the formula of Schneider-Orelli (1947),

$$\text{Corrected mortality [\%]} = \left[\frac{(t_{ij} - c_{ij})}{(100 - c_{ij})}\right] \times 100$$

where t = percentage of dead beetles in the treated group, and c = percentage of dead beetles in the control (water) treatment at the i^{th} time after treatment in replicate block j.

Results

For the fall 2005 experiment, mortality in all bioassays of disks removed 1 hour before treatment was 0%. In spring 2006 the site was flooded, but as soon as flood waters receded, further samples were taken beginning on 25 May (248 days after treatment). For the water control, mortality in bioassays was 0% in all replicates from the time of application up to and including the samples taken 70 days after application; at subsequent times of sampling, mortality was (mean ± SEM, n = 5): 4.0 ± 1.9% at 248 days, 5.0 ± 1.6% at 283 days, 4.0 ± 1.0% at 640 days and 4.0 ± 1.9% at 725 days.

The overall logistic model for the 2005 field experiment was highly significant (Table 26), and a very good fit to the data (McFadden's $\rho^2 = 0.68$). The overall effect of spray treatment on the level and temporal pattern of mortality was significant. Both insecticides differed from the water control, and there was a significant difference between chlorpyrifos and permethrin. Average corrected mortality (Fig. 25) for chlorpyrifos was 100% throughout the 2005 field experiment, which terminated in September 2007, about two years after the time of application. Corrected mortality for permethrin was 100% for the first 4 days of the trial, remained above 90% until the onset of winter, and had declined to 44 ± 4% by the May following treatment.

In the 2006 field experiment, mortality in the bioassays for water control treatments was 0% in all replicates up to and including the samples taken 140 days after application. In subsequently-taken samples, control mortality ($n = 6$) was 5.0 ± 1.3% at 248 days, 5.0 ± 2.2% at 283 days, 4.2 ± 1.5% at 640 days, 5.0 ± 1.3% at 725 days and 5.5 ± 2.2% at 1,163 days.

The overall logistic regression model for the 2006 fall experiment was significant (Table 27) and was an excellent fit to the data (McFadden's $\rho^2 = 0.67$). The overall effect of spray treatments on mortality and its temporal pattern was significant, the insecticidal treatments differed from the water control, and there were significant differences among insecticides. In the comparison between chlorpyrifos and the other three insecticides, permethrin and carbaryl both differed from chlorpyrifos, but there was no significant difference between chlorpyrifos and bifenthrin. In the chlorpyrifos treatment, corrected mortality in all bioassays was 100% from the date of application until September 2008, 725 days after application (Fig. 26); mortality in the bifenthrin treatment was 100% until

June 2008, but had declined to an average of 98.3 ± 1.1% (n = 6) by September 2008. Corrected mortality in permethrin and carbaryl treatments had declined to 90 ± 3.2% and 80 ± 1.8% respectively by the onset of winter following the applications, and by May of the following year were 42.0 ± 3.2% and 26.4 ± 4.6% respectively.

Discussion

In both field experiments, there were no pre-treatment differences in bioassay results of disks, so the post-application differences can be ascribed to the treatments. The low level of mortality in the water control treatments for disks taken ≥ 248 days after application may be attributable to desiccation of bark disks during storage. Bioassays were conducted on bark disks in the order in which they were removed from the trees, and were conducted whenever sufficient beetles were available from trap logs to test a complete replicate block for a date for all the treatments. Scarcity of beetles in 2008 forced prolonged freezer storage of bark disks, and it was the disks that were stored until beetles became available in 2009 for which there was some mortality in the controls.

Studies in different parts of Canada have shown that 0.5% chlorpyrifos is effective in controlling overwintering *H. rufipes* (Gardiner and Webb, 1980): similar demonstrations of the efficacy of 0.5% chlorpyrifos were provided by Lanier et al. (1984) and Phillipsen et al. (1986). Chlorpyrifos is particularly effective and persistent on corky bark such as that in which *H. rufipes* overwinters (Lanier et al., 1984; Pajares and Lanier, 1989). Because these studies assessed insect performance on living trees, or emergence from logs, they did not provide a detailed time course of efficacy. Jin et al. (1996) developed the bark disk bioassay method, and reported 100% mortality in 24 h bioassays from an application of 0.48% chlorpyrifos in bark disks from the day of application to the

end of their two experiments 791 and 532 days later. Such duration of efficacy allows for applications to be made in late summer or spring that kill beetles entering overwintering sites for the following two winters. This duration of efficacy was confirmed by both of my experiments, and my 2006 field experiment suggests that chlorpyrifos may be effective against beetles entering overwintering sites before the third winter after a fall application.

Permethrin is a pyrethroid insecticide that is registered in Canada for bark beetle control, although not specifically for basal applications (Health Canada, 2010). The compound is effective in prevention of elm twig feeding by *Scolytus multistriatus* (Pajares and Lanier, 1989). In trap-log studies lasting about 7 weeks, permethrin inhibits construction of brood galleries by *H. rufipes* and kills emerging new generation beetles (Phillipsen et al., 1986). However, in both the experiments in my study, the efficacy of permethrin diminished below that of chlorpyrifos within a few days of application; the lack of persistence of efficacy would require that basal applications be made every year. The persistence of efficacy of carbaryl in the 2006 field experiment was inferior to that of permethrin. Lanier et al. (1984) consider carbaryl to be inferior to chlorpyrifos for most methods of vector management for DED, although applications of carbaryl to trap logs inhibit brood gallery construction and kill emerging beetles (Phillipsen et al., 1986).

In my fall 2006 field experiment, the pyrethroid insecticide bifenthrin had almost exactly the same pattern of efficacy as did chlorpyrifos. Bifenthrin confers protection to conifers against bark beetle attack for one, and possibly two, seasons of exposure (DeGomez et al., 2006; Fettig et al., 2006). Although the scientific literature does not contain any accounts of efficacy trials of bifenthrin against elm bark beetles, rates of

0.03% and 0.06% (a.i. in water) are registered in the USA for application to the trunk of elm trees for management of elm bark beetles (Environmental Protection Agency, 2009). In Canada, the technical material is registered, but no formulations for application are registered (Health Canada, 2010). Bifenthrin is considered moderately hazardous to users (World Health Organization, 2005), but is highly toxic to aquatic organisms (Environmental Protection Agency, 2009). It is highly persistent, binds strongly to soils and sediments, and is considered unlikely to leach into ground or surface water (Fecko, 1999). However concerns about the fate of a degradation product and about non-target toxicity have recently led to withdrawal of authorization of bifenthrin as a plant protection product in Europe (European Union, 2009).

The reduction of *H. rufipes* populations by basal spraying is an important element of the management of DED (Gardiner, 1976a; Westwood, 1991a), and the long term effectiveness of chlorpyrifos on the corky bark substrate at the base of elm trees (Lanier et al., 1984; Jin et al., 1996) has made it the insecticide of choice for this purpose. In Canada, should chlorpyrifos become unavailable, permethrin could be a stop-gap substitute, as it is already registered for bark beetle control. However, my studies suggest that permethrin is not as persistent on basal elm bark in Manitoba as on the bark of branches in the crown in New York (Pajares and Lanier, 1989), that control in the season of application would be less effective than that from chlorpyrifos, and that annual applications of permethrin would be needed to approach the level of *H. rufipes* suppression currently attained with biennial applications of chlorpyrifos. I conclude that biennial basal applications of bifenthrin could provide essentially the same level of *H. rufipes* population suppression as is currently achieved with chlorpyrifos.

Table 26. Results of binary logistic regression analysis of bioassay data from disks taken after spray application in the fall 2005 field experiment.

Source	$LR\,\chi^2$	df	P
Complete model			
Time since application + main treatment effect + interactions	2176.53	5	<0.001
Overall spray treatment effects (main treatment effects + interactions)	1731.38	4	<0.001
Partitioning of spray treatment effects			
Water control versus insecticides	1172.82	2	<0.001
Chlorpyrifos versus permethrin	558.57	2	<0.001

The primary regression is on time since application; treatments affect both the location (main effect) and slope (interaction) of the regression. The adjusted α level for partitioning of treatment effects is 0.025.

Table 27. Results of binary logistic regression analysis of bioassay data from disks taken after spray application in the fall 2006 field experiment.

Source	$LR\ \chi^2$	df	P
Complete model			
Time since application + main treatment effect + interactions	6398.30	9	<0.001
Overall spray treatment effects (main effects + interactions)	5303.58	8	<0.001
Partitioning of spray treatment effects			
Water control versus insecticides	3913.52	2	<0.001
Among insecticides	1390.06	6	<0.001
Chlorpyrifos vs bifenthrin	0.37	2	0.829
Chlorpyrifos versus carbaryl	811.86	2	<0.001
Chlorpyrifos versus permethrin	29.68	2	<0.001

The primary regression is on time since application; treatments affect both the location (main effect) and slope (interaction) of the regression. The adjusted α level for partitioning of treatment effects is 0.01.

Figure 21. Basal spraying with insecticide on elm trees at the La Salle site on 15 September 2006. Photo credit: Sunday Oghiakhe.

Figure 22. Three bark pieces taken from American elm tree after spraying with bifenthrin at the La Salle site in 2006. Photo credit: Sunday Oghiakhe.

Figure 23. *Hylurgopinus rufipes* rearing chamber and glass collection jars. Photo credit: Sunday Oghiakhe.

Figure 24. Modified PVC pipes with bark disk floors used as bioassay chambers. Photo credit: Sunday Oghiakhe.

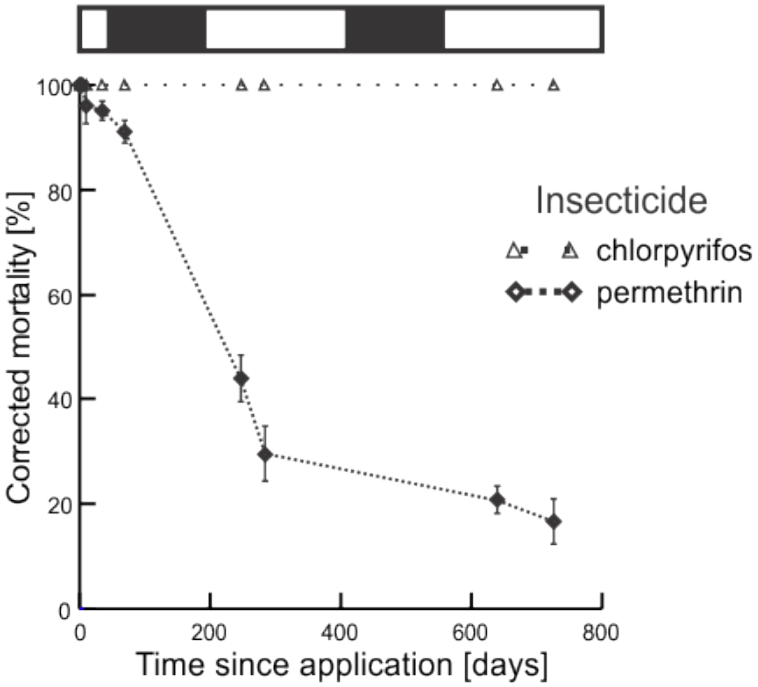

Figure 25. Mean (± SEM) corrected mortalities of *Hylurgopinus rufipes* for insecticide treatments in relation to time since application for the 2005 field experiment. The dark blocks in the bar above the main graph represent the months November−March, during which the long-term monthly average temperature is below 0 °C at the study sites.

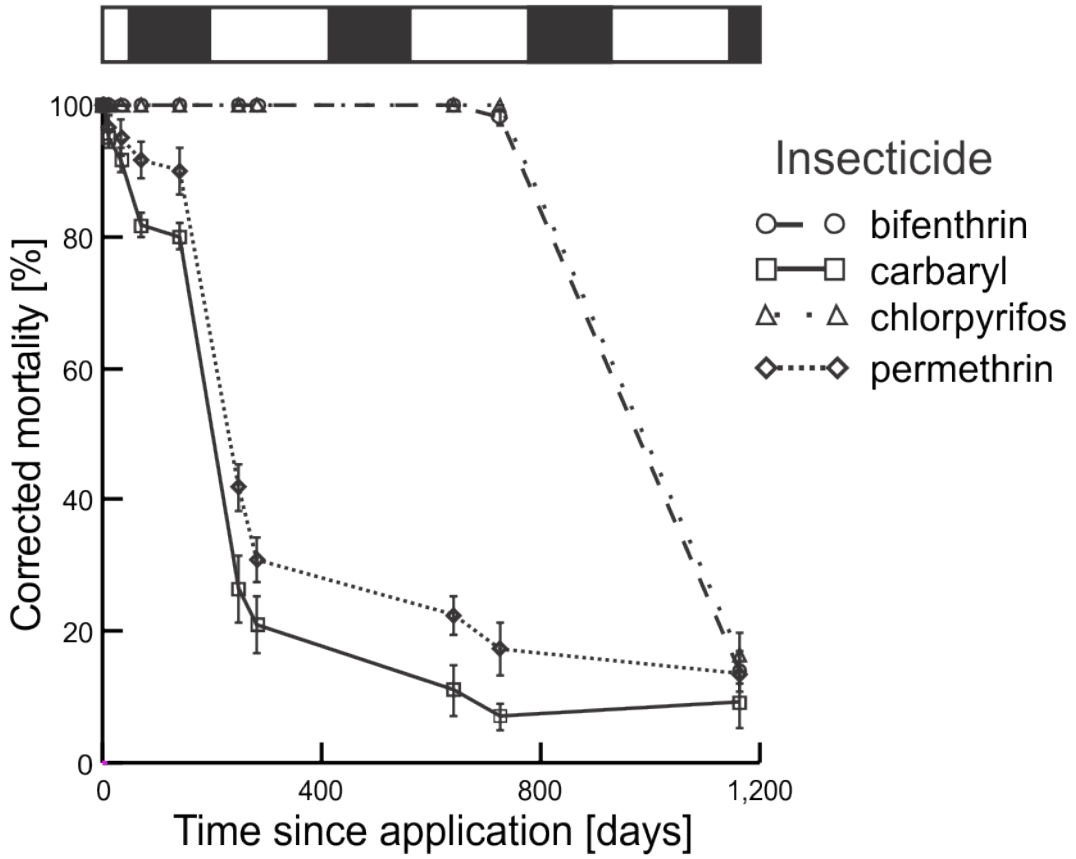

Figure 26. Mean (± SEM) corrected mortalities of *Hylurgopinus rufipes* for insecticide treatments in relation to time since application for the 2006 field experiment. The dark blocks in the bar above the main graph represent the months November−March, during which the long-term monthly average temperature is below 0 °C at the study sites.

Chapter 6: General Discussion

The aims of this research were to develop biological knowledge to improve vector and disease management in Manitoba, and to provide practical information to urban forest managers so that they can enhance the efficiency and sustainability of integrated DED control management programs that they operate. Current control methods have been in place, without any major change, since the beginning of the DED management programs in Manitoba about 38 years ago.

My first research objective was to determine whether *Hylurgopinus rufipes* can complete development in newly-diagnosed elm trees in the same growing season that symptoms become detectable, and can emerge carrying spores. The data from the 2006 and 2007 field seasons showed that newly-symptomatic trees were suitable for construction of brood galleries, can support development of a generation of *H. rufipes* and are a source of adult beetles carrying fungal spores. Emergence of adults is dependent upon the temperature of the summer, so completion of development of *H. rufipes* might not occur in all years. Nevertheless, rapid removal of newly-symptomatic trees, completed by mid-August, is a precaution to prevent the possibility that adult spore-bearing *H. rufipes* can emerge from the trees and transmit DED pathogens. The total number of *H. rufipes* in symptomatic trees was extremely variable, ranging from 1 to 41,213. Given that it is not possible for some jurisdictions to perform rapid removal of all infected trees, identifying those trees with most beetles is desirable. The relationship between presence of *H. rufipes* in stained branch sections and the total number of beetles per tree could be the basis for a diagnostic tool to help prioritize trees for rapid removal.

My second research objective was to study overwintering of *H. rufipes* in *U. americana*. Numbers and density of overwintering *H. rufipes* increased with decreasing height, and the proportion overwintering in the basal 15 cm always exceeded 70% of the population overwintering above ground. My study provides the first evidence that considerable numbers of *H. rufipes* overwinter below the soil surface, and could be unaffected by basal spraying. Frequency of spore bearing by overwintering beetles in 2008 averaged 45% for wild population and 2% for DayGlo®-marked *H. rufipes*. From the combination of spore bearing and marked beetle data, it appears that most beetles do not travel long distances from their emergence site to their overwintering site, but a small proportion may travel several kilometres.

My third objective was to study the effectiveness of insecticides for control of overwintering *H. rufipes,* using a bark disk bioassay. All beetles died when exposed to chlorpyrifos-treated disks up to 725 days after treatment. The pattern of mortality for beetles exposed to bifenthrin was very similar. In contrast, permethrin and carbaryl treatments provided relatively short-term efficacy.

My study showed that *H. rufipes* can complete their immature development in the year of DED symptom appearance and emerge carrying spores. Based on the response of development rate to temperature (Appendix 2), at average air temperatures for July and August, emergence would occur less than 8 weeks after egg laying. This fits well with my findings from dissections of trees from river-bank forests. In elms in sunlit sites, *H. rufipes* would be exposed to higher temperatures than in the shady sites from which my dissected trees came. Therefore, in parks, boulevards and other open sites, emergence

could be earlier than in my dissected trees, and the incidence of emergence in the year of symptom appearance could be higher than suggested by my study reported in chapter 3.

In Manitoba, warmer summers will reduce time for development and may prolong periods of post-emergence feeding and flight activity. Warmer winters may increase winter survivals and allow larval overwintering in brood galleries, which occurs in more temperate regions, but for which, currently, there is no evidence in Manitoba. In Minnesota, *H. rufipes* generally is univoltine and overwinters as adults in the basal 30 cm of elm trunks; in a few locations with abundant brood wood, a second generation is able to overwinter as larvae (Landwehr et al., 1982). In December samples in 2006 and 2007, 6 and 585 *H. rufipes* respectively were found in brood galleries in my tree dissections, but all appeared to be dead. Thus, in my study, there was no evidence of larval overwintering.

In 2006, adults emerged in September while in 2007, they emerged in October and not all the beetles completed development. I assume that the emerged adults fed for a while before moving to the bottom of elm trunks to overwinter. However, as feeding tunnels can be observed as early as August in Manitoba (Ellis, 1990), beetles emerging in September or October might not have sufficient time to feed to accumulate lipids before winter and may die during winter due to the lack of energy or cryoprotectants. In my research (Appendix 1) fat content in overwintering beetles was lower in February 2009 than in November 2008, and this might indicate impending energy depletion. However, Lombardero et al. (2000) observed increases and decreases in lipid content in overwintering *Ips pini* (Say), and in Nova Scotia, Rousseau et al. (2012) observed that lipids decrease through fall and winter before rising again in the spring in the spruce

beetle (*Dendroctonus rufipennis* Kirby). Declines and subsequent increases in lipids may result from conversion of lipids to cryoprotectants at the onset of cold conditions and conversion back to lipids with warmer weather (Lencioni, 2004). If this is so in *H. rufipes*, the lower levels of lipids in my February samples may not be due entirely to depletion of reserves, as some may be the result of cryoprotectant synthesis. If so, reconversion to lipids may occur. The need for cryoprotectants is less below the snow line and underground, where temperatures may be around or above zero.

The short period of adult feeding in late-emerging *H. rufipes* may not impair lipid accumulation and winter survival, as some scolytines acquire lipids during larval feeding. In Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopkins), Atkins (1967) showed that there is rapid accumulation of fat reserves in the later stages of larval development. Also, the period available for adult feeding in Manitoba may be adequate, as Anderson (1996) observed tunnel construction by *H. rufipes* until the end of October in 1992.

During the period 2005-2010 in Manitoba, the average annual prevalence of DED in communities that removed symptomatic trees shortly after symptom detection was 1.5 ± 0.2%, which was less than the 3.1 ± 0.4% in communities where removal was in winter (Veilleux et al., 2012). Thus there are benefits to rapid removal in places where the primary vector is *H. rufipes*, and the insect overwinters only in the adult stage. A major effect of rapid removal is prevention of emergence of spore-bearing *H. rufipes* adults from newly symptomatic trees. The results of Veilleux et al. (2012) are in accord with the view that *H. rufipes* emerging from newly-symptomatic trees do survive to transmit spores, although some portion of rapid removal benefit could also come from reduced root graft transmission.

In my mark-recapture study, the high frequency of marked beetles overwintering in the river bank sites where they were released suggests that beetles do not travel very far, and that many of the spore-bearing overwintering beetles must have come from local newly-symptomatic trees. Trees that had displayed symptoms in previous years had already been removed by the Urban Forestry Branch, City of Winnipeg. If the majority of *H. rufipes* in river bank sites have moved only locally, then the relatively high number of new infections in such sites was probably the result of transmission by beetles that emerged from newly-symptomatic trees in the previous fall and overwintered locally.

The success of Manitoba's integrated DED management program (Jeffrey, 1982) is indicated by the 160,000 American elm trees remaining in Winnipeg, the largest remaining stand of American elms in North America (City of Winnipeg, 2008a). Despite the vigor of the management program, elm trees continue to be lost to DED at a rate of >2% per annum (Domke, 2012), and in Winnipeg over the last 10 years the average loss is 5,000 trees per year (City of Winnipeg, 2008a). In urban management zones and buffer zones, sanitation is practised in winter to remove symptomatic trees infested with beetles carrying spores. My research suggests that this timing of removal allows spore-bearing beetles to emerge from newly-symptomatic trees. This could account for the difficulty in reducing the annual new infection rates below 2%.

I recommend that rapid removal of newly-symptomatic trees be completed by mid-August to eliminate the possibility that adult spore-bearing beetles could emerge and transmit DED pathogens. In Manitoba communities that practice rapid removal, prevalence of DED is lower than in those that practice fall/winter removal (Veilleux et al., 2012). In other places in Canada and USA with different conditions, rapid removal is

considered as the most effective DED management method (Barger, 1977; Euale et al., 1978; French et al., 1980; Hart and Kennedy, 1981; Barger et al., 1982; Magasi et al., 1993; City of Eagan, 2011; Newberger, 2012).

As a result of the lack of sufficient capacity to remove all diseased trees before beetles emerge with spores, a technique for prioritizing trees for rapid removal must be developed. My finding of preferred brood gallery sites may provide a way for prioritizing. My results showed that *H. rufipes* and galleries tended to be less numerous in the smallest and largest available branches; for example, 65% of the population of *H. rufipes* in branches in the June 2006 tree was in the 10% of branches that were of diameter 8–11cm in diameter. The relationship between the percentage of stained branch sections with *H. rufipes* present and the total number of *H. rufipes* in the tree might be the basis for a practical, effective method for identifying which symptomatic elm trees harbor high numbers of *H. rufipes*. Research is needed to develop the method, for which, sample units should be well defined, easy to collect, provide suitable precision at detecting an infestation, and be cost effective (Binns et al., 2000). In addition to providing a basis for more efficient rapid removal, such a method would be useful for identifying and delimiting the distribution of incipient outbreaks. Branch sampling has been used in Ontario, Canada to detect the emerald ash borer (*Agrilus planipennis* Fairmaire) infestations in asymptomatic trees (Ryall et al., 2011).

There are a number of reports of the effectiveness of basal applications of chlorpyrifos for management of overwintering adult populations of *H. rufipes* trees (Gardiner, 1976a; Gardiner and Webb, 1980; Landwehr et al., 1982; Lanier et al., 1984; Magasi et al., 1993). My bioassays (Chapter 5, Oghiakhe and Holliday, 2011) confirmed

the results of Jin et al., (1996) that efficacy of chlorpyrifos on elm bark lasts for two years under Manitoba conditions. However, at the time that my thesis research began, the future of chlorpyrifos was in doubt. If the full evaluation of the aggregate risk from all organophosphate pesticides, including chlorpyrifos, is concluded and the risks are deemed within acceptable levels (Environmental Protection Agency, 2002), then chlorpyrifos should be retained because of its proven persistence and efficacy in controlling overwintering *H. rufipes*. Currently, chlorpyrifos is eligible for reregistration (Environmental Protection Agency, 2002, 2007). In Canada, Dursban-T is registered for basal applications under a restricted use condition, and this registration is valid until the end of 2014 (Health Canada, 2014), when it could be renewed. Availability of chlorpyrifos for basal applications could be jeopardized by future reviews, or a corporate decision to discontinue registration. Thus, having an alternative to chlorpyrifos is desirable. Also, replacement of chlorpyrifos with a less acutely toxic alternative for basal spraying would make the public more accepting of this management tool and increase its usage. Adverse media coverage of the use of chlorpyrifos has enhanced public apprehension about basal application. Such anxiety adds to the difficulty of spraying elms on privately owned property, and the low frequency of basal applications on private property impairs the effectiveness of the overall management program. The registered insecticides permethrin and carabaryl are not suitable replacements for chlorpyrifos as, to get efficacy equivalent to alternating year applications of chlorpyrifos, two late year applications would need to be made every year.

I chose bifenthrin as one of the insecticides for evaluation because it was already registered by the Environmental Protection Agency in the United States for elm bark

beetle management, and the technical material is registered by Health Canada with an expiry date of 31 December, 2016. Economic considerations make it improbable that an insecticide that is not registered elsewhere for the same target pest would be registered for the limited market of the Canadian jurisdictions that carry out basal spraying for elm bark beetle management. Since the beginning of my research, studies of the toxicity of bifenthrin to aquatic organisms have shown that bifenthrin is the most toxic pyrethroid to freshwater Crustacea (Amweg et al. 2005) and that it has sub-lethal behavioural effects on these organisms ((Huynh et al. 2014). Bifenthrin is also lethal to aquatic insects and to a lesser degree to fish (Solomon et al. 2001; Maund et al. 2011). Although it might be thought that, if chlorpyrifos became unavailable, an emergency registration of bifenthrin with buffer zones areas around freshwater bodies could be implemented, this now seems improbable. In urban areas in California where bifenthrin is widely sprayed, storm water contributions can increase concentrations of bifenthrin in river water well above levels that influence Crustacea (Weston and Lydy, 2012). In order to reduce susceptibility due to the possible unavailability of chlorpyrifos, research needs to be carried out to find a new insecticide that is both persistent and has suitable environmental and toxicological properties.

In bioassays, beetle death occurs when chlorpyrifos or bifenthrin (Jin et al., 1996; Oghiakhe and Holliday, 2011) residues on elm bark are encountered by beetles excavating feeding or overwintering tunnels. Once beetles have penetrated the bark and are below the level of the surface residues, or after beetles have entered overwintering tunnels below ground, they are unlikely to be affected by insecticide. Anderson and Holliday (2003) reports that basal insecticide applications could be limited to the basal 55

cm of each elm rather than 2 m (Landwehr et al., 1982) or 2.5 m (Gardiner and Webb, 1980), because the number of overwintering *H. rufipes* alive above 55 cm was insignificant. The recommendation (Anderson and Holliday, 2003) of fine scale assessments between the soil surface and 55 cm to assess the wisdom of further reductions of spray height prompted my studies on the height distribution of overwintering beetles. My studies showed that indeed, the majority of *H. rufipes* overwinter very close to the ground surface, and most do so below 15 cm. However, I also provided (Chapter 4) the first evidence that considerable numbers of *H. rufipes* overwinter below the soil surface where they may be unaffected by basal spraying. No current or potential insecticide for basal applications can penetrate below ground to kill overwintering beetles. Beetles overwintering below ground likely constructed feeding tunnels higher up on the same trees, as beetles overwintering above ground are thought to do (Anderson and Holliday, 2003). It is not known whether beetles overwintering below ground normally previously feed in tunnels within the basal 50 cm of the trunk; if so, current basal applications would kill them. Reducing the upper limit of basal applications below the current 50 cm is not recommended, as considerable numbers of beetles do overwinter in the upper parts of this zone, and a reduction would increase the likelihood of insecticide avoidance of the beetles that overwinter below ground. Future research arising from my findings could include an assessment of numbers of living beetles overwintering on trees that had received basal applications; however, if that research demonstrated the wisdom of extending basal application further up the trunk, it is unlikely that regulators or public perception would allow reversal of the height reduction that reduced environmental pesticide load.

In Manitoba, DED was initially reported in 1975, and the invading pathogen was the highly aggressive and pathogenic *Ophiostoma novo-ulmi* (Hintz et al., 1993). The pathogen, the presence of a population of *H. rufipes*, and the availability of a high number of susceptible American elm trees, created ideal conditions for the disease to break out into the classic exponential phase that characterizes epidemics. As *H. rufipes* are recruited to elm trees carrying *O. novo-ulmi* by the volatiles these trees release (McLeod et al., 2005), an initial pathogen infection can efficiently acquire vectors, even when their density is low because of restricted availability of brood wood. Following this phase, we may expect a build-up in the population of the pathogen through killing more trees and an increase in vector population because of an increased supply of brood material for the beetles. Eventually, most of the accessible large elms are killed, resulting in a decline of the beetle population and that of the pathogen. However, even in the absence of management, elms do not completely disappear. Elm seedlings and root suckers regenerate and when they grow to be large enough to support beetle breeding, they are attacked by the pathogen and a chronic pattern of endemic disease emerges (Brasier, 1983a; Peterken and Mountford, 1998). For unmanaged wych elm (*Ulmus glabra* Hudson, Fl. Angl. 95. 1762) in Britain, 23 years after a DED outbreak, the disease afflicts vigorous, exposed individuals and sprouts, but not slow-growing individuals on poor sites (Peterken and Mountford, 1998). Similarly in a well-preserved Danish forest reserve affected by storm damage and DED, trees in smaller diameter classes were less affected by the disease and grew vigorously (Emborg and Heilmann-Clausen, 2007).

The patterns in unmanaged elm stands in Europe (Peterken and Mountford, 1998; Emborg and Heilmann-Clausen, 2007) are similar to those in Manitoba following the

arrival of DED. Dutch elm disease initially severely affected the trees killing most large diameter elms in unmanaged stands in Manitoba, but now there is regeneration in these stands. However, host, pathogen and vector dynamics have not occurred uniformly in Manitoba, because of effective disease management programs in urban areas, and because susceptible elms in rural areas are generally restricted to moister sites (Scoggan, 1957; Caners and Kenkel, 1997), particularly narrow strips of river bottom forest (Shay, 1986; Waters and Shay, 1995).

Urban elms in Manitoba continue to persist with good management. Without DED management, annual urban elm losses of approximately 18% are normal in North America (Domke, 2005) instead of annual loss rates of about 3% or less observed in urban areas in Manitoba (City of Winnipeg, 2008a; Veilleux et al., 2012). Relative to an unmanaged situation, Manitoba's urban areas have high populations of susceptible elm trees, and pathogen and vector populations are maintained lower than under natural dynamics by disease management. In Saskatchewan, despite shifts in responsibility, DED management still occurs in all the major cities and in 17 smaller communities (McIntosh, 2013). Relaxation of DED management activities in smaller communities in Saskatchewan (ForestTalk, 2010) and in Alberta (St. Albert Gazette, 2013) could lead to a rapid increase in DED because the high density of susceptible hosts would favour rapid increases in pathogen and vector populations.

In Manitoba, wild elms grow along riverbanks. In unmanaged situations many large elms have succumbed to DED, and most probably will do so, but there are small regenerating elms. Disease dynamics in these types of sites will likely follow the pathway outlined above: as regenerating elms grow big enough to support vector populations,

pathogen and vector populations will resurge and a pattern of endemic disease will prevail. Such sites are potential sources for pathogens and vectors to spread to urban areas. Spread could be by the flight of vectors but, as riverbank stands in Manitoba are prone to spring floods, carriage downstream on flood-borne trees and debris is also possible. It is not known what proportion of the 2–3% of urban elm trees that currently become infected each year in urban areas comes from transmission within the built-up area, and what proportion results from transmission from less managed rural sites.

The riverbank sites in which I worked were in a disease management buffer zone around the City of Winnipeg that the Government of Manitoba implemented to reduce the disease pressure on the city's elm trees. Buffer zones are along rivers or creeks where there are large numbers of elm trees, and are designed to reduce the emigration along waterside corridors of pathogens and vectors from rural unmanaged sites to urban areas with disease management (Westwood, 1991a). Disease management operations in buffer zones are expected to lower populations of beetles carrying spores relative to unmanaged sites. Comparison of yearly elm loss rates in Cost Shared Agreement communities with or without watercourses and buffer zones shows that, communities not located on a river or creek suffer an average annual elm loss rate of 1.02%, those on a river or creek with no buffer zone lost an annual average rate of 4.76% while towns or cities on a river or creek with a buffer zone experience an average annual elm loss rate of 1.46% (Westwood, 1991a). Thus comparable communities with buffer zones have significantly lower loss rates than those without buffer zones.

In the buffer zone in which my sites were located, symptomatic trees were removed but basal spraying was not practised. Some large elm trees remained in my sites,

and there were high populations of *H. rufipes* mostly carrying spores of *O. novo-ulmi*. Each year, considerable numbers of elms trees were infected with *O. novo-ulmi*, showed the characteristic flagging symptoms starting in spring, and were removed the following winter.

Pines and Westwood (2008) recommended that 1 km be the minimum width of buffer zones, as marked beetles were recaptured on the farthest trap from the release site, a distance of 1 km. My studies showed that, although most marked *H. rufipes* did not travel far from their emergence site to their overwintering location, some travelled considerable distances: 17 marked beetles released at the La Salle site were recovered at the Camp Amisk site, a straight line distance of 4.8 km or about 7.5 km along the river. Also, 47% of overwintering beetles carried *O. novo-ulmi* spores when the nearest detected source of spores was >300 m away, suggesting that a substantial proportion of the population may travel distances of the order of 1 km. The establishment of buffer zones has helped to reduce DED in urban areas with disease management. However, some portion of the >2% residual annual infection rate in urban areas (Domke, 2012) is probably due to penetration of buffer zones by beetles, so I recommend that minimum buffer zone width be increased to 4.8 km. In recommending 4.8 km as the minimum width, I am cognizant of the increased cost of maintaining much larger buffer zones, and recognize that most beetles presumably fly much less than 4.8 km. Neither my work nor that of Pines and Westwood (2008) allows characterization of the frequency distribution of distance travelled by beetles, and so the additional cost of 4.8 km buffers could confer little extra advantage. However managers, in using 1 km buffer zones should understand that they are not stopping all beetles from flying through the buffer zone.

Based on my results, I recommend the following changes to improve the efficacy of DED management in Manitoba:

1. The Forestry Branch of Manitoba Conservation and Water Stewardship and the City of Winnipeg Urban Forestry Branch, should implement rapid removal of newly-symptomatic American elm trees, completed by mid-August, in their respective jurisdictions.
2. A diagnostic tool, possibly based on the relationship I found between presence of *H. rufipes* in stained branch sections and the total number of beetles per tree, should be developed to help prioritize symptomatic trees infested with high numbers of beetles for rapid removal. This will require development of a standardized sampling protocol.
3. As a priority, urban foresters should press for research to find an alternative insecticide for basal spraying to kill overwintering *H. rufipes* in the event that chlorpyrifos is no longer available. The alternative should be sufficiently persistent, that it can be sprayed in alternating years, and like chlorpyrifos, should be applied to the bottom 55 cm portion of each elm trunk.
4. The minimum width for buffer zones should be 4.8 km, to prevent beetles from entering urban forests from surrounding unmanaged areas.

If my recommendations are adopted, I expect that in the next 10 years DED management in the managed zones will show significant improvement. Based on my research, I recommend that there be future research to address:

1. Seasonal patterns of cold hardiness and cryoprotectant profiles in *H. rufipes* in Manitoba. This would provide information on composition of the lipid content of

overwintering beetles in relation to their time of emergence, ability to survive winter and the freezing temperature for the beetle.

2. Studies of the rate of development of native elm bark beetles in relation to temperature. Results would help to develop a model of emergence time under field conditions that could be used to adjust DED management programs in response to climate change.
3. Identification and screening of suitable insecticides that can kill overwintering beetles including those below the ground surface. These could include alternative residual insecticides or, for high value trees, injected systemic compounds.

Summary of main findings of my thesis research:

1. In some years, newly-symptomatic trees are suitable for construction of brood galleries, can support development of a generation of *H. rufipes* and are a source of adult beetles carrying fungal spores.
2. Emergence of *H. rufipes* adults is dependent upon the temperature of the summer; in years with temperature closer to the long term average, completion of development might not occur for *H. rufipes*.
3. Rapid removal of newly-symptomatic trees, completed by mid-August, would prevent the possibility that adult spore-bearing *H. rufipes* can emerge from these trees and transmit DED pathogens.
4. The total number of *H. rufipes* in symptomatic trees was extremely variable.
5. In general, the lower trunks and small and large diameter branches tended to be underutilized by broods of *H. rufipes* and for gallery construction, and most *H. rufipes* occur in areas with xylem staining.
6. The relationship between presence of *H. rufipes* in stained branch sections and the total number of beetles per tree could be the basis for a diagnostic tool to help prioritize elm trees for prompt removal.
7. Numbers and density of overwintering *H. rufipes* increased with decreasing height; beetles showed a definite preference for the lower portion of the elm trunk.
8. Considerable numbers of *H. rufipes* overwinter below the soil surface and may be unaffected by basal spraying.
9. Two percent of marked *H. rufipes* that were plated had spores of *O. novo-ulmi*, in contrast to 45% of unmarked beetles in the same samples that carried spores. This

suggests that the majority of spores on overwintering beetles are acquired in the brood wood, rather than after leaving the brood site and before entering the wintering tree.

10. A total of 17 marked *H. rufipes* were found at Camp Amisk on 4 February 2008; these beetles flew at least 4.8 km. However there is evidence many beetles overwinter close to their brood tree.
11. Chlorpyrifos provided 100% control of *H. rufipes* for two seasons and was not significantly different from bifenthrin. Control with permethrin or carbaryl was relatively poor.

Appendices

In this section of my thesis, I have included studies that I started and due to one reason or another, could not complete. Nevertheless, I got useful results from the studies and would like to document them here. I believe that inclusion of these studies would enhance the overall quality of my thesis and would supplement some of the information provided therein. Some of the studies require further work and my results would provide a starting reference point. These studies were excluded from the thesis itself based on the advice of my advisor and committee members.

Appendix 1. Variations in fat content in overwintering native elm bark beetle, *Hylurgopinus rufipes* (Coleoptera: Curculionidae) in Manitoba

Introduction

In Manitoba, native elm bark beetle, *Hylurgopinus rufipes* adults overwinter at the bottom of the trunks in American elm trees, *Ulmus americana* (Anderson and Holliday, 2003) and occasionally in Siberian elm trees, *Ulmus pumila* (Anderson and Holliday, 2000). These overwintering adults emerge in summer from brood galleries and move to healthy elms where they first construct feeding tunnels in the trunk and large branches (Becker, 1937), before moving to overwintering sites in the trunk in late fall (Swedenborg et al., 1988; Anderson and Holliday, 2003). Spring feeding by adult beetles occurs in late April or early May (Anderson, 1996), when beetles crawl or fly to the crowns of healthy American elm trees, often in the same tree as they overwintered (Kaston, 1939). During feeding the spores of the fungus may be transferred to the water-conducting vessels of the tree (Takai et al., 1979). My data suggest that most *H. rufipes* beetles emerging from newly-symptomatic trees would do so in September or October, and, it appears that some of them might have insufficient time to feed to accumulate lipids before winter (see Chapter 3 on brood development of *H. rufipes*). So contributions of beetles that emerge after late August to the transmission of DED is not known because of the little time they have to feed and accumulate the necessary lipids before overwintering, and ignorance about the effect of energy reserves on the survival of overwintering *H. rufipes*. Metabolic reserves affect insect fitness attributes, such as

reproductive rate and survival (Crespi, 1989). Lipid content in bark beetles is correlated with increased survival, dispersal, and competitive abilities (Hagen and Atkins, 1975, Anderbrant et al., 1985, Anderbrant, 1988) and directly involved in the biosynthesis of cryoprotectants (Sømme, 1964, 1982; Miller and Werner, 1987; Lombardero et al., 2000). Most of the research on fat content of overwintering bark beetles has been on species that feed on coniferous trees (Truchan and Butcher, 1970; Hagen and Atkins, 1975; Lombardero et al., 2000; Graf et al., 2012; Rousseau et al., 2012); there is a dearth of information in the literature on the fat content of overwintering elm bark beetles. A thorough knowledge of the fat content and the ability of *H. rufipes* to survive winter and emerge in summer to transmit DED are needed to improve beetle management. The aim of this study was to investigate variations in fat content in overwintering *H. rufipes* in Manitoba.

Materials and methods

This study was done at the La Salle site in Winnipeg. On 17 November 2008 and on 17 February 2009, seven randomly-selected healthy American elm trees of different DBH were felled and the basal 55 cm removed to the laboratory, stored at 5 °C in a cooler (refer to Chapter 4 on overwintering of *H. rufipes* for details) and debarked to collect overwintering *H. rufipes* for fat analysis. Individual fresh masses of adults were measured using a CAHN 25 automatic electrobalance and the insects were stored singly in labeled glass tubes, oven dried for five days at 55 °C and reweighed. Fat content of overwintering beetles was measured as described by Zhou et al. (1995) and Mills (1981). Each beetle was extracted in fresh 3 ml of petroleum ether for five days at 38 °C, oven-dried for five

days at 55 °C and reweighed. Formulae used to calculate percent moisture and lipid content were:

$$Percentage\ moisture\ content\ =\ 100\ \times\ \left[\frac{fresh\ mass - dry\ mass}{fresh\ mass}\right]$$

$$Fat\ mass =\ Initial\ dry\ mass -\ dry\ mass\ after\ fat\ extraction$$

$$Percent\ lipid\ =\ 100\ \times\ \left[\frac{Fat\ mass}{Initial\ dry\ mass}\right]$$

I analysed whether there was a significant difference in measures between November 2008 and February 2009, and also assessed whether there was a relationship between DBH and the calculated measures within dates, using general linear modelling. All analyses were carried out using Systat 13 (Systat, 2009) and the alpha level for significance was 0.05.

Results

Table 28 shows DBH and number of beetles tested for each stump, and the fresh and dry mass, and moisture and lipid content of adult *H. rufipes* from stumps removed from the La Salle site on 17 November 2008 and on 17 February 2009. There were significant differences between dates in DBH of stumps ($P < 0.01$) and between dates and lipid content of overwintering beetles ($P < 0.05$). There were no significant differences between dates and fresh mass, dry mass and moisture content of overwintering *H. rufipes*.

There were highly significant differences ($P < 0.001$) within dates in dry mass, moisture content (%) and fat content (%) of overwintering beetles. Fresh masses of overwintering beetles within dates were statistically significant ($P < 0.01$). Mean (± SE) fat content of overwintering beetles from the 17 November 2008 stumps ranged from

5.83 ± 0.42% to 11.17 ± 0.47%, while the percentage fat content of overwintering beetles collected from 17 February 2009 stumps ranged from 5.04 ± 0.40% to 7.96 ± 0.52%. Within these dates, there was no significant relationship between lipid content and DBH for either date individually of for both dates pooled (Table 28).

Discussion

The trees used in February 2009 were smaller than all the trees used in November 2008 because of a randomization error in tree selection at the La Salle site. This confounding variable (DBH) was important. However, despite the significant difference ($P < 0.01$) in DBH between dates, fresh mass, dry mass and moisture content of overwintering *H. rufipes* were not significantly different. This probably means that smaller and bigger trees provided an essentially similar moist and suitable micro-environment in winter for *H. rufipes*.

The reasons for the significantly higher percentage lipid content in November 2008 than in February 2009 does not appear to be an effect of beetles with higher fat choosing larger DBH trees, or beetles on larger trees having different fat metabolism. If this were so, the within date relationships of lipid content and DBH would have been significant. As they were not, I conclude that fat content of overwintering *H. rufipes* decreased as wintering progressed. Stored body fat is used in insects to survive winter through production of cryoprotectants (Williams and Lee, 2011). Lipids are usually the main energy reserve for overwintering insects (Izadi et al., 2011) and in freeze-tolerant and intolerant insects, lipids and glycogen are food reserves accumulated during summer and transformed into cryoprotectants for winter (Lencioni, 2004).

In the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (Coleoptera: Curculionidae: Scolytinae), water and lipid reserves in *D. ponderosae* decrease in tandem through autumn and winter before rising again in the spring (Graf et al., 2012). From August to October lipid content in *Ips pini* (Say) concomitantly declines in the field and the laboratory, and then in both situations, lipid content rises by February (Lombardero et al., 2000). These changes suggest that metabolism of lipids may be associated with seasonal changes in cold hardiness in the beetle (Lombardero et al., 2000). In Nova Scotia, Rousseau et al. (2012) report a decrease in water and lipid reserves through autumn and winter before a rise in the spring in the spruce beetle, *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae). Thus, the decline in lipids I observed in *H. rufipes* might not indicate a utilization of fat reserves as a substrate for metabolism, but could be evidence of a reversible conversion to cryoprotectants.

From my brood development studies of *H. rufipes* in Manitoba (see chapter 3), it is not clear that *H. rufipes* beetles have sufficient time to develop into adults or accumulate fat reserves in bigger elm trees before the onset of winter. This small study showed that lipid content is lower in mid-winter than in early winter but, as no measurements of metabolites and supercooling points were made, further research should be conducted with respect to *H. rufipes* lipid content and cold hardiness in Manitoba.

Table 28. Mass, moisture content and fat content of adult overwintering *Hylurgopinus rufipes* from stumps removed from the La Salle site on two dates in winter 2008–9.

Removal date	Stump ID	DBH (cm)	Number of beetles tested	Fresh mass (mg) Mean ± SE	Dry mass (mg) Mean ± SE	Moisture content (%) Mean ± SE	Fat content (%) Mean ± SE
17 Nov 2008	Brady 2	14.5	30	1.59 ± 0.05	0.69 ± 0.02	55.6 ± 0.57	9.43 ± 0.71
	Brady 3	20.9	30	1.57 ± 0.04	0.65 ± 0.02	58.4 ± 0.64	5.83 ± 0.42
	Brady 4	25.2	28	1.66 ± 0.04	0.70 ± 0.02	57.6 ± 1.10	8.12 ± 0.38
	Brady 5	22.3	30	1.37 ± 0.04	0.52 ± 0.02	61.9 ± 0.47	7.15 ± 0.53
	Brady 6	19.3	30	1.62 ± 0.04	0.66 ± 0.02	58.9 ± 0.57	8.91 ± 1.08
	Brady 7	47.6	31	1.56 ± 0.05	0.62 ± 0.02	60.3 ± 0.32	11.17 ± 0.47
	Average	25.1 ± 0.8	179	1.56 ± 0.02	0.64 ± 0.01	59.0 ± 0.29	8.45 ± 0.29
17 Feb 2009	Brady 8	5.7	31	1.52 ± 0.05	0.63 ± 0.02	58.7 ± 0.44	5.04 ± 0.40
	Brady 9	4.8	19	1.38 ± 0.09	0.60 ± 0.02	53.1 ± 3.67	5.10 ± 0.79
	Brady 10	8.0	21	1.42 ± 0.06	0.56 ± 0.02	59.5 ± 2.72	6.12 ± 0.98
	Brady 11	6.0	17	1.54 ± 0.07	0.62 ± 0.03	59.3 ± 0.51	7.96 ± 0.52
	Brady 13	4.8	21	1.48 ± 0.05	0.60 ± 0.03	59.9 ± 0.66	6.31 ± 0.47
	Brady 14	5.1	31	1.50 ± 0.03	0.65 ± 0.01	56.6 ± 0.43	6.66 ± 0.37
	Average	5.7 ± 0.1	140	1.48 ± 0.02	0.61 ± 0.01	57.8 ± 0.68	6.11 ± 0.25
Analysis of variance							
Between dates *F* (df = 1,10)		13.5**	—	3.3 n.s	1.1 n.s.	0.8 n.s.	5.9 *
Among stumps within dates *F* (df = 10, 307)		—	—	2.9**	7.2***	3.6 ***	6.2 ***
DBH regressions within dates							
November 2008 (df = 1,4)		—	—	0.0 n.s.	0.2 n.s.	1.0 n.s.	2.2 n.s.
February 2009 (df = 1,4)		—	—	0.1 n.s.	2.6 n.s.	1.1 n.s.	0.0 n.s.
Both dates (df = 2, 8)		—	—	0.0 n.s.	0.5 n.s.	1.1 n.s.	1.6 n.s.

* P <0.05, ** P <0.01, *** P <0.001, ns. = not significant

Appendix 2. Temperature dependent development of immature stages of the native elm bark beetle, *Hylurgopinus rufipes* (Coleoptera: Curculionidae) in Manitoba

Introduction

In Manitoba, the native elm bark beetle, *Hylurgopinus rufipes* Eichoff (Coleoptera: Curculionidae: Scolytinae) is the major vector of DED (DED) fungal pathogens, *Ophiostoma ulmi* and *O. novo ulmi* (Hildahl, 1977). Knowledge of the biology of *H. rufipes* is important in designing an integrated management program for the disease. Precise information on the life history of *H. rufipes* is rare because beetles are relatively inaccessible and their activities cannot be readily observed (Kaston, 1939). The few available studies on *H. rufipes* life history at different temperatures were conducted in the United States (Kaston, 1939) and in southwestern Ontario, Finnegan (1957) carried out field studies on seasonal pattern of the beetle.

The effects of temperature of sapwood on the growth and survival of *H. rufipes* in symptomatic elm trees have not been examined. Therefore, the aim of this study was to collect reliable quantitative data on the effect of temperature on development and survival of immature *H. rufipes* in Manitoba, and to characterize the summer temperatures of sapwood of symptomatic elm trees in Manitoba.

Materials and methods

In 2007 and 2008, slabs of elm bark, approximately 15 x 30 cm were removed from healthy elm trees, and placed in a rearing device (Figs. 27–29) (Kaston and Riggs, 1937; Wermelinger and Seifert, 1998) in which the underside of the bark was clamped firmly

against a Plexiglass plate, and the exterior of the bark opened into the remainder of the cage, into which adult beetles could be introduced. Up to 20 beetles were introduced to the exterior side of the bark, and brood galleries that were constructed under the bark were observed from the side of the bark clamped to the Plexiglass plate. These cages were placed in the dark in incubators at different temperatures, and examined daily to record the location and stage of eggs, larvae, pupae, and emerged adults resulting from the reproduction of the introduced adults. A range of five constant temperatures (12, 16, 20, 24 and 28 °C) was used, and the resulting data on duration of development of different stages were used to calculate rates of development.

The temperature regime beneath the bark of trunks was characterized between 16 June and 30 September 2008 in a river bank forest with natural elm population at the La Salle site. Hobo® data loggers were used to measure the temperature beneath the bark of trunks in symptomatic American elm trees where native elm bark beetle adults lay their eggs and produce brood galleries. Six symptomatic American elm trees with different DBH measurements were selected (Table 29): three trees were naturally exposed to sunlight, while the other three trees were in a shaded part of the forest. Data logger probes were inserted at a height of 2 m in the space between phloem (inner bark) and sapwood (xylem layer) to measure temperature at 15 minute intervals from 16 June to 30 September 2008. To prevent loss of data, temperature readings were downloaded at biweekly intervals into an Excel database. At the end of the study, temperature measurements from under the bark were compared to values obtained at Winnipeg airport (Environment Canada, 2013) for the same period.

Results

Percentage survival of different stages of *H. rufipes* showed a progressive increase with temperature (Table 30). From 16 °C to 28 °C, temperature treatment significantly affected egg survival ($LR\chi^2$ = 25.54, df = 3, P < 0.001), larval survival ($LR\chi^2$ = 19.08, df = 3, P < 0.001) and overall survival ($LR\chi^2$ = 36.92, df = 3, P < 0001). In all these cases, survival was highest at the highest temperature. The effect of temperature treatment on pupal survival was not significant ($LR\chi^2$ = 4.35, df = 3, P = 0.23), although again, there was a tendency for survival to be higher at higher temperatures. There were no survival data for 12 °C as, at this temperature, introduced adult beetles died within nine days without any oviposition; most adults were inactive at one end of a tunnel in the bark.

At temperatures where development occurred, the total number of days from egg laying to adult emergence decreased with increasing temperature (Table 31). The same trend was evident for egg, larval and pupal development separately. When expressed as rates of development, there was a progressive increase in development rate of the various stages *of H. rufipes* with increasing temperature (Fig. 30).

Data loggers placed between the inner phloem and xylem of symptomatic elm trees showed wide daily temperature fluctuations between sun-exposed and shaded trees. Out of the 12 Hobo data loggers set out on 16 June 2008, it was discovered on 29 July 2008 that 11 had malfunctioned and did not record data. During this period, only one data logger worked properly and recorded data. The data loggers were subsequently reprogrammed and redeployed on 6 August 2008. Temperatures recorded from 6 August to 20 August 2008 ranged from 5.0 °C to 44.5 °C, while Environment Canada air temperatures ranged from 11.7 °C to 29.2 °C. Temperatures recorded between 20 August

and 28 September, 2008 ranged from 2.8 °C to 31.0 °C, while Environment Canada air temperatures ranged from 7.3 °C to 25.0 °C (Table 32).

Discussion

Rate of development of insects normally increases between the lower lethal limit and a temperature slightly below the upper lethal limit, and then declines slightly as temperature approaches the upper lethal limit (Taylor, 1981). I did not get any development below 16 °C, but this was due to failure of females to lay eggs, and may not represent limitations on development of immature stages. My results and those of Kaston (1939) are generally similar. In my experiments, hatching took place about 7 days after eggs were laid at 24 °C, whereas in Kaston (1939) hatching occurs in 5 or 6 days at a temperature of 25 °C. In Kaston (1939) the average duration of the pupal period was about 11 days at 20 °C, 7.3 days at 24.5 °C and 5.4 days at 30 °C, and pupal development did not occur at temperatures below 8.8 °C. Between 10 °C and 30 °C, the rate of development was related to temperature in a rectilinear way (Kaston, 1939). Emergence of young beetles from logs kept at 24.5 °C occurs 57 days after exposure to attack, with peak emergence occurring at about 74-75 days (Kaston, 1939). In my studies, emergence took 44 days at 24 °C.

Limitations to using my data to develop a model of *H. rufipes* development include there being only four temperatures at which development rate could be estimated, and that the highest of these appeared to be below the temperature at which development rate is maximum. In order to complete this study, a range of temperatures is needed to enable the development of a full scale model that runs from lower to upper lethal limit. Currently, it is clear that my data are mostly at the low end.

Based on the average of maximum and minimum temperatures in table 32, from 6 to 20 August, sun-exposed trees had mean temperatures between inner phloem and xylem on the south side of 27.0 °C and on the north side of 25.0 °C, both of which were much warmer than the mean air temperature (20.4 °C) recorded by Environment Canada. Temperature minima on sunlit trees were not very different from minimum air temperatures; however, even on the north side of sunlit trees, maxima were considerably higher than corresponding maximum air temperatures. For trees in shade, mean temperatures between the inner phloem and xylem from 20 August to 28 September were 15.9 °C on the south side and 14.9 °C on the north side; these values were similar to the mean air temperature recorded by Environment Canada for the same period (15.2^{o}C). Mean minimum temperatures between inner phloem and xylem of the shaded trees were lower than minimum temperatures recorded by Environment Canada. Conversely, mean maximum temperatures between inner phloem and xylem for the shaded trees were a few degrees higher than maximum air temperature for the same period.

Vermunt et al. (2012) recorded under-bark temperatures on ash trees in winter (December–February) and spring (March–May) in several locations in Ontario, and found great variability in under-bark temperatures among trees. In winter, mean daily temperatures under bark on the south and north sides of trees were warmer than air temperatures. In spring, daily maxima under bark on the south side—but not on the north side—of trees were also significantly warmer than air temperature maxima (Vermunt et al., 2012). As in my study, under-bark temperatures on the south side of trees were higher than on the north side (Vermunt et al., 2012), Although the spring temperatures were cooler than my late summer temperatures, they were within the range in which insects

could develop, and models of emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) development predicted shorter development times on the south side of urban trees than if predictions were based on air temperature (Vermunt et al., 2012).

In late August and early September, *H. rufipes* development on the shaded trees in my study would likely have been slow: my laboratory study shows that at the average temperature of about 16 °C on south side almost 17 days would be required for pupae to complete development. In the sun-exposed trees that were monitored in the middle two weeks of August, average temperatures would have allowed pupal development to be completed in <10 days, and development from egg laying to adult emergence in <43 days. The average temperatures measured in the sun-exposed trees, even if they were not higher earlier in August and in July, would certainly have allowed completion of brood development of *H. rufipes* in American elm trees newly diagnosed with DED, as I observed to occur in the study reported in chapter 3. As the upper lethal limit for *H. rufipes* is not known, it is unclear whether the observed daily maxima under bark of >40 °C would be detrimental to the insects.

My study in chapter 3 was conducted in river bank forest where trees were mostly shaded and so, based on my temperature measurements, would likely have had average under-bark temperatures similar to air temperatures. In that study, a higher proportion of *H. rufipes* successfully completed development in the warm summer of 2006 than in the cooler 2007. My finding that average under-bark temperatures in sunlit trees were considerably higher than corresponding air temperatures, suggests that in newly-

symptomatic trees on boulevards or other sunlit locations, *H. rufipes* might be able to complete development in average, or even cooler than average, years.

Table 29. Distribution of Hobo data loggers on six elm trees at the La Salle site in 2008.

Tree number	DBH (cm)	Direction	Growing Condition
1a	44.3	South	sunlight
1b	44.3	North	sunlight
2a	14.5	South	sunlight
2b	14.5	North	sunlight
3a	20.9	South	shade
3b	20.9	North	shade
4a	25.2	North	sunlight
4b	25.2	South	sunlight
5a	22.3	South	shade
5b	22.3	North	shade
6a	47.6	South	shade
6b	47.6	North	shade

Table 30. Percentage survival of different stages of *Hylurgopinus rufipes* at constant temperatures.

Temperature (°C)	Eggs		Larvae		Pupae		Overall	
	Survival (%)	N	Survival (%)	N	Survival (%)	N	Survival (%)	N
16	60.5	86	57.7	52	76.7	30	27.9	86
20	85.2	27	87.0	23	75.0	20	55.5	27
24	90.6	32	89.7	29	88.5	26	71.9	32
28	95.2	42	90.0	40	91.7	36	78.6	42

Table 31. Duration of development *of Hylurgopinus rufipes* at constant temperatures.

Temperature (°C)	Days to develop (Mean± SE)			
	Laying to hatch	Hatch to pupation	Pupation to emergence	Laying to emergence
16	8.5±0.30	35.0±0.03	16.9±0.47	61.5±0.12
20	7.5±0.27	31.9±0.07	12.9±0.57	53.2±0.43
24	7.1±0.05	27.1±0.05	9.4±0.11	43.7±0.10
28	3.3±0.08	18.3±0.08	8.4±0.17	30.4±0.09

Table 32. Temperatures recorded by Hobo data loggers using probes placed between inner phloem and xylem on the south and north sides of American elm trees growing in the sun and shade at the La Salle site between 6 and 20 August and 20 August and 28 September, 2008 and minimum and maximum temperatures for Winnipeg recorded by Environment Canada at Richardson International Airport for the same period.

Date range	Direction and exposure				Environment Canada temperatures for Winnipeg	
	South (sunlight)		North (sunlight)			
	Min temp °C	Max temp °C	Min temp °C	Max temp °C	Min temp °C	Max temp °C
6–8 Aug	5.0	40.7	10.5	36.5	11.7	27.6
8–10 Aug	12.5	44.0	13.2	41.2	12.2	28.6
10–12 Aug	12.5	43.7	13.3	38.0	14.0	23.0
12–14 Aug	14.1	39.2	17.0	22.0	13.2	24.2
14–16 Aug	13.5	39.5	14.0	36.4	15.1	29.2
16–18 Aug	12.0	44.4	14.5	38.5	14.7	29.0
18–20 Aug	13.0	44.0	12.3	42.5	14.6	28.2
Mean	**11.8**	**42.2**	**13.5**	**36.4**	**13.6**	**27.1**
Date range	South (shade)		North (shade)			
	Min temp °C	Max temp °C	Min temp °C	Max temp °C		
20–29 Aug	7.1	31.0	6.3	29.3	12.1	25.0
29 Aug– 8 Sep	5.0	27.2	5.3	21.6	8.5	20.3
8–18 Sep	2.8	24.1	5.6	21.0	7.9	20.5
18–28 Sep	4.5	24.8	7.2	22.6	7.3	20.1
Mean	**4.9**	**26.8**	**6.1**	**23.6**	**9.0**	**21.5**

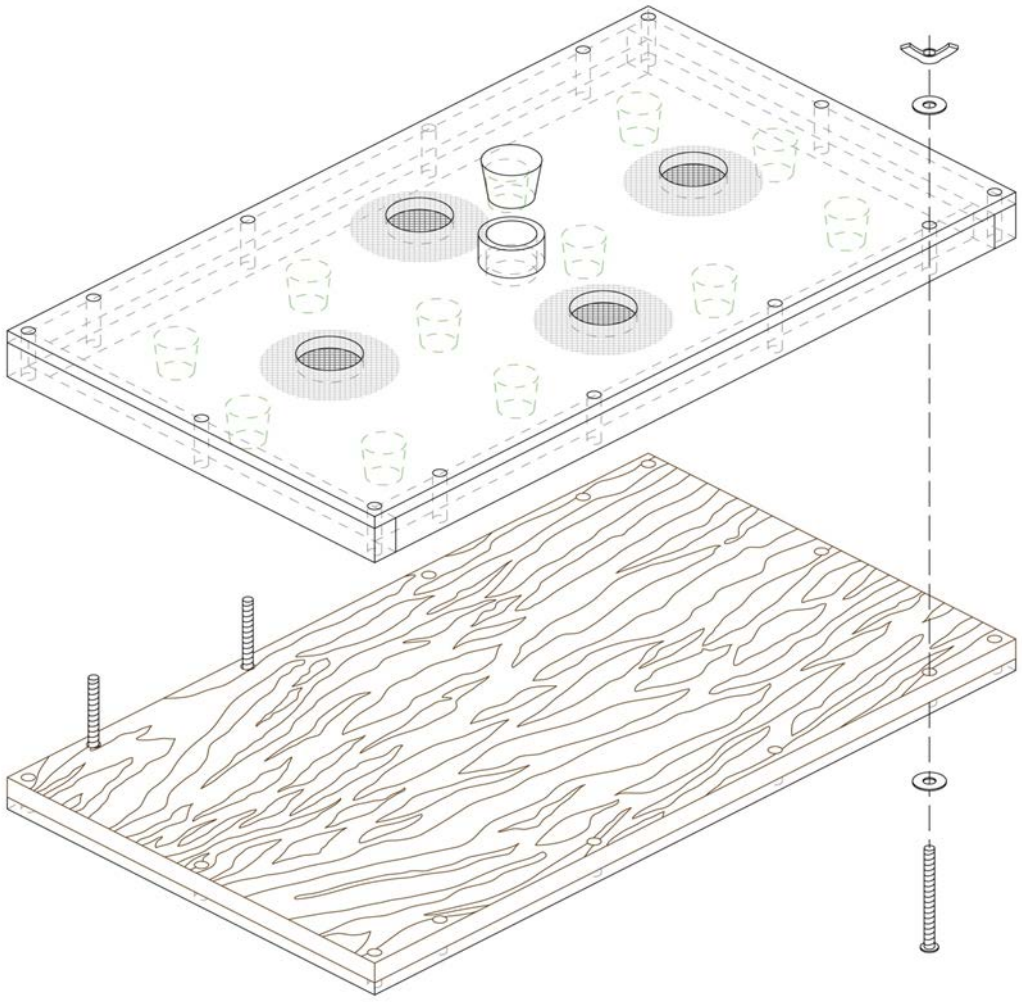

Figure 27. Schematic diagram of rearing chamber. Image credit: Jonathan Veilleux. Used with permission.

Figure 28. Picture of rearing chamber showing exterior side of elm slab. Photo credit: Sunday Oghiakhe.

Figure 29. Picture of rearing chamber showing inner bark (secondary phloem) of elm slab. Photo credit: Sunday Oghiakhe.

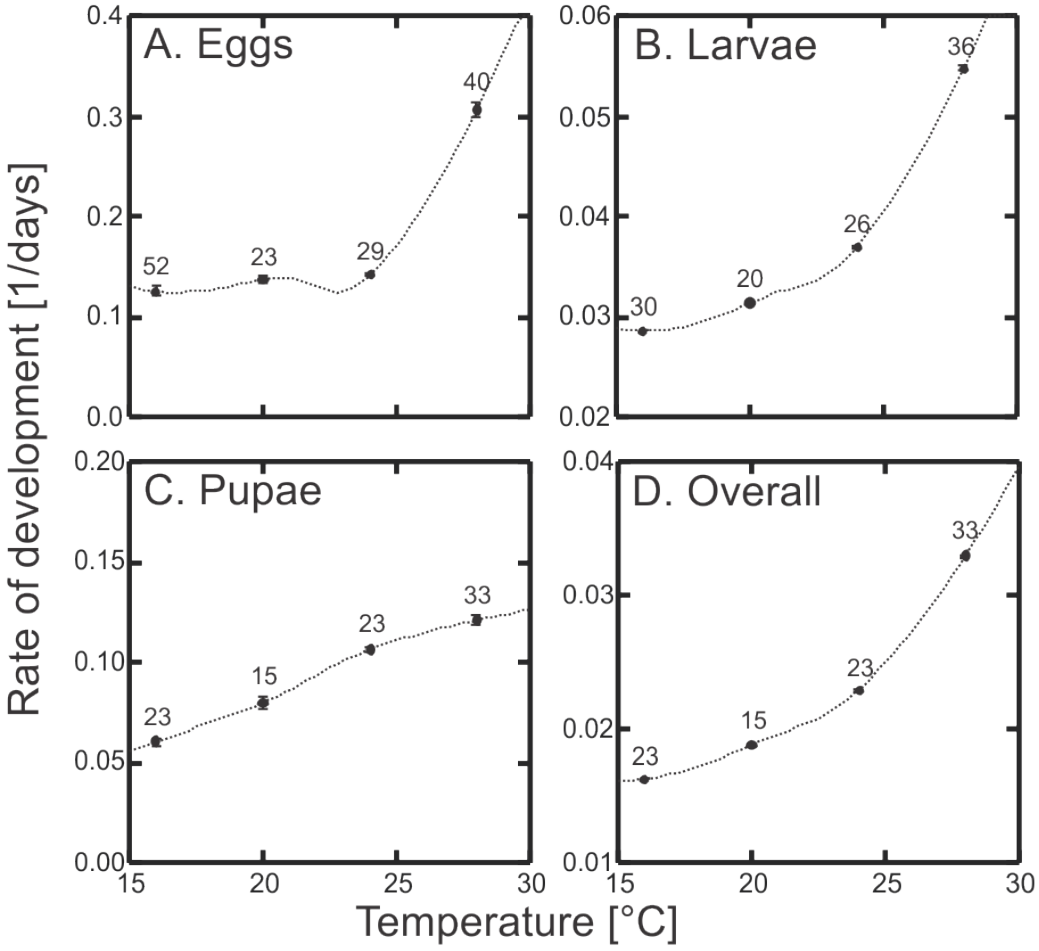

Figure 30. Mean (± SE) rates of development at four constant temperatures for A. Eggs, B. larvae, C. pupae and D pooled immature stages of *Hylurgopinus rufipes*. Trend line is fitted by distance-weighted least squares regression and numbers above points indicate the number of individuals contributing to the mean. Note vertical axes have different scales.

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