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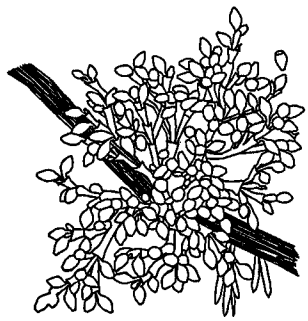

Biology of the dwarf mistletoe, *Arceuthobium americanum* Nutt. ex Engelm., on jack pine (*Pinus banksiana* Lamb.) in Alberta, Canada

by
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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Forest Biology and Management

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ABSTRACT

Lodgepole pine dwarf mistletoe (*Arceuthobium americanum*) is an important pest of *Pinus contorta* var. *latifolia* and *Pinus banksiana* in western Canada. While many aspects of the *A. americanum–P. contorta* var. *latifolia* pathosystem have been investigated, this is not true of the *A. americanum–P. banksiana* pathosystem. This study reports five investigations on the latter pathosystem: collecting and storing of seed; adhesives for inoculation of host seedlings; seed germination, and host penetration and infection; effect of extreme cold temperatures on seeds and germinants; and the life cycle of the parasite on *P. banksiana*. (1) Stockinet was an effective collection material, intercepting 62% of seeds; the maximum collection rate was 1160 seeds per hour of stockinet placement and recovery. Aseptic seed storage in the dark at 2°C in jars with an internal relative humidity of 75% was effective at storing seeds without molding. (2) Three adhesives, anhydrous lanolin, hydroxypropylcellulose, and polyvinyl acetate, and the seed's natural viscin were used for host inoculations. Percentage of infected seedlings was significantly higher with hydroxypropylcellulose compared with polyvinyl acetate, lanolin, and with the seed's viscin. (3) Timing of seed germination varied considerably while differences in seed germination did not vary between years and among seed sources. Germinated seeds were significantly larger than those that did not germinate. Host penetration began during June–August and usually continued until June–July of the next year. Twig swelling near the penetration point usually occurred 13–15 months after germination. (4) Germinative ability of overwintering seeds increased with increasing simulated winter temperatures between –39 to –35°C. Exposure period also influenced germination rates. Exposure to temperatures between –38 and –53°C for 96 or 144 hr was almost always lethal. At –37°C, germination was greater after 48 hr than after 96 hr, but still significantly lower than controls. Temperatures down to –6°C did not reduce germinant survival. (5) Based on 56 plants that developed from 175 inoculations in a jack pine plantation, 32 were pistillate plants, 15 were staminate plants, six died, and three were sexually immature as of October 2003. Most plants flowered in the fourth year after inoculation. Pistillate plants produced seed in the fifth year, although one plant produced seed in four years. Thus, *A. americanum* has a five-year life cycle on jack pine.

Dedication

To my parents,
Josef and Josephine Brandt,
who taught me to work hard
and let the rest fall into place

and

Donald J. Pluth
5 February 1936 – 12 January 2004
A mentor, a friend, and someone who was
always a pleasure to work with.

PREFACE

One of the motivating factors for conducting the research reported in this dissertation was the unique nature and fascinating parasitic habit of *A. americanum*. Another motivating factor was a desire to begin developing an understanding of some of the factors that affect the distribution of this interesting plant on the landscape of the western Canadian interior (Chapter 5). As I began my work I realized there was a need to develop some techniques in order to conduct my experimental work. Chapters 2 and 3 are the result of these activities. From my many trips to dwarf mistletoe-infested jack pine stands I also developed a sense that perhaps the *A. americanum*–jack pine pathosystem was different in some respects to what was described in the literature for the same dwarf mistletoe on lodgepole pine. Chapters 4 and 6 are the result of these ideas. At the time of defense and publication of this dissertation Chapter 2 had been accepted by Canadian Journal of Plant Pathology, Chapter 3 had been published in Canadian Journal of Botany, and Chapter 5 had been published in Canadian Journal of Forest Research. My intent is to submit Chapters 4 and 6 after collecting more field data in 2004; Canadian Journal of Forest Research and Canadian Journal of Botany are candidates for submission. Appendix 1 provides a glossary of dwarf mistletoe terms used in this dissertation. Appendix 2 provides ANOVA tables for statistical analyses reported in Chapters 3, 4, and 5. Appendix 3 is the result of analyses on temperature and precipitation data originally collected for but not included in Chapter 4. The results contained in this appendix were not particularly informative but could be of use to future investigations relating germination performance in the field to temperature and precipitation. For this reason these results have been included in this dissertation as an appendix.

This dissertation uses the paper format as described by the *Thesis handbook: a manual of regulations and guidelines for thesis preparation* (2003) of the University of Alberta's Faculty of Graduate Studies and Research. In the paper format the prefatory pages of the dissertation are followed by the body of text, which includes: (i) an introductory chapter to the entire thesis with its own bibliography; (ii) subsequent chapters presented in paper format without an abstract but with its own bibliography; and (iii) a final chapter that provides a general discussion and conclusions relating the separate studies described in the earlier chapters to each other and with its own bibliography.

ACKNOWLEDGMENTS

First, I wish to thank my wife, C. van Eden, for her support over the last few years while I completed my Ph. D. research. I am also grateful to C. Van Eden for laying-out the thesis and assisting in the design of the life cycle illustration (Fig. 6.3). I thank my young son, Willem, for providing the much-needed breaks from the demands of my research. My supervisors, originally D.J. Pluth and Y. Hiratsuka, and then in the past year, D.J. Pluth and V.J. Lieffers, have provided an environment conducive for developing and refining ideas as well as guidance in the research and writing aspects of my studies. K.I. Mallett, as a member of my supervisory committee, provided useful advice and support along the way. I am also grateful to S.S. Malhotra and T.E. Sterner of the Canadian Forest Service for their support of both my education leave and my research.

In terms of my research activities, I am grateful to G. Hood and other staff of Wood Buffalo National Park, S. Schwartz of Alberta Sustainable Resource Development, and A. Hare of Alberta Agriculture, Food and Rural Development (AAFRD) for permission to collect dwarf mistletoe seeds at the four seed collection sites and protecting them from disturbance; L. Elmes of the University of Alberta and J. Wu of AAFRD for use of freezers; R. Carr for assistance in compiling the Edmonton climate data; B. Kochtubajda of the Meteorological Service of Canada for providing climate data from several meteorological stations; N. Doesken and O. Bliss for providing the Red Feather Lakes daily temperature data; and B. Tomm, R. Brett, J. Weber, M. Michaelian, J. Hammond, T. Young, B. Vroom, R. Raypold, A. Engel, and D. Sherling for collecting and processing dwarf mistletoe seeds and other field work.

P.V. Blenis (Chapters 2, 3, & 5), P.E. Crane (Chapter 3), and E.H. Hogg (Chapter 5) provided helpful suggestions for improvement of earlier drafts of several chapters prior to their submission to journals. I also thank the anonymous reviewers of the Canadian Journal of Botany, Canadian Journal of Forest Research, and Canadian Journal of Plant Pathology for their contribution in improving these published chapters.

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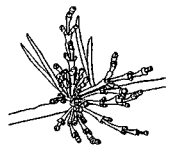

CHAPTER ONE

Biology of dwarf mistletoes

INTRODUCTION

Lodgepole pine dwarf mistletoe (*Arceuthobium americanum* Nutt. ex Engelm.) is a parasitic angiosperm belonging to the family Viscaceae. This parasite is the most widely distributed dwarf mistletoe and occurs throughout much of western North America on its two principal hosts: jack pine (*Pinus banksiana* Lamb.) and lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm., *P. contorta* Dougl. ex Loud. var. *murrayana* [Greville & Balfour] Engelm.) (Fig. 1.1) (Hawksworth and Wiens 1996). *Arceuthobium americanum* occurs rarely on *P. contorta* Dougl. ex Loud. var. *contorta* (Smith and Wass 1979). The northern limit of *A. americanum* is near 59° N, where it occurs on jack pine in Alberta (Brandt et al. 1998). The southern limit occurs on lodgepole pine in central California near 35° N (Hawksworth and Johnson 1989). Recently three races within *A. americanum* were identified: on jack pine in the western Canadian interior; on lodgepole pine (*P. contorta* var. *murrayana*) in the Sierra Nevada and Cascade Mountain ranges of the western United States; and on lodgepole pine (*P. contorta* var. *latifolia*) in the western United States and Canada (Jerome and Ford 2002).

Arceuthobium americanum presumably evolved on lodgepole pine in southwestern North America and transferred to jack pine only relatively recently, perhaps in the last 15 000–10 000 years B.P. near the end of the Wisconsin glaciation (Yeatman 1967). It was following the retreat of the continental glaciers that jack pine moved north and west from refugia in central and eastern North America and contact was made with lodgepole pine as it advanced northward from areas south of the glacial maximum in western North America (Critchfield 1985). The post-glacial history of both the parasite and its hosts has likely influenced the formation of the current genetic races within *A. americanum* (Jerome and Ford 2002).

Successful germination, penetration, and infection by the parasite cause stem and branch swellings and witches' brooms in trees. As infections intensify within the host, branches and tree tops die and the tree eventually succumbs (Fig. 1.2). Other than decay organisms, *A. americanum* is the most important pest of pine in the western Canadian interior. Regional estimates of annual growth losses for dwarf mistletoe-infected jack pine growing in the three prairie provinces are 314 000 m^3, while mortality losses are 1 478 000 m^3 (Brandt et al. 1998). Growth losses for dwarf mistletoe-infected lodgepole pine in Alberta and Saskatchewan are 486 000 m^3, while mortality losses are 69 000 m^3 (Brandt et al. 1998). These losses are not trivial considering that the standing volume of pine in the same area is 1.1 billion m^3 on timber-productive forest lands (Canadian Council of Forest Ministers 1997).

Factors affecting the development of *A. americanum* are uncertain. Dwarf mistletoe seeds disperse over short distances by forcible discharge from fruits and over long distances by various vectors (Hinds et al. 1963; Hawksworth and Hinds 1965; Punter and Gilbert 1989; Hawksworth and Wiens 1996). Climate, topography, soil type, and fire are

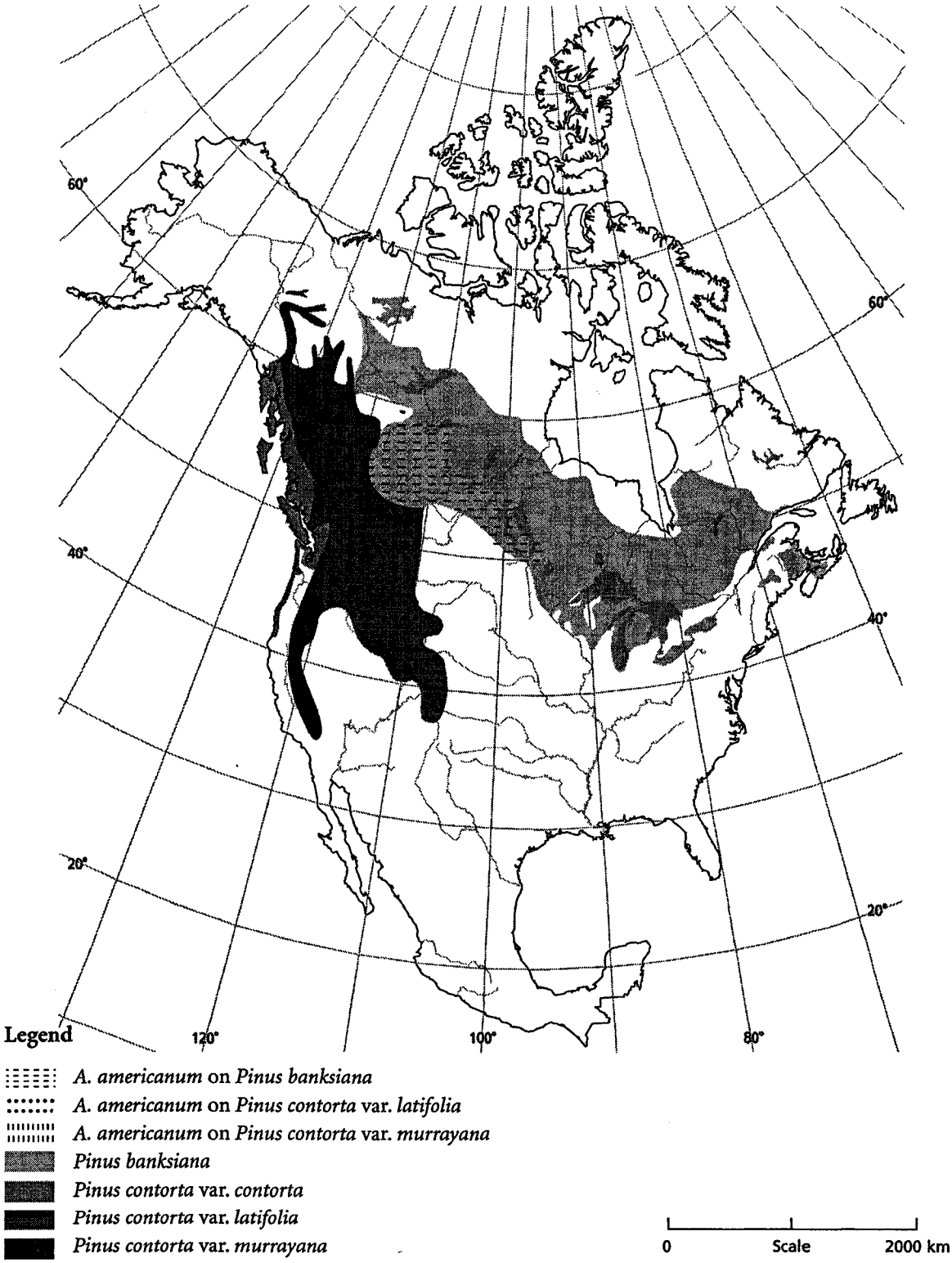

FIGURE 1.1 Distribution of *Pinus banksiana* and *Pinus contorta* in North America and the distribution of the three genetic races of *Arceuthobium americanum* for the same area (modified from Critchfield and Little 1966; Farrar 1995; Hawksworth and Wiens 1996; Jerome and Ford 2002).

FIGURE 1.2 Severely infested jack pine stands: (a) an infection center west of McClelland Lake (57° 31'N, 111° 24'W) north of Fort McMurray, Alberta, with infected regeneration; and (b) 40-year-old jack pine trees about 2–3 m in height and <5 cm in diameter near Hodge Lake (57° 49'N, 109° 24'W) south of Lake Athabasca in northwestern Saskatchewan.

important ecological factors in the biology and occurrence of various dwarf mistletoes but the influence of these factors is not well understood (Hawksworth and Wiens 1996).

Prior to 1972 substantial revisions were made to the taxonomy of *Arceuthobium*. The genus is considered taxonomically difficult because of the extreme morphological reduction related to the group's parasitic nature and the morphological similarities among species (Hawksworth and Wiens 1972, 1996). There are many morphological, physiological, phenological, palynological, and cytogenetical characters useful as taxonomic criteria in the classification of the genus (Hawksworth and Wiens 1972). The first taxonomic monograph of the genus described five species, with two of the species having several host forms (Gill 1935). The first subgeneric classification of the genus, including classification of several new species and assignment to full species rank for Gill's host forms was presented by Hawksworth and Wiens (1972) and revised later (Hawksworth and Wiens 1984). With the development of isozyme analysis and DNA sequencing, an alternative classification was presented by Nickrent (1996) using the previously accepted nomenclature for species. This classification was accepted by Hawksworth and Wiens (1996). In the following literature review and subsequent chapters of this dissertation, the nomenclature used follows that of Hawksworth and Wiens (1996). Consequently, for studies examining species within the *Arceuthobium campylopodum* complex and published prior to Hawksworth's and Wiens' 1972 classification, the nomenclature used in this dissertation (Table 1.1) will not necessarily match that used originally by the author(s) of the cited material because of the taxonomic revisions within this complex.

The studies reported in this dissertation deal with *A. americanum* infecting jack pine or with seeds collected from *A. americanum* plants growing on jack pine. The following literature review, however, is intended to provide a broader context by summarizing findings of all pertinent studies on the genus as these relate to the major topics investigated and reported in the subsequent chapters.

LITERATURE REVIEW

Collecting and storing seeds

Various materials and techniques have been employed to collect dwarf mistletoe seeds. Kraft paper bags placed over female plants with mature fruit were used effectively to collect *Arceuthobium occidentale* seeds (Scharpf and Parmeter 1962). Cheesecloth wrapped around brooms was used for collecting *Arceuthobium pusillum* seeds (Bonga 1965; Livingston and Blanchette 1986). In a comparison of paper bags, plastic bags, cheesecloth, and sausage-casing bags for collecting seeds of *A. americanum*, *Arceuthobium abietinum* f. sp. *concoloris*, *A. campylopodum*, *Arceuthobium douglasii*, *Arceuthobium laricis*, and *Arceuthobium tsugense*, sausage-casing bags were preferable to the other types because they were reusable and allowed gaseous and aqueous exchange with the environment (Wicker 1967a).

The environmental regime for storage of dwarf mistletoe seeds has received considerable attention. Seeds of *A. occidentale* retained viability longer at temperatures near freezing and low relative humidity than at temperatures above freezing and high relative humidity where molds can be problematic (Scharpf and Parmeter 1962). Optimum conditions for storage of *A. americanum*, *A. abietinum* f. sp. *concoloris*, *A. campylopodum*, *A. douglasii*, *A. laricis*, and *A. tsugense* seeds in the laboratory was achieved in the dark at 4–5°C and 35–45% relative

TABLE 1.1 **Nomenclature associated with taxonomic revisions within the *Arceuthobium campylopodum* complex by cited references published prior to 1972.**

Reference	Nomenclature used by author(s) of reference	Host(s) specified by author(s)	Species name used in dissertation based on host and nomenclature of Hawksworth & Wiens (1996)
Beckman & Roth 1968	*A. campylopodum* f. *campylopodum*	*Pinus ponderosa*	*A. campylopodum*
Cohen 1963	*A. campylopodum*	not specified	*A. campylopodum*
Knutson 1969	*A. campylopodum* f. sp. *campylopodum*	not specified	*A. campylopodum*
Knutson 1971	*A. campylopodum*	*P. ponderosa*	*A. campylopodum*
Kuijt 1960	*A. campylopodum*	*Pinus sabiniana*	*A. occidentale*
Peirce 1905	*A. occidentale*	*Pinus radiata*	*A. littorum*
Scharpf 1963	*A. campylopodum* f. *abietinum*	*Abies magnifica* *Abies concolor*	*A. abietinum* f. sp. *magnificae* *A. abietinum* f. sp. *concoloris*
	A. campylopodum f. *typicum*	*P. sabiniana*	*A. occidentale*
Scharpf 1965	*A. campylopodum* f. *abietinum*	*A. magnifica* *A. concolor*	*A. abietinum* f. sp. *magnificae* *A. abietinum* f. sp. *concoloris*
	A. campylopodum f. *campylopodum*	*P. sabiniana* *P. radiata* *P. ponderosa*	*A. occidentale* *A. littorum* *A. campylopodum*
	A. campylopodum f. *blumeri*	*Pinus lambertiana*	*A. californicum*
Scharpf 1969	*A. campylopodum* f. *campylopodum*	*P. sabiniana* *P. radiata*	*A. occidentale* *A. littorum*
Scharpf 1970	*A. abietinum*	*A. magnifica* *A. concolor*	*A. abietinum* f. sp. *magnificae* *A. abietinum* f. sp. *concoloris*
	A. occidentale	*P. sabiniana* *P. radiata*	*A. occidentale* *A. littorum*
Scharpf & Parmeter 1962	*A. campylopodum*	*P. sabiniana*	*A. occidentale*
Scharpf & Parmeter 1967	*A. campylopodum* f. *abietinum*	*A. magnifica* *A. concolor*	*A. abietinum* f. sp. *magnificae* *A. abietinum* f. sp. *concoloris*
Wicker 1967[a]	*A. campylopodum* f. *laricis*	*Larix occidentalis*[a]	*A. laricis*
	A. campylopodum f. *campylopodum*	*P. ponderosa*	*A. campylopodum*
	A. campylopodum f. *abietinum*	*Abies amabilis*	*A. abietinum* f. sp. *concoloris*

[a] *Hosts listed in E.F. Wicker's Ph.D. dissertation (1965), not in Wicker (1967a).*

humidity (Wicker 1967a). Seeds of *A. campylopodum* retained high viability when stored for 10 months in kraft paper bags within cardboard boxes outdoors or in the laboratory at 1.5±1°C and 34–75% relative humidity (Beckman and Roth 1968). Between 82 and 97% of *A. campylopodum* seeds germinated after storage at 1°C and relative humidities of 1, 7, 9, or 75% (Knutson 1969). Highest germination of *A. abietinum* f. sp. *concoloris*, *Arceuthobium abietinum* f. sp. *magnificae*, *Arceuthobium littorum*, and *A. occidentale* was attained when seeds were stored at 2°C and 0% relative humidity; high relative humidity often resulted in molding of seeds (Scharpf 1970). Seeds of these same species stored in natural light had significantly lower viability than seeds stored in dark or under artificial light; however, the storage temperatures of 13–16°C in this study were higher than optimum, as noted by Scharpf (1970). Humidity had no effect on germination of seeds of these same species stored up to 62 d at 15 or 25°C (Scharpf 1970). Optimum conditions for storage of *A. abietinum* f. sp. *concoloris*, *A. americanum*, *A. campylopodum*, *A. douglasii*, *A. laricis*, and *A. tsugense* seeds were at 1–2°C and 75% relative humidity (Knutson 1971, 1974) while those for *Arceuthobium apachecum*, *Arceuthobium blumeri*, and *Arceuthobium microcarpum* seeds were at the same temperature but 20–30% relative humidity (Mathiasen 1978). *Arceuthobium pusillum* seeds germinated best when stored at –10°C (Livingston and Blanchette 1986). It appears that temperatures slightly above freezing and a relative humidity of about 75% would be suitable for long-term storage of *A. americanum* seeds collected from dwarf mistletoe plants growing on jack pine provided molding of seeds can be prevented at this higher level of humidity.

Several types of closed containers have been used to store dwarf mistletoe seeds. Seeds of *A. campylopodum* were suspended on Scotch brand filament tape in Erlenmeyer flasks over either NaOH, $ZnCl_2$, or NaCl; the latter solution worked best, consistently maintaining relative humidity at 75% (Knutson 1971). *Arceuthobium abietinum* f. sp. *concoloris*, *A. americanum*, *A. campylopodum*, *A. douglasii*, *A. laricis*, and *A. tsugense* seeds were stored on 9-cm filter paper disks, 200 seeds per disk, stacked 1 cm apart on a rod and in a glass jar containing 300 mL of saturated NaCl solution (Knutson 1974). *Arceuthobium americanum*, *A. douglasii*, *A. laricis*, and *A. tsugense* seeds were successfully stored in petri dishes for 1–2 months at 5°C (Smith 1974). The container and apparatus described by Knutson (1974) are preferable to those of the others described because glass jars are relatively inexpensive and, depending on their size, can store relatively large numbers of seeds.

Artificial inoculation techniques

Only two studies have examined techniques for routinely producing infected conifer tree seedlings with *Arceuthobium* on a continuous, year-round basis (Knutson 1974; Mathiasen 1978). Knutson (1974) developed such a technique for *A. abietinum*, *A. americanum*, *A. campylopodum*, *A. douglasii*, *A. laricis*, and *A. tsugense* on their principal hosts (while Knutson did not list the host species inoculated, lodgepole pine was the most likely host in the case of *A. americanum*). Prior to inoculation, dwarf mistletoe seeds were placed in sterilized water for 3 d at 15°C followed by placement in 2% hydrogen peroxide until the radicles of the seeds ruptured the endocarp crests. Once germinated, seeds were transferred to open petri dishes containing sterilized water and allowed to air dry for several days. Dry individual germinants were placed at the base of a needle so that the radicular tip was in the leaf axil. Seeds were held in place using a drop of an adhesive described as water-soluble polyvinyl acetate (PVA). Knutson obtained infection rates of 60–80% for the dwarf mistletoes on the various tree species used in his greenhouse study. Mathiasen (1978) achieved infection rates of about 50% with PVA and a similar technique to Knutson's for inoculations with

A. apachecum, *A. blumeri*, and *A. microcarpum* seeds on bristlecone pine (*Pinus aristata*), limber pine (*Pinus flexilis*), southwestern white pine (*Pinus strobiformis*), and Engelmann spruce (*Picea engelmannii*).

Seed germination, host penetration, and infection

Timing of germination of *Arceuthobium* seeds depends on the species and its environment; some species germinate in fall while others germinate in spring. Establishment of *Arceuthobium* on its host consists of three stages: (i) an initial stage of linear radicle growth following seed germination; (ii) cessation of linear growth and formation of a holdfast; and (iii) production of one or more infection pegs from the holdfast, which then penetrate the host and begin the infection process (Scharpf 1970). The dwarf mistletoe germinant must infect its host prior to exhaustion of its limited endogenous reserves. Dwarf mistletoe seeds can photosynthetically fix small amounts of carbon dioxide and this likely contributes substantially to food reserves of germinants during germination and penetration (Muir 1975).

Germination of *A. pusillum* seeds from different seed sources did not vary significantly (Bonga 1969). Seeds of *A. abietinum* f. sp. *concoloris*, *A. abietinum* f. sp. *magnificae*, *A. littorum*, and *A. occidentale* from four different seed sources each and collected in different years exhibited no differences in germination (Scharpf 1970). Scharpf also observed little variation in germination of seeds collected from the same dwarf mistletoe plant.

Under artificial conditions, germination of seeds of several western dwarf mistletoes can be stimulated by soaking seeds in 1–5% hydrogen peroxide (Wicker 1962; Knutson 1974; Mathiasen 1978). *Arceuthobium pusillum* seeds germinated equally well whether pre-treated with hydrogen peroxide or not (Bonga 1965).

Arceuthobium spp. do not require stimuli from the host for seed germination (Gill 1935). *Arceuthobium oxycedri* germinated on both organic and inorganic substrates (Heinricher 1915, 1917), *A. occidentale* germinated on plastic (Kuijt 1960), and *A. littorum* germinated on dead leaves and fence boards (Peirce 1905). Seeds of *A. pusillum* germinated on several types of organic and inorganic media; addition of host twig extracts did not promote radicle growth (Bonga 1965; Bonga and Chakraborty 1968). Radicle growth of *A. occidentale* and *A. littorum* was significantly greater on water agar compared to host tissue media (Scharpf 1970). While not directly studied in *A. americanum*, the conclusion of Gill (1935) that host stimuli are unnecessary likely applies to *A. americanum* because other species within the genus germinated on a wide range of substrates and were not stimulated by host extracts.

Tissue age where the germinant penetrates was initially thought to have some importance on infection success (Weir 1916, 1918) but this was found not to be the case as lodgepole pine stems as old as 58 years can be infected by *A. americanum* (Hawksworth 1954). Hawksworth speculated that bark thickness could be important. On jack pine, there was no relationship between tissue age and susceptibility to infection by *A. americanum* (Robinson and Punter 2001). Infections usually occur on needle-bearing twigs as this is where most seeds are intercepted and adhere using the seed's natural viscin (Gill and Hawksworth 1961; Hawksworth 1965; Roth 1959; Wicker 1967b).

The radicle of a germinant grows along the surface of the host until its food supply is exhausted or until it is obstructed by a needle base or bud (Peirce 1905; Weir 1916; Gill 1935; Kuijt 1960; Gill and Hawksworth 1961; Hawksworth 1961; Cohen 1963; Scharpf and Parmeter 1967; and Scharpf 1970). A holdfast usually develops at the radicle tip at this point. An obstruction was not a prerequisite for holdfast development in *A. pusillum* growing on artificial media (Bonga and Chakraborty 1968; Bonga 1971). The radicle of *A. tsugense* germinants grew under old bud scales and host epidermal fragments without holdfast development but when the radicular apex met a needle base, the base provided a sufficient obstruction to initiate a holdfast (Hunt et al. 1996).

Gill and Hawksworth (1961), in their literature review of mistletoes, reported that both chemical and mechanical forces play a role in host penetration, but the reviewed studies examined genera other than *Arceuthobium*. Penetration of fir (*Abies*) by *A. abietinum* f. sp. *magnificae* and *A. abietinum* f. sp. *concoloris* was strictly a mechanical process as no evidence of enzyme activity was observed based on histochemical tests and microscopic examinations (Scharpf and Parmeter 1967). Mechanical forces were identified as important in penetration of western hemlock (*Tsuga heterophylla*) by *A. tsugense* (Hunt et al. 1996), although no test for enzymatic activity was conducted.

Little information is available on duration of the germination, penetration, and infection stages in the life cycles of dwarf mistletoes. Germination, from emergence of the radicle from the endocarp through formation of the holdfast, took 1–2 months for *A. occidentale* on digger pine (*Pinus sabiniana*) (Scharpf 1963; Scharpf and Parmeter 1967). Penetration took at least 2–3 months for *A. abietinum* f. sp. *magnificae* on red fir (*Abies magnifica*) and *A. occidentale* on digger pine (Scharpf 1963; Scharpf and Parmeter 1967). Duration of the germination, penetration, and infection stages taken together is generally recognized as prolonged as evidenced by the descriptions of the life cycles of various *Arceuthobium* spp. (Scharpf and Parmeter 1967; Baranyay and Smith 1972; Hawksworth and Wiens 1996). Because these stages are prolonged, the germinant, its growing radicle, and the penetrating structures must be able to survive changing environmental conditions (Scharpf 1972).

Influence of climatic factors on dwarf mistletoe biology

Temperature

Cold temperatures negatively affect dwarf mistletoes. In Colorado and Wyoming, the upper elevational limits of infection for *A. americanum* growing on lodgepole pine coincided with the –1°C mean annual temperature isotherm (Hawksworth 1956). In British Columbia, *A. americanum* seeds on lodgepole pine did not survive one winter when the extreme minimum temperature reached –39°C (Smith and Wass 1979). Overwintering seeds of *A. americanum* infecting lodgepole pine growing in the Rocky Mountains of Colorado were killed near –33°C (Becwar 1980). Low temperature resulted in reduced viability of *A. americanum* pollen (Gilbert and Punter 1991). Fall frosts damaged the fruit of *A. americanum*, *A. douglassii*, and *A. pusillum* (Tunnock et al. 1966; Hudler and French 1976), and a temperature of –3.9°C for 2.3–4.8 h caused permanent damage to *A. americanum* and *A. tsugense* fruits (Baranyay and Smith 1974).

Temperature also influences dwarf mistletoe's life cycle in other ways. Optimum temperatures for *in vitro* germination of *A. americanum* seeds collected from plants infecting jack pine were 15–20°C (Robinson 1995). Optimum constant temperatures for germination of *A. campylopodum* seeds were 17–19°C while optimum alternating night-day temperatures were 12 hr at 5°C and 15°C (Beckman and Roth 1968). Germination rates of *A. abietinum* f. sp. *concoloris*, *A. abietinum* f. sp. *magnificae*, *A. littorum*, and *A. occidentale* seeds increased between 0 and 13°C and decreased between 13 and 22°C (Scharpf 1970). Growth of *A. pusillum* embryos, small seedlings, and seeds *in vitro* was highest at 15°C (Bonga and Chakraborty 1968). Highest radicle survival, penetration, and infection for *A. occidentale* and *A. littorum* germinants occurred at 13°C outdoors compared to temperatures up to 32°C, although other undetermined factors could have been involved (Scharpf 1969). At temperatures above 30°C, radicles of *A. abietinum* f. sp. *concoloris*, *A. abietinum* f. sp. *magnificae*, *A. littorum*, and *A. occidentale* germinants either failed to grow or died; radicle growth was greatest at 19–25°C (Scharpf 1970).

Infection of ponderosa pine (*Pinus ponderosa*) seedlings by *A. campylopodum* was greatest under a regime of low temperature (20°C, day; 15°C, night) and high light (195 $\mu mol\ s^{-1}\ m^{-2}$) (Knutson 1984). Once *A. campylopodum* was established, however,

higher temperature (25°C, day; 20°C, night) and lower light (91 μmol s^{-1} m^{-2}) favored biomass production.

Temperature also has an important influence on phenology of flowering and seed dispersal. Early and delayed flowering or seed dispersal was correlated with above and below average monthly temperatures, respectively, for *A. abietinum* f. sp. *magnificae*, *A. abietinum* f. sp. *concoloris*, *A. californicum*, *A. campylopodum*, *A. littorum*, and *A. occidentale* in California (Scharpf 1965). Interannual differences in timing of seed dispersal of *A. tsugense* and *A. laricis* appeared to be related to differences in accumulation of heat units from one year to the next in British Columbia (Smith 1966).

Water

Liquid water and water vapor are critical to dispersal, destiny, and germination of dwarf mistletoe seeds. Seeds are dispersed from mature fruit by an explosive discharge mechanism, which occurs when the fruit's hull absorbs water and creates a high hydrostatic pressure in a layer of viscin encircling the seed (Hawksworth and Hinds 1965; Hinds and Hawksworth 1965). The seed's viscin is a specialized layer of cells composed largely of polysaccharides (Gedalovich-Shedletzky et al. 1989). Air-dried viscin acts as a natural adhesive (Gill and Hawksworth 1961) while hydrated viscin serves as a lubricant, allowing seeds to slide down needles on the upper sides of twigs to the twig surface where they penetrate and infect the host (Hawksworth 1965). Seeds intercepted by needles on the lower sides of twigs, however, will usually slide off the needle, fall to the ground, and die (Wicker 1967b). *Arceuthobium* seeds require water to germinate (Gill 1935). Based on a series of experiments, Heinricher (1915, 1917) initially observed germination of *A. oxycedri* only on organic substrates but later observed germination on both organic and inorganic substrates; he related these differences to the presence or absence of water. Relative humidity had no significant effect on germination of *A. abietinum* f. sp. *concoloris* and *A. abietinum* f. sp. *magnificae* seeds but humidity approaching saturation or free water provided adequate moisture to stimulate germination (Scharpf 1970). Similarly, *A. pusillum* seeds germinated more rapidly and produced longer radicles in liquid water than when exposed to 90 or 100% relative humidity (Bonga 1972). Dry periods interspersed with wet periods had only a small, negative effect on germination, provided that the dry periods were of sufficiently short duration (Bonga 1972). The geographical distribution of *Arceuthobium vaginatum* subsp. *crytopodum* and *A. pusillum* appears to be related to availability of water during seed germination (Hawksworth 1967; Bonga 1969, 1972).

Life cycle of *Arceuthobium americanum* on lodgepole pine

A generalized life cycle is depicted for *A. americanum* infecting *P. contorta* var. *latifolia* in Colorado (Fig. 1.3). Seeds are forcibly discharged from mature fruit during 3–4 weeks from late July to early October, with peak dispersal between late August and late September in Colorado (Hawksworth and Johnson 1989) and about 1–2 weeks earlier in southwestern Alberta (Muir 1977). As noted earlier, most infections occur on needle-bearing twigs as this is where the majority of seeds land (Hawksworth 1965). Seeds overwinter there until they germinate the next spring. Germination of seeds on lodgepole pine occurs in mid-April in southwestern Alberta (Muir 1977) and in May in northern Colorado (Hawksworth 1965). Penetration of the host presumably occurs during the summer of the same year. The first symptom of infection, defined as penetration of the host cortex by the growing embryo and development of the endophytic system (Baranyay et al. 1971), is a localized swelling of host tissue at the point of penetration. Hawksworth and Wiens (1996), citing Hawksworth and Johnson (1989), reported that about two-thirds of dwarf mistletoe shoots emerge 3–4 yr after "infection". Hawksworth and Johnson (1989), however, reported that about two-thirds of

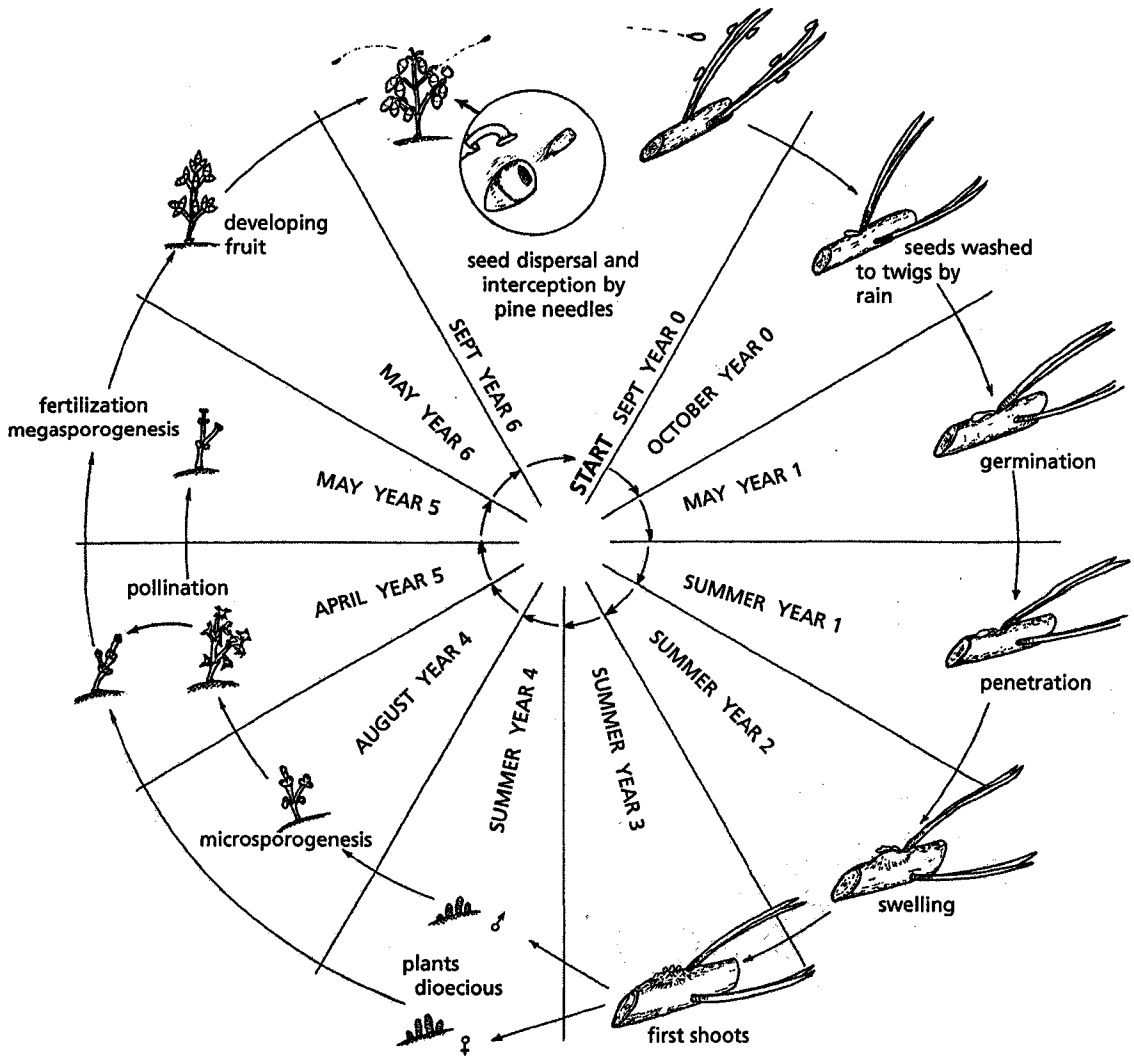

FIGURE 1.3 Generalized life cycle of *Arceuthobium americanum* on *Pinus contorta* var. *latifolia* in Colorado (Hawksworth and Wiens 1972, 1996; used with permission).

dwarf mistletoe shoots emerge 3–4 yr after "seed dispersal" and that production of first shoots may range from 2 to 8 yr. Only two percent of infections resulted in production of shoots two years after seed dispersal (Hawksworth and Johnson 1989). Male and female flowers develop on dioecious dwarf mistletoe plants by spring of the sixth year. Flowering may begin as early as March but usually peaks between early May and mid June in Colorado (Hawksworth and Johnson 1989). In Alberta, *A. americanum* on lodgepole pine flowers during April and May (Baranyay 1970). Fruit require more than 1 yr to mature after pollination of female flowers. On average, time from seed dispersal to flowering requires a minimum of 6 years (Hawksworth and Johnson 1989). Hawksworth and Wiens (1996), however, reported that minimum time from infection to initial seed production averages 6 years. My interpretation of the previous two reports is that *A. americanum* requires, on average, 7 years to complete its life cycle on lodgepole pine from seed dispersal to development of mature seeds, which is one year longer than what is depicted in Fig. 1.3.

STUDY OBJECTIVES AND RATIONALE FOR RESEARCH

The study objectives addressed in the subsequent chapters and the rationale for the research are as follows:

1. Estimate the productivity of *A. americanum* seed collection using stockinet at widely separated jack pine sites; estimate the efficacy of this material for trapping seeds; and evaluate the germinative ability of seeds in long-term storage using my materials and techniques.

An effective technique for collecting and storing large quantities of *A. americanum* seeds is critical for year-round experimental work. Witches' brooms on jack pine are large, often 1–3 m in circumference and frequently consist of 100 or more branchlets. Also problematic from a collecting perspective is the fact that pistillate plants are often not near distal portions of branches and seed discharge of *A. americanum* occurs during 2–3 weeks in late summer. In preliminary evaluations of various collection materials and techniques, paper bags (Scharpf and Parmeter 1962) were useful for collecting relatively small quantities of seeds at one or two sites by one or two people but they were unsatisfactory for collecting large quantities from widely separated sites at the same time during the seed discharge period. Paper bags were also unsuitable in wet conditions (i.e., rain or dew) and, therefore, could not be left on pistillate plants for later removal. Cutting and washing brooms from jack pine using the technique described by Bonga (1965) also proved unsatisfactory as it was labor-intensive, used large quantities of water, and yielded too few seeds. Sausage-casing bags (Wicker 1967a) available from local suppliers were only 10 cm in diameter and 50 cm long; far too small for most individual pistillate plants or isophasic infections of large brooms on jack pine. These bags were closed at one end and were also relatively rigid making them suitable for bagging only those pistillate plants close to distal ends of small branches. Stockinet is a soft, circular-knit, cotton fabric in stockinette stitch that has considerable elasticity and is manufactured in several sizes. Because this product is available in long lengths of tubing it has the potential to provide a suitable alternative for collecting *A. americanum* seeds from jack pine.

Seed storage of most western dwarf mistletoes has been achieved using various types of closed containers kept at 1–5°C and at a relative humidity of

30–75% (Beckman and Roth 1968; Knutson 1971, 1974; Mathiasen 1978). *Arceuthobium americanum* collected from lodgepole pine was stored effectively in jars kept at 2°C and 75% relative humidity (Knutson 1974). No one has reported on the effectiveness of these techniques and conditions for long-term storage of *A. americanum* seeds collected from jack pine.

2. Compare the effectiveness of three synthetic chemical compounds and the natural viscin of the *A. americanum* seed to act as adhesives during artificial inoculation of jack pine seedlings.

Research aimed at improving our understanding of factors such as jack pine resistance to *A. americanum*, influence of jack pine nutrition on infection rates, and effect of temperature and humidity on the life cycle of *A. americanum* on jack pine in a controlled environment is limited by our ability to consistently infect the host through artificial means. It is important to understand the influences of these factors on the host–parasite pathosystem in order to improve management of this economically important parasite of jack pine. Because of the nature of attachment between the parasite and its host, it is necessary to compare alternative adhesives for artificially inoculating jack pine seedlings with seeds of *A. americanum* for three reasons. First, although the seed's viscin acts as a natural adhesive, heavy rainfall or repeated wetting and drying cycles can reduce the viscin's adhesiveness (Roth 1959; Wicker 1967b); such conditions are typical in a greenhouse or growth chamber where seedlings are usually watered from above. Second, I was unable to replicate the technique of Knutson (1974) with the water-soluble PVA because PVA is a thermoplastic high polymer ($[-CH_2CH(OOCCH_3)-]_n$) that is insoluble in water but soluble in low molecular weight alcohols (Lewis 2001). Consequently, I wanted to confirm the effectiveness of PVA dissolved in alcohol as an adhesive and compare its effectiveness in relation to other adhesives. As a solvent for PVA, alcohol increases the viscosity of the adhesive over that of emulsions, accelerates setting speed, and increases wet tack (Jaffe et al. 1990), all desirable qualities in my application of the PVA adhesive. Third, Knutson's technique was developed using *A. americanum* seeds collected from dwarf mistletoe plants on lodgepole pine, and lodgepole pine was the inoculated host. I expected infection rates different from those he obtained because I was infecting jack pine with *A. americanum* seeds collected from dwarf mistletoe plants on jack pine.

3. Working with seeds from four populations of *A. americanum* infecting jack pine along a north-south gradient, determine the timing and nature of germination of the parasite's seeds and subsequent penetration and infection of the parasite's host.

Based on anatomical studies, the development of the dwarf mistletoe germinant and the mode of penetration and infection have been described by Cohen (1963) for *Arceuthobium* in general, by Scharpf and Parmeter (1967) for *A. abietinum* f. sp. *magnificae* and *A. occidentale*, and by Hunt et al. (1996) for *A. tsugense*. There is little information, however, on the timing and duration of the germination, penetration, and infection stages in the life cycles of dwarf mistletoes. *Arceuthobium americanum* seeds on lodgepole pine germinate in the spring (Hawksworth 1965; Muir 1977) while some *A. americanum* seeds on jack pine germinate in the fall in central Alberta (Dowding 1929). Germination of *A. occidentale* takes 1–2 months while penetration by *A. abietinum* f. sp. *magnificae* and *A. occidentale* takes about 2–3 months (Scharpf 1963; Scharpf and Parmeter 1967). Penetration of lodgepole pine occurs during the same summer as germination (Hawksworth and Wiens 1972). An understanding of the timing and nature of germination of *A. americanum* seeds and penetration and infection of the parasite's host will improve our knowledge of the these critical stages in the establishment of *A. americanum* on jack pine and may provide an alternative opportunity for controlling this damaging parasite.

4. Working with four populations of *A. americanum* infecting jack pine along a north-south gradient, determine the minimum winter temperature that prevents overwintering dwarf mistletoe seeds from germinating during the following spring, and determine if exposure to late spring frosts kill the embryo and radicle of dwarf mistletoe germinants.

Gaps exist in our knowledge of the basic biology of *A. americanum* and how the parasite responds to changes in temperature as most of the work to date has examined species of *Arceuthobium* other than *A. americanum* or has been conducted in study areas climatically distinct from the boreal climate of the western Canadian interior. Cold temperatures during winter and frosts during the growing season are important environmental factors limiting the productivity and distribution of plants (Sakai and Larcher 1987). If the northern limit of *A. americanum* is presently governed by extreme low temperatures, then climatic warming could lead to a northward expansion of the parasite, with reduced growth and increased mortality of jack pine in the more northerly boreal regions. Increasing our understanding of the influence of climate and its effect on dwarf mistletoe and its host has the potential to improve management of the parasite, to assess areas of non-infected pine at risk within dwarf mistletoe's current distribution, and to predict how the distribution of this parasitic plant might change in a changing environment.

5. Determine the life cycle of *A. americanum* growing on jack pine.

Most of our knowledge of the biology of *A. americanum* is based on the work of Hawksworth and his colleagues working on populations of the parasite infecting *P. contorta* var. *latifolia* in Colorado (Hawksworth 1965; Hawksworth and Wiens 1972, 1996; Hawksworth and Johnson 1989). The generalized life cycle for *A. americanum* infecting *P. contorta* var. *latifolia* has been described but there are discrepancies in the literature as to the timing of shoot emergence and the length of the life cycle. To date no one has conducted a detailed study of the life cycle of *A. americanum* infecting jack pine, presumably because researchers have assumed it is the same or similar to that on lodgepole pine. Gilbert and Punter (1990, 1991) provide detailed information on the pollination biology of *A. americanum* on jack pine in Manitoba while some limited aspects of the biology of *A. americanum* infecting jack pine in Alberta are reported by Dowding (1929). The recent discovery of three races within *A. americanum* (Jerome and Ford 2002), however, provides stimulus for a reevaluation of past research and a review of the assumption that the life cycle of *A. americanum* infecting jack pine is the same as that on lodgepole pine.

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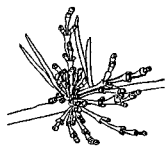

CHAPTER TWO

Collecting and storing seeds of *Arceuthobium americanum* from *Pinus banksiana*[1]

INTRODUCTION

Lodgepole pine dwarf mistletoe (*Arceuthobium americanum*) is second only to decay organisms in the amount of damage caused to jack pine (*Pinus banksiana*) in the western Canadian interior. Annual volume losses for dwarf mistletoe-infected jack pine growing in Alberta, Saskatchewan, and Manitoba are 1.8 million m^3 (Brandt et al. 1998). A rapid, consistent, and effective technique for collecting and storing large quantities of *A. americanum* seeds is critical for year-round experimental work.

Witches' brooms on jack pine are large, often 1–3 m in circumference and frequently consist of 100 or more branchlets. Also problematic from a collecting perspective is the fact that pistillate plants are often not near distal portions of branches and seed discharge of *A. americanum* occurs during 2–3 weeks in late summer. In preliminary evaluations of various collections materials and techniques, paper bags (Scharpf and Parmeter 1962) were useful for collecting relatively small quantities of seeds at one or two sites by one or two people but they were unsatisfactory for collecting large quantities from widely separated sites during the seed discharge period. Paper bags were also unsuitable in wet conditions (i.e., rain or dew) and, therefore, could not be left on pistillate plants for later removal. Cutting and washing brooms from jack pine using the technique described by Bonga (1965) was labor-intensive, used large quantities of water, and yielded too few seeds. Sausage-casing bags (Wicker 1967) available from local suppliers were only 10 cm in diameter and 50 cm long; far too small for most individual pistillate plants or isophasic infections of large brooms on jack pine. These bags were closed at one end and were also relatively rigid making them suitable for bagging only those pistillate plants close to distal ends of small branches. Stockinet is a soft, circular-knit, cotton fabric in stockinette stitch that has considerable elasticity. It is typically used to wrap and hang meat during smoking or to wrap and secure bandages on patients in hospitals. Because stockinet (available from Cottonia Products Inc. [Montreal, Quebec]) comes in long lengths of tubing it has the potential to provide a suitable alternative for collecting *A. americanum* seeds from jack pine.

Optimum seed storage of most western dwarf mistletoes has been achieved using various types of containers kept at 1–5°C and at a relative humidity of 30–75% (Beckman and Roth 1968; Knutson 1971, 1974; Mathiasen 1978). *Arceuthobium americanum* collected from lodgepole pine was stored effectively in jars kept at 2°C and 75% relative humidity (Knutson 1974). No one has reported the effectiveness of these containers and conditions for long-term storage of *A. americanum* seeds collected from jack pine.

The objectives of this study were to (i) estimate the productivity of *A. americanum* seed collection using stockinet at widely separated jack pine sites; (ii) estimate the efficacy of this material for trapping seeds; and (iii) evaluate the germinative ability of seeds in long-term storage using my materials and techniques.

[1] *A version of this chapter has been accepted for publication. Brandt, J.P., Hiratsuka, Y., and Pluth, D.J. Canadian Journal of Plant Pathology.*

MATERIALS AND METHODS

To estimate productivity of bagging plants with stockinet, the number of person-hours required for bagging and the number of seeds collected were recorded in 1999, 2000, and 2001 when *A. americanum* seeds were collected for other studies. Collection sites were in stands of infected jack pine at four locations within Alberta: north of Peace Point (59° 11'N, 112° 23'W, 260 m above m.s.l.), west of McClelland Lake (57° 31'N, 111° 24'W, 300 m above m.s.l.), west of the Logan River (55° 21'N, 111° 56'W, 670 m above m.s.l.), and southeast of Smoky Lake (54° 05'N, 112° 20'W, 610 m above m.s.l.). At each site, orchard ladders, ranging in height from 4.3 to 4.9 m, were used to access pistillate plants on pine branches near ground level to about 6 m high (Fig. 2.1a, b). These branches were wrapped in stockinet during late July prior to fruit maturation. Stockinet from my supplier was available in two unstretched diameters: 10 or 24 cm. Regardless of the diameter, thread thickness of the stockinet was 0.23 mm and weave density was 14 threads/cm in the longitudinal direction and 10 threads/cm in the other. Stockinet was cut into appropriate lengths (usually 30–120 cm) to cover a pistillate plant without damage. When stockinet was placed on the end of a branch, the end of the stockinet at the distal end of the branch was closed using an overhand knot while the other end was secured with a reef knot (Budworth 1999), ensuring the branch was between the first and second crossing of the knot. Stockinet placed in the middle of a branch was closed and secured to the branch using a reef knot at both ends as above. Bagged plants were periodically monitored until most seeds discharged. Stockinet sections with seeds were removed during mid-September, placed in kraft paper bags, and kept at 2°C for 3–4 weeks until seeds were processed for long-term storage.

To estimate productivity of seed picking and processing prior to storage, the number of hours required for picking and processing and the number of seeds picked and processed were recorded in 1999, 2000, and 2001. Picking and processing, modified from methods described by Knutson (1971, 1974) and Bonga (1965), involved several steps. Seeds were carefully removed from stockinet using tweezers (Fig. 2.1c,d). Seeds were surface-sterilized in batches of 300 by shaking for 30 min in a 60-mL vial containing 40 mL of 3% H_2O_2. Sterilized seeds were transferred aseptically using flamed tweezers in a sterile containment cabinet to autoclaved shelving units (Fig 2.1e); 50 seeds per paper disk were held in place by their viscin. A shelving unit (250 seed capacity) consisted of five paper disks spaced 2 cm apart on three upright polycarbonate rods (dia. 5 mm) glued to a 6-mm thick polycarbonate disk (dia. 9 cm) 3 cm from the base of the rods (Fig. 2.1f). The lowest paper disk was at least 2 cm above the lower polycarbonate disk to avoid splashes of NaCl, which was toxic to the seeds. The upper ends of the rods were affixed to a second polycarbonate disk. Paper disks were supported on rods by two 19-mm fold-back paper clips. Each shelving unit was placed in a 4 L wide-mouth jar and autoclaved for 20 minutes. Before returning shelving units with seeds to jars, 300 mL of saturated NaCl solution was added to each jar to maintain an internal relative humidity of 75% when stored at 2°C (Winston and Bates 1960; Knutson 1971). Seeds were stored in the dark at 2°C for up to 24 months.

To determine the seed-trapping efficacy of stockinet, a test was conducted on 100 pistillate plants southeast of Smoky Lake. In the test, the number of fruit on pistillate plants was counted prior to bagging and then compared to the number of seeds trapped after seed discharge was complete. All aspects of stockinet placement and securement were as described for the 1999–2001 seed collection years.

To evaluate the germinative ability of seeds in relation to storage duration, eight (in 1999) or sixteen (in 2000 and 2001) paper disks with 50 seeds each were removed at random from storage at 30 d intervals and germinated using the method of Wicker (1962). A germinated seed was defined as one in which the endocarp crest was ruptured by the elongating radicle.

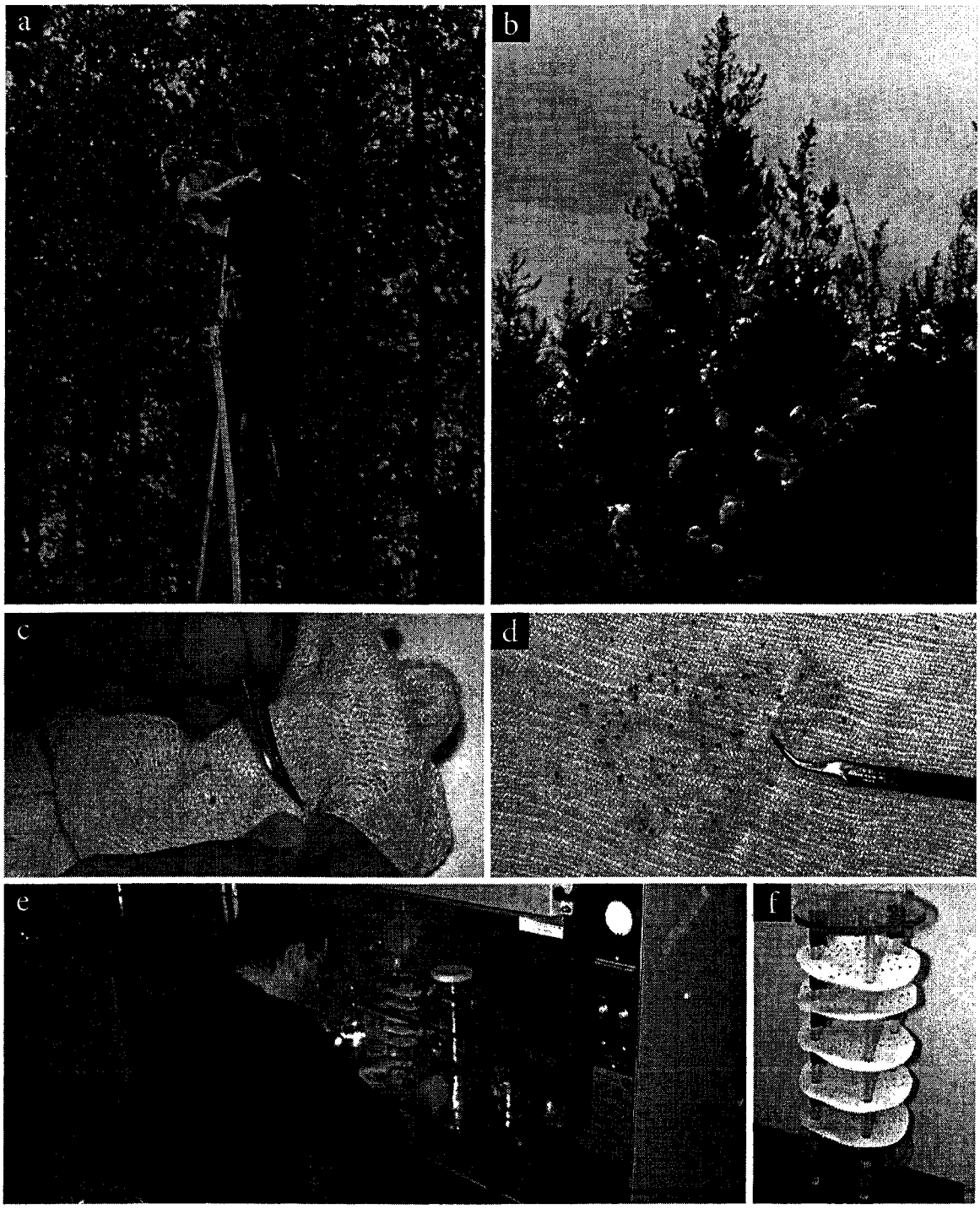

FIGURE 2.1 The collection and storage of *Arceuthobium americanum* seeds from pistillate plants infecting jack pine: (a) placing stockinet on a high branch with the aid of an orchard ladder; (b) jack pine trees with large stockinet; (c) removing seeds from stockinet; (d) a 10×14 cm piece of stockinet with about 65 adhered seeds; (e) aseptic placement of seeds onto shelving units in a sterile containment cabinet; and (f) a shelving unit with seeds.

RESULTS AND DISCUSSION

Collection of seeds can be thought of as a three-step process: bagging of pistillate plants, picking seeds from stockinet in the laboratory, and seed processing prior to storage (Fig. 2.1). Maximum productivity for the three stages occurred in 2001 (Table 2.1). The substantial increase in productivity of bagging during the three collection years was due to the use of only the large stockinet after the first year, increased familiarity with sites, improved knot-tying skill, and a small increase in fecundity of individual pistillate plants in 2000 and 2001. In 2000, the number of stockinet bags and number of trees with at least one bagged pistillate plant were recorded for each site. At Peace Point, 30 800 seeds were wrapped in 494 bags on 202 trees; at McClelland Lake, 30 900 seeds in 635 bags on 280 trees; at Logan River, 29 700 seeds in 322 bags on 173 trees, and at Smoky Lake, 28 600 seeds in 999 bags on 551 trees. About 14 000 *A. pusillum* seeds were collected from 23 brooms on an unspecified number of black spruce (*Picea mariana*) trees using cheesecloth (Livingston and Blanchette 1986). An increase in productivity for processing of seeds prior to storage was accomplished by ensuring an uninterrupted supply of seeds for placement on the shelving units (i.e., placing a batch of seeds requiring surface sterilization on the shaker about every 40 min).

While both large and small stockinet can be used for collecting seeds from *A. americanum* on jack pine, large (24 cm diameter) stockinet was more effective. Large stockinet can be stretched to > 40 cm in diameter, pulled over several branches in a broom at once, and easily secured. Pistillate plants not at distal ends of branches were also easy to wrap by simply rolling the stockinet into a ring, pulling it over the branch to where the plant was located, and unrolling and securing it. Large stockinet was also less likely to damage the pistillate plant during the lengthy seed discharge period. Stockinet, like the sausage-casing bags used by Wicker (1967), allowed atmospheric moisture to the pistillate plant, which enhanced seed discharge; and it dried quickly, which reduced risk of molding once seeds discharged.

On average, stockinet trapped 62% of the total number of seeds on pistillate plants, with a range of 7-221% among the 100 bags. Some sections of stockinet trapped more than 100% of the potential seeds because of interceptions from adjacent, non-bagged plants. Cheesecloth intercepted about 80% of the discharged *A. pusillum* seeds from small brooms on black spruce trees but was ineffective for large, dense brooms (Bonga 1965).

Using the materials, processing techniques, and environmental conditions described, *A. americanum* seeds were successfully stored for at least 24 months. Evaluations of germinative ability during three seed collection years indicated that germination peaked at 61–74% between 210 and 300 d in storage and then

TABLE 2.1 **Productivity estimates during 3 years for stages in the collection of *Arceuthobium americanum* seeds from jack pine.**

	Bagging plants with stockinet		Picking seeds from stockinet		Seed processing prior to storage	
Year	No. of seeds	Seeds per person-hour	No. of seeds	Seeds per person-hour	No. of seeds	Seeds per person-hour
1999	45700	320	24000	200	24000	170
2000	119400	520	60500	200	60500	410
2001	143100	1160	70500	240	55800	450

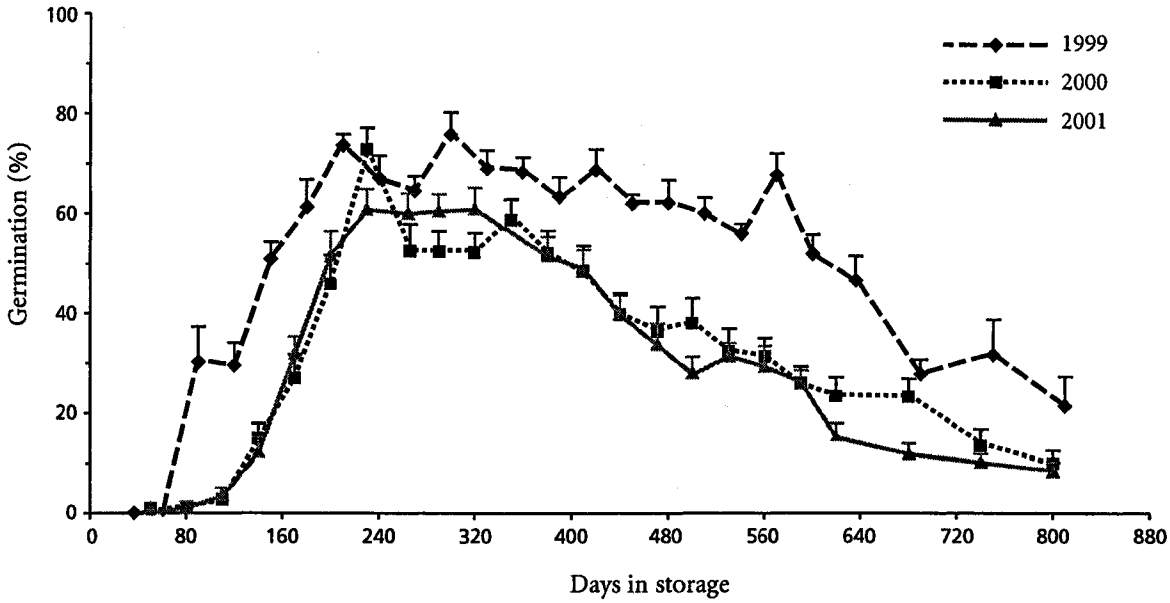

Figure 2.2 Germinative ability of *Arceuthobium americanum* seeds in relation to storage duration. Seeds were collected in each of 3 years from pistillate plants infecting jack pine. Bars represent standard errors (1999, n = 8; 2000 and 2001, n = 16).

declined slowly to 11–30% at 24 months (Fig. 2.2). At 450 d, germination was 40–63% for the three collection years; these values are comparable to the 58% of *A. campylopodum* seeds that germinated after storage for the same number of days and environmental regime (Knutson 1971). For each collection year, tens of thousands of seeds were successfully stored without molding. Molding of dwarf mistletoe seeds during storage can be a serious problem (Scharpf and Parmeter 1962; Bonga 1965; Scharpf 1970). Lack of molding in this study may be attributed to four features of processing prior to storage: surface sterilization of seeds for 30 minutes (Bonga 1965); autoclaving the jars with the shelving units prior to their receiving seeds (my modification); aseptic transfer of seeds using flamed tweezers (Bonga 1965) in a sterile containment cabinet (my modification); and maintenance of relative humidity at 75% at the 2°C storage temperature (Knutson 1971).

In conclusion, stockinet provides a valuable alternative for collection of *A. americanum* seeds from pistillate plants causing both isophasic and anisophasic infections on jack pine. This material is easy to place over pistillate plants on jack pine branches, it can be left on plants at widely separated sites for several weeks, and it traps 62% of the discharged seeds. Stockinet was effective for collecting more than 300 000 seeds during the three seed trapping years at a maximum rate of 1160 seeds per person-hour of stockinet placement and recovery. Although this technique was tested only for *A. americanum* on jack pine, it should be of interest to researchers studying other dwarf mistletoes and requiring large numbers of seeds. By modifying the materials and techniques described by Bonga (1965) and Knutson (1971, 1974), *A. americanum* seeds were stored in the dark at 2°C in sealed jars with an internal relative humidity of 75% to maintain viable seeds for up to 24 months without molding.

SUMMARY

An effective technique for collecting and storing *A. americanum* seeds from dwarf mistletoe-infected jack pine is critical for year-round experiments on this pathosystem. The required technique should also be easy to scale up to collect many seeds efficiently at widely separated sites. While several dwarf mistletoes have been stored long-term, no one has reported the effectiveness of these techniques for storage of *A. americanum* seeds collected from jack pine. This study estimated productivity of seed collection at widely separated sites using stockinet as a collection material, determined efficacy of this material for collecting seeds, and evaluated germinative ability of seeds in long-term storage. Stockinet trapped more than 300 000 seeds during three seed collection years at a maximum rate of 1160 seeds per person-hour of stockinet placement and recovery. Stockinet intercepted 62% of discharged seeds. Based on evaluations of germinative ability during three seed collection years, germination peaked at 61–74% between 210 and 300 d in storage and then declined slowly to 11–30% at 24 months. Aseptic storage of seeds in the dark at 2°C in sealed jars with an internal relative humidity of 75% was effective at storing more than 140 000 seeds without molding.

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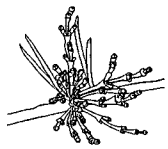

CHAPTER THREE

Adhesives for seed placement during artificial inoculation of *Pinus banksiana* seedlings with *Arceuthobium americanum*[2]

INTRODUCTION

Other than decay organisms, lodgepole pine dwarf mistletoe (*Arceuthobium americanum*) is the most important pest of jack pine (*Pinus banksiana*) in the western Canadian interior. Annual growth and mortality losses for dwarf mistletoe-infected jack pine growing in Alberta, Saskatchewan, and Manitoba are 314 000 m^3 and 1 478 000 m^3, respectively (Brandt et al. 1998). Additionally, dwarf mistletoes (*Arceuthobium* spp.) are parasitic upon several economically important members of the Pinaceae and Cupressaceae (Hawksworth and Wiens 1996). Dwarf mistletoe seeds disperse by forcible discharge from mature fruits in late summer. Most infections occur on needle-bearing twigs of the host as this is where the majority of dwarf mistletoe seeds land and adhere using the seed's natural viscin (Gill and Hawksworth 1961; Hawksworth 1965). Successful infection by the parasite causes stem and branch swellings and witches' brooms in trees. As infections intensify within the host, branches and tree tops die and the tree eventually succumbs. Research aimed at improving our understanding of factors such as host resistance, influence of host nutrition on infection rates, and effect of temperature and humidity on the life cycle of the parasite is limited by our ability to consistently infect the host through artificial means. It is important to understand the influences of these factors on the host–parasite pathosystem and to improve management of this economically important parasite of jack pine.

Only two studies have examined techniques for routinely producing infected conifer tree seedlings with *Arceuthobium* on a continuous, year-round basis (Knutson 1974; Mathiasen 1978). Knutson (1974) developed such a technique for *A. abietinum*, *A. americanum*, *A. campylopodum*, *A. douglasii*, *A. laricis*, and *A. tsugense* on their principal hosts (while Knutson did not list the host species inoculated, lodgepole pine (*Pinus contorta* var. *latifolia*) was the most likely host in the case of *A. americanum*). Knutson obtained infection rates of 60%–80% using polyvinyl acetate (PVA) as an adhesive for the dwarf mistletoes on the various species of tree seedlings used in his study. According to Knutson, the PVA used was dissolved in water. Lewis (2001) described PVA as a thermoplastic high polymer ($[-CH_2CH(OOCCH_3)-]_n$) that is insoluble in water but soluble in low molecular weight alcohols. After further inquiry, it was determined that Knutson used a type of Elmer's® adhesive of unknown formulation (personal communication, D. Knutson, February 2003). Elmer's Products Inc.

[2] *A version of this chapter has been published. Brandt, J.P., Hiratsuka, Y., and Pluth, D.J. 2003. Canadian Journal of Botany. 81: 1039-1043.*

produces many types of adhesives based on PVA or polyvinyl alcohol (Elmer's Products Inc. 2000). Polyvinyl alcohol is closely related to PVA but is water-soluble and is obtained by the alcoholysis of polyvinyl acetate (Lewis 2001). Many PVA-based adhesives are emulsions, but individual formulations vary depending on the polymer emulsions, film formers, and other additives (Jaffe et al. 1990).

The objective of this study was to compare the effectiveness of three synthetic chemical compounds and the natural viscin of the *A. americanum* seed to act as adhesives during artificial inoculation of jack pine seedlings. There were three reasons for comparing alternative adhesives for artificially inoculating jack pine seedlings with seeds of *A. americanum*. First, although the seed's viscin acts as a natural adhesive, heavy rainfall or repeated wetting and drying cycles can reduce the viscin's adhesiveness (Roth 1959; Wicker 1967); such conditions are typical in a greenhouse or growth chamber where seedlings are usually watered from above. Second, I was unable to replicate Knutson's technique with the unknown PVA-based adhesive. Consequently, I needed to confirm the effectiveness of PVA dissolved in alcohol as an adhesive and compare its effectiveness in relation to other adhesives. As a solvent for PVA, alcohol increases the viscosity of the adhesive over that of emulsions, accelerates setting speed, and increases wet tack (Jaffe et al. 1990), all desirable qualities in my application of the PVA adhesive. Third, Knutson's technique was developed using *A. americanum* seeds collected from dwarf mistletoe plants on lodgepole pine, and lodgepole pine was the inoculated host. I expected infection rates different from those he obtained because I was infecting jack pine with *A. americanum* seeds collected from dwarf mistletoe plants infecting jack pine. *Arceuthobium americanum* on jack pine in the western Canadian interior is a distinct genetic race from two other races found on *Pinus contorta* var. *latifolia* and *P. contorta* var. *murrayana* in western North America (Jerome and Ford 2002).

Materials and Methods

Inoculation trials were conducted to develop and test various methods of applying adhesives to dwarf mistletoe germinants and jack pine seedlings. Subsequently, a greenhouse experiment utilizing a completely randomized design examined the effectiveness of three chemical compounds as adhesives and the natural adhesive of dwarf mistletoe seeds, viscin, for infecting jack pine seedlings.

A. americanum seed source

Seeds of *A. americanum* growing on jack pine were collected west of the Logan River, Alberta (55° 21'N, 111° 56'W, 670 m above m.s.l.). Seeds were collected and stored according to methods described by Brandt et al. (2004). Germination rates of seeds collected from plants infecting jack pine vary between 50 and 75% (data not included). Seeds were germinated under natural light and 18–20°C by immersion in sterile distilled water for four days, followed by 2% hydrogen peroxide for six days, and finally placed back into enough sterile distilled water to cover the now germinated seeds. After the water evaporated, germinants were culled based on their apparent vigor: vigorous germinants consisted of dark green seeds and reddish-green radicles with turgor; less vigorous germinants consisted of pale green or yellowish seeds with similarly colored but desiccated radicles.

Experimental design

Each of fifteen jack pine seedlings planted in one 35 cm × 60 cm styrofoam tray (one seedling per 1000-mL cavity) was inoculated with a single dwarf mistletoe germinant using one of the synthetic adhesives or the seed's viscin. Seedlings were grown from seeds collected near Nipawin, Saskatchewan and were 4–7 cm high and 18 weeks old when inoculated. One tray of inoculated seedlings represented an experimental unit; a unit was replicated four times per adhesive treatment. The experiment of 240 inoculated seedlings in 16 trays was conducted in a greenhouse from April 2002 through January 2003. Greenhouse temperature was automatically regulated to a 10:15°C night–day temperature, although temperatures occasionally exceeded 25°C during the summer months.

Adhesives and positioning of *A. americanum* on seedlings

The synthetic adhesives used for inoculation were anhydrous lanolin (LAN) (Stanley Pharmaceuticals Ltd., North Vancouver, British Columbia), Klucel® hydroxypropylcellulose (HPC) (Hercules Inc., Wilmington, Delaware), and polyvinyl acetate (PVA) (Aldrich Chemical Company, Inc., Milwaukee, Wisconsin, avg. MW approx. 12 800). These synthetic adhesives were prepared as follows in order to obtain suitable viscosity. The gelatinous LAN required heating to about 58°C. About 1 g of granular HPC was ground to a fine powder using a mortar and pestle, mixed with sterile distilled water to make about 10 mL of gel, and then allowed to stand for one day for complete dissolution. About 6 g of small irregularly shaped PVA pellets were dissolved during 1–2 d in 10 mL of 95% ethyl alcohol to make about 10 mL of liquid.

Germinant positioning in needle axils depended on the curvature and the length of the 1–3 mm radicle. Most radicles departed from the extended longitudinal axis of the oblong seed. A correctly positioned germinant contacted the seedling stem with its endocarp and had its elongating radicle tip in contact or < 1 mm away and oriented towards the needle base (Fig. 3.1). Where the acute angle between needle and stem was small, the germinant would be in contact with both seedling stem and needle.

For germinants held in place artificially, synthetic adhesives were applied once germinants were optimally positioned. A micropipette with a 0.1–10 µL ultramicro tip (certified universal-fit pipette tips, Ultident Scientific, St. Laurent, Quebec) was used to apply adhesives. Applied volume varied with adhesive compound and its apparent viscosity: LAN was applied as a drop formed by touching the pipette tip to the surface of the 58°C adhesive; HPC as a 10-µL drop; and PVA as a 2.5-µL drop because it was less viscous than HPC. Synthetic adhesives were applied to the endocarp in contact with the stem and opposite from where the radicle emerged from the endocarp crest (Fig. 3.1). No adhesives contacted radicles. Germinants were adhered to stems instead of needles (Fig. 3.1) because juvenile needles frequently cast as seedlings develop.

Data analysis

Counts were made of successfully infected seedlings as determined by emergence of aerial shoots. This response variable was expressed as a percentage of the number of infected seedlings out of the total number of seedlings inoculated per tray and was tested for normality. Treatment effects were assessed using a one-way ANOVA. Pairwise comparisons were made using the least significant difference procedure (SAS Institute Inc. 1999) and a significance level of $\alpha = 0.05$.

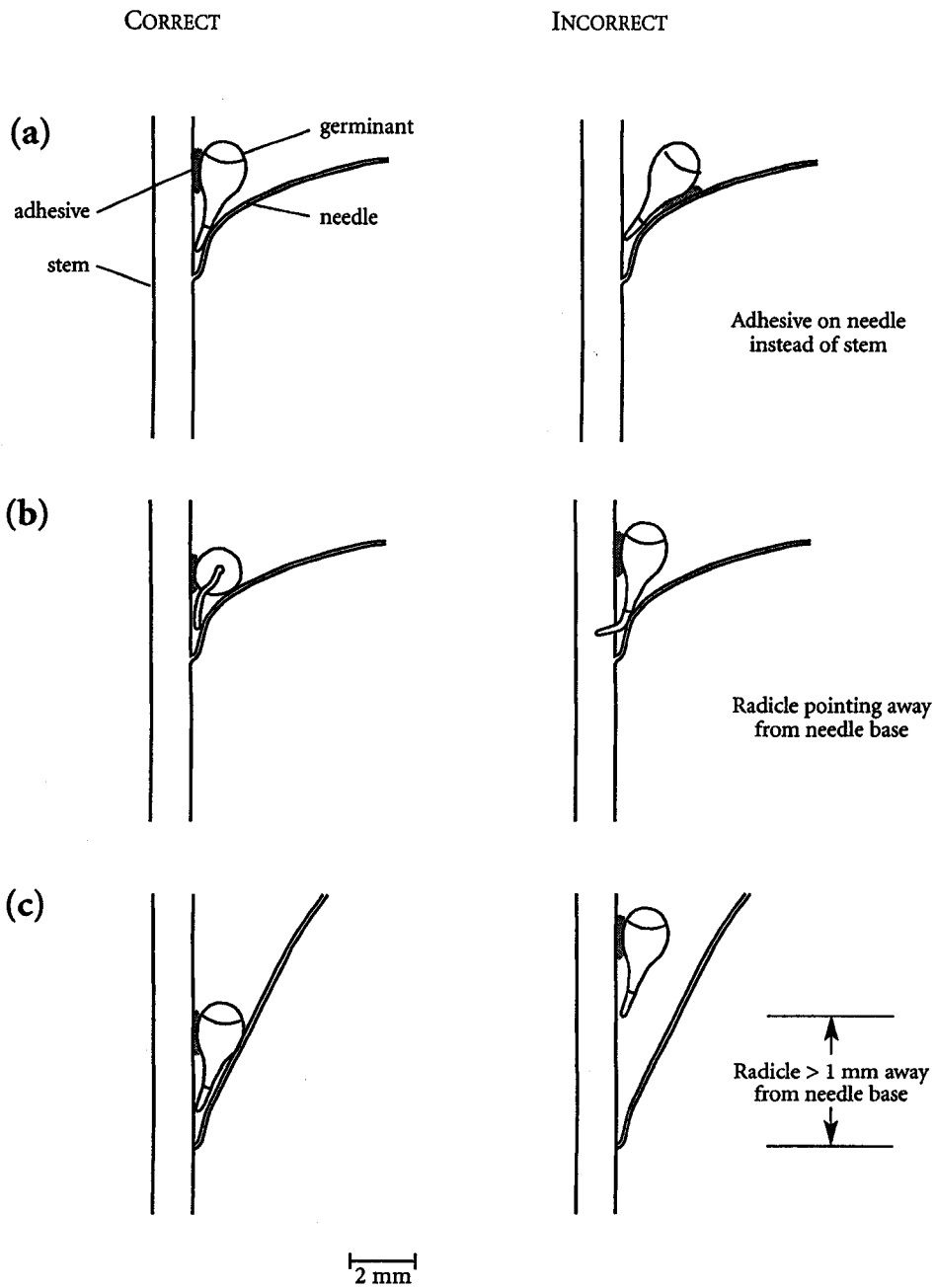

FIGURE 3.1 Correct and incorrect positioning of germinant and synthetic adhesive in relation to seedling stem and needle when inoculating jack pine with *Arceuthobium americanum*: (a) germinant with a short radicle elongating along the longitudinal axis of the seed; (b) germinant with a longer radicle departing from the longitudinal axis of the seed; and (c) germinant with a short radicle and a small, acute angle between the needle and stem. Most germinants in this study had curved radicles (b).

Results and Discussion

Percentage of infected jack pine seedlings was highest with HPC at 67 ± 6.5 (SE)% (see Table A.1, Appendix 2, for ANOVA table). This was significantly higher ($P \leq 0.05$) than the 43 ± 9.0% obtained with PVA, the 40 ± 7.4% with the seed's viscin, and the 35 ± 6.9% obtained with LAN. I was unable to attain infection rates as high as 80% reported by Knutson (1974). Rates for HPC in this study for the four replicates were 47, 73, 73, and 73%. Mathiasen (1978) achieved infection rates of about 50% with PVA (the same type of Elmer's glue used by Knutson, personal communication, R. Mathiasen, February 2003) and a similar technique to Knutson's for inoculations with *A. apachecum*, *A. blumeri*, and *A. microcarpum* seeds on bristlecone pine (*P. aristata*), limber pine (*P. flexilis*), southwestern white pine (*P. strobiformis*), and Engelmann spruce (*Picea engelmannii*) in a greenhouse. An infection rate of about 25% was obtained with *A. tsugense* on western hemlock (*Tsuga heterophylla*) using lanolin smeared on twigs near needles and placing seeds singly in the paste with the radicular end pointing to the needle base (Smith 1972); these artificial inoculations were conducted outdoors where other factors could have influenced infection rate. Viscin of *A. americanum*, *A. campylopodum*, *A. douglasii*, *A. laricis*, and *A. tsugense* held wetted seeds to needle bases of inoculated hosts growing outdoors; an infection rate of 12.5% was achieved for *A. americanum* on lodgepole pine (Smith 1974).

In the trials prior to the experiment, a small 1.5 mm wide spatula was used to apply the three adhesives. This tool would not deposit a small enough volume of adhesive, which resulted in low infection rates. The pipette with the ultramicro tip proved superior for all synthetic adhesive preparations because of the control it afforded in the placement and the volume deposited. Viscosity of the three synthetic adhesives was important as well because it affected the volume of adhesive applied. This was especially critical in the case of the phytotoxic PVA dissolved in alcohol. Knutson (1974) noted radicles died after contact with PVA dissolved in water. No evidence of phytotoxicity with HPC was observed; however, application of excessive amounts of this adhesive displaced germinants.

Orientation of a germinant's radicle relative to the needle axil influenced infection rate during initial trials. Placing the germinated seed so that the radicular end was pointed towards the needle base was incorrect if the elongating radicle departed from the longitudinal axis of the seed (Fig. 3.1b). A radicle tip not pointed towards the needle base often elongated until it either reached another needle base, which occurred infrequently, or it died.

I concur with Knutson (1974) and Mathiasen (1978) that evaporative drying of germinants in distilled water prior to inoculation and selection of vigorous germinants are critical to successful inoculation of seedlings. Radicles of less-vigorous germinants failed to extend > 0.5 mm or the germinants died shortly thereafter (data not included). Intuitively, culling will increase infection rates.

LAN at room-temperature was difficult to apply in a small enough volume to prevent its encapsulation of a germinant. LAN at about 58°C diminished this difficulty but did not eliminate it. Regardless of the preparation temperature of LAN, I suspect that the compound might interfere with the seed's and radicle's ability to photosynthesize because of LAN's opaqueness. This is likely the reason for the low infection rates obtained with this adhesive. Dwarf mistletoe seeds photosynthetically fix carbon dioxide and this ability likely contributes to the food reserves of seeds during the long germination and penetration periods (Muir 1975).

The major drawback to using the seed's viscin for adherence is that the repeated wetting and drying of this material (when watering inoculated jack pine seedlings from above) diminishes the adhesive-

ness of the viscin. In this situation, retention of inoculated seeds decreased; similar observations were made by Roth (1959) and Wicker (1967). A major component of viscin is polysaccharidic, including xylose and arabinose, but uronic acids and proteins are also present (Gedalovich-Shedletzky et al. 1989). Just under 50% of the total seeds inoculated using viscin detached; this was significantly higher than that observed for the three synthetic adhesives. While no such information has been reported by others developing artificial inoculation techniques, similar losses have been reported for seeds inoculated on trees outdoors and held in place with viscin (Smith 1977; Carpenter et al. 1979).

In conclusion, the findings of this study suggest that HPC is the superior adhesive when inoculating jack pine seedlings with seeds of *A. americanum* collected from jack pine. The infection rate of 67%, while lower than that reported by Knutson (1974), should be high enough to conduct research aimed at improving our understanding of *A. americanum* on jack pine. In addition, because seeds of all members within *Arceuthobium* and most other mistletoes use viscin to adhere to the host, the use of HPC in this study should be of interest to researchers studying these other parasites on artificially inoculated hosts in a greenhouse or growth chamber; the only requirement would be to determine the volume per seed of HPC required and the best technique of application. For the neophyte inoculating seedlings with mistletoes, HPC has the added advantage of not being phytotoxic like PVA and allows better control than LAN over the amount applied.

SUMMARY

Lodgepole pine dwarf mistletoe (*Arceuthobium americanum*) is a serious pest of jack pine. Research investigating factors such as host resistance, influence of host nutrition on infection rates, and effect of temperature and humidity on the life cycle of the parasite is limited by our ability to consistently infect the host through artificial means. A greenhouse experiment utilizing a completely randomized design with four replicates was conducted to test the effectiveness of three chemical compounds and the natural viscin of the dwarf mistletoe seed to act as adhesives during artificial inoculation of jack pine seedlings. Synthetic adhesives used were anhydrous lanolin (LAN), hydroxypropylcellulose (HPC), and polyvinyl acetate (PVA). The percentage of infected seedlings was significantly higher with HPC compared with that of PVA, LAN, and with the seed's viscin. HPC, as the superior adhesive, and the techniques described should allow consistent production of dwarf mistletoe-infected seedlings for research, regardless of dwarf mistletoe species involved. Problems encountered during the testing of the adhesives are discussed in relation to the nature of the adhesives and their application.

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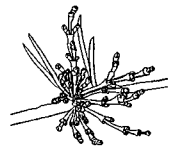

CHAPTER FOUR

Germination, penetration, and infection by *Arceuthobium americanum* on *Pinus banksiana*

INTRODUCTION

Lodgepole pine dwarf mistletoe (*Arceuthobium americanum*) is second only to decay organisms in the amount of damage caused to jack pine (*Pinus banksiana*) and lodgepole pine (*Pinus contorta* var. *latifolia*) in the western Canadian interior. Infection by the parasite causes witches' brooms and malformed stems in host trees. As these infections intensify, branches and tree tops die and the tree eventually succumbs. Regional estimates of annual growth losses for dwarf mistletoe-infected jack pine growing in the three prairie provinces are 314 000 m^3, while mortality losses are 1 478 000 m^3 (Brandt et al. 1998). Annual growth losses for dwarf mistletoe-infected lodgepole pine in Alberta and Saskatchewan are 486 000 m^3, while mortality losses are 69 000 m^3 (Brandt et al. 1998).

Establishment of *Arceuthobium* on its host consists of three stages: (i) an initial stage of linear radicle growth following seed germination, which is defined as rupture of the seed's endocarp crest by the radicle; (ii) cessation of linear radicle growth and formation of a holdfast; and (iii) production of one or more infection pegs from the holdfast, which then penetrate the host and begin the infection process (Scharpf 1970). Infection occurs after successful penetration when the parasite establishes its endophytic system (Baranyay et al. 1971). While the dwarf mistletoe germinant can photosynthetically fix small amounts of carbon dioxide (Muir 1975), it must infect its host prior to exhaustion of its limited endogenous carbohydrate reserves during the prolonged germination and penetration stages.

Based on anatomical studies, development of the dwarf mistletoe germinant and mode of penetration and infection have been described by Cohen (1963) for *Arceuthobium* in general, by Scharpf and Parmeter (1967) for *A. abietinum* f. sp. *magnificae* and *A. occidentale*, and by Hunt et al. (1996) for *A. tsugense*. Little information, however, is available on the timing and duration of the germination, penetration, and infection stages in the life cycles of dwarf mistletoes. *Arceuthobium americanum* seeds on lodgepole pine germinate in the spring (Hawksworth 1965; Muir 1977) while some *A. americanum* seeds on jack pine germinate in the fall in central Alberta (Dowding 1929). Penetration of lodgepole pine presumably occurs during the same summer as germination (Hawksworth and Wiens 1972). Germination, from emergence of the radicle to formation of the holdfast, takes 1–2 months for *A. occidentale* on digger pine (*Pinus sabiniana*) (Scharpf 1963; Scharpf and Parmeter 1967). Penetration takes at least 2–3 months for *A. abietinum* f. sp. *magnificae* on red fir (*Abies magnifica*), and *A. occidentale* on digger pine (Scharpf 1963; Scharpf and Parmeter 1967). Duration of the three stages taken together is generally recognized as prolonged as suggested by descriptions of the life cycles of various species of *Arceuthobium* (Scharpf and Parmeter 1967; Baranyay and Smith 1972; Hawksworth and Wiens 1972, 1996) but details on the methodology used in these studies are lacking.

Working with seeds from four populations of *A. americanum* infecting jack pine along a north-south gradient in Alberta, the objectives of this study were to determine the timing and nature of germination of the parasite's seeds and subsequent penetration and infection of the parasite's host, jack pine.

MATERIALS AND METHODS

The timing and nature of *A. americanum* seed germination were assessed using germination tests conducted in the laboratory, measurements of seed size, and observations of seeds placed on jack pine trees at three study sites. The timing and nature of host penetration and infection were assessed using histological observations of germinants and observations of seeds placed on jack pine trees at the same three study sites.

A. americanum seed sources

In 2000 and 2001, seeds of *A. americanum* growing on jack pine were collected from four sites in Alberta: north of Peace Point (59° 11'N, 112° 23'W, 260 m above m.s.l.), west of McClelland Lake (57° 31'N, 111° 24'W, 300 m above m.s.l.), west of the Logan River (55° 21'N, 111° 56'W, 670 m above m.s.l.), and southeast of Smoky Lake (54° 05'N, 112° 20'W, 610 m above m.s.l.) (Fig. 4.1). Seeds were collected and stored using the methods described by Brandt et al. (in press).

Germination tests

Germination tests were conducted on seeds collected in 2000 and 2001 from all four sources as follows: Four groups of 50 seeds per group from each seed source were selected at random at 30 d intervals from the total number of seeds remaining in storage (beginning 50 d after collection of seeds in mid-September). Each group was placed in sterile distilled water for 24 hr followed by 10 d in 3% hydrogen peroxide (Wicker 1962). Seeds were then assessed for germination, which was defined as rupture of the endocarp crest by the elongating radicle. Percentage of seeds that germinated was recorded for each group.

Seed measurements

The lengths and diameters of 1000 seeds were measured in 2000 and 2001. Seeds were selected at random in groups of 50 seeds from those sampled during the germination tests (one group from each seed source at each of the five consecutive 30-day intervals between 140 d and 260 d). Measurements were made at ×10 magnification using an ocular scale and a microscope. For each measured seed the germination status (positive or negative) was recorded as well. Seed volume was estimated by using the seed's radius, dividing the seed's length into thirds, substituting these values into the volume formulae for a cone (distal pole), cylinder, and half of a sphere (proximal pole), and then adding the three resultant volumes.

Histology of germinants

In the fall of 2000, 19 jack pine trees north of Bruderheim (53° 52'N, 112° 57'W, 625 m above m.s.l.) were inoculated with 11 dwarf mistletoe seeds each from the Smoky Lake seed collection site. A seed was positioned such that it contacted the host twig with its endocarp and had its distal pole oriented towards the needle base. Inoculated seeds were held in place by their natural viscin. Inoculated branches were flagged and marked with a grease pencil 2–3 cm from the seed. Seeds and germinants were visually monitored at two-week intervals beginning in April 2001 and continuing until they were destructively sampled for histological examinations. At each assessment date and inoculated branch, the developmental stage of the seed or germinant and symptom of infection were noted

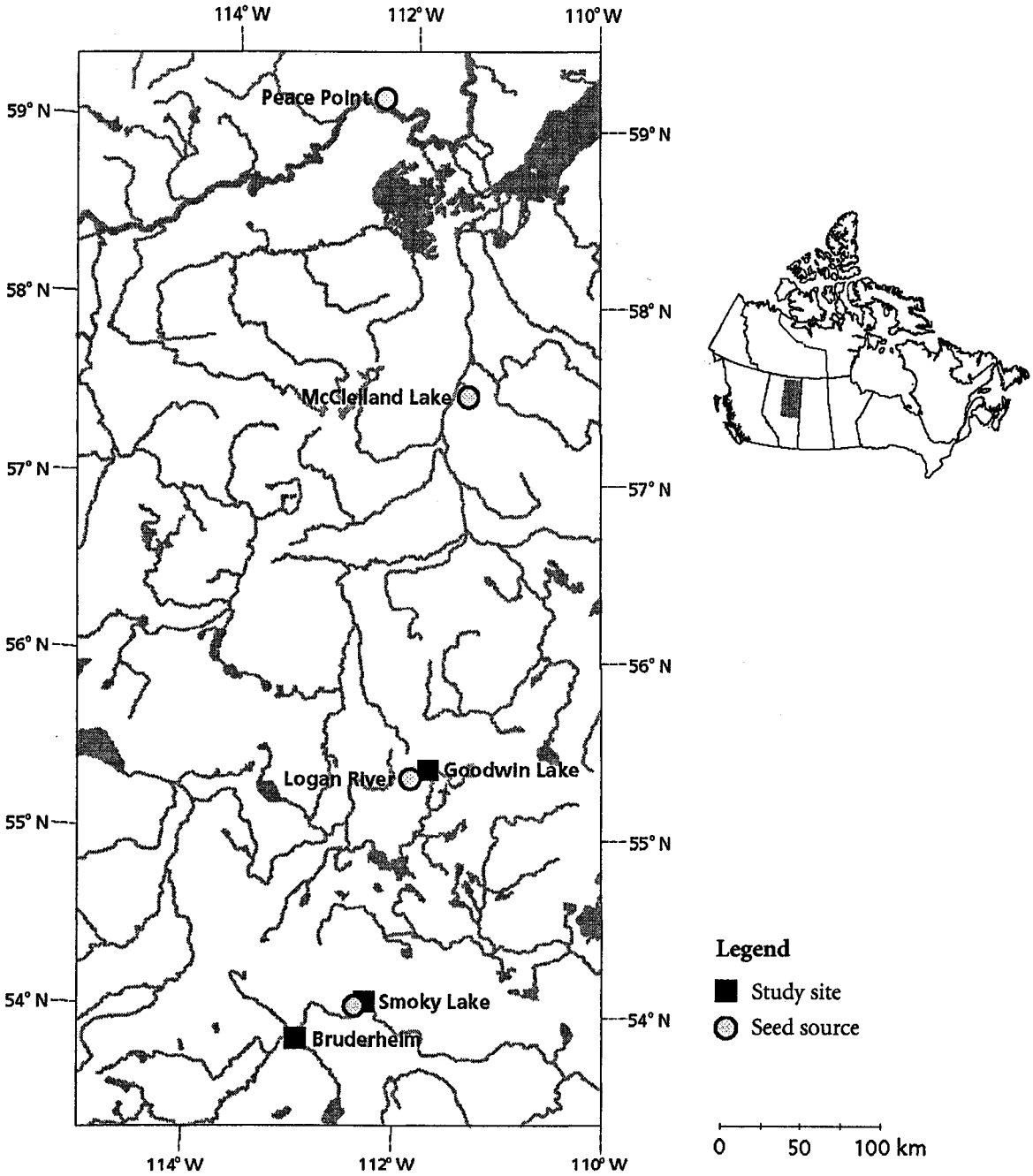

FIGURE 4.1 Study sites where jack pine trees were inoculated with *Arceuthobium americanum* seeds and *Arceuthobium americanum* seed sources.

(e.g., germination; holdfast development; withered, pale green or yellowish radicle, which was apparently desiccated; stem swelling near holdfast).

Portions of host twigs bearing germinants with holdfasts were destructively sampled at 6-week intervals from June through September 2001 (three sampling dates) and then once in June 2002. Twig–germinant samples were selected at random from the total number of living germinants available at each sampling date. Sample sizes were not equal among sampling dates because some germinants withered and apparently died during the intervening periods resulting in a variably sized sample population. The twigs with withered germinants were sampled at each sampling date after the first. Twig–germinant samples were fixed in formalin-acetic acid alcohol (FAA) and imbedded in paraffin (Paraplast X-tra®, Sherwood Medical, St. Louis, Missouri) (Johansen 1940). Longitudinal and transverse sections 15–20 μm thick were cut on a rotary microtome. Sequential sections of each twig–germinant sample were then affixed to microscope slides with Haupt's adhesive in formalin (Bissing 1974), stained with safranin-fast green, and mounted in Permount (Johnasen 1940; Sass 1958; O'Brien and McCully 1981). Slides were examined with a light microscope at magnifications of ×126–640.

Field observations of germinant development

In the fall of 2000 and 2001, 100 jack pine trees at each of three study sites in Alberta were inoculated with four dwarf mistletoe seeds per tree (one seed per seed source) using the seeds' viscin to hold them in place. Study sites were located west of Goodwin Lake (55° 24'N, 111° 44'W, 661 m above m.s.l.), southeast of Smoky Lake (54° 05'N, 112° 20'W, 610 m above m.s.l.), and north of Bruderheim (Fig. 4.1). The seeds used for inoculations were from the four seed collection sites. Inoculations and *in situ* visual monitoring were conducted as described for histology of germinants. Monitoring began in April 2001 and continued until October 2003.

Data analysis

For the germination tests conducted in the laboratory, variation in the percentage of seeds that germinated (near peak germination) was analyzed using an ANOVA with seed source as a fixed effect and year and its interaction with seed source as random effects (PROC GLM, SAS Institute Inc. 1999). A contrast was used to test for a linear trend in seed germination in relation to latitude of seed source. The significance level was set at $\alpha = 0.05$ for these tests as well as all other tests described subsequently. Seed volumes required transformation to natural logarithms because these data were not normally distributed. Variation in seed volume was assessed using an ANOVA with germination status as a fixed effect and year, seed source, and all two- and three-way interactions as random effects.

For the study sites, variation in the percentage of seeds that germinated and the percentage of germinants that developed holdfasts were analyzed using an ANOVA with seed source as a fixed effect and year, site, and all two-way interactions as random effects. A contrast was used to test for a linear trend in seed germination and development of holdfasts in relation to latitude of seed source.

Fisher's exact test (SAS Institute Inc. 1999) was used to test for an association between duration of germinant survival and infection by seed source and study site because some expected cell counts were less than five. Calculation of odds was based on the overall association between duration of germinant survival and infection for all seed sources and sites.

RESULTS

Seed germination

Germinative abiltiy of seeds removed from long-term storage at 2°C varied between years and among the four seed sources (Fig. 4.2). For both seed collection years and all seed sources, the percentage of seeds that germinated was generally <5% until 110 d after placement of seeds into storage. For year 2000, the percentage of seeds that germinated of all four seed sources peaked at 230 d in storage. For year 2001, germination of Peace Point and Smoky Lake seeds peaked at 230 d in storage but that of the other two seed sources peaked at 320 d.

For the germination tests conducted in the laboratory, the interaction between year and seed source ($P < 0.0001$) was a significant source of variation in germination of seeds (near peak germination); seed source ($P = 0.89$) and year ($P = 0.86$) were not significant sources of variation (see Table A.2, Appendix 2, for ANOVA table). Based on seeds collected in 2000 and germinated in the laboratory, the percentage of seeds that germinated was 37% for Peace Point seeds, 56% for McClelland Lake, 55% for Logan River, and 63% for Smoky Lake. Based on seeds collected in 2001, the percentage of seeds that germinated was 66% for Peace Point seeds, 64% for McClelland Lake, 47% for Logan River, and 43% for Smoky Lake. There was no significant linear trend in seed germination in relation to latitude of seed source ($P = 0.87$).

Germination status ($P = 0.024$) and the interaction between year and seed source ($P = 0.041$) were significant sources of variation in seed volume; year ($P = 0.53$), seed source ($P = 0.19$), two-way interactions between germination status and seed source ($P = 0.22$) and germination status and year ($P = 0.10$), and the three-way interaction ($P = 0.29$) were not significant sources of variation (see Table A.3, Appendix 2, for ANOVA table). Seeds that germinated were significantly larger in volume than seeds that did not germinate, which was consistent in both years and within each seed source. Overall, seeds that germinated (2.87 mm^3) were 48% larger in volume than those that did not germinate (1.94 mm^3).

For seed germination in the field, the interaction between year and seed source ($P = 0.006$) was a significant source of variation; site ($P = 0.39$) year ($P = 0.81$), seed source ($P = 0.94$), and the interactions between year and site ($P = 0.88$) and site and seed source ($P = 0.38$) were not significant sources of variation (see Table A.4, Appendix 2, for ANOVA table). Based on seeds collected in 2000 and germinated under field conditions, the percentage of seeds that germinated was 28% for Peace Point seeds, 62% for McClelland Lake, 50% for Logan River, and 53% for Smoky Lake. Based on seeds collected in 2001, the percentage of seeds that germinated was 68% for Peace Point seeds, 48% for McClelland Lake, 40% for Logan River, and 50% for Smoky Lake. There was no linear trend in seed germination in relation to latitude of seed source ($P = 0.96$) under field conditions.

Based on biweekly field observations in 2001, artificially inoculated *A. americanum* seeds germinated about 18 May at Goodwin Lake (range: 9 May–6 June), 22 May at Smoky Lake (8 May–18 June), and 25 May at Bruderheim (18 April–6 July). In 2002, seeds germinated about 14 June at Goodwin Lake (range: 28 May–24 July), 13 June at Smoky Lake (27 May–29 July), and 12 June at Bruderheim (23 May–1 August).

Results of the ANOVA on the percentage of germinants that developed holdfasts in the field indicated that the interaction between site and year ($P = 0.021$) was a significant source of variation; year ($P = 0.48$), seed source ($P = 0.25$), site ($P = 0.29$), and the interactions between year and seed source ($P = 0.39$) and site and seed source ($P = 0.33$) were not significant (see Table A.5, Appendix 2, for ANOVA table). Overall, 74% of germinants developed holdfasts. For seeds collected in 2000, the percentage of germinants that developed holdfasts was

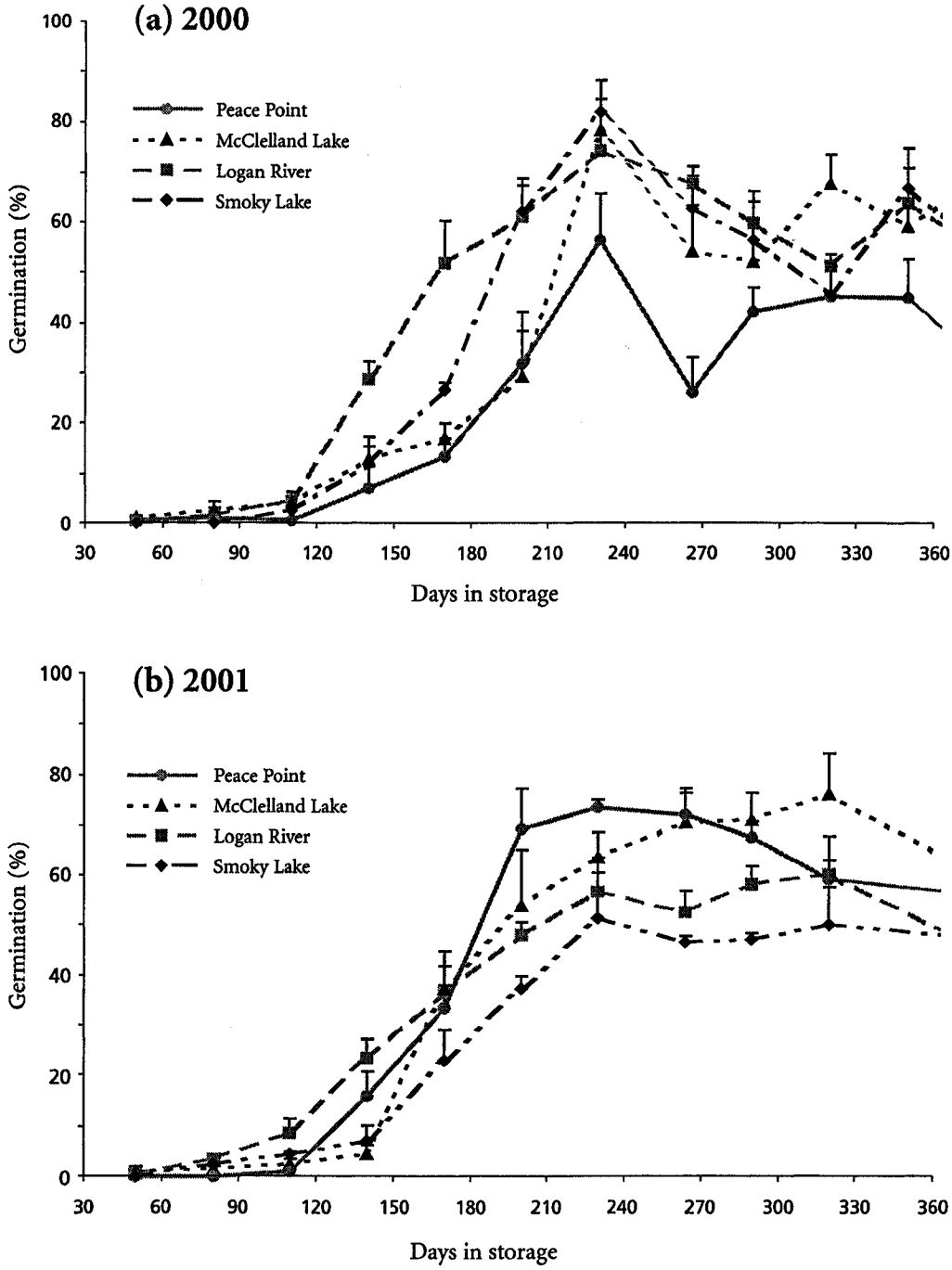

FIGURE 4.2 Germinative ability of *Arceuthobium americanum* seeds from four seed sources in relation to storage duration. Graphs depict data for seeds collected in (a) 2000 and (b) 2001. Data points beyond 360 d are not displayed. Bars represent standard errors (n = 4).

80% for Peace Point, 74% for McClelland Lake, 74% for Logan River, and 80% for Smoky Lake. For seeds collected in 2001, the percentage of germinants that developed holdfasts was 75% for Peace Point, 59% for McClelland Lake, 74% for Logan River, and 75% for Smoky Lake. There was no significant linear trend in development of holdfasts in relation to latitude of seed source ($P = 0.96$) under field conditions.

Host penetration and infection

At Bruderheim, 209 *A. americanum* seeds were placed on jack pine trees for the microscopy work. Of these, 143 seeds failed to germinate, or they germinated but failed to develop holdfasts, or they were dislodged from the host. The remainder of the seeds germinated, developed holdfasts and were sampled at one of the four sampling dates.

Based on histological observations of twelve germinants sampled on 25 June, no germinants had penetrated the host. *In situ* observations prior to sectioning indicated that each germinant had a well-developed holdfast, which was tightly appressed to the host's tissue at the base of the needle where it connected to the twig (e.g., Fig. 4.3b, d). In the sectioned material from these germinants, holdfasts were close to (Fig. 4.3a) and occasionally in contact with host tissue. In cases where the holdfast was in contact with the host, no attachment of the parasite to the host by means of groups of surface cells (as illustrated in Cohen (1963)) was observed.

On the second sampling date of 7 August, 24 germinants were collected. Of these, nine germinants had vigorous, reddish-green radicles and holdfasts with turgor (e.g., Fig. 4.3d) and 15 had pale yellow and withered radicles and holdfasts, which were apparently dead. Five of the nine vigorous germinants were penetrating the host (Fig. 4.4b, c) but none had an endophytic system. None of the withered germinants had penetrated the host. In all the samples where the parasite was penetrating the host, penetration was occurring at the extreme base of the needle where it connected to the twig (Fig. 4.4b).

On the third sampling date of 21 September, 19 germinants were collected. Of these, ten germinants were vigorous and nine were withered. Eight of the vigorous germinants were penetrating the host (Fig. 4.5) but, again, none had an endophytic system. As in the previous sampling date, penetration was occurring at the extreme base of the needle. In five of the withered germinants penetration appears to have begun but failed.

On the final sampling date on 25 June of the second growing season, 11 samples were collected. The twig of the host was swollen in three samples indicating established endophytic systems (Fig. 4.6c, d). Three other vigorous germinants were still in the process of penetration because parasite tissue was not well-advanced into the host (Fig. 4.6a). The remainder of the germinants apparently failed to penetrate and infect the host because no parasite cells could be found within the host in the sections of these samples.

Based on the field observations of germinant development at Bruderheim, Smoky Lake and Goodwin Lake, the timing and nature of holdfast development and the apparent death of some germinants throughout June, July, and August of 2001 and 2002 were similar to observations made on the twig-germinant samples collected for histological examinations in 2001 at Bruderheim. Based on the infections arising from the fall 2000 inoculations at the three sites, there was a highly significant association between length of germinant survival and infection ($P < 0.0001$), regardless of seed source or site (a similar analysis was not completed for the fall 2001 inoculations because infection data for these inoculations were not complete as of October 2003). The odds of infection when the germinant survives until the second growing season was 55 times greater than the odds of infection when the radicle withered in the first growing season. In most cases, germinants that remained alive during the first winter infected their host whereas germinants that withered during the first growing season had rarely

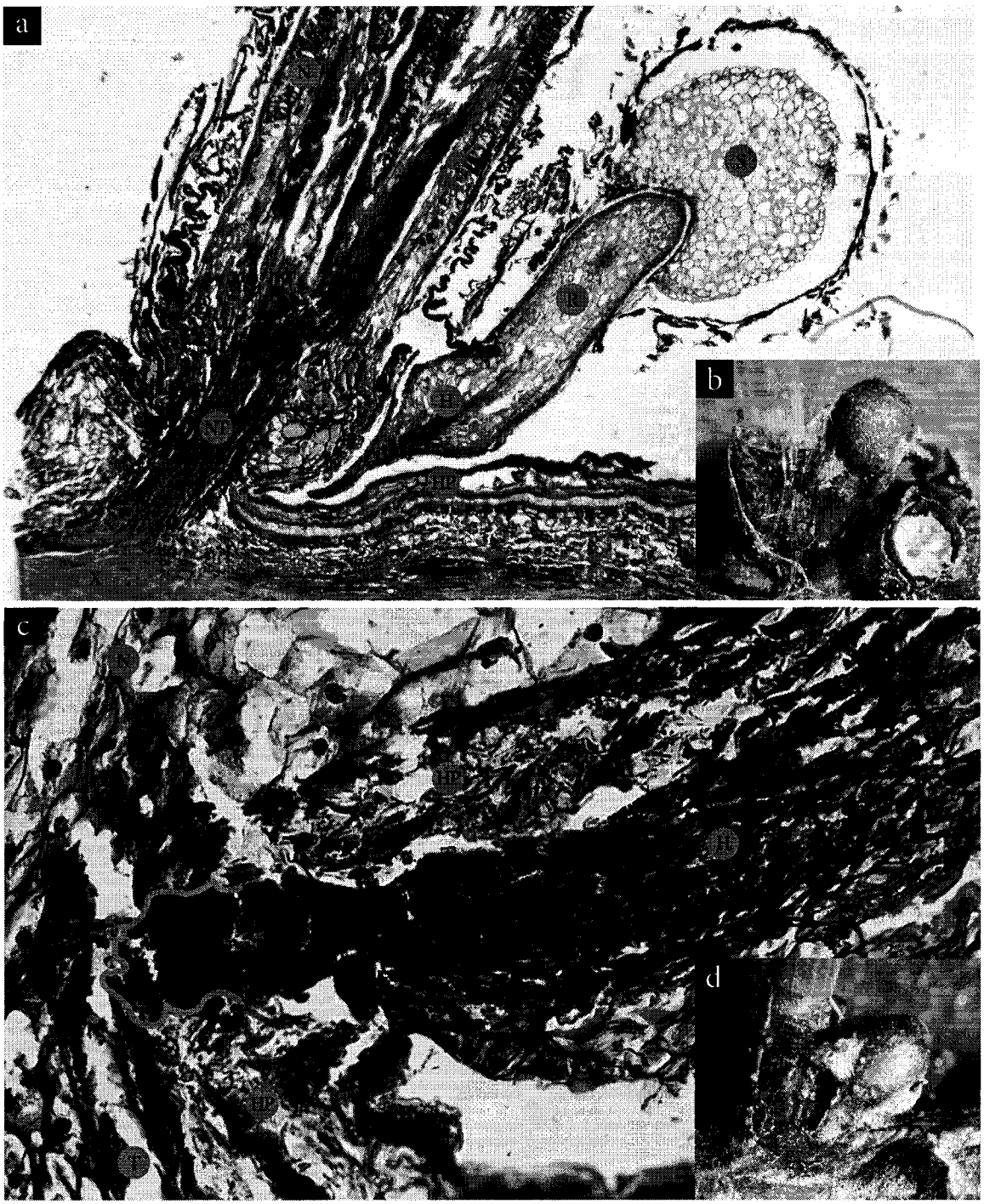

FIGURE 4.3 Matched pairs of photographs of germinated *Arceuthobium americanum* seeds. No penetration of host tissues has occurred. (a) Longitudinal section of seed sampled 25 June 2001 about 30–45 d after germination. (b) The same seed as in (a) with a holdfast formed at the base of a jack pine needle but prior to sectioning. (c) Longitudinal section of seed sampled 7 August 2001 about 72–87 d after germination. (d) The same seed as in (c) prior to sectioning. Yellow line indicates boundary between parasite and host cells (solid: high confidence; dashed: lower confidence). H – holdfast, HP – host periderm, N – jack pine needle, NT – needle trace, R – radicle, S – seed, T – twig, X – twig xylem.

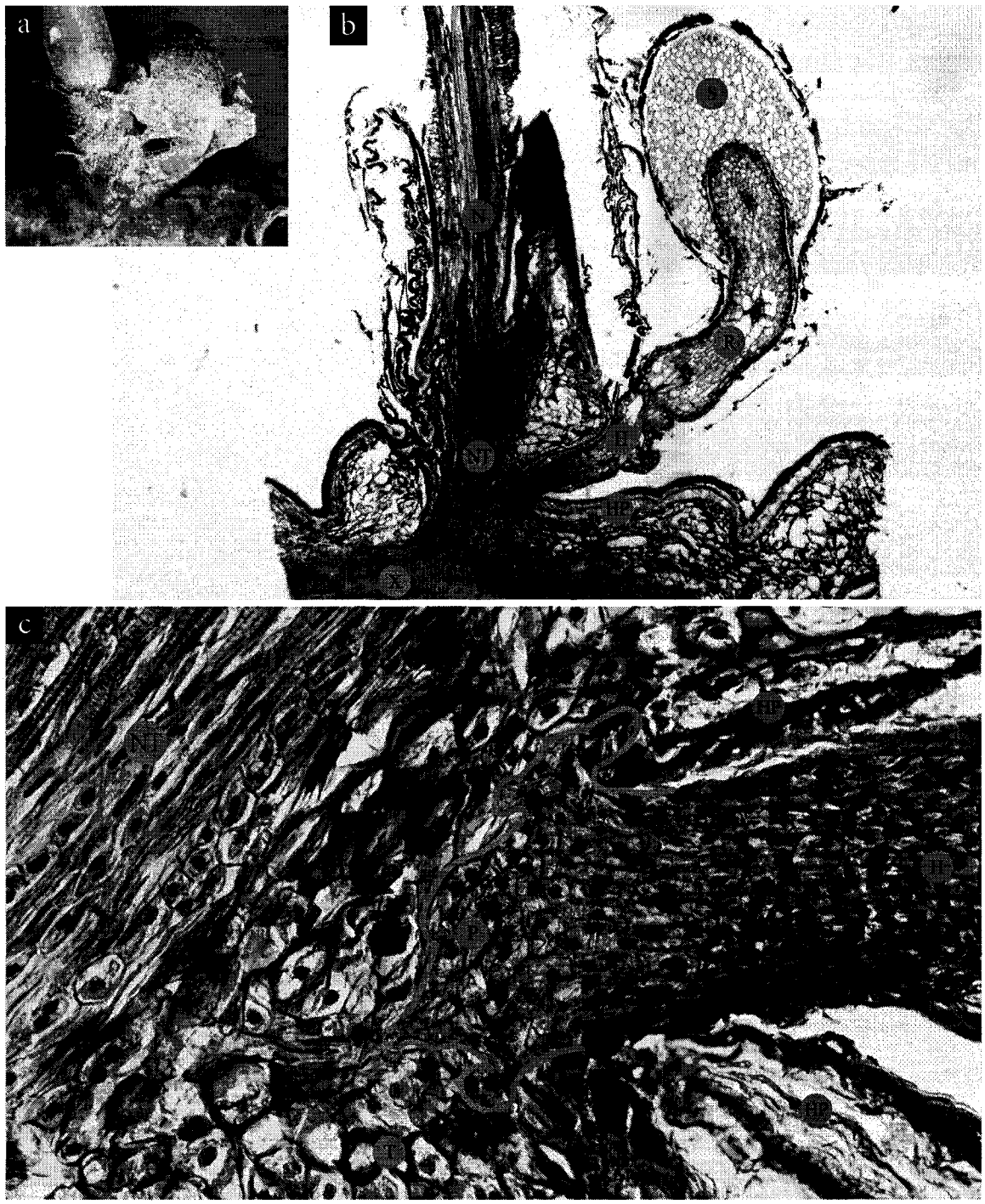

FIGURE 4.4 Matched photographs of a germinated *Arceuthobium americanum* seed sampled 7 August 2001 about 72–87 d after germination: (a) a germinated seed with a holdfast formed at the base of a jack pine needle; (b) same seed as in (a) but in longitudinal section; and (c) same longitudinal section but at higher magnification. Yellow line indicates boundary between parasite and host cells (solid: high confidence; dashed: lower confidence). H – holdfast, HP – host periderm, N – jack pine needle, NT – needle trace, P – penetration, R – radicle, S – seed, T – twig, X – twig xylem.

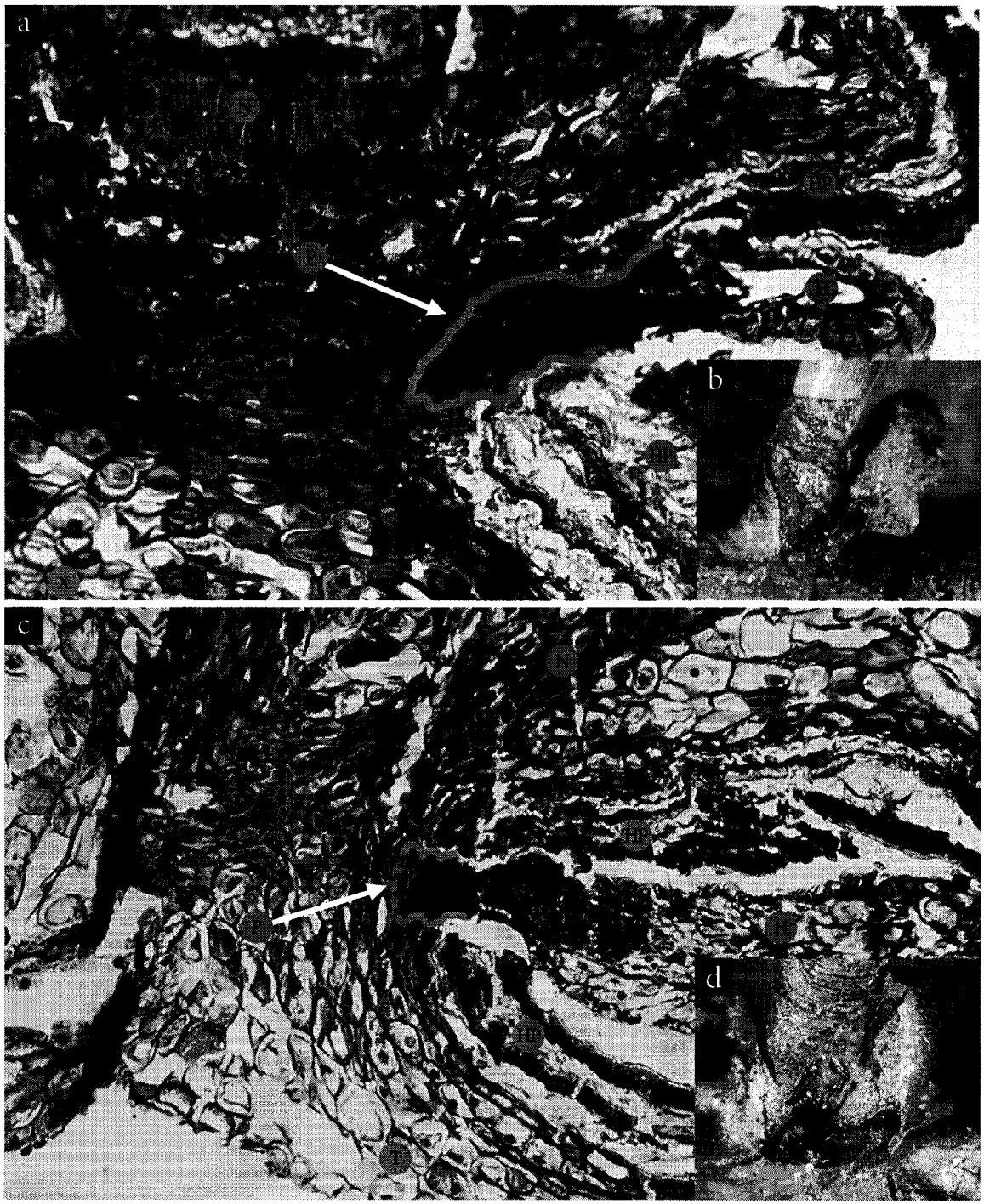

FIGURE 4.5 Matched pairs of photographs of germinated *Arceuthobium americanum* seeds sampled 21 September 2001 about 114–129 d after germination. For each matched pair, the smaller image depicts a germinated seed with a holdfast formed at the base of a jack pine needle while the larger image depicts the same seed but in transverse (a) or longitudinal (c) section. In both (a) and (c) penetration of host tissues has begun. Yellow line indicates boundary between parasite and host cells (solid: high confidence; dashed: lower confidence). H – holdfast, HP – host periderm, N – jack pine needle, NT – needle trace, P – penetration, T – twig, X – twig xylem.

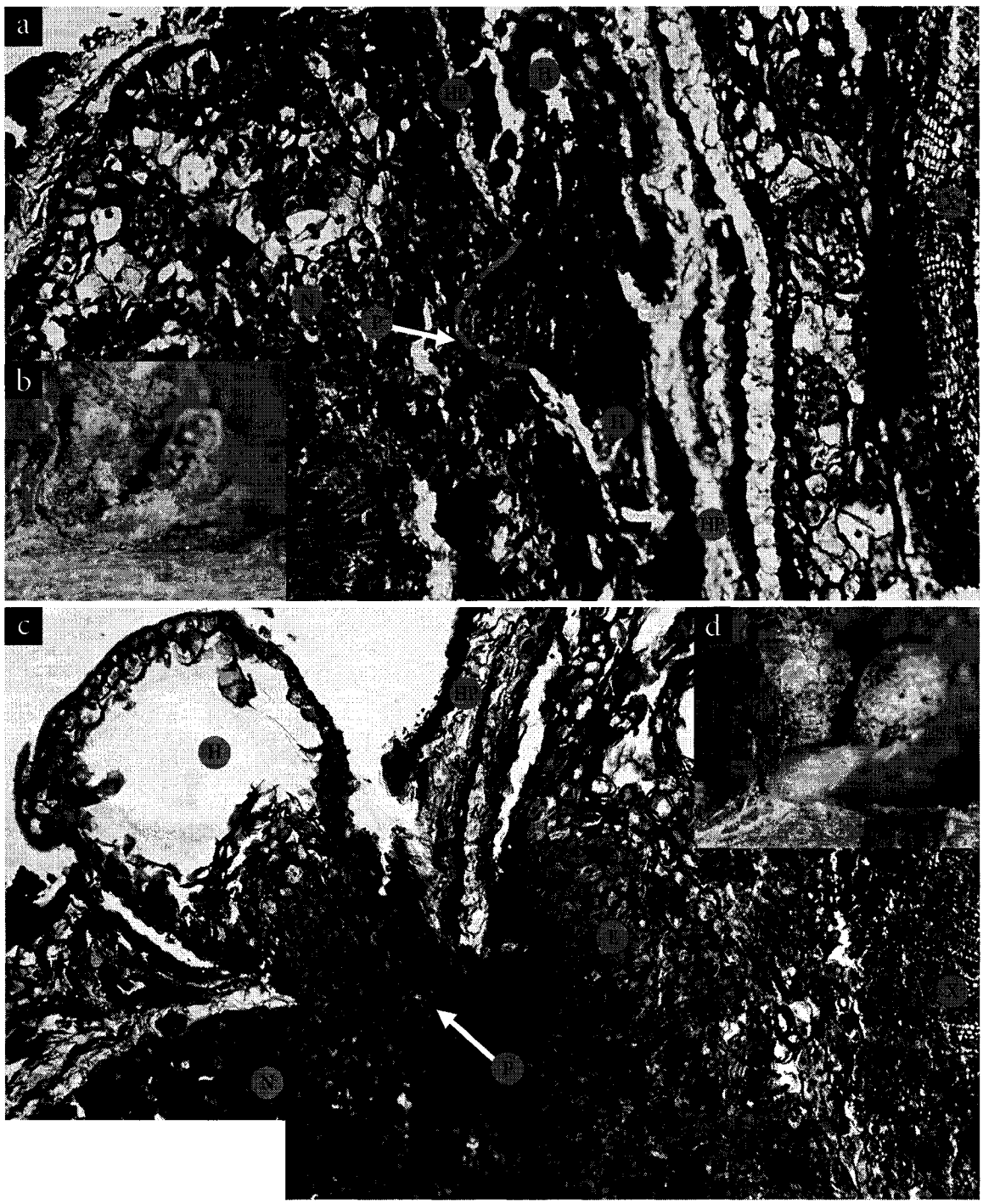

FIGURE 4.6 Matched pairs of photographs of *Arceuthobium americanum* seeds sampled 25 June 2002 about 13 months after germination. For each matched pair, the smaller image depicts a germinated seed with a holdfast formed at the base of a jack pine needle while the larger image depicts the same seed but in transverse section. In both (a) and (c) penetration of host tissues has occurred. The endophytic system is established in (c). Yellow line indicates boundary between parasite and host cells (solid: high confidence; dashed: lower confidence). E – endophytic system, H – holdfast, HP – host periderm , N – jack pine needle, P – penetration, X – twig xylem.

infected their host. Because of the cryptic nature of penetration, this stage could not be observed on germinants *in situ*. The first symptom of successful penetration and infection that could be reliably observed in the field was a localized swelling of the twig near the point of holdfast attachment. In most cases, this occurred 13–15 months after germination between June and August of the second growing season and was consistent for all four seed sources and three study sites. In a few cases, swellings did not occur until the third growing season. Interestingly, many of these infections arose from the germinants in which the radicle and holdfast withered during the first growing season.

DISCUSSION

Dowding (1929) reported that some *A. americanum* seeds germinate on jack pine in the fall soon after seed discharge. However, germination rates of seeds in this study were near zero until about 110 days after discharge. Thus, it appears that an after-ripening period is required to break dormancy for *A. americanum* seeds collected from jack pine. Seed dormancy has been reported for *A. campylopodum* (Beckman and Roth 1968; Knutson 1984) but is lacking in *A. abietinum* f. sp. concoloris, *A. abietinum* f. sp. magnificae, *A. littorum*, *A. occidentale*, and *A. pusillum* (Scharpf and Parmeter 1962; Scharpf 1970; Livingston and Blanchette 1986). Beckman and Roth (1968) speculated that a chemical inhibitor associated with the endocarp or viscin cells existed for *A. campylopodum*, although the work of Scharpf (1970) on *A. occidentale* does not support this conjecture. Another possibility is the accumulation during dormancy of growth promoters such as gibberellins or cytokinins, which are important in many cases for seeds of other plants (Salisbury and Ross 1992). None of the 1200 seeds artificially inoculated on the jack pine trees at the three study sites germinated prior to April or May of the next growing season. Seeds stored artificially for up to 24 months at 2°C are capable of surviving and germinating (Brandt et al. in press), and it is possible that the seeds Dowding (1929) observed germinating in the fall were discharged more than a year prior to her observations. The frequency of observations is unclear in Dowding's paper of 1929. At Goodwin Lake, Smoky Lake, and Bruderheim where seeds were visually monitored biweekly, seeds that did not germinate in the spring following inoculation did not germinate later that year or in the next spring. The discrepancy between the observations in this study and those of Dowding emphasize the importance of frequent observations on seeds and germinants and the potential uncertainty associated with infrequent monitoring.

There is high interannual variation in the timing of germination. Germination in 2002 was considerably later than in 2001: 27 d later at Goodwin Lake, 21 d later at Smoky Lake, and 17 d later at Bruderheim. The 2002 spring was late with temperatures much lower throughout April and May when compared to the same months in 2001. Interestingly, seeds in the laboratory reached peak germination rates about the same time as the seeds placed on the jack pine trees at the three study sites.

Interannual variation in *A. americanum* seed germination is low as is variation in seed germination from different *A. americanum* seed sources. Germination of *A. pusillum* seeds from two seed sources did not vary (Bonga 1969). Seeds of *A. abietinum* f. sp. *concoloris*, *A. abietinum* f. sp. *magnificae*, *A. littorum*, and *A. occidentale* from four different seed sources each and collected in different years exhibited no differences in germination (Scharpf

1970). The results of this study, however, indicate that there is an interaction between year in which seeds were collected and seed source based both on results from the laboratory and the field. Seeds collected from Peace Point performed poorly in 2000 with 37% of the seeds germinating in the laboratory and 28% in the field; in the next year seed germination increased significantly to 66% in the laboratory and 68% in the field. The Peace Point seed collection site is at the northern limit of *A. americanum* (Fig. 5.1; Brandt et al. 1998). Others have demonstrated that extreme cold temperatures reduce overwintering seed survival, impair seed discharge, and damage pollen in *A. americanum* (Baranyay and Smith 1974; Gilbert and Punter 1991; Brandt et al. 2004). Perhaps unfavorable weather conditions at Peace Point during the fruit and seed maturation period in 1999 and 2000 negatively impacted some aspect of the seeds, which ultimately resulted in their poor germination performance.

Seeds that germinated were considerably larger than those that did not germinate. This relationship was consistent for the McClelland Lake, Logan River, and Smoky Lake seed sources. Peace Point seeds, however, were smaller in 2001 compared to 2000 but a significantly and substantially higher percentage of seeds germinated in 2001 compared to the previous year. Other unknown factors must be affecting germination performance. As mentioned above, weather conditions during fruit and seed maturation could be a factor. Vigor of the pistillate plants on which the seeds developed during this same period could also be a factor.

Even though holdfasts were well-developed and appeared to be tightly appressed to the host in June, no penetration occurred based on the histological examinations in this study. Penetration of the host does not begin until as late as August. The histological and field evidence of this study strongly supports the conclusion that penetration is not complete until the spring of the next growing season for most germinants because few infections result from germinants that wither in the first growing season. The findings of this study are in contrast to those of Scharpf and Parmeter (1967), who reported that penetration took at least 2–3 months for *A. abietinum* f. sp. *magnificae* on red fir and *A. occidentale* on digger pine based on histological observation. Scharpf and Parmeter (1967) also removed germinants of *A. abietinum* f. sp. *magnificae* from the host at different times. No infection resulted from seeds removed in June or July of the first growing season. Penetrating germinants removed in August infected the host at a 5% infection rate while those removed in September resulted in a 3% infection rate. Seeds left in place as controls infected the host at a rate of 8%. The importance of overwintering survival to *A. americanum* germinants that successfully penetrate and infect their host can be interpreted in two ways. In one, the radicle and holdfast remains hydrated and living after the parasite has infected the host but serves no apparent purpose to the endophytic system. According to the literature, however, disassociation of the radicle and holdfast occurs after successful penetration of the host and establishment of the endophytic system (Kuijt 1960; Cohen 1963). Another interpretation, and the most likely one, is that the penetration process requires more than one growing season for establishment of the endophytic system. In this interpretation, the penetrating cells of the parasite need reserves from the seed's endosperm and from the sugars produced by the photosynthesizing seed and radicle. The potential importance of the seed's and radicle's ability to photosynthesize in relation to germination, successful penetration, and infection has been recognized by others (Muir 1975; Knutson 1983). The second interpretation is also supported by the following experimental work. A regime of 20°C/15°C day–night and high light (195 $\mu mol\ s^{-1}\ m^{-2}$) resulted in high infection rates on ponderosa pine seedlings by *A. campylopodum* (Knutson 1984). High infection rates on digger pine by *A. occidentale* occurred under half or full

sunlight; little infection occurred in absence of light, although there may have been an interaction with temperature because it increased in the black bags used to exclude light (Scharpf 1972).

The form of the penetrating structure and the location where it penetrates the host in this study is in contrast with much of the literature. In all the sectioned samples examined, the penetrating structure consisted of many cells formed into a narrow cone and located at the distal end of the holdfast (Fig. 4.3c; Fig. 4.5c). Penetration always occurred at the base of the needle where it connected with the twig. Cohen (1963) shows several photomicrographs of penetrating holdfasts for *Arceuthobium* spp. In each photomicrograph, the penetrating cells occur at the base of the disk-like holdfast and are ramifying through the bark of the twig. Scharpf and Parmeter (1967) show a similar form of penetration in their photomicrographs. Hunt et al. (1996) observed penetration by *A. tsugense* through the base of needles on western hemlock. Penetration at the base of the needle as opposed to penetration through the bark of the twig provides support for Hawksworth's speculation that bark thickness could be an important factor in penetration (Hawksworth 1954).

In most cases, the penetration and infection processes took about 12–13 months because swelling of the twig at the point of infection (a reliable symptom of infection) occurred 13–15 months after germination. Scharpf (1972) noted that the germinated seed, growing radicle, and penetrating structure of dwarf mistletoes must be able to survive in a regime of changing environmental conditions. This is especially true for *A. americanum* infecting jack pine in Alberta where the germinant often survives, in summer, daily maximum temperatures above 30°C and periods of no rain, and, in winter, extreme cold temperatures. The most noteworthy ability of *A. americanum* germinants is their hardiness to drought. The epidermal cells of the radicle and the endosperm of the seed of *Arceuthobium* spp. have a thick cuticle (Kuijt 1960), and this, in part, likely plays a role in drought hardiness. No attempt was made in this study to identify the mechanism used by the germinant to deal with drought, although this would be a interesting avenue of research. Plants hardy to drought stress either avoid or tolerate drought using a variety of mechanisms; these have been reviewed by Levitt (1980).

The long penetration period could provide an opportunity for an alternate method of control because the germinant is potentially vulnerable to biotic or abiotic agents prior to infection of the host. Mold fungi can be a serious problem to dwarf mistletoe seeds (Scharpf and Parmeter 1962; Bonga 1965; Scharpf 1970). Perhaps a suitable fungal agent could be found to act as an effective biological control agent for germinants. Research aimed at identifying such fungal agents or other pathogens and testing their efficacy against *A. americanum* germinants would be beneficial and could ultimately provide another tool for management of this damaging pest of pines.

SUMMARY

Information on the establishment of *Arceuthobium americanum* on its host, *Pinus banksiana*, is lacking but is required to improve knowledge of this critical stage and improve management of the pest. A combination of laboratory and field observations on four populations of *A. americanum* seeds collected in 2000 and 2001 and histological observations on one population were used to determine timing and nature of parasite establishment on jack pine. Germination rates of seeds from four populations were near zero until 110 d after placement of seeds into storage; germination rates peaked after 230 d in 2001 and between 230–320 d in 2002. There was high interannual variation in germination timing at study sites: mean of 25 May 2001 and 12 June 2002. Interannual variation in seed germination, however, was low as was seed germination from different seed sources. Germinated seeds of each seed source were significantly larger (48%) than those that did not germinate. Host penetration, which always occurred at the base of needles, began between June and August and continued, in most cases, until at least June or July of the next growing season based on field and histological evidence. The odds of infection when the germinant survives until the second growing season was 55 times greater than the odds of infection when the radicle withered in the first growing season. Swelling of the twig near the penetration point was a reliable symptom of infection in the field; in most cases this occurred 13–15 months after seed germination and was consistent for all seed sources and sites.

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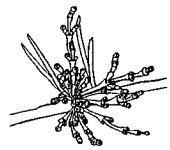

CHAPTER FIVE

Extreme cold temperatures and survival of overwintering and germinated *Arceuthobium americanum* seeds[1]

INTRODUCTION

Lodgepole pine dwarf mistletoe (*Arceuthobium americanum*) is distributed throughout much of western North America, where it occurs on its two principal hosts, lodgepole pine (*Pinus contorta* var. *latifolia*, *P. contorta* var. *murrayana*) and jack pine (*P. banksiana*) (Hawksworth and Wiens 1996). The northern limit of *A. americanum* is near 59° N, where it occurs on jack pine in Alberta (Brandt et al. 1998). The southern limit occurs on lodgepole pine in central California near 35° N (Hawksworth and Johnson 1989). Recently three races within *A. americanum* were identified: on jack pine in the western Canadian interior; on lodgepole pine (*P. contorta* var. *murrayana*) in the Sierra Nevada and Cascade Mountain ranges of the western United States; and on lodgepole pine (*P. contorta* var. *latifolia*) in western United States and Canada (Jerome and Ford 2002).

Arceuthobium americanum presumably evolved on lodgepole pine in southwestern North America and transferred to jack pine only relatively recently, perhaps in the last 15 000–10 000 years B.P. near the end of the Wisconsin glaciation (Yeatman 1967). It was following the retreat of the continental glaciers that jack pine moved north and west from refugia in central and eastern North America and contact was made with lodgepole pine as it advanced northward from areas south of the glacial maximum in western North America (Critchfield 1985). The post-glacial history of both the parasite and its hosts has likely influenced the formation of the current genetic races within *A. americanum* (Jerome and Ford 2002).

Dwarf mistletoe seeds disperse by forcible discharge from mature fruits in August and September. Most infections occur on needle-bearing twigs as this is where the majority of *A. americanum* seeds land (Hawksworth 1965). Seeds overwinter there until they germinate the following spring. Successful germination, penetration, and infection by the parasite causes stem and branch swellings and witches' brooms in trees. As infections intensify within the host, branches and tree tops die and the tree eventually succumbs. Other than decay organisms, *A. americanum* is the most important pest of pine in the western Canadian interior. Regional estimates of annual growth and mortality losses for jack pine growing in the three prairie provinces are 314 000 m^3 and 1 478 000 m^3, respectively, while those for lodgepole pine in Alberta and Saskatchewan are 486 000 m^3 and 69 000 m^3, respectively (Brandt et al. 1998).

Gaps exist in our knowledge of the basic biology of *A. americanum* and how the parasite responds to changes in temperature as most of the work to date has examined species of *Arceuthobium*

[1] *A version of this chapter has been published. Brandt, J.P., Hiratsuka, Y., and Pluth, D.J. 2004. Canadian Journal of Forest Research. 34: 174-183.*

other than *A. americanum* or has been conducted in study areas climatically distinct from the boreal climate of the western Canadian interior. In Colorado and Wyoming, the upper limits of infection for *A. americanum* growing on lodgepole pine coincided with the –1°C mean annual temperature isotherm (Hawksworth 1956). In British Columbia, *A. americanum* seeds on lodgepole pine did not survive one winter when the extreme minimum temperature reached –39°C (Smith and Wass 1979). Over-wintering seeds of *A. americanum* infecting lodgepole pine growing in the Rocky Mountains of Colorado were killed near –33°C (Becwar 1980). Temperature also influences dwarf mistletoe's life cycle in other ways. *Arceuthobium americanum* pollen was damaged by low temperature (Gilbert and Punter 1991). Fall frosts damaged the fruit of *A. americanum*, *A. douglassii*, and *A. pusillum* (Tunnock et al. 1966; Hudler and French 1976), and a temperature of –3.9°C for 2.3–4.8 h caused permanent damage to *A. americanum* and *A. tsugense* fruits (Baranyay and Smith 1974). The recent discovery of three genetic races within *A. americanum* may require a reevaluation of some of the above studies on *A. americanum*.

Cold temperatures during winter and frosts during the growing season are important environmental factors limiting the productivity and distribution of plants (Sakai and Larcher 1987). Increasing our understanding of the influence of climate and its effect on dwarf mistletoe and its host has the potential to improve management of the parasite, to assess areas of non-infected pine at risk within dwarf mistletoe's current distribution, and to predict how the distribution of this parasitic plant might change in a changing environment. If the northern limit of dwarf mistletoe is presently governed by extreme low temperatures, then climatic warming could lead to a northward expansion of the parasite, with reduced growth and increased mortality of jack pine in the more northerly boreal regions. Working with four populations of *A. americanum* infecting jack pine along a north-south gradient, the objectives of this study were to determine (i) the minimum winter temperature that prevents overwintering dwarf mistletoe seeds from germinating during the following spring; and (ii) if exposure to late spring frosts kill the embryo and radicle of dwarf mistletoe germinants.

Materials and Methods

Experimental design

There were four experiments in this study: three examined the effect of extreme cold winter temperatures on germination rates of overwintering *A. americanum* seeds, and one examined the effect of freezing spring temperatures on the survival of *A. americanum* germinants. The first experiment was repeated in two different years with dwarf mistletoe seeds collected in 2000 and 2001, while the others were conducted once with seeds collected in 2001. Each experiment consisted of a factorial treatment structure in a randomized complete block design. Treatments consisted of combinations of several different dwarf mistletoe seed sources, freezing temperatures, and exposure periods (Table 5.1).

Seeds of *A. americanum* growing on jack pine were collected from four sites in Alberta: north of Peace Point (59° 11'N, 112° 23'W), west of McClelland Lake (57° 31'N, 111° 24'W), west of the Logan River (55° 21'N, 111° 56'W), and southeast of Smoky Lake (54° 05'N, 112° 20'W) (Fig. 5.1). At each site, jack pine branches with female plants were

TABLE 5.1 **Levels of factors used in experiments on *Arceuthobium americanum* examining the effect of extreme cold winter temperatures on the germination rates of overwintering seeds and the effect of freezing spring temperatures on the survival of germinants.**

	Factor		
Experiment[a]	Dwarf mistletoe seed source and latitude	Target treatment temperature (°C)	Exposure (hr)
Extreme cold winter temperatures			
1	Peace Point, 59.2° N McClelland Lake, 57.5° N Logan River, 55.4° N Smoky Lake, 54.1° N	control, –39,–46, –53	144
2	As above	control, –30, –34	144
3	As above	control, –39, –46	48, 96
Freezing spring temperatures			
4	As above	control, –1, –3, –7	3

[a] *Experiment 1 replicated in 2001 and 2002 using seeds collected in 2000 or 2001, respectively. All other experiments were conducted in 2002 only using the seeds collected in 2001.*

wrapped in sections of cotton stockinet (Cottonia Products Inc., Montreal, Quebec) during late July prior to fruit maturation and seed dispersal. The sections of stockinet with trapped seeds were collected during mid-September. Seeds were then transferred to and stored at 2°C in sealed, 4.0 L wide-mouth glass jars according to the methods described in Chapter 2.

Extreme cold winter temperatures

In November, seeds were removed from cool storage, soaked in distilled water for 30 minutes, and then placed on a 15×15-cm screened aluminum frame in groups of 50 or 100 seeds. Each seed's viscin held it firmly to the screen once the viscin dried. Frames were double screened with one layer of fine-mesh fibreglass screen on top and one layer of fine-mesh cloth beneath to prevent seeds from falling through both layers. Seeds on each screened frame were exposed to one treatment combination. Blocks of each experiment consisted of 18×18×100-cm wooden boxes made from 19-mm spruce plywood. Each rectangular box was open but screened on two of its four opposing vertical sides. This allowed free movement of air and water but prevented small animals from entering. Depending on the experiment, 16 or 24 horizontal slots were cut equidistantly into the two other opposing vertical sides of the box in order to receive and support the screened frames with seeds. Four boxes were suspended by ropes 120 cm above the ground in different locations in a small jack pine plantation (<1 ha, 669 m above m.s.l.) located near the laboratory in Edmonton.

At the beginning of February, the screened frames with seeds were removed from the wooden boxes and placed in cardboard boxes. Two slits were cut into two of the opposing vertical sides of each box to allow free air movement. Frames were stacked flat with two small wooden slats between frames for air movement. Cardboard boxes were closed and then placed in a walk-in cooler for three days at –10°C followed by three days at –25°C in

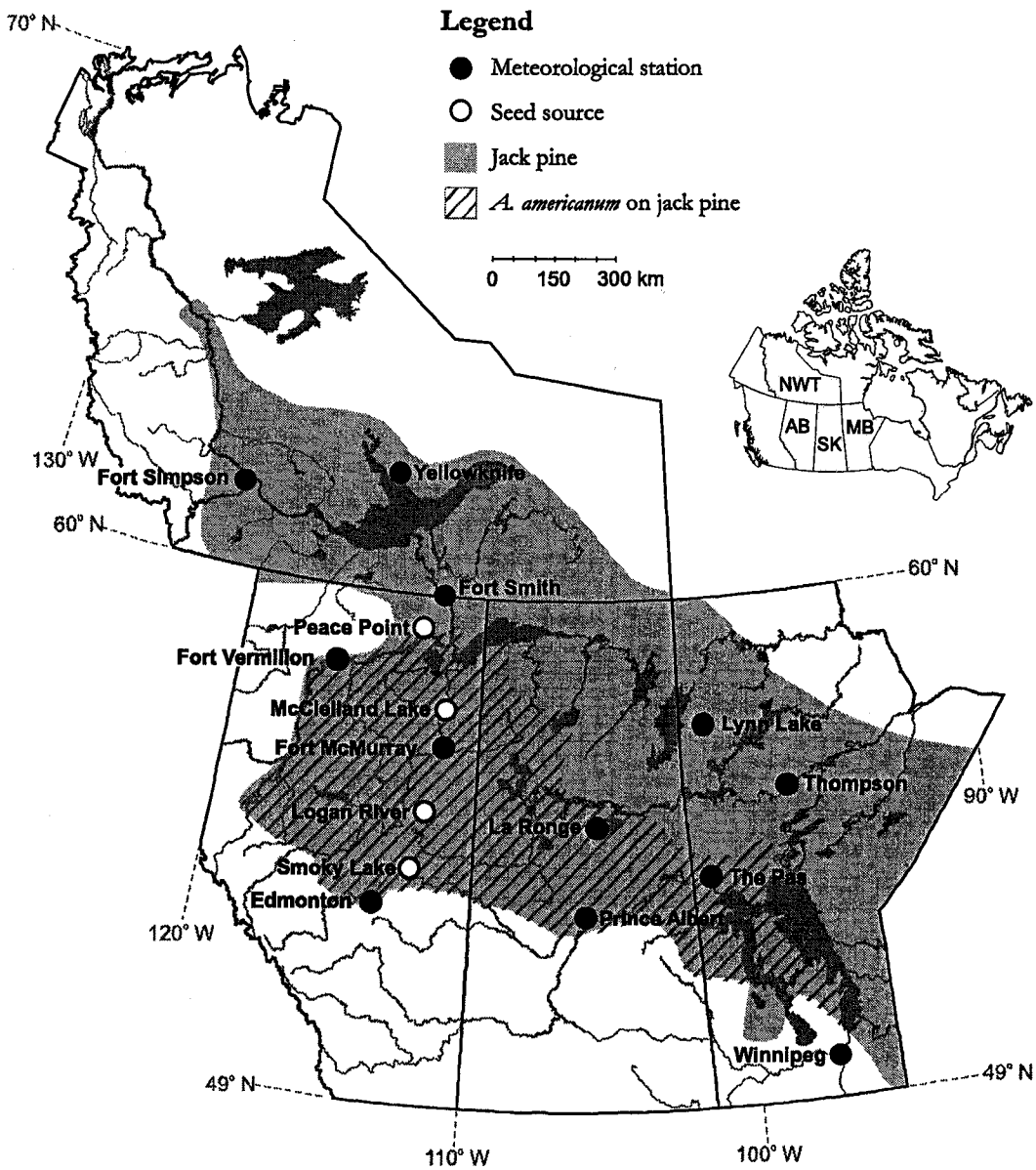

FIGURE 5.1 Distribution of jack pine in Alberta, Saskatchewan, Manitoba, and the Northwest Territories, Canada (Farrar 1995), and the distribution of *Arceuthobium americanum* infecting jack pine for the same area (modified from Brandt et al. 1998). Jack x lodgepole pine hybrids, which may be infected by *Arceuthobium americanum*, occur in Alberta in the western portions of jack pine's range, but the distribution of these hybrids is not depicted.

order to acclimatize the seeds. Temperature-exposure time controls were also removed from the wooden boxes, placed in cardboard boxes, and acclimated but were returned outdoors to the wooden boxes.

The cardboard boxes with seeds were removed from the –25°C walk-in cooler and placed in freezers set to treatment temperatures for 48, 96, or 144 hours depending on the experiment. After exposure to low temperatures, cardboard boxes were removed and screened frames were returned outdoors to the wooden boxes. During the last week of May, dwarf mistletoe seeds were germinated (Wicker 1962).

A data logger (Campbell Scientific Inc. CR23X or 21X) with two thermocouples per freezer recorded temperature every 60 seconds and calculated hourly averages. Temperature data from the Edmonton Municipal Airport (10 km north, 671 m above m.s.l.) and Edmonton International Airport (21 km south, 715 m above m.s.l.) meteorological stations were also obtained for the period 1 November through 30 April for both exposure years.

Freezing spring temperatures

Seeds from the four seed sources were overwintered outdoors on twigs of jack pine seedlings using the seeds' natural viscin to hold them in place. During the first week of May these seeds were collected and germinated by soaking in sterilized distilled water for two days. Seeds were then aseptically transferred in groups of 50 to wetted, sterilized filter paper in labeled glass petri dishes with one dish per seed source × temperature treatment combination. Petri dishes with seeds were incubated in environmental chambers set to a 9°C night : 16°C day temperature regime and an 8 hr dark : 16 hr light illumination regime until seeds were exposed to treatment temperatures at the end of June. Prior to treatment, temperatures were reduced stepwise at 2-hr intervals from 16 to 12 to 8 to 4°C, and then seeds were exposed to either –1°C or –3°C for 3 hr. For the coldest treatment, temperature was decreased at 2-hr intervals from 16 to 10 to 4 to –3°C, and then seeds were exposed to –6°C for 3 hr. Controls were decreased to 4°C. Following treatment, all petri dishes containing seeds were returned to their original environmental chambers and conditions. The temperature regime of the environmental chamber prior to treatment, the stepwise reduction of temperatures in the hours before the treatment, and the duration of the exposure to the treatment were all consistent with a typical frost event (personal communication, B. Kochtubajda, Environment Canada, Meteorological Service of Canada). Seeds were assessed for visual signs of necrotic or desiccated tissue on the surface of radicles and for mortality at 6 and 20 days after treatment. A data logger (Campbell Scientific Inc. CR23X or 21X) equipped with two thermocouples per chamber recorded temperature every minute and produced hourly averages. To compare temperatures within the environmental chambers to temperatures within the petri dishes containing the seeds, two trials were conducted. In the trials, one temperature thermocouple measured the temperature within the chamber, and the second measured the temperature on the surface of the filter paper within the closed petri dish.

Data analysis

Results from the experiments were expressed as a percentage or count of seeds that germinated for Experiments 1–3, or as a count of germinants that survived for Experiment 4. Percentages of germinated seeds obtained from Experiment 1 were highly skewed for the different treatment combinations with many percentages having a value of zero. Thus, the Mantel-Haenszel chi-square statistic (FREQ procedure, SAS Institute Inc. 1999) was used to test for an association between temperature (with the four treatment temperatures as levels) and germination status. The extended Mantel-Haenszel correlation statistic was used to test for an overall association between the same variables after controlling for seed source. The significance level was set at $\alpha = 0.05$ for these tests as well as all other tests described subsequently.

Percentages of germinated seeds from Experiment 2 were normally distributed. The model for the analysis of variance included three sources of variation and an interaction term: block, with four levels; seed source, with four levels; temperature, with three levels; and a seed source × temperature term. Orthogonal contrasts were used to test for linear, quadratic, and cubic trends in latitude of seed source.

Percentages of germinated seeds obtained from the results of Experiment 3 were also highly skewed, except for normally distributed data for the control and the treatment of –39°C for 48 hr. Fisher's exact test (SAS Institute Inc. 1999) was used to test the association between germination status and exposure period separately for the two temperature treatments because some expected cell counts were less than five. The extended Mantel-Haenszel correlation statistic was used to test for an overall association between these variables while controlling for seed source. An analysis of variance assessed effects for the normally distributed data. The model for the analysis of variance included block, with four levels; seed source, with four levels; temperature, with two levels; and a seed source × temperature term. Orthogonal contrasts were used to test for linear, quadratic, and cubic trends in latitude of seed source. Tukey's studentized range test (SAS Institute Inc. 1999) was utilized to test for pairwise differences in means of germination rates.

RESULTS

Extreme cold winter temperatures

Freezer temperatures differed slightly from the target temperatures (Tables 5.2, 5.3, and 5.4 versus Table 5.1). Temperature variability was low, with all but four of the standard deviations being less than ± 0.9°C.

There was a highly significant ($P < 0.0001$) association between germination status and temperature for each dwarf mistletoe seed source based on count data obtained from Experiment 1 (Table 5.2). The overall association, adjusting for seed source, was also highly significant. Regardless of seed source for the 144 hr exposure period, the percentage of germinated seeds decreased significantly as temperatures dropped from –15.1 or –23.5°C of the control to about –39°C and then leveled off to near-zero germination rates through to about –53°C.

For seeds exposed for 144 hr to temperatures between –10°C (control) and –34°C in Experiment 2, there was no effect of temperature ($P = 0.47$), seed source ($P = 0.23$), or their interaction ($P = 0.34$) (Table 5.3) (see Table A.6, Appendix 2, for ANOVA table). There was also no evidence of a cubic ($P = 0.54$), quadratic ($P = 0.85$), or linear ($P = 0.051$) relationship in germination rates among the four seed sources.

At about –44°C in Experiment 3, germination rates were near-zero and similar ($0.061 < P < 0.61$) for exposure periods of 48 and 96 hr (Table 5.4). At –37°C, significantly fewer seeds germinated when exposed for 96 hr than when exposed for 48 hr. Results of the ANOVA for the control and the treatment of –37°C for 48 hr indicated no effect of block ($P = 0.67$) or the interaction between seed source and temperature ($P = 0.55$) (see Table A.7, Appendix 2, for ANOVA table). There was a temperature ($P = 0.045$) and seed source effect ($P = 0.0001$). The overall mean germination rate of 44.6% for seeds exposed to –36.7°C for 48 hr was significantly lower than the overall mean of 51.7% for the control. Germination rates of seeds from the three more southerly seed sources were not significantly different, whereas the germination rate of seeds from Peace Point was significantly higher than the others. The

TABLE 5.2 **Mean percent germination of *Arceuthobium americanum* seeds in Experiment 1, in which overwintering seeds from four seed sources were exposed to four temperatures for 144 hr. The experiment was repeated in two consecutive years.**

	Control[a]		Treatment temperature[b]					
Dwarf mistletoe seed source and latitude	−23.5°C (2001)[c]	−15.1°C (2002)[d]	−39.3°C±0.1°C (2001)	−38.4°C±1.5°C (2002)	−47.4±0.2°C (2001)	−45.0±0.7°C (2002)	−53.7±1.1°C (2001)	−52.6±1.4°C (2002)
Peace Point, 59.2° N	53.7	68.8	0.5	0.0	0.0	0.0	0.3	0.0
McClelland Lake, 57.5° N	42.4	63.9	0.4	1.0	0.3	3.9	0.0	0.5
Logan River, 55.4° N	52.2	52.0	3.7	0.0	0.0	0.5	0.0	0.0
Smoky Lake, 54.1° N	60.1	44.5	15.8	0.0	0.3	0.5	0.5	0.5
Overall	52.1	57.3	5.1	0.3	0.2	1.2	0.2	0.3

Note: Each value in the matrix of the table is the mean of four groups of 100 and 50 seeds for the experiments conducted in 2001 and 2002, respectively. For both years, the germination rates for all treatment temperatures were always lower than the control ($\alpha = 0.05$) regardless of whether comparisons were done separately for the different seed sources or combined over seed sources.

a Control temperatures are the average of the extreme minimum temperature recorded at the Edmonton Municipal Airport and Edmonton International Airport meteorological stations during the extreme temperature treatments.

b Treatment temperatures (mean ± SD) were measured in the freezers with two thermocouples.

c Winter period 2000–2001.

d Winter period 2001–2002.

TABLE 5.3 **Mean percent germination of *Arceuthobium americanum* seeds in Experiment 2, in which overwintering seeds from four seed sources were exposed to three temperatures for 144 hr.**

	Control[a]	Treatment temperature[b]	
Dwarf mistletoe seed source and latitude	−10.5°C	−30.3 ± 0.3°C	−34.4 ± 0.2°C
Peace Point, 59.2° N	64.9	59.4	53.0
McClelland Lake, 57.5° N	56.2	55.3	64.4
Logan River, 55.4° N	53.0	59.7	45.5
Smoky Lake, 54.1° N	53.0	54.3	49.1
Overall	56.8	57.2	53.0

Note: Each value in the matrix of the table is the mean of four groups of 50 seeds each. Germination rates did not differ among seed sources, temperatures, or their interaction ($\alpha = 0.05$).

a Control temperature is the average of the extreme minimum temperature recorded at the Edmonton Municipal Airport and Edmonton International Airport meteorological stations during the extreme temperature treatments.

b Treatment temperatures (mean ± SD) were measured in the freezers with two thermocouples.

TABLE 5.4 **Mean percent germination of *Arceuthobium americanum* seeds in Experiment 3, in which overwintering seeds from four seed sources were exposed to three temperatures for 48 or 96 hr.**

Dwarf mistletoe seed source and latitude	Control[a]	Treatment temperature[b]			
	−15.1°C	−36.7 ± 0.8°C for 48 hr	−37.8 ± 1.4°C for 96 hr	−44.3 ± 0.8°C for 48 hr	−44.7 ± 0.7 °C for 96 hr
Peace Point, 59.2° N	65.4	59.8	0	0	0.5
McClelland Lake, 57.5° N	44.9	32.0	7.3	1.0	3.0
Logan River, 55.4° N	50.8	40.3	2.5	0	1.0
Smoky Lake, 54.1° N	46.0	46.3	3.5	2.5	0
Overall	51.7	44.6	3.3	0.9	1.1

Note: Each value in the matrix of the table is the mean of four groups of 50 seeds each. The overall mean germination rate for seeds exposed to −37°C for 48 hr was significantly lower than the overall mean for the control ($\alpha = 0.05$). At −37°C, significantly fewer seeds germinated when exposed for 96 hr than when exposed for 48 hr. At −44°C, germination rates were not significantly different for the 48 and 96 hr exposure periods.

[a] *Control temperature is the average of the extreme minimum temperature recorded at the Edmonton Municipal Airport and Edmonton International Airport meteorological stations during the extreme temperature treatments.*

[b] *Treatment temperatures (mean ± SD) were measured in the freezers with two thermocouples.*

linear ($P = 0.010$), quadratic ($P = 0.0006$), and cubic ($P = 0.028$) trends in germination rates as a function of seed source latitude were significant.

Freezing spring temperatures

Exposure for three hours to –1, –3, or –6°C had no effect on survival of dwarf mistletoe germinants assessed 6 and 20 days following the exposure period. None of the germinants died and there were no visual signs of surface injury such as necrotic or desiccated tissue on the germinant radicles. Actual mean temperatures and standard deviations were –1.2 ± 0.5°C, –2.8 ± 0.1°C, and –6.0 ± 0.1°C, respectively. Measured temperatures within the environmental chambers and within their petri dishes were similar except for an initial 10–20 minute lag in temperatures on the surface of the filter paper during the temperature reduction period.

DISCUSSION

For a given period of exposure, an extreme cold temperature threshold exists for germinative ability of overwintering seeds collected from *A. americanum* plants infecting jack pine. This threshold was consistent across the four locations ranging from 54 to 59° N in Alberta where seeds were collected in this study. Germination rates of dwarf mistletoe seeds were reduced to almost zero when temperatures reached –38°C or colder for 96 hr or more. These findings provide strong evidence for the existence of a temperature threshold consistent with the observations of Smith and Wass (1979), who noted that *A. americanum* seeds collected from and artificially inoculated on lodgepole pine did not survive one winter when the extreme minimum temperature reached –39°C. The threshold observed in this study is substantially lower than the –33°C found by Becwar (1980) using differential thermal analysis on overwintering *A. americanum* seeds collected from lodgepole pine growing in the Rocky Mountains of Colorado.

The evidence provided by this study suggests that extreme cold winter temperature plays an important role in the current distribution of dwarf mistletoe in the western Canadian interior and could be sufficient to explain the absence of the parasite on jack pine from northern areas that are commonly exposed to prolonged winter cold spells with temperatures below about –40°C. Jack pine's distribution extends substantially farther north than dwarf mistletoe's distribution (Fig. 5.1). Experimentally, jack pine buds and needles are hardy to at least –70°C (Sakai 1983). An examination of temperature records from selected meteorological stations in Alberta, Saskatchewan, Manitoba, and the Northwest Territories reveals that air temperatures do commonly fall to the extreme temperatures tested in this study (Table 5.5). Temperatures typically reach these extremes between November and March, with the lowest temperature being –53.9°C recorded at Fort Smith in the Northwest Territories. Within the range of *A. americanum*, cold spells of four or more consecutive days in which the minimum temperature reached –38°C are infrequent, ranging from 2 to 35% of the winters within the period of record (Table 5.5). In contrast, at the meteorological stations examined north of the range of *A. americanum*, 52–81% of the winters within the period of record had such cold spells.

Cold temperatures are known to affect other stages in the life cycle of *A. americanum*. Spring frosts reduced germination rates of pollen collected from *A. americanum* plants infecting jack pine (Gilbert and Punter 1991). Exposure to –3.9°C for

TABLE 5.5 **Extreme minimum temperatures (°C) recorded during a given month for selected meteorological stations located throughout the range of jack pine in western Canada (Environment Canada 1993a, 1993b). Also shown for each station is the percentage of winters within the period of record with at least one cold spell when minimum temperatures reached –38°C or colder on four or more consecutive days (source data: Environment Canada).**

Meteorological station	Period of record	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Winters within period of record with at least one cold spell (%)
NWT														
Yellowknife	1942-90	–51.2	–51.2	–43.3	–40.6	–22.8	–4.4	0.6	–0.6	–9.7	–28.9	–44.4	–48.3	75
Fort Simpson	1963-90	–50.0	–53.3	–42.2	–38.3	–15.2	–2.2	–1.1	–3.7	–20.6	–27.7	–41.7	–50.6	81
Fort Smith	1943-90	–49.4	–53.9	–44.4	–40.6	–19.4	–6.1	–3.3	–5.6	–12.8	–27.9	–40.6	–48.3	72
Alberta														
Fort Vermilion	**1938-85**	**–51.7**	**–52.2**	**–47.2**	**–37.8**	**–12.2**	**–4.4**	**–0.6**	**–3.3**	**–12.8**	**–32.0**	**–40.0**	**–49.4**	**35**
Fort McMurray	**1944-90**	**–50.0**	**–50.6**	**–44.4**	**–34.4**	**–13.3**	**–4.4**	**–3.3**	**–2.8**	**–15.6**	**–24.5**	**–37.8**	**–47.2**	**33**
Edmonton	**1937-90**	**–44.4**	**–46.1**	**–36.1**	**–26.6**	**–12.2**	**–1.1**	**0.6**	**0.6**	**–11.7**	**–25.0**	**–34.1**	**–48.3**	**2**
Saskatchewan														
La Ronge	**1959-90**	**–48.3**	**–45.6**	**–39.4**	**–30.5**	**–10.9**	**–2.8**	**1.1**	**–3.4**	**–7.8**	**–18.3**	**–37.6**	**–44.3**	**29**
Prince Albert	**1942-90**	**–50.0**	**–46.1**	**–45.6**	**–33.9**	**–12.8**	**–4.4**	**1.1**	**–3.7**	**–15.6**	**–26.1**	**–38.9**	**–44.3**	**21**
Manitoba														
Thompson	1967-90	–48.9	–47.2	–48.3	–34.4	–18.3	–5.0	–1.1	–3.5	–11.1	–25.7	–41.1	–47.6	52
Lynn Lake	1968-90	–46.7	–46.1	–45.0	–33.0	–15.5	–5.6	2.2	–2.8	–10.0	–28.9	–37.7	–47.1	59
The Pas	**1943-90**	**–45.0**	**–49.4**	**–39.4**	**–30.0**	**–12.8**	**–3.3**	**1.4**	**0.6**	**–7.2**	**–15.6**	**–35.5**	**–44.4**	**4**
Winnipeg	**1938-90**	**–42.2**	**–45.0**	**–37.8**	**–26.3**	**–11.1**	**–3.3**	**1.1**	**0.6**	**–7.2**	**–17.2**	**–34.0**	**–37.8**	**2**

Note: *Meteorological stations in bold are within or south of the natural range of A. americanum.*

about 2.3 hr permanently damaged mature fruit of *A. americanum* infecting lodgepole pine (Baranyay and Smith 1974). The impact of these negative effects and the effect on germination rates of overwintering seeds in the present study for any given area would depend on the number of cold events during early spring, late summer, or winter and the degree of damage caused to the affected pollen grains, fruits, or overwintering seeds. Based on work to date, it is difficult to assess the relative importance of each negative effect but all three likely contribute to restricting the northern limit of the parasite's distribution.

Other ecological factors may also play an important role in the distribution of *A. americanum* on the landscape of the western Canadian interior. Such factors could include fire, soil type, and topography, which are listed as important factors affecting the distribution of dwarf mistletoes in general (Hawksworth and Wiens 1996). None of these factors, however, has received much attention by researchers. It is unknown whether the current distribution of *A. americanum* is expanding, relatively static, or decreasing, as no paleontological studies have been conducted on *A. americanum* in areas where it infects jack pine. If the parasite's distribution is not static then one of the primary reasons the parasite has not moved farther north and northeast is that there has not been enough time since its introduction onto jack pine following the retreat of the continental glaciers.

These findings have implications for models describing dwarf mistletoe spread rates. Dwarf mistletoe will potentially spread faster in areas where extreme cold temperatures occur infrequently during winter. The amount of *A. americanum* seeds produced within a given infested area does not vary appreciably from year to year (J. Brandt, unpublished data), although the fungal parasite *Caliciopsis arceuthobii* is known to cause local seed reductions (Hawksworth et al. 1977). Outdoors, dwarf mistletoe seeds that do not germinate in the spring following discharge are not known to survive until the second spring (Hawksworth and Wiens 1996), but seeds stored artificially are capable of surviving such lengths of time (J. Brandt, unpublished data). Regardless of the amount of seed present, extreme cold winter spells have the potential to kill all seeds overwintering in a given year. Such events should be incorporated into dwarf mistletoe spread models under development.

No attempt was made in this study to identify the mechanism of freezing injury or survival in overwintering seeds and germinants exposed to extreme cold temperatures. Cell death in plants exposed to freezing temperatures can result from either dehydration due to extracellular freezing of water, or from formation of intracellular ice, which damages cell membranes (Levitt 1980). It is generally accepted that cellular membranes are the primary site of freezing injury (Levitt 1980; Ziegler and Kandler 1980). Plants utilize three strategies to survive freezing: plants can tolerate extracellular freezing, plants can avoid freezing by supercooling, or plants can undergo extraorgan freezing (Sakai and Larcher 1987). Boreal and alpine plants, which are the most low temperature-tolerant species, exhibit extracellular freezing as opposed to supercooling (Becwar et al. 1981; George et al. 1982; DeHayes 1992). Because *A. americanum* likely evolved outside the boreal bioclimatic zone on lodgepole pine at lower elevations in warmer southwestern North America and transferred to jack pine only relatively recently, the parasite's seeds probably utilize a supercooling mechanism. Becwar (1980) detected a low temperature exotherm near –33°C for overwintering seeds collected from lodgepole pine in Colorado and, as a consequence, concluded that *A. americanum* seeds avoid freezing by supercooling. However, Becwar's methods involved taking seeds stored at –2°C and cooling them at a rate of 1°C/minute until the low temperature exotherm was reached. This rate of cooling rarely occurs in nature and has been identified as a factor influencing

results of studies attempting to ascertain the freezing survival strategy used by a plant (Sakai and Larcher 1987). Various studies have demonstrated that if the rate of cooling is slowed to a rate similar to that which occurs in nature, low temperature exotherms can disappear or they can occur at a much lower temperature (Ishikawa and Sakai 1981,1982; Gusta et al. 1983). Plants utilizing supercooling are restricted to areas where temperatures do not fall substantially below –38°C (Burke et al. 1976; Becwar et al. 1981; George and Burke 1981; George et al. 1982). As indicated earlier in the discussion, temperatures in the northern parts of the Canadian prairie provinces commonly fall to –38°C or colder. Based on experiments of this study, dwarf mistletoe seeds collected from jack pine can survive temperatures between –38°C and –46°C for at least 48 hr but no more than 96 hr. This is colder than the low temperature exotherm found by Becwar (1980) but still within the limits of supercooling. Extraorgan freezing, however, cannot be dismissed as the strategy used by dwarf mistletoe seeds either. The freezing limits of some plants utilizing this strategy generally fall between –35°C and –50°C as well (Sakai and Larcher 1987). Obviously, further study is warranted to clarify the freezing survival strategy utilized by *A. americanum* seeds.

There appears to be a trend in germination rates of seeds from the different seed sources along the north-south gradient with increasing seed cold hardiness northward (Fig. 5.2), but the form of this trend is inconclusive. In Experiment 2, the linear trend was not significant, although a *P*-value of 0.051 suggests a possible relationship. The lack of a significant trend in this experiment could be due to the relatively warm treatment temperatures, which

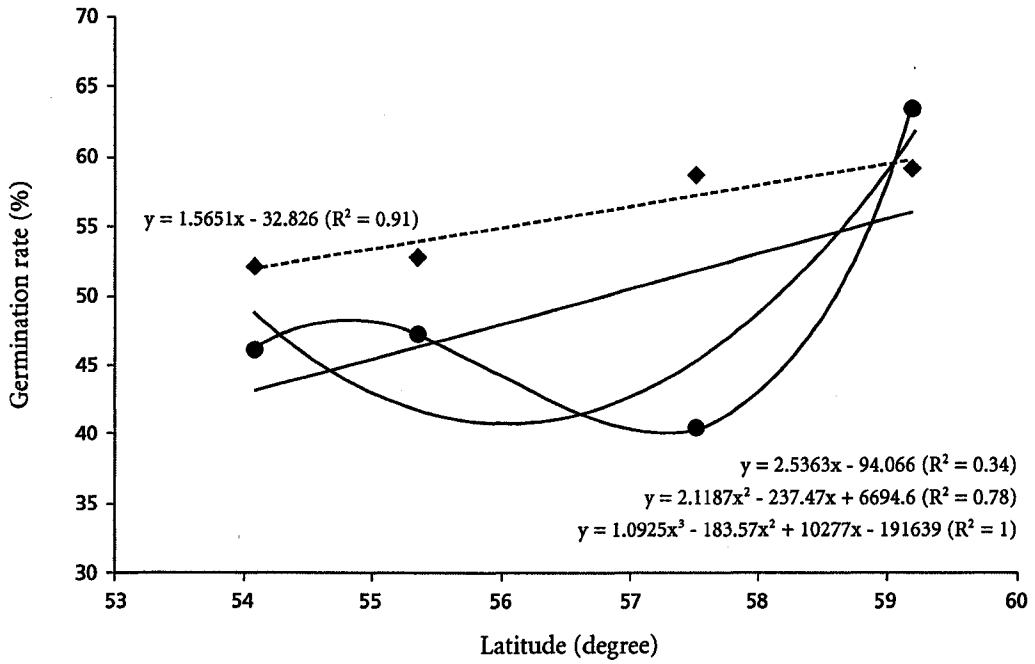

Figure 5.2 Trends in mean germination rates in relation to latitude of four *Arceuthobium americanum* seed sources. The linear trend is depicted for the pooled results of Experiment 2 (shown in dashed black), in which overwintering seeds were exposed to three temperatures for 144 hr (control, –30, –34°C). The linear, quadratic, and cubic trends are depicted for the pooled results of the control and the 48 hr exposure to –37°C treatment in Experiment 3 (shown in solid black).

had no impact on germination rates in comparison to the control. In Experiment 3, the linear, quadratic, and cubic trends were all significant, suggesting that a linear trend exists but other factors may be responsible for a deviation from linearity. Germination performance and seed size within a seed source varied between years for the seeds collected in 2000 and 2001 based on a series of germination tests conducted at 30-day intervals on seeds in long-term storage at 2°C (J. Brandt, unpublished data). Results of tests on the 2001 seeds support a linear north-south trend in germination rates while results of tests on the 2000 seeds do not.

Germinants of *A. americanum* were hardy to simulated spring frosts over the range of temperatures tested, –1 to –6°C for 3 hr. This result was unexpected. Many plants have very little resistance to the cold of episodic spring frosts when in an active vegetative state (Sakai and Larcher 1987). Consequently, such frosts generally present the greatest freeze threat to plants and affect the regional and microtopographic distribution of herbaceous and woody plants (Sakai and Larcher 1987). The range of freezing temperatures tested in this study are consistent with those that have occurred historically within the region during the months of June and July (Table 5.5) when dwarf mistletoe seeds would be germinating *in situ*. It is also important to note that the recorded temperatures from the meteorological stations are usually measured in treeless areas close to the ground (usually 1.5 m) and are more extreme than air temperatures within tree crowns in a closed or partially closed canopy (Oke 1978). Most germinating dwarf mistletoe seeds occur within crowns of jack pine trees.

There are several avenues of research worth pursuing that have been alluded to in this discussion. One avenue would be to develop a model to predict the effects of temperature and exposure period on germination rates of overwintering dwarf mistletoe seeds. Such a model could then be used in a spatial analysis of the distribution of the host, the parasite, and climate patterns to assess areas of jack pine at risk. This spatial analysis and model could also be used to assess the impact of the parasite under different climate-change scenarios. Research aimed at identifying the mechanism of freezing survival in dwarf mistletoe seeds and germinants would also be beneficial. Another avenue would be to more clearly define the trend in germination rates of seeds along a north-south gradient following exposure to a given extreme cold treatment, including temperature-exposure period cycling. Assessing differences in the freezing threshold of seeds from different locations among and within the three genetic races within *A. americanum* would be important to evaluate as well.

Large areas of jack pine forests in the Northwest Territories and northern Saskatchewan and Manitoba are presently free of this devastating parasite. Pine in these areas, however, is not being utilized to a great extent by the forest industry. This will likely change in the future if wood supply shortages occur or the value of the fibre resource increases. The distribution of the parasite may also change in response to a warming climate if the frequency of prolonged, extreme cold spells during winter decreases. Such situations have implications on the utilization and management of these northern pine forests.

SUMMARY

Lodgepole pine dwarf mistletoe is one of the most damaging parasites of jack pine in western Canada. Jack pine forests in the colder, more northerly areas, however, are free of dwarf mistletoe, suggesting the parasite is limited by low temperature. The effect of extreme cold temperatures on germination rates of overwintering dwarf mistletoe seeds and survival of dwarf mistletoe germinants was evaluated. Germinative ability of overwintering seeds increased with increasing simulated winter temperatures between –39 to –35°C, regardless of seed source. Exposure period also strongly influenced germination rates. Exposure to temperatures near –38, –46, or –53°C for 96 or 144 hr was almost always lethal. At –37°C, germination was greater after 48 hr than after 96 hr, although it was still significantly lower than the controls. Temperatures down to –6°C in late spring did not reduce germinant survival. Overall, these results may explain dwarf mistletoe's absence from northern areas commonly exposed to periods in winter with minimum temperatures below about –40°C. These areas are potentially at risk from the parasite if the climate of Canada's northern interior continues to warm as it has over the last several decades.

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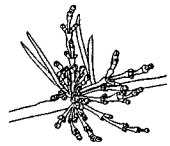

CHAPTER SIX

Life cycle of *Arceuthobium americanum* on jack pine (*Pinus banksiana*)

INTRODUCTION

Lodgepole pine dwarf mistletoe (*Arceuthobium americanum*) is distributed throughout much of western North America where it occurs on its two principal hosts, jack pine (*P. banksiana*) and lodgepole pine (*Pinus contorta* var. *latifolia*, *P. contorta* var. *murrayana*) (Hawksworth and Wiens 1996). The northern limit of *A. americanum* occurs on jack pine in Alberta near 59° N, (Brandt et al. 1998). The southern limit occurs on lodgepole pine in California near 35° N (Hawksworth and Johnson 1989). Other than decay organisms, *A. americanum* is the most important pest of pine in the western Canadian interior. Regional estimates of annual growth losses for dwarf mistletoe-infected jack pine growing in the three prairie provinces are 314 000 m^3, while mortality losses are 1 478 000 m^3 (Brandt et al. 1998). Annual growth losses for dwarf mistletoe-infected lodgepole pine in Alberta and Saskatchewan are 486 000 m^3, while mortality losses are 69 000 m^3 (Brandt et al. 1998).

Most of our knowledge of the biology of *A. americanum* is based on the work of Hawksworth and his colleagues working on populations of the parasite infecting *P. contorta* var. *latifolia* in Colorado (Hawksworth 1965; Hawksworth and Johnson 1989; Hawksworth and Wiens 1996). Figure 1.3 illustrates the generalized life cycle for *A. americanum* infecting *P. contorta* var. *latifolia* based on this work. This illustration may not be entirely accurate, however, because there are discrepancies in the literature as to timing of shoot emergence and length of the life cycle. Hawksworth and Wiens (1996), citing Hawksworth and Johnson (1989), reported that about two-thirds of *A. americanum* plants produced shoots 3–4 years after "infection". Hawksworth and Johnson (1989), however, reported that about two-thirds of plants produced shoots 3–4 years after "seed dispersal" and that production of first shoots ranged from 2 to 8 years. Only two percent of infections resulted in production of shoots two years after seed dispersal (Hawksworth and Johnson 1989). This apparent discrepancy between the two reports in timing of shoot emergence is important. On average, time from seed dispersal to flowering required a minimum of six years (Hawksworth and Johnson 1989). Hawksworth and Wiens (1996), however, reported that minimum time from infection to initial seed production averaged six years. Because mature fruit are usually produced 15–17 months after flowers are pollinated (Hawksworth and Johnson 1989) and infection occurs at least 11-21 months after seed dispersal (Chapter 4), it appears that *A. americanum* requires at least seven years to complete its life cycle on lodgepole pine from seed dispersal to development of mature seeds, which is one year longer than illustrated.

In addition to the apparent discrepancies in the life cycle of *A. americanum* on lodgepole pine, no one has examined this parasite's life cycle on jack pine, presumably because researchers and for-

est managers have assumed that it would the same or similar to that on lodgepole pine. This assumption requires scrutiny because *A. americanum* on jack pine in the western Canadian interior is a distinct genetic race from two other races found on *Pinus contorta* var. *latifolia* and on *P. contorta* var. *murrayana* in western North America (Jerome and Ford 2002). In order to address these uncertainties, the objective of this study was to determine the life cycle of *A. americanum* on jack pine based on inoculations conducted outdoors.

MATERIALS AND METHODS

Study site

Host material for the study were young, 4–6 m high jack pine trees planted in 1989 in a small plantation (<1 ha) located on the grounds of the Northern Forestry Center (53° 30'N, 113° 35'W, 669 m above m.s.l.) in Edmonton, Alberta. Source seeds for the trees were from several provenances in Manitoba. Jack pine trees were dwarf mistletoe-free prior to inoculations. The soil at the site is a black chernozem derived from lacustrine parent material. Site surface expression is nearly level.

Inoculations

In 1998, *A. americanum* seeds were collected from pistillate plants infecting jack pine north of Bruderheim (53° 52'N, 112° 57'W, 625 m above m.s.l.) using paper bags as described by Scharpf and Parmeter (1962) and kept at 2°C until seeds were required for inoculations. In 1999, seeds were collected from southeast of Smoky Lake (54° 05'N, 112° 20'W, 610 m above m.s.l.) and stored as described in Chapter 2. In early October 1998 and 1999, jack pine trees were inoculated with *A. americanum* seeds by wetting seeds with water and placing the radicular end of individual seeds at bases of needles where they attached to twigs. In 1998, three branches on each of 25 trees selected at random were inoculated with one seed per branch; in 1999, four branches on each of 25 trees selected at random were inoculated. Branches were flagged and marked with a grease pencil 2–3 cm from the inoculated seed. Inoculants or the points of inoculation (in cases where seeds or germinants were displaced or died) were visually monitored every two weeks for the following two growing seasons and every four weeks for subsequent growing seasons until dwarf mistletoe plants that developed from these inoculations flowered (staminate plants) or produced mature fruit (pistillate plants). At each assessment, developmental stage and symptom of infection were recorded for each inoculation. For mature pistillate plants, the number of fruit produced in each year were counted at the end of July prior to seed discharge. Once pistillate plants produced seeds they were monitored annually at the end of July for seed production.

Climate data

Daily temperature and precipitation data from the Edmonton Municipal Airport (10 km north, 671 m above m.s.l.) and Edmonton International Airport (21 km south, 715 m above m.s.l.) meteorological stations were obtained for the period 1 October 1998 through 30 September 2003. Mean daily temperature (°C), total growing degree days (≥5°C; GDD), and total precipitation (mm) were averaged for the two stations for each month of the study period (Table 6.1). The number of GDD required from the first March after inoculation to initial emergence of aerial shoots was determined for each inoculation that resulted in infection of the host.

TABLE 6.1 **Interpolated monthly mean daily temperature (°C), total growing degree days (base temperature of 5°C), and total precipitation (mm), respectively, between October 1998 and September 2003 for the study site (source data: Environment Canada).**

Month	1998-99	1999-00	2000-01	2001-02	2002-03
October	6.1	5.4	4.6	3.1	1.3
	71.1	50.3	35.5	25.9	16.7
	29.0	11.5	4.3	20.7	24.1
November	−5.4	−2.3	−4.5	−1.5	−0.6
	0.0	0.0	0.0	4.6	1.5
	27.1	12.9	7.9	14.9	12.6
December	−11.4	−3.3	−13.2	−11.0	−5.8
	0.0	3.0	0.0	0.0	0.0
	18.3	10.2	10.0	4.6	9.9
January	−13.6	−13.2	−3.7	−10.6	−12.0
	0.0	0.0	0.0	0.0	0.0
	45.4	22.9	1.9	7.3	37.5
February	−7.9	−9.1	−11.5	−5.4	−9.4
	0.0	0.0	0.0	0.0	0.0
	5.3	9.3	5.6	7.0	24.3
March	−4.3	−2.4	−1.1	−13.2	−8.1
	0.0	3.2	1.2	0.0	0.8
	10.5	22.1	9.0	25.2	24.9
April	5.7	4.3	5.0	−1.2	4.0
	59.9	61.2	62.8	10.1	53.5
	27.4	23.4	4.2	33.3	48.9
May	9.3	9.3	11.8	9.1	9.8
	136.3	134.6	211.4	150.7	178.8
	100.5	62.8	31.8	8.1	50.6
June	13.9	13.8	13.8	16.5	14.2
	267.7	262.7	262.6	343.7	276.0
	48.8	88.6	71.1	18.8	50.9
July	15.1	17.2	16.8	18.4	17.8
	312.3	375.6	365.1	414.8	396.5
	96.0	96.6	168.6	54.8	70.7
August	16.9	15.0	17.0	15.0	17.3
	368.2	309.2	372.0	308.6	381.6
	57.2	38.9	28.3	45.2	43.7
September	10.8	10.4	11.9	9.9	10.6
	184.5	171.5	206.5	152.2	177.3
	13.8	47.6	26.0	11.8	17.7

Note: *Individual values for mean daily temperature and total precipitation (mm) represent the average of these parameters recorded at the Edmonton Municipal Airport and Edmonton International Airport meteorological stations. Growing degrees days were calculated using the average daily maximum and minimum temperatures at the same stations according to the methods of Environment Canada (1993).*

RESULTS

Based on the 75 inoculations conducted in 1998, 91% of the inoculated seeds overwintered on their host at the point of inoculation. Fifty-six percent of the overwintered seeds germinated, from late April to early June 1999 but predominantly in May. Of these germinants, 63% developed holdfasts. Sixty-three percent of the germinants that developed holdfasts infected their host and produced aerial shoots. All but two of these developing dwarf mistletoe plants produced shoots during August and September 2000. One slow-developing pistillate plant produced shoots in May 2001; the other was a staminate plant that produced shoots in May 2002. Of the 15 plants that developed from the 1998 inoculations, 12 were pistillate plants, two were staminate plants, and one plant died before its sex could be determined because the host branch died. Ten of the 12 pistillate plants produced seed in 2003, the fifth year of their life cycle. Potentially, one of the other two pistillate plants will not produce seed until 2004 because most of its shoots died in 2002; the other plant was on a branch that died before the dwarf mistletoe plant could produce seed. The three staminate plants flowered during April and May 2003. Figure 6.1 provides a photographic chronosequence of stages in the life cycle of a pistillate *A. americanum* plant on jack pine arising from a single inoculation in 1998 while Fig. 6.2 provides analogous photographs for a staminate plant.

Based on the 100 inoculations conducted in 1999, 92% of the inoculated seeds overwintered on their host at the point of inoculation. Eighty-three percent of the overwintered seeds germinated, occurring mostly in May 2000. Seventy-five percent of these germinants developed holdfasts. Seventy-two percent of the germinants that developed holdfasts infected their host and produced aerial shoots. Sixty-eight percent of these developing dwarf mistletoe plants produced shoots between June and October 2001, 24% of the plants produced shoots in 2002, and the remainder produced shoots in 2003. Of the 41 plants that developed from the 1999 inoculations, 20 were pistillate plants, 13 were staminate plants, three were sexually immature at the end of the 2003 growing season, and the sex of five plants could not be determined because their immature shoots died and cast, presumably infected by *Colletotrichum gloeosporioides*. One pistillate plant produced fruit in 2003, the fourth year of its life cycle, while this plant and 13 others were fertilized in 2003 and will produce seed in 2004. Nine of the 13 staminate plants flowered in May 2003 while the remaining four staminate plants have flower buds and should flower during Spring 2004.

The ten mature pistillate plants from the 1998 inoculations produced 425 seeds in total in 2003, their first seed production year. Seed production per plant averaged 43 but ranged from 4–210. These same ten plants produced 1384 seeds in their second seed production year, with an average of 138 seeds per plant and a range of 20–279. The one pistillate plant that should produce seed in 2004 had 11 immature seeds as of October 2003.

The one pistillate plant from the 1999 inoculations that completed its life cycle in 4 years produced two seeds in 2003. This plant and the 13 other pistillate plants that should produce seed in 2004 had a total of 1207 immature seeds as of October 2003. Seed production per plant will average about 81 but will range from 5–228.

For the infections arising from the 1998 inoculations, 2682±467 (SD) GDD were required for initial emergence of aerial shoots, which was not significantly different than the 2661±907 (SD) GDD for the 1999 inoculations (t-test, α=0.05). The overall mean for emergence of shoots was 2666±808 GDD.

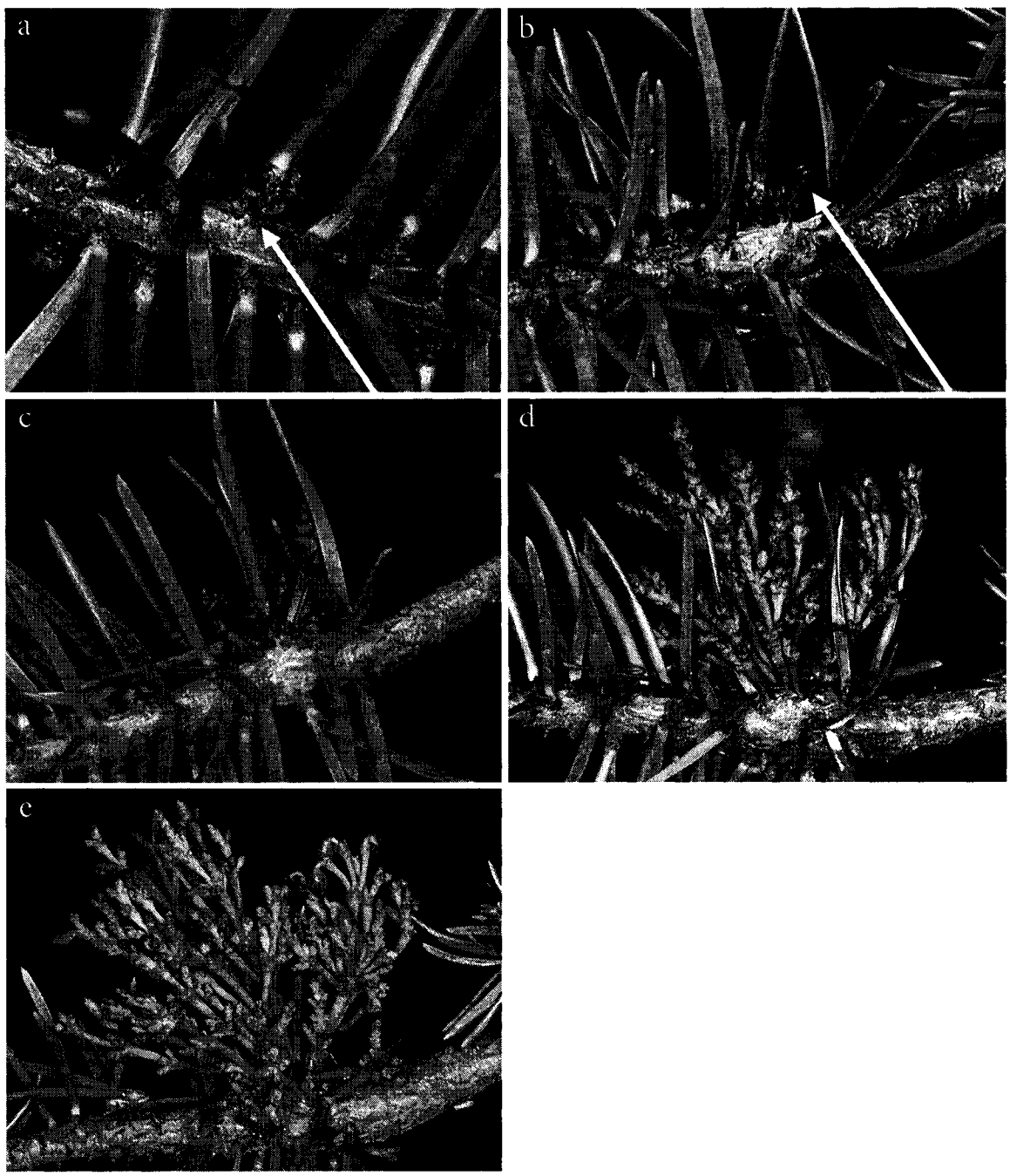

FIGURE 6.1 Photographic chronosequence of stages in the life cycle of a pistillate *Arceuthobium americanum* plant on jack pine arising from a single inoculation in October 1998. (a) Germinant penetrating host in July 2000. Localized swelling at point of infection is evident. Arrow indicates a small patch of reddish tissue where aerial shoots are beginning to emerge from host. (b) Aerial shoots in June 2001. (c) Aerial shoots in August 2001. (d) Mature pistillate plant in October 2002 with developing fruit resulting from pollination the previous May. (e) Pistillate plant with mature and immature fruit in August 2003.

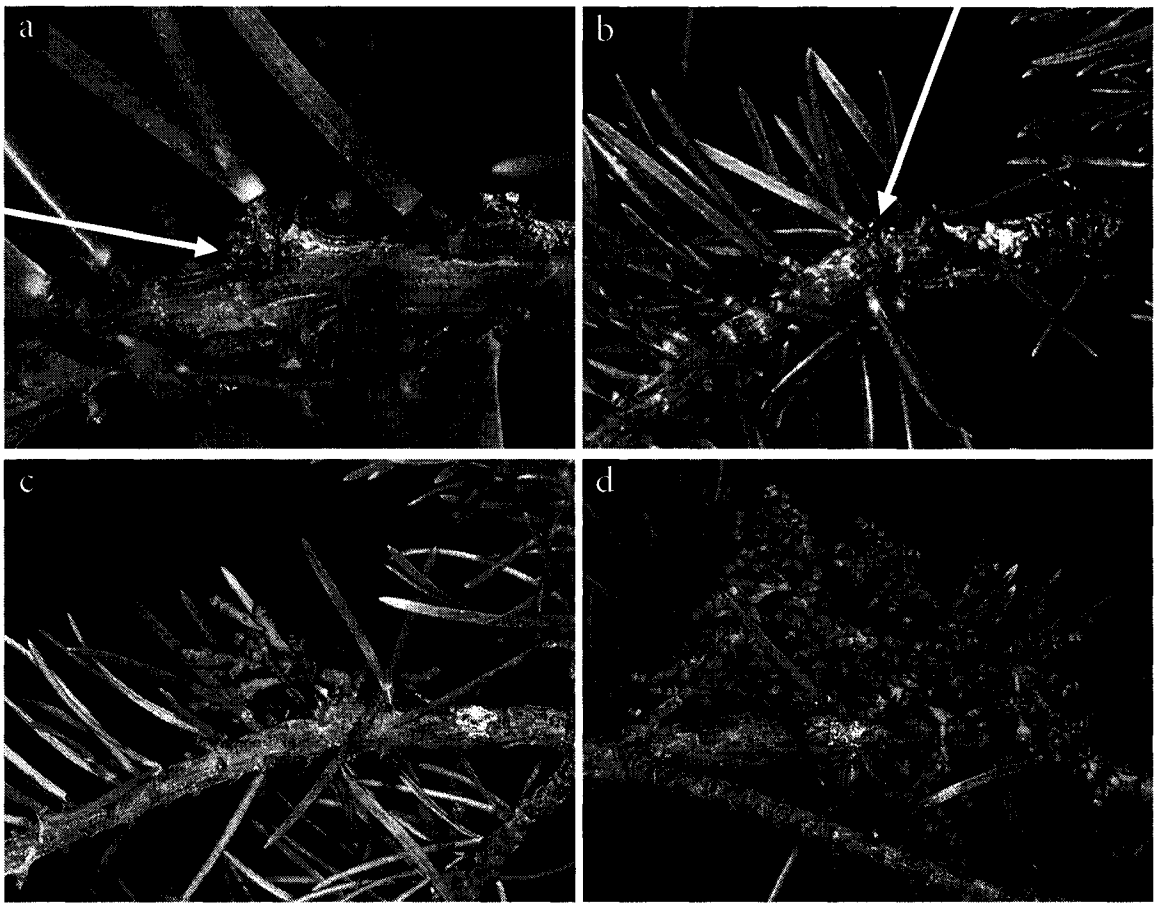

FIGURE 6.2 Photographic chronosequence of stages in the life cycle of a staminate *Arceuthobium americanum* plant on jack pine arising from a single inoculation in October 1998. (a) Localized swelling at point of infection in July 2000. Arrow indicates the desiccated seed, radicle and holdfast. (b) Aerial shoots emerging from host twig in August 2001. (c) Mature plant with staminate flower buds in October 2002. (d) Plant in August 2003.

DISCUSSION

Arceuthobium americanum on plantation jack pine growing in Edmonton requires five years on average to complete its life cycle, illustrated in Fig. 6.3. This is in contrast to the presumed seven years for *A. americanum* on lodgepole pine in Colorado as reported by Hawksworth and Wiens (1972, 1996) and Hawksworth and Johnson (1989). Penetration and infection of jack pine by *A. americanum* germinants usually required more than one growing season (Chapter 4). This is considerably longer than the 2–3 months reported in the literature previously for *Arceuthobium* spp. (Scharpf 1963; Scharpf and Parmeter 1967; Knutson 1983). However, the incubation period (from infection to emergence of aerial shoots) is considerably shorter for *A. americanum* on jack pine than it is on lodgepole pine. Hawksworth and Wiens (1996), citing Hawksworth and Johnson (1989), reported that about two-thirds of *A. americanum* plants produced shoots 3–4 yr after infection. This is likely an error because Hawksworth and Johnson (1989) reported that about two-thirds of plants produced shoots 3–4 yr after seed dispersal but initial shoot production ranged from 2 to 8 yr. Based on both inoculation years in this study, 73% of the *A. americanum* plants produced shoots less than 2 yr after seed dispersal, 20% required 2–3 yr after seed dispersal, and the remaining 7% produced shoots 3–4 yr after seed dispersal. An extended incubation period for *A. americanum* on jack pine as reported for the same dwarf mistletoe species on lodgepole pine was not observed in this study. The time required from emergence of aerial shoots to fruit maturation and seed dispersal (Fig. 6.3) in this study is the same as previously reported (Fig.1.3, Hawksworth and Wiens 1972, 1996).

There are several possible reasons that could explain the shorter life cycle of *A. americanum* on jack pine in Alberta compared with that on lodgepole pine in Colorado, among them differences in host resistance, parasite virulence, and climate of the study sites. *Arceuthobium americanum* presumably transferred to jack pine only relatively recently, perhaps in the last 15 000–10 000 years B.P. after the end of the Wisconsin glaciation (Yeatman 1967). With the retreat of the continental glaciers, jack pine moved north and west from refugia in central and eastern North America and made contact with lodgepole pine as it advanced from areas south of the glacial maximum in western North America (Critchfield 1985). Thus, *A. americanum* on jack pine represents a relatively new pathosystem, one in which the host has had little time to develop resistance to the parasite. In contrast, the *A. americanum*–lodgepole pine pathosystem is relatively old because the two species co-evolved in southwestern North America (Hawksworth and Wiens 1996). The race of *A. americanum* on jack pine could also be more virulent than the race on lodgepole pine and, therefore, more capable of infecting its host and developing faster. Temperature and precipitation are important to the phenology and development of plants and differences in these two parameters between the Edmonton and Colorado study sites could account for the shorter life cycle on jack pine.

It appears that the basis for much of the life cycle information for *A. americanum* on lodgepole pine was from inoculations conducted on *P. contorta* var. *latifolia* in Roosevelt National Forest, Colorado (Hawksworth and Johnson 1989). Unfortunately, study site information was not published. According to B.W. Geils (personal communication, November 2003), the inoculation site was near Hawksworth's (1965) Lone Pine Creek study site, which is on a plateau at an elevation of 2804 m above m.s.l. and 6.4 km west of Red Feather Lakes (40° 47'N, 105° 33'W). A comparison of monthly normals reveals that the annual growing degree days at the Red Feather Lakes meteorological station (Table 6.2; 2489 m above m.s.l.) were 14% less than

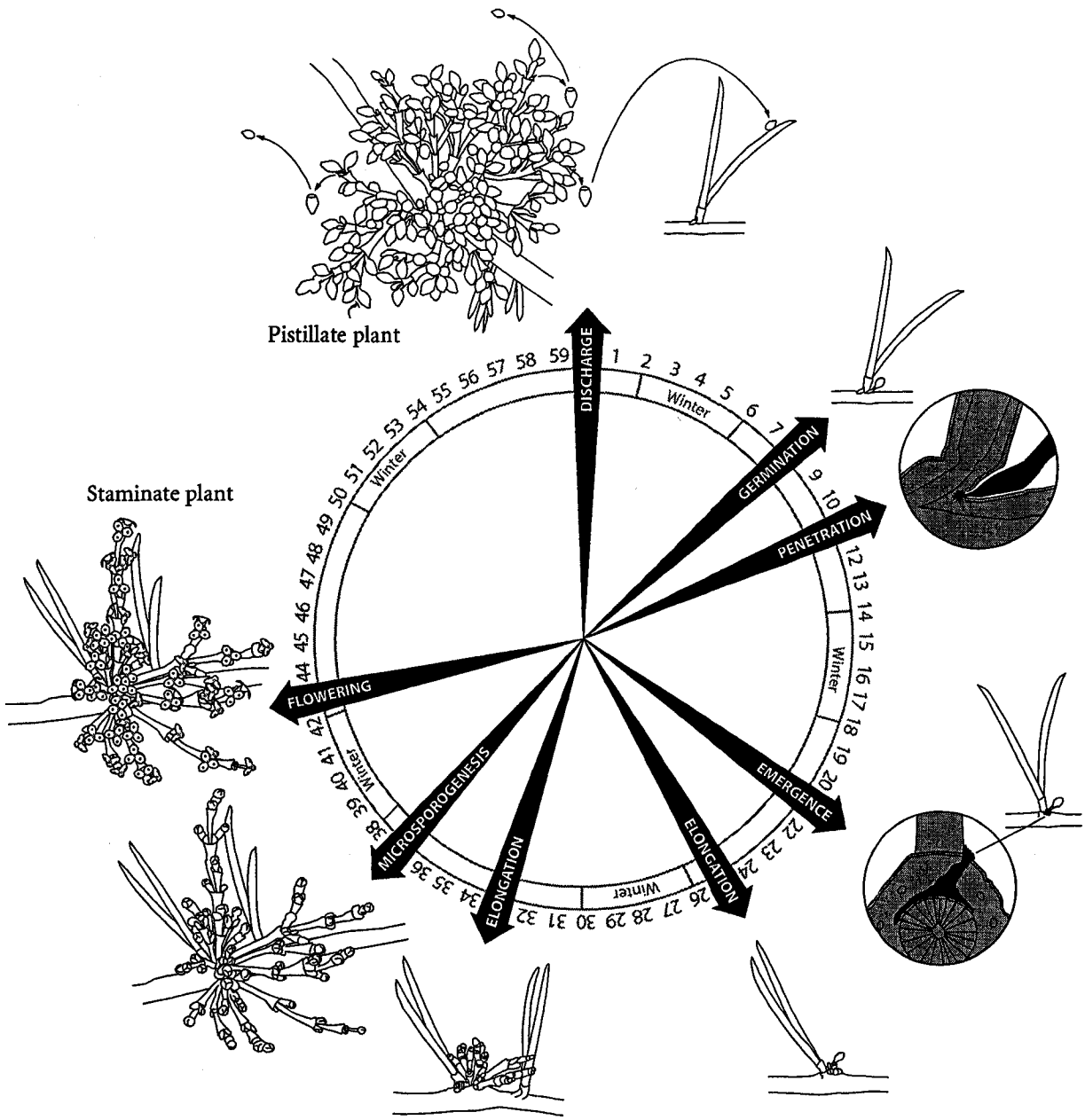

FIGURE 6.3 Generalized life cycle of *Arceuthobium americanum* infecting *Pinus banksiana* in Alberta.

Month 0: Discharged seed lands on needle. Seed slides down the needle to the twig using the seed's wetted viscin as a lubricant during fall and winter. Dried viscin acts as an adhesive. **Month 8:** Most seeds germinate in May (range: April–June). About one month later a holdfast has developed at the radicle tip where it has been obstructed. **Month 11:** Host penetration has begun by August. **Months 21-22:** First symptom of infection is a swelling of the twig at the point of penetration. Aerial shoots emerge about one month later, usually near the holdfast (if holdfast is still attached). The parasite's endophytic system is well-established. **Month 25:** Shoots have elongated several millimetres by end of second growing season. **Months 33-37:** During third growing season, shoots continue to elongate and, by end of season, shoots of both sexes are mature. **Months 43-44:** Pistillate and staminate plants flower during April–May and pollination occurs. **Month 60:** Fruit are mature on pistillate plants about 16 months after pollination. These fruit fall from the plant in August and September and discharge their seeds.

TABLE 6.2 **Monthly normals for the period 1961–1990 for Red Feather Lakes (40° 47'N, 105° 33'W), Colorado[a] (plain type), and Edmonton[b] (bold).**

Climate parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Daily max. temperature (°C)	1.0	2.2	4.2	8.6	13.9	20.2	24.2	22.8	18.2	12.6	5.6	1.4	11.2
	–8.5	**–4.7**	**0.6**	**10.2**	**17.5**	**21.1**	**22.8**	**22.0**	**16.6**	**11.3**	**–0.3**	**–6.9**	**8.5**
Daily min. temperature (°C)	–12.7	–11.9	–10.1	–5.4	–0.6	3.4	6.6	5.6	1.2	–3.0	–8.8	–12.2	–4.0
	–18.4	**–15.2**	**–9.8**	**–1.7**	**4.5**	**8.7**	**10.7**	**9.6**	**4.4**	**–0.8**	**–9.8**	**–16.3**	**–2.8**
Daily mean temperature (°C)	–5.8	–4.9	–2.9	1.6	6.7	11.8	15.4	14.2	9.7	4.8	–1.6	–5.4	3.6
	–13.4	**–9.9**	**–4.5**	**4.3**	**11.0**	**14.9**	**16.8**	**15.8**	**10.5**	**5.3**	**–5.0**	**–11.4**	**2.9**
Growing degree days (5°C)	1	1	5	22	94	221	337	301	175	67	7	2	1233
	0	**0**	**3**	**50**	**190**	**297**	**365**	**335**	**174**	**66**	**3**	**0**	**1483**
Total precipitation (mm)	14.2	16.0	32.5	41.7	54.6	47.8	59.2	46.7	37.6	25.7	18.8	14.0	408.7
	23.1	**16.2**	**16.5**	**22.0**	**43.2**	**78.0**	**97.7**	**68.3**	**44.6**	**17.5**	**16.1**	**20.7**	**463.9**

[a] *All climate data except growing degree days are the National Climatic Data Center (NCDC) normals available from the Western Regional Climate Center (Reno, Nevada, http: //www.wrcc.dri.edu). Growing degree days (base temperature of 5°C) were calculated according to the methods of Environment Canada (1993) using 1961–1990 daily temperature data for Red Feather Lakes obtained from the Colorado Climate Center.*

[b] *Individual values for the various climate parameters listed for Edmonton represent the average of these parameters recorded at the Edmonton Municipal Airport and Edmonton International Airport meteorological stations. Source data from Environment Canada.*

at Edmonton during the five years of this study (Table 6.1; October 1998 to September 2003). Also, Hawksworth's inoculation site near Lone Pine Creek being at a higher elevation, presumably had a colder growing-season climate than the lower, nearby meteorological station. During October 1998 to September 2003, annual growing degree days (range: 1371–1517 GDD) were not substantially different than Edmonton's 30-yr normals (1483 GDD). Annual precipitation was higher at Edmonton than at Red Feather Lakes (464 mm versus 409 mm), particularly during the months of June, July, and August (244 mm versus 154 mm). Because Red Feather Lakes meteorological station is a few kilometres away and at lower elevation than Hawksworth's site, it is not possible to make a direct comparison of climates between this study's site and Hawksworth's site. However, because of the differences in climate between this study's site and Red Feather Lakes (compare Tables 6.1 and 6.2), at least some of the differences in the length of the life cycle on the two hosts are likely due to differences in climate between the sites.

In the prairie provinces, damage severity on jack pine due to *A. americanum* is generally greater than that observed on lodgepole pine (Hiratsuka 1987; Brandt 1995; Brandt et al. 1998), with volume losses due to growth reductions and tree mortality of 53–70% in jack pine stands in Manitoba (Baker et al. 1992) compared with 18–32% reduction in growth and 0–26% loss due to mortality in lodgepole pine stands in Alberta (Baranyay and Safranik 1970). With a shorter life cycle on jack pine, the ability of the parasite to intensify infections within the host are enhanced, including vertical rate of spread. Studies have suggested that most trees escape severe damage if rate of height growth of the host exceeds vertical rate of spread of the dwarf mistletoe, restricting the parasite to the lower crown (Childs 1963; Richardson and van der Kamp 1972; Scharpf and Parmeter 1976; Parmeter 1978; Roth and Barrett 1985). Vertical rate of spread has not been studied for *A. americanum*. Horizontal rate of spread to nearby host trees is also potentially greater than that reported for the parasite on lodgepole pine; this has implications on spread models under development and buffer zones used to prevent or slow the advance of the parasite from infested stands into adjacent regenerating stands. Horizontal rate of spread for *A. americanum* in lodgepole pine stands is about 0.3–0.5m/year (Hawksworth 1958). Recommended buffer zone widths in jack pine stands where *A. americanum* is a serious pest are 30 m in Alberta and 20 m in Saskatchewan and Manitoba (personal communications: H. Ono, 25 November 2003; K. Knowles, 7 November 2003; R. MacIntosh, 5 January 2004). In buffer zones where jack pine regenerates naturally, *A. americanum* could spread through these zones quicker than what would be expected based on spread rates for the parasite in lodgepole pine.

There is no information in the literature regarding seed production in *A. americanum*. Average initial seed production of 62 seeds/plant for both inoculation years was not high. However, a few plants resulting from both inoculation years produced over 200 seeds in their first production year. Also, seed production of plants from the 1998 inoculations tripled between their first and second seed production years. Based on observations at several locations in Alberta, the range in number of seeds/plant from naturally occurring pistillate plants arising from anisophasic infections in severely infested jack pine stands is similar to the range observed in this study (J. Brandt, unpublished data). However, I have occasionally observed isophasic infections causing large brooms in jack pine and more than a thousand fruits on pistillate shoots throughout the broom.

Jerome and Ford (2002) identified three races of *A. americanum*, one each on *P. banksiana*, *P. contorta* var. *latifolia*, and *P. contorta* var. *murrayana*. They speculated that the degree of speciation in the parasite may be greater than race-level alone and

suggested further study. The findings of this study provide additional evidence of differences between at least two of the races, the one on *P. banksiana* and the one on *P. contorta* var. *latifolia*. While results of this study provide strong evidence for a shortened life cycle for *A. americanum* on jack pine compared with that for lodgepole pine, more detailed studies examining a wider range of jack pine sites and different *A. americanum* seed sources are needed. Other aspects of the parasite's life cycle, such as flowering and seed dispersal phenology, might also be reviewed in light of this study's findings and those of Jerome and Ford (2002). A direct comparison of developmental times for *A. americanum* on both jack and lodgepole pine and natural hybrids of these genotypes at the same site would be valuable in order to provide a definitive answer to the question of whether differences between the two pathosystems are related to the host, the parasite, or environment.

SUMMARY

While *Arceuthobium americanum* is one of the most damaging pests of jack pine in western Canada, no studies have examined the life cycle of the parasite in this pathosystem. Twenty-five jack pine trees in a plantation in Edmonton were inoculated with seeds of *A. americanum* in the fall of 1998 and 1999 and then these inoculants were monitored until the dwarf mistletoe plants that arose completed their life cycle. Ninety-two percent of inoculated seeds overwintered on their host. Seventy percent of these seeds germinated, mostly in May. Of these germinants, 69% developed holdfasts. Sixty-eight percent of germinants that developed holdfasts infected their host and produced shoots, primarily between July and August in the second season after inoculation. Of the 56 plants that developed from the 175 inoculations, 32 were pistillate plants, 15 were staminate plants, six died because the host branch died, and three were sexually immature as of October 2003. Most pistillate and staminate plants flowered in the fourth year after inoculation. Pistillate plants produced seed in the fifth year, although one plant produced seed in the fourth year. Thus, *A. americanum* has a five-year life cycle on plantation jack pine, which has implications on the management of dwarf mistletoe-infested jack pine stands in western Canada.

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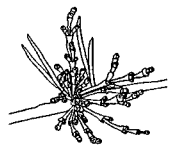

CHAPTER SEVEN

Discussion, conclusions, and future research

The primary goal at the outset of these studies was to begin to develop an understanding of the influences of some of the factors affecting the distribution of *A. americanum* on the landscape of the western Canadian interior. Before long a second goal was added: to understand the life cycle of *A. americanum* on jack pine, one of the two principal hosts of the parasite. These goals have been achieved based on the significant findings reported in this dissertation.

First, germination rates of *A. americanum* seeds were reduced to almost zero when temperatures reached –38°C or colder for 96 hr or more. Thus, an extreme cold temperature threshold exists for overwintering seeds collected from *A. americanum* plants infecting jack pine. This suggests that this factor plays an important role in the current distribution of the parasite in the western Canadian interior and could be sufficient to explain its absence on jack pine from northern areas that are commonly exposed to prolonged winter cold spells with temperatures below about –40°C. Second, *A. americanum* germinants were hardy to simulated spring frosts over the range of temperatures tested, –1 to –6°C for 3 hr. Both of these findings were consistent across the four populations ranging from 54 to 59° N in Alberta where seeds were collected.

Another significant finding is that the establishment phase of *A. americanum* on jack pine is much longer than what has been reported in the literature for the parasite on lodgepole pine and for other dwarf mistletoe species in general. Based on the histological observations of this study, penetration of the host did not begin until as late as August and penetration was not complete, in most cases, until the spring of the next growing season. Most infections resulted from *A. americanum* germinants that survived the first winter after germination while few infections resulted from germinants that withered during the first growing season. The penetration and infection processes entail about 12–13 months.

The shorter life cycle for *A. americanum* on jack pine compared to what is reported in the literature for lodgepole pine is another significant finding. Although several factors, among them climate, parasite virulence, and host resistence or susceptibility, could be working independently or in concert to affect the developmental time, the fact remains that the life cycle of the parasite on jack pine is 28% shorter than what is described for the *A. americanum*–lodgepole pine pathosystem. The shorter generation time for the parasite in this pathosystem is mainly due to the brief period between infection and emergence of aerial shoots.

Two new and valuable techniques have also been tested and documented: stockinet as an effective seed collection material, and hydroxypropylcellulose as an adhesive for seed placement during artificial inoculation of jack pine seedlings. Both should be of value to other researchers working on *Arceuthobium* spp. or other mistletoes.

The significant findings reported will have an impact on management of this important pest of jack pine. Many of the tools and techniques used for management of the parasite on jack pine are based on data from the *A. americanum*–lodgepole pine pathosystem. Because *A. americanum* in infested jack pine stands is usually managed using sanitizing clear-cuts, spread models for the parasite and

buffer-zone widths between regenerating stands and dwarf mistletoe-infected residuals should take the findings of this study into consideration. Vertical and horizontal rates of spread are likely higher in the *A. americanum*–jack pine pathosystem than in the *A. americanum*–lodgepole pine pathosystem and should be determined so that these can be incorporated into spread models under development. The influence of climate must also be factored into spread models because spread rates will be affected by winter temperatures.

There are several unique features about the distribution of *A. americanum* on jack pine that are noteworthy and raise interesting questions about factors affecting the occurrence of the parasite as well as the level of speciation among the races of parasite. First, while common throughout jack pine stands south of the Canadian Shield, the distribution of *A. americanum* does not extend far beyond this boundary, which runs in a north-west to south-east direction across Alberta, Saskatchewan, and Manitoba. The host, jack pine, is abundant over much of the Shield on mesic to xeric sites. There is an air temperature gradient across this boundary, and the findings of this study suggest that this factor, at least in part, influences the distribution of *A. americanum*. There are also differences in soil types between the Canadian Shield and the plains of the western Canadian interior. The Canadian Shield is an area of shallow soils and exposed bedrock. Soils on the Shield generally have coarse-textured acidic parent material derived from igneous and metamorphic bedrock while those of the plains generally have fine-textured calcareous parent material derived largely from shale and limestone bedrock. An additional factor that could be different between the Shield and the plains is the frequency of stand-replacing (and in the case of *A. americanum*, sanitizing) wildfires.

Another unique feature about the distribution of *A. americanum* is the apparent rapid spread of the parasite across most of the western range of jack pine (Fig. 5.1) following the end of the Wisconsin glaciation. After the retreat of the continental glaciers, jack pine moved north and west from refugia in central and eastern North America and contact was made with lodgepole pine as it advanced northward from areas south of the glacial maximum in western North America (Critchfield 1985). The eastern limit of the parasite was near Lac Seul, Ontario (50° 27'N, 93° 03'W) (Larsen and Gross 1970), but this population was extirpated by fire and logging. In some of the literature on jack and lodgepole pine, the apparent rapid spread of *A. americanum* following the retreat of the continental glaciers is interpreted both as evidence for and against refugia for jack and lodgepole pine in northwestern North America or in an ice-free corridor between the Laurentide and Cordilleran ice sheets somewhere in what is now western Alberta (Yeatman 1967; Zavarin et al. 1969; Critchfield 1985). The presence of both of these possible refugia seems unlikely (Critchfield 1985). Evidence now exists for a migration of lodgepole pine from areas south of the glacial maximum to its current northern limit in the Yukon, where it has arrived only relatively recently (MacDonald and Cwynar 1985). Also, geological evidence does not support the presence of an ice-free corridor in Alberta during the glacial maximum (Dyke and Prest 1987). Jerome and Ford (2002) suggest that the existence of a refugium with dwarf mistletoe-infected lodgepole pine in western Alberta could explain some of the observed genetic differentiation in results they obtained for various populations of *A. americanum* but I think they have misinterpreted the available paleontological and geological data in the published literature as reviewed above. Thus, *A. americanum* likely spread from the area where jack pine first encountered dwarf mistletoe-infected lodgepole or the parasite was vectored by birds, which has been documented for dwarf mistletoes (Nicholls et al. 1984; Punter and Gilbert 1989). The apparent spread rate of *A. americanum* in the intervening period between present time and the end of the Wisconsin is not possible by tree-to-tree spread even if the generation period of this study is taken into account.

Another noteworthy feature is the presence of the parasite in most of the outlier pockets of jack pine near the contact zone of jack and lodgepole pine (Brandt et al. 1998). Many of these outlying jack pine stands are infested with *A. americanum* but the closest stands of lodgepole pine are not infested. Examples of this are the many jack pine stands and stands of jack×lodgepole pine hybrids near the Athabasca River in Alberta between Whitecourt and Smith (Brandt et al. 1998). These stands are infested with the parasite but lodgepole pine stands in the adjacent Swan Hills are parasite-free. There is no wide separation between the stands of the two tree species and it is unlikely that the separation that does exist could account solely for the lack of the parasite in the lodgepole pine stands. The maximum elevation of the Swan Hills region is well below the upper elevational limit of *A. americanum* on lodgepole pine in the front ranges of the Rocky Mountains in western Alberta. Additionally, outlier pockets of lodgepole pine to the north and northeast of its main distribution (Birch Mountains, Caribou Mountains, and Thickwood Hills) are parasite-free whereas nearby jack pine stands are infested (Brandt et al. 1998). According to Hawksworth and Wiens (1996), many of the outlying populations of lodgepole pine to the south of the glacial maximum are presently infested with the parasite. Jerome and Ford (2002) speculate that the degree of speciation between the three races of the parasite could be greater than race-level alone. Results of this study on the life cycle of *A. americanum* on jack pine provide additional phenotypic evidence of differences between at least two of the races, the ones on *P. banksiana* and on *P. contorta* var. *latifolia*. In a search of the literature, no reference could be found to any work in which seed from *A. americanum* populations on jack pine inoculated lodgepole pine or vice versa. Based on the geographic distribution of *A. americanum* in Alberta, the recent discovery of three races within the species (Jerome and Ford 2002), and the apparent differences in the length of the life cycle of the parasite on the different hosts, research is warranted to ascertain the degree of speciation and determine if host barriers exist among the different *A. americanum* races. Separate from the studies reported in this doctoral thesis, a small cross-inoculation trial was conducted. In the trial seeds of *A. americanum* collected from pistillate plants infecting jack pine (Smoky Lake seed source) were used to inoculate both jack and lodgepole pine (30 trees each). The proportion of lodgepole pine trees infected was significantly less than the proportion of jack pine trees infected. A factorial experiment is planned for 2004.

There are a few other avenues of research that could be pursued. A climate profile could be developed for *A. americanum* based on the results of my extreme cold temperature experiments and life cycle studies, as well as the work of Baranyay and Smith (1974) and Gilbert and Punter (1991). The effects of extreme cold temperatures in winter on overwintering survival of seeds, spring frosts on pollen, late summer frosts on fruit, and growing degree days on emergence of aerial shoots have been documented. This information could be used to develop a model to assess the impact of changes in these parameters on the distribution of the parasite. Such a model could also be used to assess areas of jack pine presently parasite-free but at risk. The model could also predict changes in the distribution of the parasite under various climate change scenarios. An experiment is also required to determine developmental times of *A. americanum* on jack pine and on lodgepole pine at the same site. The influence of edaphic factors on infection rates of *A. americanum* on jack pine would be useful to evaluate as well, especially calcium and aluminum which are known to vary considerably in soils derived from acidic parent material compared to soils derived from calcareous parent material. Finally, in light of this study's findings on both the effect of cold temperatures and the life cycle of the parasite on jack pine, studies should be conducted to estimate rates of spread in jack pine stands infested with *A. americanum*.

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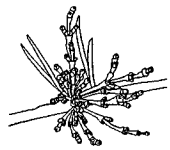


APPENDIX ONE

GLOSSARY

The following glossary primarily uses the definitions of terms provided in the *Glossary of dwarf mistletoe terms* by J.A. Baranyay, F.G. Hawksworth, and R.B. Smith published in 1971 by the Department of the Environment, Canadian Forest Service, Pacific Forest Research Centre, Victoria, British Columbia. The term "germinant"was defined by the author while "dwarf mistletoe" is based on Hawksworth and Wiens (1996).

aerial shoot Stem-like portion of dwarf mistletoe plant outside the host bark. Its primary function is reproduction. Frequently referred to collectively as an aerial plant.

anisophasic infection (syn.: non-systemic infection) An infection in which the endophytic system is generally restricted to the swollen portion of the host.

buffer zone An artificial obstacle to the local spread of dwarf mistletoe, such as a road, railroad or powerline right-of-way, or plantation of immune tree species.

cortical strand A structure that ramifies throughout the inner bark of the host from which the aerial shoots and sinkers are derived.

distal pole The pointed end of the dwarf mistletoe seed (where the radicle emerges) farthest from the pedicel of the fruit.

dwarf mistletoe A small flowering plant within the genus *Arceuthobium* characterized by stem-like shoots with reduced, scale-like leaves. Dwarf mistletoes are obligate parasites and rely on their host for most of their carbon requirements.

endophytic system The root-like parts of dwarf mistletoe within the host tissues. The endophytic system consists of cortical strands within the bark and sinkers which are embedded in successive layers of xylem. Formerly referred to as the haustorial root system or haustorial system.

germinant A germinated dwarf mistletoe seed in which the radicle has ruptured the endocarp crest.

holdfast A disc-like swelling at the distal end of the radicle through which infection of the host takes place. Formerly referred to as haustorial disc.

hyperplasia Abnormal growth of host tissues due to increased number of cells.

hypertrophy Abnormal growth of host tissues due to increase in size of cells.

incubation period The time between infection and emergence of aerial shoots.

infection (i) That process in which dwarf mistletoes successfully penetrate host tissues and initiate establishment of the endophytic system. (ii) The whole dwarf mistletoe plant (aerial shoots and endophytic system) developing from a single seed, plus associated host symptoms.

infection peg Structure that develops from the holdfast and initiates the infection process. Formerly referred to as haustorial wedge or primary haustorium.

intensification Increase in the number of dwarf mistletoe infections in a tree.

isophasic infection (syn.: systemic infection) An infection in which the endophytic system is in the terminal bud and keeps pace with the apices of the host. There is generally little hypertrophy or hyperplasia.

localized infection See anisophasic infection.

non-systemic infection See anisophasic infection.

principal host The main host of a particular taxon. Infection factor is at least 90% and usually nearly 100%. Although some trees may show little infection within 6-metre zone, uninfected trees are seldom found unless they are suppressed. A dwarf mistletoe species may have several principal hosts.

proximal pole The blunt end of the dwarf mistletoe seed closest to the pedicel of the fruit.

radicle The germinating root-like structure of a dwarf mistletoe seed. Frequently but incorrectly referred to as a hypocotyl.

seed A propagating structure of dwarf mistletoe made up of endosperm and embryo, lacking a true seed coat but encased in the endocarp of the fruit.

seed discharge Forceful ejection of dwarf mistletoe seeds from the fruit as a result of internal pressure.

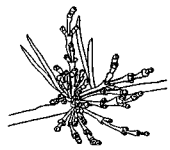
sinker Structures, composed of tracheary and parenchyma elements, that originate from dwarf mistletoe cortical strands and grow centripetally to the cambium where they are embedded by successive layers of xylem.

systemic infection See isophasic infection.

viscin Mucilaginous material contained in the viscin cells of dwarf mistletoe fruit, which acts as the means of attachment of the seed to the host.

viscin cells Elongated mucilaginous cells that cover most of the dwarf mistletoe seed.

witches' broom An abnormally profuse, dense mass of host branches. This is a common symptom induced by dwarf mistletoes, as well as other parasites and abiotic agents. Witches' brooms caused by dwarf mistletoes are of two basic types: systemic or non-systemic.



APPENDIX TWO

ANOVA TABLES

TABLE A.1 **ANOVA table for infected tree seedling data (Chapter 3) in the experiment testing the effectiveness of three synthetic adhesives and the viscin of the dwarf mistletoe seed for inoculation of jack pine seedlings with *Arceuthobium americanum*.**

Source of variation	Degrees of freedom	Sum of squares	Mean Square	F value	Probability
Adhesive	3	2363.0	787.7	3.56	0.0474
Error	12	2652.8	221.1		
Total	**15**	**5015.8**			

TABLE A.2 **ANOVA table for data on the percentage of *Arceuthobium americanum* seeds that germinated in the laboratory (Chapter 4) using PROC GLM in SAS and a mixed model, with seed source as a fixed effect and year and its interaction with seed source as random effects.**

Source of variation	Degrees of freedom	Sum of squares	Mean Square	F value	Probability
Year (Y)	1	230	229.6	0.03	0.8636
Error	3.0083	19760	6568.6		
Seed source (SS)	3	4162	1387.4	0.20	0.8884
Error	3	20493	6830.9		
Y × SS	3	20493	6830.9	29.98	<0.0001
Error	291	66307	227.9		

TABLE A.3 **ANOVA table for data on volume of *Arceuthobium americanum* seeds (Chapter 4) using PROC GLM in SAS and a mixed model, with germination status as a fixed effect and year, seed source, and all two- and three-way interactions as random effects.**

Source of variation	Degrees of freedom	Sum of squares	Mean Square	F value	Probability
Year (Y)	1	0.97	0.97	0.50	0.5252
Error	3.3805	6.59	1.95		
Seed source (SS)	3	12.80	4.27	2.66	0.1923
Error	3.7464	6.02	1.61		
Germination status (GS)	1	73.86	73.86	81.44	0.0241
Error	1.5777	1.43	0.91		
Y × SS	3	4.17	1.39	10.76	0.0410
SS × GS	3	1.04	0.35	2.68	0.2196
Error	3	0.39	0.13		
Y × GS	1	0.69	0.69	5.34	0.1038
Error	3.0029	0.39	0.13		
Y × SS × GS	3	0.39	0.13	1.25	0.2913
Error	1984	205.46	0.10		

TABLE A.4 **ANOVA table for data on the percentage of *Arceuthobium americanum* seeds that germinated in the field (Chapter 4) using PROC GLM in SAS and a mixed model, with seed source as a fixed effect and year, site, and all two-way interactions as random effects.**

Source of variation	Degrees of freedom	Sum of squares	Mean Square	F value	Probability
Year (Y)	1	60.2	60.2	0.07	0.8111
Error	2.5833	2222.5	860.3		
Site (ST)	2	1278.6	639.3	19.53	0.3905
Error	0.4123	13.5	32.7		
Seed source (SS)	3	353.8	117.9	0.12	0.9398
Error	3.1194	2957.1	947.9		
Y × SS	3	2776.5	925.5	12.27	0.0057
Y × ST	2	20.6	10.3	0.14	0.8751
ST × SS	6	587.4	97.9	1.30	0.3800
Error	6	452.8	75.5		

TABLE A.5 **ANOVA table for data on the percentage of *Arceuthobium americanum* germinants that developed holdfasts (Chapter 4) using PROC GLM in SAS and a mixed model, with seed source as a fixed effect and year, site, and all two-way interactions as random effects.**

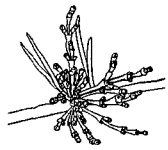
Source of variation	Degrees of freedom	Sum of squares	Mean Square	F value	Probability
Year (Y)	1	247.0	247.0	0.75	0.4766
Error	2.0542	680.0	331.0		
Site (ST)	2	1570.8	785.4	2.30	0.2893
Error	2.1973	750.8	341.7		
Seed source (SS)	3	499.5	166.5	2.46	0.2540
Error	2.7057	182.8	67.6		
Y × SS	3	147.5	49.2	1.19	0.3908
Y × ST	2	646.6	323.3	7.81	0.0214
ST × SS	6	358.9	59.8	1.44	0.3332
Error	6	248.4	41.4		

TABLE A.6 **ANOVA table for germination data from Experiment 2 (Chapter 5), in which overwintering *Arceuthobium americanum* seeds from four seed sources were exposed to three temperatures for 144 hr.**

Source of variation	Degrees of freedom	Sum of squares	Mean Square	F value	Probability
Model	14	1663.2	118.8	1.08	0.4099
Block	3	217.0	72.3	0.66	0.5845
Seed source (SS)	3	499.4	166.5	1.51	0.2297
Temperature (T)	2	171.1	85.5	0.78	0.4682
SS × T	6	775.7	129.3	1.17	0.3439
Error	33	3634.6	110.1		
Total	**47**	**5297.8**			

TABLE A.7 **ANOVA table for germination data for the control and the treatment of –37°C for 48 hr in Experiment 3 (Chapter 5).**

Source of variation	Degrees of freedom	Sum of squares	Mean Square	F value	Probability
Model	10	4521.5	452.1	3.59	0.0021
Block	3	199.0	66.3	0.53	0.6670
Seed source (SS)	3	3350.1	1116.7	8.86	0.0001
Temperature (T)	1	540.6	540.6	4.29	0.0454
SS × T	3	267.2	89.1	0.71	0.5543
Error	37	4664.9	126.1		
Total	**47**	**9186.4**			



APPENDIX THREE

Germination of *Arceuthobium americanum* seeds in young *Pinus banksiana* stands in relation to growing degree days and precipitation

INTRODUCTION

Climate is an important factor affecting the development of plants, with extreme summer and winter temperatures and drought often limiting their geographic distribution. Hawksworth and Wiens (1996) identified several factors, including climate, that affect the distribution of dwarf mistletoes but they also noted that the influence of climate has not been studied thoroughly. One of the key stages in the life cycle of dwarf mistletoes is the germination of the seed on the host tree. Optimum conditions of temperature and moisture for germination have been determined experimentally under controlled conditions in the laboratory for several *Arceuthobium* spp. (Beckman and Roth 1968; Bonga and Chakraborty 1968; Scharpf 1970; Bonga 1972; Robinson 1995). No studies, however, have attempted to determine the influence of temperature and moisture on germination under field conditions. Such information has been developed for germination and development of fungi on their hosts and has been critical for modeling epidemics of several serious plant pathogens (Agrios 1997). The objective of this study was to determine the influence of growing degree days and precipitation on the germination of *A. americanum* seeds from four seed sources under field conditions.

MATERIALS AND METHODS

A. americanum seed sources

In 2000 and 2001, seeds of *A. americanum* growing on jack pine were collected from four sites in Alberta: north of Peace Point (59° 11'N, 112° 23'W, 260 m above m.s.l.), west of McClelland Lake (57° 31'N, 111° 24'W, 300 m above m.s.l.), west of the Logan River (55° 21'N, 111° 56'W, 670 m above m.s.l.), and southeast of Smoky Lake (54° 05'N, 112° 20'W, 610 m above m.s.l.) (Fig. 4.1). Seeds were collected and stored using the methods described in Chapter 2.

Field observations of germinant development

In the fall of 2000 and 2001, 100 jack pine trees at each of three study sites in Alberta were inoculated with four dwarf mistletoe seeds per tree (one seed per seed source) using the seeds' viscin to hold them in place. A seed was positioned such that it contacted the host twig with its endocarp and had its distal pole oriented towards the needle base. Study sites were located west of Goodwin Lake (55° 24'N, 111° 44'W, 661 m above m.s.l.), southeast of Smoky Lake (54° 05'N, 112° 20'W, 610 m above m.s.l.), and

north of Bruderheim ((53° 52'N, 112° 57'W, 625 m above m.s.l.) (Fig. 4.1). The seeds used for inoculations were from the four seed collection sites. Inoculated branches were flagged and marked with a grease pencil 2–3 cm from the seed. Seeds and germinants were visually monitored at two-week intervals in the spring and summer of 2001 and 2002. At each assessment date and inoculated branch, the developmental stage of the seed or germinant and symptom of infection were noted.

Climate data

Historical daily temperature and precipitation data from the nearest 1–3 meteorological stations were obtained for each of the three study sites. For the Goodwin Lake site, these stations included primarily Round Hill Lookout (19 km southwest, 750 m above m.s.l.), but also Lac La Biche (72 km south, 567 m above m.s.l.) and Fort McMurray (142 km north, 369 m above m.s.l.). For the Smoky Lake site, the stations included primarily the Alberta Tree Improvement & Seed Centre (7 km east, 600 m above m.s.l.), but also Andrew (9 km southeast, 610 m above m.s.l.) and Lac La Biche (79 km north, 567 m above m.s.l.). For the Bruderheim site, the meteorological stations were Redwater (21 km northwest, 617 m above m.s.l.), Elk Island National Park (21 km south, 716 m above m.s.l.), and Fort Saskatchewan (22 km southwest, 620 m above m.s.l.). The number of growing degree days (≥5°C) from the first of March until seed germination was interpolated for each germinant at each site in the 2001 and 2002 growing seasons. Total precipitation from the first of April until seed germination was also interpolated for each germinant at each site.

Data analysis

Differences in growing degree days and precipitation in 2001 and 2002 among study sites and seed sources were assessed using an ANOVA with site, seed source, and their interaction as sources of variation. Least-square means were calculated and used for pairwise comparisons with the Tukey-Kramer method (SAS Institute Inc. 1999). The significance level was set at $\alpha = 0.05$ for these tests.

RESULTS

In 2001, the number of growing degree days prior to germination varied by site ($P < 0.001$), seed source ($P = 0.001$), and their interaction ($P = 0.007$). Growing degree days prior to seed germination did not vary significantly among the four seed sources at each of the three study sites. Thus, overall, there were 117 growing degree days prior to germination at Goodwin Lake, 144 at Smoky Lake, and 177 at Bruderheim. For seeds from the Smoky Lake seed source, growing degree days were significantly fewer at Goodwin Lake (107) than at either the Smoky Lake (146) or Bruderheim (184) study sites; the latter two sites were similar. For seeds from the Logan River seed source, growing degree days were significantly fewer at Goodwin Lake (110) than at Bruderheim (151) but were similar between Goodwin Lake and Smoky Lake (128) and Smoky Lake and Bruderheim. For seeds from the McClelland Lake seed source, growing degree days were significantly fewer at Goodwin Lake (126) than at either Smoky Lake (167) or Bruderheim (164); the latter two sites were similar. For seeds from the Peace Point seed source, growing degree days were significantly fewer at Goodwin Lake (129) and at Smoky Lake (137) when compared with Bruderheim (217) but were similar between Goodwin Lake and Smoky Lake.

In 2002, seed source ($P < 0.001$) and the interaction between site and seed source ($P = 0.005$) were significant sources of variation in number of growing degree days prior to germination; site ($P = 0.73$) was not significant. Growing degree days prior to seed germination did not vary among seed sources at both Goodwin Lake (228 growing degree days on average across the four seed sources) and Bruderheim (231 growing degree days on average) but did at Smoky Lake. At this site, the Smoky Lake seeds germinated after 346 growing degree days, which was significantly more than seeds from Peace Point (210), McClelland Lake (227), or Logan River (190). Growing degree days of these latter three seed sources were similar. In contrast to 2001, growing degree days in 2002 prior to seed germination within a seed source were similar among the three study sites. On average across the three study sites, Smoky Lake seeds germinated after 296 growing degree days, Logan River seeds germinated after 212 growing degree days, McClelland Lake seeds germinated after 217 growing degree days, and Peace Point seeds germinated after 212 growing degree days.

In 2001, site ($P < 0.001$), seed source ($P = 0.036$), and their interaction ($P = 0.008$) were significant sources of variation in the amount of precipitation prior to germination. The amount of precipitation prior to seed germination did not vary among seed sources at the three study sites. Thus, mean precipitation prior to germination was 44.4 mm at Goodwin Lake, 16.1 mm at Smoky Lake, and 12.8 mm at Bruderheim. For seeds from the Smoky Lake seed source, precipitation was significantly more at Goodwin Lake (42.7 mm) than at either Smoky Lake (16.5 mm) or Bruderheim (14.0 mm) but was similar at the latter two sites. The same pattern was observed for seeds from the Logan River seed source: 43.2 mm of precipitation at Goodwin Lake versus 13.7 mm at Smoky Lake and 9.3 mm at Bruderheim. There were no significant differences in precipitation between the latter two sites. For seeds from the McClelland Lake seed source, precipitation was significantly more at Goodwin Lake (45.7 mm) than at either Smoky Lake (19.9 mm) or Bruderheim (19.5 mm); precipitation was significantly different between the latter two sites as well. For seeds from the Peace Point seed source, precipitation was significantly more at Goodwin Lake (46.1 mm) than at either Smoky Lake (14.9 mm) or Bruderheim (19.5 mm) but were similar at the latter two sites.

In 2002, the results of the ANOVA on precipitation were the same as 2001 in terms of significant sources of variation. Precipitation prior to seed germination did not vary among seed sources at both the Bruderheim (10.4 mm on average across the four seed sources) and Smoky Lake (13.3 mm on average across the four seed sources) sites but did at Goodwin Lake. At this site, the amount of precipitation prior to germination of Smoky Lake seeds (40.4 mm) was significantly more than the amount for seeds of Peace Point (20.1 mm), McClelland Lake (25.1 mm), or Logan River (20.9 mm). For seeds from the Smoky Lake seed source, precipitation was significantly more at Goodwin Lake (40.4 mm) than at either Smoky Lake (16.1 mm) or Bruderheim (11.7 mm); precipitation prior to germination was significantly different at the latter two sites as well. The same pattern in precipitation was observed for seeds from Peace Point, McClelland Lake, and Logan River.

DISCUSSION

The importance of growing degree days to *A. americanum* germination appears to be limited. Interannual variation in growing degree days prior to germination was expected to be minor. Trends related to differences among the four seed sources was also expected. This was not the case, however, as there were substantial differences between seeds that germinated in 2001 and 2002 and there was no clear relationship among the seed sources. Temperature appears to have some influence because germination was delayed in the late, cold spring of 2002. The observed differences in growing degree days between years likely were real and not due to extrapolation from distant meteorological stations. Climate data for the Bruderheim study site were interpolated from the three nearest stations, all just over 20 km distant; variability among these stations in terms of temperature and precipitation was low. Climate data for the Smoky Lake study site were obtained primarily from a station 7 km to the east; early season data and missing data were primarily supplemented with data from another station 9 km to the southeast. Climate data for the Goodwin Lake study site were obtained primarily from Round Hill Lookout only 19 km to the southwest. Early season data were interpolated from Lac La Biche (72 km south) and Fort McMurray (142 km north).

Other than this study, no one has examined the relationship between germination and accumulation of heat during the growing season. Germination rates of *A. abietinum* f. sp. *concoloris*, *A. abietinum* f. sp. *magnificae*, *A. littorum*, and *A. occidentale* seeds increased between 0–13°C and decreased between 13–22°C (Scharpf 1970) while optimum temperatures for *in vitro* germination of *A. americanum* seeds collected from plants infecting jack pine were between 15–20°C (Robinson 1995). Optimum temperatures for germination of *A. campylopodum* seeds were 17–19°C (Beckman and Roth 1968).

Germination of *A. americanum* seeds on jack pine trees outdoors appears to be dependent on adequate moisture. In 2001, study site was a significant source of variation in the amount of precipitation prior to germination and was consistent for all four seed sources. Total precipitation was substantially more at Goodwin Lake than at the other two sites and it occurred earlier. These findings support previous research that has demonstrated the importance of water to seed germination in *Arceuthobium* spp. Relative humidity approaching saturation or free water provided adequate moisture to stimulate germination *A. abietinum* f. sp. *concoloris* and *A. abietinum* f. sp. *magnificae* seeds (Scharpf 1970). Similarly, *A. pusillum* seeds germinated more rapidly and produced longer radicles in water than when exposed to 90 or 100% relative humidity (Bonga 1972). The geographical distribution of *A. vaginatum* subsp. *crytopodum* and *A. pusillum* appears to be related to availability of water during seed germination (Hawksworth 1967; Bonga 1969, 1972).

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