Some aspects of the reproductive biology of *Arceuthobium americanum* in Manitoba

by

© Jeannie Anne Gilbert

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
in
Botany

Winnipeg, Manitoba, 1988

ISBN 0-315-48032-7

SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY OF *Arceuthobium americanum* IN MANITOBA

BY

JEANNIE ANNE GILBERT

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

ABSTRACT

Dwarf mistletoe, Arceuthobium americanum (Viscaceae), a damaging parasite of jack pine, Pinus banksiana, was studied at Grand Beach Provincial Park, north of Winnipeg, Manitoba. In previous phenological studies of Arceuthobium spp. an important role for microclimatic variables has often been implied. However, past records of these variables have been too sporadic and unreliable to draw firm conclusions. Consequently, it was appropriate to reexamine some aspects of the reproductive biology of Arceuthobium americanum in conjunction with the use of dataloggers to monitor temperature, relative humidity, windspeed and direction, and rainfall. Atmospheric pollen loads were recorded on a volumetric spore trap and flower movements by time-lapse photography.

The male flowers opened 6 April 1987 after a mild winter but 18 April 1985, after a winter in which February temperatures were lower than average. Nectar with a mean sugar concentration of approximately 30%, was always apparent on male flowers under conditions of high humidity. On the other hand, large, highly concentrated nectar droplets were seen on the female flowers only in the hot, dry spring of 1987. Although readings of 60-65% sugar were obtained, few measurements could be taken on account of the high concentration. Anther opening in response to rising temperatures and falling relative humidity, and closing

under the reverse conditions, was observed for the first time in Arceuthobium. The mechanism effectively retains pollen during unfavourable conditions, and further, helps to explain the fluctuations in atmospheric pollen loads observed in earlier studies. The high levels of activity of large Diptera, evident in the hot spring weather of 1987 and the measurable air-borne pollen concentrations provide evidence that pollination is effected by both insects and wind. Germination of the pollen was examined for the first time in Arceuthobium. The percentage germination fluctuated with changes in the temperature and generally increased as the season progressed.

The over-winter survival of maturing fruits was 88% and 94% in 1985/6 and 1986/7, respectively. Losses of approximately 30% of the fruits occurred over the summer, and a further decline in numbers was attributed to the high incidence of the fungal hyperparasites, Wallrothiella arceuthobii and Colletotrichum gloeosporioides in addition to the hot, dry spring of 1987.

Seed dispersal started 10 days earlier in 1987 than in 1986 as a result of the warm, early start to the growing season. Peak daily dispersal occurred between 0900 and 1100 CST and was associated with rising temperatures. Seeds were dispersed for distances up to 18 m, but there was no linear relationship between the logarithm of the seeds dispersed and distance, as reported for other Arceuthobium spp. While

explosive seed discharge accounts for local spread and intensification, small mammals may carry seed into uninfected parts of the forest and birds are potential vectors of long- and medium-range seed dispersal, depending on their migration habits. No seeds were found on any of the 193 mammals examined, but over 5% of the birds mist-netted in each year carried seed. In addition to gray jays and juncos, a brown creeper, a red-breasted nuthatch and a Swainson's thrush were recorded for the first time carrying seed.

During seed dispersal 41 of 73 naturally deposited seeds were washed from needles. Three of these later slid on to host twig tissue, whereas 7 of 27 that had originally landed on twigs were lost. In the same interval 3 of 25 seeds artificially placed on twigs of various ages were lost. After fall rains, over-winter retention of the surviving seeds was high, but losses occurred during the summer. The frequency of production of radicles was 88% in 1986, a spring characterised by persistent rain and high humidities, but only approximately 50% in the dry conditions in 1987. When seeds were examined in September few radicles had even penetrated the bark of the jack pine, an essential preliminary step in the infection process.

ACKNOWLEDGEMENTS

Many people have contributed in various ways to the thesis and their help and encouragement is acknowledged.

My deepest appreciation is extended to Dr. D. Punter who supervised the project and provided the guidance and unfailing support essential to the successful completion of the study.

The members of my committee, Dr D. W. French, Dr. T. D. Galloway, Dr. S. C. Jay, Dr. S. R. Rimmer, and Dr. J. M. Shay are thanked for their help and constructive criticism. Many members of the Department of Botany have given their time and attention to my questions and my welfare. Dr. A. Olchowecki helped with many aspects of photography. Garry Burgess gave technical advice and assistance in embedding procedures, and was instrumental in the production of the plates. I wish also to thank my colleagues in the laboratory, G. Esposito, G. Gyda Eyjolfsdottir, and G. Hausner who have given generously of their time and resources and brightened many days with their ready wits.

Interesting and helpful discussions on various points in the thesis resulted from correspondence with Dr. F. Baker, Dr. F. G. Hawksworth, Dr. J. Kuijt, and Dr. D. Wiens. Members of the Provincial Forestry Branch provided valuable assistance in choice and clearing of study sites, in particular, Mr. K. Knowles, Coordinator of Pest Management, Dr. T. Meyer, and Mr. M. Slivitzky. The assistance and

companionship of L. Lywak, M. Gray, and A. Hay made many hours of field work pass quickly and pleasantly. Their help is gratefully acknowledged.

Finally sincere gratitude is extended to my husband, Paul, who collaborated in the solution of several practical aspects of the field work, and provided help with the drafting of several figures.

Material support, which made the project feasible, came in the form of a loan of equipment and a research contract from the Manitoba Department of Natural Resources and a postgraduate scholarship from the Natural Sciences and Engineering Research Council.

TABLE OF CONTENTS

PAGE

PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

Dwarf mistletoes (*Arceuthobium*, Viscaceae), are highly specialized dioecious angiosperms that parasitize members of the Pinaceae and Cupressaceae. Five of the thirty-three taxa that occur in North America are found in Canada. Of these, three, *Arceuthobium tsugense* (Rosendahl) G.N. Jones, *A. laricis* (Piper) St. John and *A. douglasii* Engelmann, are restricted to British Columbia. *Arceuthobium americanum* is one of the most widely distributed dwarf mistletoes in North America. The literature on the species is extensive, numbering more than 1100 publications (Hawksworth, personal communication). *Arceuthobium pusillum* Peck has the widest range in Canada, occurring in all provinces except Alberta and British Columbia, followed by *A. americanum* Nutt. ex Engelm. which is found in the five western provinces, British Columbia to Ontario. Each dwarf mistletoe is associated with one or more principal hosts, but some may parasitize secondary, occasional or rare hosts. *Arceuthobium americanum* is common on lodgepole pine in Alberta and British Columbia, on the jack pine-lodgepole pine hybrid in Alberta, and on jack pine from Alberta to Manitoba. *Arceuthobium americanum* was also reported from Lac Seul, north of Dryden in Ontario (Larson and Gross, 1970), but that infection has since burned.

Many stands of jack pine, from Cormorant Lake north of The Pas to Agassiz Provincial Forest in the south of Manitoba, are heavily infected with A. americanum. Symptoms of infection include fusiform trunk swellings, witches' brooms, retarded growth and early mortality. The parasite seriously reduces both the potential yield of commercial stands, and the aesthetic appeal of resort areas on the southern shores of Lake Winnipeg where jack pine is the dominant tree species on sandy soils.

Numerous studies have examined some part of the life-history of Arceuthobium spp. including megasporogenesis, the ontogeny of male and female flowers, the life-cycle , the pollination biology, and most extensively, such topics as seed discharge, dispersal, survival and germination, and the ecology of seed storage. The influence of the microclimate on many of these aspects has frequently been questioned, but explanations remained speculative in the absence of reliable and continuous monitoring of the environment. The effects of mistletoe on jack pine may be more damaging than on lodgepole pine (Hawksworth, personal communication) for which host-pathogen combination the majority of research has been conducted. In order to understand the epidemiology of the parasite on its host in Manitoba, its reproductive potential was examined. Earlier studies on the pollination biology of A. americanum

in Manitoba (Gilbert and Punter, 1984), showed that air-borne pollen concentrations fluctuated, and that losses of maturing fruits were very high. A lack of adequate records precluded any attempt to correlate the results with climatic variables. The objectives of the study were:

1. To determine the effect of microclimatic factors, especially temperature and relative humidity, on various stages of the reproductive biology of A. americanum.
2. To assess the contribution of certain major stages or events in the life-cycle of A. americanum to the overall reproductive potential of the pathogen.
3. To determine the relative importance of explosive discharge and avian vectors in seed dispersal.
4. To provide a database for prediction and epidemiological modeling for use in the management of dwarf mistletoe-infected stands in Manitoba.

For the sake of convenience, the dispersal unit in Arceuthobium spp. is commonly termed a "seed", although technically it does not fit the definition of a seed. Lacking a seed coat, the embryo is embedded in a chlorophyllous endosperm which is surrounded by the pericarp.

Study site

The site was located 75 km north of Winnipeg on Highway 59 in Grand Beach Provincial Park. It included the west side of sections 35 and 26 and crossed over into the eastern portions of sections 34 and 27 of Township 18 (Lat.50° 35′, Long. 96° 35′). Except in the case of survival of maturing fruits in 1985/6, and explosive discharge and dispersal of the seeds in 1986 and 1987, all studies were conducted in the severely infected margin of one even-aged Pinus banksiana stand. Details of height, age, dwarf mistletoe rating (Hawksworth, 1977), tree density, and distance between trees by the point quarter method (20 points) are given in Table I. Arctostaphylos uva-ursi (L.) Spreng and mosses provided the greatest cover in the herb layer while Vaccinium angustifolium Ait. predominated in the shrub layer.

Table 1. Comparison of data for margin and interior of study stand of Pinus banksiana in Grand Beach Provincial Park, Manitoba.

	Margin	Interior
Mean dbh (cm)	9.8	18.0
Mean height (m)	11.0	16.6
Mean age (yr)	49.1	57.3
Dwarf mistletoe rating	4.6	2.7
Tree density (ha)	307.8	816.3
Mean distance between trees (m)	5.7	3.5

CHAPTER ONE

Pre-fertilisation Phenomena

Introduction

While the available information on many aspects of the biology of Arceuthobium americanum and its relationship to its hosts is rapidly expanding, the response of the flowers to the microclimatic variables in their surroundings has not been thoroughly examined. A number of studies have investigated the role of insects and wind in the dispersal of pollen, but no conclusive evidence has emerged that favours one mechanism to the exclusion of the other. There are no published data concerning germination and viability of A. americanum pollen. The first part of the study examined the factors relating to pollination, including the phenology and biology of flowering, and pollen release, dispersal, and germination and viability.

Section One

Phenological Observations and Floral Biology

Literature Review

Gilbert (1984) summarized the papers which refer to the onset of flowering in dwarf mistletoe. While Gill (1935)

suggested that flowering appeared to show little response to climatic or other environmental factors, Gilbert (1984) reported a difference, between seasons, of 29 days in the time of opening of the male flowers of Arceuthobium americanum in Manitoba. Scharpf (1965) reported that in successive years, the start of flowering in A. campylopodum Engelm. differed by as much as month. Both flowering and seed dispersal occurred earlier in years in which the mean monthly temperatures were near or above normal, and later when temperatures were below normal. There is also a report by Baker et al. (1985) that opening of the flowers of A. pusillum Peck in Minnesota appears to be related to temperature sums with base temperatures of 7.2 C and 12.7 C.

The peak period of anthesis for A. americanum in Colorado and Utah occurs from mid-April to mid-May (Gregor et al., 1974). Penfield et al. (1976) reported the beginning of the flowering period to be late March in Colorado in 1972, with the peak of anthesis occurring in the third week of April. The length of the flowering period for A. americanum was very long that year, extending to the third week of June. In Manitoba, male flowers are not usually seen after the third or fourth week of May although in 1981 a few male flowers were found each week until early July (Gilbert, 1984).

Nectar production by both male and female plants is characteristic of Arceuthobium, and has been observed in all species examined (Hawksworth, personal communication). In flowers of A. americanum, nectar in the central cushions of the males and on the stigmas of the females was observed when temperatures ranged from approximately 10 to 20 C (Gregor et al. 1974). Nectar has also been observed on flowers of A. pusillum (Baker, 1981; Baker et al., 1985) and on A. douglasii Engelm. (Player, 1979).

The quantity and concentration of nectar secreted by plants varies with species, previous history of the plant, age of the flower, size and degree of exposure of the nectary, air temperature, relative humidity, sunlight, wind, soil moisture and soil composition (Free, 1960). Probably nectar secretion by A. americanum flowers is affected by many of the above factors in the same general way as nectar secretion of other species, although little relevant information is available.

For plants in general, the greatest amount of nectar is secreted within the first few days of opening and only persists until the flower is fertilized (Free, 1960). On the other hand, in flowers of fruit trees such as cherry, apple, and orange, the mean nectar concentration increases with the age of the flower and bee visitors are abundant at this time (Free, 1960). Nectar concentration fluctuates widely in

accordance with relative humidity where nectaries are well-exposed. The mean nectar concentration of orange flowers, for example, varied from 20% when the atmospheric humidity was high, to 50% when humidities were low. In addition, where flowers have an open structure the nectar becomes diluted by rain or dew (Free, 1960).

Free (1970) reported that the threshold temperature necessary for nectar production varies with species, but that irrespective of temperature, nectar secretion is greater on a sunny than a dull day, reflecting the photosynthetic origins of nectar sugars. Soil moisture and composition might affect dwarf mistletoe nectar production, but only indirectly, and in as much as they affected the nutrient and water status of the host.

The nectar of shallow flowers with unprotected nectaries frequently contains little sucrose, mostly fructose and glucose (Percival, 1961). There is only one study of the nectar of Arceuthobium spp., which analyzed the sugars of A. abietinum Engelm. nectar (Brewer et al., 1974). The major components of the highly concentrated nectar (58-92% total solids, expressed as sugars) were glucose (48%), fructose (39%), and sucrose (11%).

Baker (1981) found that the number of female flowers per shoot of A. pusillum did not differ significantly from

year to year, ranging from 4.8 to 5.1. There are no published reports concerning the potential number of fruits per shoot for other Arceuthobium species. The sex ratio of A. americanum is reported to be 1:1 (Hawksworth, 1972).

Materials and Methods

Observations were made beginning in late March for the years 1984 to 1987 to determine the onset of flowering. Data loggers (CR21, Campbell Scientific Canada Corp.) were implemented in the spring of 1985 to 1987 with sensors to monitor the temperature (101 Thermistor probe) and leaf surface wetness (231 leaf wetness sensor) within brooms, and relative humidity (201 Thermistor and RH probe), wind speed (013A Met-one wind speed sensor) and direction (023A Met-one wind direction sensor), and rainfall (RG2501 Sierra tipping bucket raingauge), close to dwarf mistletoe infections (Plate 1a).

In 1985 a Minolta X-570 camera with a multi function back, 50 mm/3.5 MD macrolens and auto electroflash (Macro 80PX set) was programmed to take time-lapse exposures every 1 or 1.5 h on Ektachrome 200 ASA Daylight film, to monitor both flower movement and anther dehiscence of one male shoot in response to changing microclimatic variables. Branches of Pinus banksiana were brought into a cold room (4 C) and left in water under continuous light of low intensity

Plate 1a Datalogger and sensors for monitoring microclimatic variables.

Plate 1b Camera array for time-lapse photography.