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Development of Dwarf Mistletoe Infections on Inoculated Western Hemlock Trees in Southeast Alaska

Abstract

In the autumn of 1977, 1978, and 1979, mature seeds of hemlock dwarf mistletoe (*Arceuthobium tsugense*) were placed on needles and twigs of young western hemlock trees growing on a clearcut in Juneau, Alaska to provide information on seed retention and germination, infection development and survival, and generation time. Retention over one winter of 1,956 placed seeds averaged 65 percent while germination of retained seeds was 33 percent. Two hundred and sixty infections developed, 78 percent of which produced dwarf mistletoe shoots; however, over the 11 to 12 years that infections were observed, no seeds were produced and shoots were frequently broken or damaged. Plant damage, branch death, pollination biology, and environmental conditions are discussed as possible reasons for the lack of seed production and failure to complete the life cycle. Comparisons are made to other inoculation studies with *A. tsugense*. These comparisons indicate that the initial process of infection development, after mistletoe seeds are captured on needles or twigs, does not appear to account for the markedly lower infection levels that have been noted between stands in southeast Alaska and those further south.

Introduction

Hemlock dwarf mistletoe (*Arceuthobium tsugense* (Rosendahl) G. N. Jones) is widespread in old-growth stands of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) in southeast Alaska (Laurent 1974). The disease is, however, regarded as less of a problem in managed, young stands of western hemlock in southeast Alaska than in similar stands farther south in coastal British Columbia, Washington, or Oregon (Shaw 1982, Hennon and Shaw 1988, Shaw and Hennon 1991). There are several hypotheses as to why this difference exists (Drummond and Hawksworth 1979, Shaw 1982, Shaw and Hennon 1990). A primary reason appears to be that spread and intensification of dwarf mistletoe in infested stands, even beneath infected residual trees where young hemlocks are highly prone to infection (Shaw 1982, Shaw and Hennon 1991), is markedly lower in southeast Alaska than in similar stands elsewhere.

Why fewer infections are present in these stands than in similar stands in coastal British Columbia, Washington, or Oregon is unknown, but many possibilities exist. For example, there may be differences in pollination biology, seed production, seed viability, seed dispersal, seed retention, seed germination, infection development, infection survival, or generation time (Hawksworth and Weins 1972). This paper reports results of field inoculation studies designed to provide information on these latter five factors and compare results with those reported from similar studies conducted on *A. tsugense* in other locations.

Methods

Mature seeds of *A. tsugense* were collected from infected western hemlock trees within the City and Borough of Juneau, Alaska in September of 1977, 1978, and 1979. Soon after each year's collection, moistened seeds were placed on foliage and branches of young western hemlock trees growing in the city near Lemon Creek. This dense, unthinned stand of young-growth western hemlock and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) developed after clearcut logging in 1958-60. It is on a gentle, south-facing slope and is rather typical of young stands throughout southeast Alaska (Harris and Farr 1974).

In 1977, 963 seeds were placed on twigs and one-year-old needles in lower and middle crown branches, as follows: 160 on individual needles with a positive angle of attachment to the twig, 160 on individual needles with a negative angle of attachment to the twig, 50 on needles with a near zero (horizontal) angle of attachment to the twig, and 593 directly on twigs (Figure 1). Only one placement type was used per branch. In 1978, 783 seeds were placed: 200 on needles with a

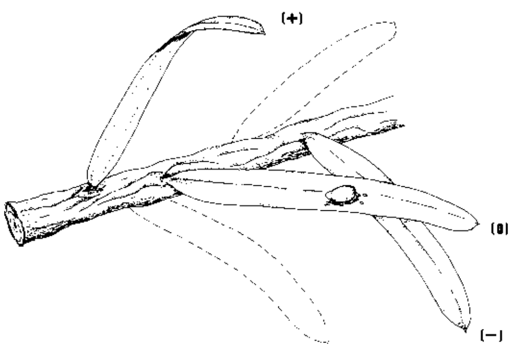

Figure 1. Side view of a western hemlock twig showing positive (+), near horizontal (0), and negative (−) angles of needle attachment. Placement of a dwarf mistletoe seed is shown on the (0) needle.

positive angle of attachment, 163 on needles with a negative angle, 168 on horizontal needles, and 252 directly on twigs. In 1979, 210 seeds were placed on twigs. There was no other source of dwarf mistletoe seed within several hundred meters of the inoculation sites.

All inoculations were examined at irregular intervals every few months during the first 4 years after seed placement and the percentage of seeds retained was calculated for each type of inoculation site after the first winter. Percent germination was calculated for these retained seeds for each year of inoculation. For the needle sites, retention included seeds that moved, primarily via rain droplets, to twigs and needle bases as these sites are the primary infection courts (Roth 1959). Seeds were placed directly on twigs and needles with different orientations to simulate actual interception of seeds ejected from explosive fruits on mature, female infections (Hawksworth and Wiens 1972). Examination of 45, one-year-old needles on each of 10 different lower crown branches (450 total needles) in the study stand showed that 52% had a positive angle of attachment, 28% were horizontal, and 20% had a negative angle of attachment.

Inspections were made approximately biannually after 1983 until 1986 and then annually until 1988. A cursory examination of the 1978 and 1979 inoculations was made in March 1990 to assess shoot production, seed production, and branch mortality. The proportion of retained seeds that developed into infections was determined by noting where branch swellings formed at inoculation sites or wherever the seeds finally rested. The time period for infections to first be detected as noticeable swellings, the temporal pattern of production and retention of dwarf mistletoe shoots and fruits on female shoots, the appearance of witches' brooms (Baranyay *et al.* 1971), and infection mortality also were calculated.

Results

Seed retention and germination

Table 1 shows the proportion of seeds retained after one winter for each inoculation type. Needles with a negative angle of attachment had the lowest level of retention while those with a positive angle of attachment had the highest. Direct twig placements and horizontal needles had intermediate levels of seed retention. The percentage of retained seeds that germinated for the 1977, 1978, and 1979 inoculations was 29 percent, 38 percent, and 25 percent, respectively.

Infection development

By September 1983, 260 infections had developed from the 1,956 seeds placed (1,265 retained) over the 3-year inoculation period (Table 2). Over this period, 147 different branches had been used in the experiments; 65, or 44 percent of these branches were dead by 1986 and only 211 viable infections remained. The 122 branches used for inoculations in 1977 and 1978 were reexamined in 1988; 61 (50%) were dead. Nineteen of the 23 branches inoculated in 1979 were dead in 1990; prior to death, infections had developed on 11 of these branches. By this time, continued tree growth had relegated most inoculated branches to interior portions of the lower crown. Thus, mortality was associated with natural pruning of lower-crown branches through shading, not the development of infections per se.

Most infections readily produced dwarf mistletoe shoots (Table 2); however, regardless of month of examination, these shoots were consistently immature and frequently broken so that only basal cups remained. This constant scarcity of mature shoots precluded accurate sex determination of dwarf mistletoe plants.

Branch swelling, the initial symptom of infection, was generally detectable from 32-35 months after inoculation. The earliest swellings appeared about 24 months after inoculation, but some did not appear for 72 months. For the successful inoculations that produced shoots, the time after seed placement to shoot production for 1977, 1978,

TABLE 1. Retention of dwarf mistletoe seeds in southeast Alaska one winter after inoculation in 1977, 1978, and 1979.

	Type of inoculation site[1]					
year	positive needle angle	negative needle angle	horizontal needle angle	twig	total retention	# of seeds placed
1977	82%	9%	30%	72%	61%	963
1978	87%	16%	43%	83%	62%	783
1979 (all placed on twigs)				92%	92%	210
mean	84%	13%	30%	79%	65%	–
# of seeds placed	360	323	218	1055	–	1956

[1]see Figure 1 for clarification of needle angles

TABLE 2. Development of dwarf mistletoe infections in southeast Alaska by September 1983 for inoculations in 1977, 1978, and 1979.

Year	No. of infections	% of placed seeds that resulted in infection	% of retained seeds that resulted in infection[1]	% of germinated seeds that resulted in infection	% of infections with plants or basal cups
1977	156	16%	27%	92%	72%
1978	81	10%	17%	44%	86%
1979	23	11%	12%	48%	87%
Total	260	13%	21%	65%	78%

[1]retained over one winter

and 1979 inoculations approximated, respectively, 40 months (range 32-72 months), 42 months (range 36-72), and 48 months (range 36-60 months). We did not statistically analyze these apparent annual differences because our irregular times of examination could have accounted for the difference. At no time during the 11-12 years that these infections were examined did any mature seeds develop. Thus, the life cycle was not completed and a generation time could not be calculated.

Individual inoculation points were 5 to 10 cm apart when seeds were originally placed on twigs or needles. Because of seed movement, many infections developed adjacent to each other. Nineteen infections had developed characteristics of incipient witches' brooms by 1986. Although it was difficult to verify conclusively, most of these broomed infections appeared to have developed through the merging of two or more adjacent infections.

Discussion

The 65 percent retention of seeds over the first winter after placement is nearly identical to the 66 percent reported from a comparable study in Oregon that also spanned a 3-year period of inoculation (Carpenter *et al.* 1979). The 92 percent retention for seeds placed on twigs in the 1979 inoculations is similar to the 96 percent observed for twig inoculations in southern British Columbia (Smith 1974). Our overall infection success of 13 percent (260 infections developing from 1,956 placed seeds) was, however, markedly higher than the 5.3 percent that occurred in Oregon. This difference occurred even though the germination success we observed for retained seeds (25-38%) was considerably lower than the 45 percent observed in both Oregon (Carpenter *et al.* 1979) and southern British Columbia (Smith 1974). Germination success in all three of these studies conducted in predominately cool but wet forests were markedly lower than the 67% germination reported for seeds of *A. tsugense* stored *in vitro* for 130 days under dry, cool, and dark conditions (Wicker 1974).

Twenty-one percent of the 1,265 seeds that were retained over one winter developed into infections (Table 2). This frequency compares to 8 percent in Oregon (Carpenter *et al.* 1979) and

18 percent and 22 percent in two studies in southern British Columbia (Smith 1971, 1974). Infection success, based on retained seeds that germinated, was 65 percent in our study (Table 2), but only 17 percent in Oregon (Carpenter *et al.* 1979).

This striking difference may result from use of dissimilar criteria in judging germination, although this seems unlikely since radicle emergence is a generally accepted definition of germination for dwarf mistletoe seeds (Gill 1935, Beckman and Roth 1968) and is relatively easy to ascertain. Another possibility is that some extreme environmental conditions developed at the Oregon inoculation site after germinants had formed, but before they were able to establish infections. This scenario also seems unlikely, however, as the inoculations spanned a 3-year period and the probability of occurrence of such events in 3 successive years is rather low.

We consider the most probable explanation for the difference to be that there was, for unknown reasons, a higher level of germinant mortality in Oregon than in southeast Alaska due primarily to fungal attack and insect predation, common causes of seed loss for dwarf mistletoes (Hawksworth 1961, Wicker 1974, Carpenter *et al.* 1979). At any rate, the process of infection development, after seeds are captured on needles or twigs, does not appear to account for the marked difference in infection levels that has been noted between stands in southeast Alaska and those farther south (Shaw 1982, Shaw and Hennon 1991).

The inoculation studies with *A. tsugense* in Oregon (Carpenter *et al.* 1979) and in southern British Columbia (Smith 1971, 1974) suggest a generation time necessary to complete the life cycle (production of seeds by female plants) of 4 to 5 years. This generation time is typical for species of dwarf mistletoe (*Arceuthobium*) (Hawksworth and Wiens 1972). The time necessary for the Alaskan infections to develop noticeable swellings and produce plants was comparable to that recorded in the above mentioned studies. However, for up to 11-12 years after our inoculations, no seeds were produced, even though shoot production was common.

The lack of seed production may have resulted from the limited number of dwarf mistletoe infections in the stand as these experimental inoculations were the only infections present and thus pollen may have been scarce. Contrarily, the ever present damage to plants through breakage, insect feeding, or other unknown causes could account for the lack of seed production. Environmental conditions, such as reduced light in the lower crowns of trees in this dense stand or its nearness to the northern limit of this mistletoe species (Drummond and Hawksworth 1979) might also be important. Even with the excessive damage that occurred to shoots and the possibility of adverse environmental conditions, the pollen hypothesis deserves particular attention as no information is available on pollen dispersal in *A. tsugense.*

Pollen of other species of *Arceuthobium* is dispersed by wind and/or insects for distances up to 500 m, but most pollen is deposited within 10 m of the source plant (Hawksworth and Wiens 1972, Penfield *et al.* 1976, Player 1979, Gilbert and Punter 1984, Coppola 1989). The effective distance of pollen dispersal—that is, the proportion of flowers pollinated in relation to distance from the pollen source—has not been determined for any species of *Arceuthobium.* Several hundred meters separated our study stand from any natural source of *A. tsugense* pollen.

Neither the Oregon nor Canadian studies apparently followed infections for a long enough period of time to record data on infection mortality. Inoculation studies in California, however, with fir dwarf mistletoe (*A. abietinum* Engelm. ex Munz) on white fir (*Abies concolor* (Gord. & Glendl.) Lindl.) and red fir (*A. magnifica* A. Murr.) resulted in considerable mortality of infections 23-28 years after inoculation (Parmeter and Scharpf 1989). This infection mortality was also associated with branch death, but Parmeter and Scharpf (1989) attributed their demise to attack by Cytospora canker (*Cytospora abietis* Sacc.) or girdling by rodents rather than lower crown shading. Gnawing by rodents has been observed on naturally established infections of hemlock dwarf mistletoe in southeast Alaska (Shaw and Hennon 1991), but not Cytospora canker.

The lack of seed production from well established infections and the death of numerous infections through natural pruning of lower crown branches could explain the limited number of dwarf mistletoe infections that develop naturally in young stands in southeast Alaska (Shaw 1982, Shaw and Hennon 1991). Before acceptance of this conclusion, however, the development of infections from naturally disseminated seed of

A. tsugense needs to be monitored for several years to validate these experimental results. Furthermore, the levels of pollen produced, mechanisms of pollen dispersal, attributes of fruit development, and consistency of seed production on natural infections need to be determined.

Acknowledgments

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