The phenology and impact of *Caliciopsis arceuthobii* on lodgepole pine dwarf mistletoe, *Arceuthobium americanum*[1]

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Abstract: *Arceuthobium americanum* Nuttal ex Engelmann in Gray (lodgepole pine dwarf mistletoe) causes significant losses to the timber industry. The fungus *Caliciopsis arceuthobii* (Peck) Barr shows high specificity to *Arceuthobium* spp., and therefore infection of *A. americanum* was monitored for 4 years to determine the effect of this fungus on fruit production. The contribution of stand-level and locally produced inoculum in the infection process was also studied to better understand the epidemiology of the pathogen. Over the period of the study, *C. arceuthobii* caused an average annual fruit reduction of 58%. When the contribution of local and stand-level inoculum was modelled, the results suggested that on *A. americanum* infections with ≥ 4 ascospore-producing immature fruit, more than half of the new *C. arceuthobii* infections on the same plant are derived from locally produced ascospores. The effect of the fungus on the host suggests that it has potential as a biological control agent for *A. americanum*, and further studies are needed to attempt to induce inoculum production in culture.

Key words: Arceuthobium, biological control, *Caliciopsis arceuthobii*.

Résumé : L'*Arceuthobium americanum* Nuttal ex Engelmann in Gray (faux-gui du pin lodgepole) cause des pertes significatives à l'industrie forestière. Le champignon *Caliciopsis arceuthobii* (Peck) Barr montre une grande spécificité envers les *Arceuthobium* spp., ce pourquoi, les auteurs ont suivi l'infection de l'*A. americanum* pendant quatre années, afin de déterminer l'effet du champignon sur la production des fruits. Ils ont également étudié sa contribution à l'échelle du peuplement ainsi que le processus d'infection causé par l'inoculum produit localement, afin de mieux comprendre l'épidémiologie de ce champignon pathogène. Au cours de la période d'étude, le *C. arceuthobii* a causé une réduction moyenne de 58 % dans la production annuelle des fruits. Les résultats de la modélisation de la contribution de l'inoculum local et à l'échelle du peuplement suggèrent que sur l'*A. americanum* portant des fruits immatures, lors de la venue de ≥ 4 ascospores, plus de la moitié des infections par le *C. arceuthobii* sur les mêmes plantes provient d'ascospores produites localement. L'effet de ce champignon sur l'hôte suggère qu'il s'agisse d'un agent de lutte biologique potentiel contre l'*A. americanum* et qu'on doit conduire d'autres études visant à développer la production d'inoculum en culture.

Mots-clés : Arceuthobium, lutte biologique, *Caliciopsis arceuthobii*.

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Introduction

Caliciopsis arceuthobii (Peck) Barr (ex *Wallrothiella arceuthobii* (Peck) Saccardo) was first described infecting *Arceuthobium pusillum* Peck in 1873 by Peck (in Weir 1915) as *Sphaeria arceuthobii* Peck. This pathogen displays extreme host specificity, infecting only the pistillate flowers of the spring-flowering dwarf mistletoes *A. pusillum*, *Arceuthobium americanum* Nuttal ex Engelmann in Gray, *Arceuthobium douglasii* Engelmann, and *Arceuthobium vaginatum* (Willdenow) Presl in Berchtold, subsp. *cryptopodum* (Engelmann) Hawksworth & Weins (Kuijt 1969; Hawksworth et al. 1977). While the pathogen was first described in eastern North America (Weir 1915), it is more abundant in western North America on *A. americanum* and *A. douglasii* (Kuijt 1963, 1969; Hawksworth et al. 1977). Ascospores are released from shiny black perithecia on infected female flowers in the spring around the time of pollination (Wicker and Shaw 1968). The mechanism of ascospore dissemination is unknown, but splash dispersal (Weir 1915; Dowding 1931), wind (Weir 1915), and insects (Dowding 1931; Kuijt 1969) have all been suggested. The fungus in-

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fects the stigma of the flower and prevents normal fruit development (i.e., the embryo fails to develop). By August, 3 months after infection, immature perithecia appear clustered around the distal end of the immature fruit. Perithecia mature in the spring, one year after infection, and sporulation coincides with dwarf mistletoe pollination (Dowding 1931; Kuijt 1969). The fungus has been successfully inoculated onto *A. douglasii* (Knutson and Hutchins 1979). Since infection by this pathogen reduces the reproductive potential of the infected dwarf mistletoe plant, it has been suggested that the fungus exerts a significant level of biological control on the infected host (Weir 1915; Wicker and Shaw 1968; Knutson and Hutchins 1979).

Dwarf mistletoe management is an important silvicultural activity that is necessary to reduce volume losses associated with dwarf mistletoe infection in stands being managed for timber production. Traditionally, dwarf-mistletoe management has been effectively achieved by clearcutting, eradicating all infected advance regeneration, and placing clearcut boundaries in "mistletoe safe" locations (Baranyay and Smith 1972). Currently, forest practices in British Columbia are shifting to include more partial harvesting regimes. Partial harvesting may leave a greater abundance of dwarf mistletoe in the overstorey and thereby increase the infection of the regenerating seedlings below. Biological control is one approach that is being investigated as a potential way of reducing dwarf mistletoe impact in such stands (Shamoun et al. 2004; Ramsfield et al. 2005).

In British Columbia, *C. arceuthobii* has been observed throughout the range of *A. americanum* and *A. douglasii* (Wood 1986), but there is a large gap in the distribution in the western United States (Hawksworth et al. 1977). The fact that this pathosystem represents a native fungus on a native host (Hawksworth et al. 1977) and the inability of the fungus to colonize the dwarf mistletoe endophytic system (Wicker and Shaw 1968; Kuijt 1969) have been cited as factors that would limit the ability of *C. arceuthobii* to be used as a biological control agent for the spring flowering dwarf mistletoes. There has been only one study that quantifies the actual impact of *C. arceuthobii* on spring flowering dwarf mistletoes (Knutson and Hutchins 1979). With renewed interest in alternatives for dwarf mistletoe management, this study was conducted to quantify the impact of *C. arceuthobii* on *A. americanum*, and to describe the relationship between *C. arceuthobii* inoculum produced on dwarf mistletoe plants and the resulting degree of parasitism on such plants.

Materials and methods

The stand selected for this study was located within the Knife Creek Block of the Alex Fraser Research Forest, near 150 Mile House, British Columbia (52°02′54″N, 121°48′45″W, 1023 m above sea level). The site was classified as the dk3 variant of Interior Douglas-Fir zone (IDFdk3) under the biogeoclimatic ecosystem classification system (Steen and Coupé 1997). Prior to spacing to 1500 stems per hectare in 1990, the stand was assessed at 4699 coniferous stems per hectare (93% lodgepole pine, 3% Douglas-fir, and 2% interior spruce). In 1998, the average height of the lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) component was 8 m, and the average diameter at breast height was 7.4 cm.

In 1998, 30 pistillate *A. americanum* plants were randomly selected and tagged. *Caliciopsis arceuthobii* was present on the flowers of 22 of the 30 selected plants. Every pistillate *A. americanum* plant selected bore a crop of fruit when the trial was initiated. *Arceuthobium americanum* plants were located on the branches and stems of the host trees and all plants were located in the lower crown.

The initial data were recorded 15 May 1998 and assessment occurred 28 July 1999 and 17 August of 2000 and 2001. At every assessment, the number of fruit and number of flowers bearing *C. arceuthobii* perithecia on each pistillate plant were counted. During the 2001 assessment, the number of healthy fertilized flowers was counted to estimate fruit production in 2002.

The impact of *Caliciopsis* was calculated by assuming that *Caliciopsis*-infected immature fruit remain attached, and are all present and bearing perithecia a year later, and that all such infected immature fruit would have produced normal fruit had they not been infected. For the first year of the study (observation of May 1998), *Caliciopsis* infection was calculated assuming that immature fruit bearing perithecia (α) were derived from flowers that were infected by the parasite at anthesis in May 1997, and that the healthy immature fruit (β) also originated from May 1997 flowers. The number of immature fruit with *Caliciopsis* perithecia in the spring of 1998 was then expressed as the percentage of the total number of immature fruit, both healthy (β) and infected (α), present on a dwarf mistletoe plant using the formula

$$[1] \qquad \text{Percentage} = \left[\frac{\alpha}{(\alpha+\beta)}\right] \times 100$$

where α = number of immature fruit with perithecia in May 1998, β = number of healthy fruit present in May 1998.

The percent reduction in fruit production in 1999 could not be calculated because the 1998 cohort of *C. arceuthobii* perithecia on immature fruit (present by August) could not be determined during the May 1998 assessment. The percent fruit reduction for 2000 and 2001 was quantified assuming that fruit present in August of year x (Fig. 1*c*) are the result of flowers that escaped infection in May of year x–1, and that *C. arceuthobii* observed on immature fruit in August year x (Fig. 1*a*) will cause a reduction in fruit production in year $x + 1$ using the formula

$$[2] \qquad \text{Percentage} = \left[\frac{\alpha}{(\alpha+\chi)}\right] \times 100$$

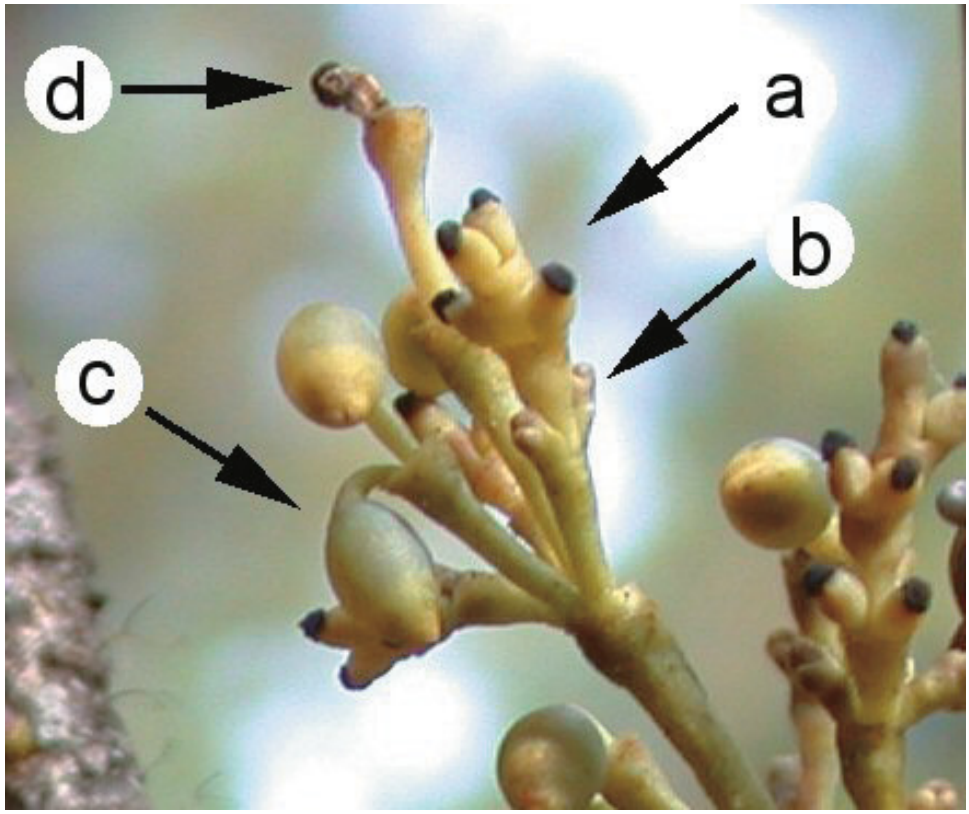
where α = number of immature fruit with perithecia in year x–1, χ = number of fruit present at year x.

The percent reduction in fruit production for 2002 was predicted based on the number of *C. arceuthobii*-infected flowers and the number of *C. arceuthobii*-free flowers using the formula

$$[3] \qquad \text{Percentage} = \left[\frac{\alpha}{(\alpha+\delta)}\right] \times 100$$

where α = number of immature fruit with perithecia in 2001, δ = number of immature fruit that escaped *C. arceuthobii* infection in 2001.

Fig. 1. *Arceuthobium americanum* infected by *Caliciopsis arceuthobii*. Photo taken 17 August 2001 at the Knife Creek block of the Alex Fraser Research Forest, near 150 Mile House, B.C. (*a*) *Caliciopsis arceuthobii* perithecia present on immature fruit infected in 2001. (*b*) Immature fruit that escaped infection in 2001. (*c*) Maturing fruit that escaped infection in 2000. (*d*) Flower infected by *C. arceuthobii* in 2000.



The dispersal of ascospores was assessed, and the relative importance of local inoculum and stand-level inoculum was modelled based on the following assumptions: (*i*) the spore concentration surrounding an individual dwarf mistletoe flower is a result of both stand-level and local inoculum, (*ii*) if wind is the primary vector, local inoculum will have a greater contribution to new infections than stand-level inoculum, owing to proximity of infected and uninfected flowers and dilution of ascospores in the air, (*iii*) if the ascospores are primarily insect vectored, it is by the same insects that pollinate dwarf mistletoe flowers, and the spore load they carry will reflect stand-level inoculum. The contribution of stand-level inoculum and local inoculum concentrations to new infections in the following year was assessed by analyzing a total of 28 pistillate plants that had flowers and fruit in consecutive years. As uninfected flowers were not counted in 2000, the number of healthy fruit in 2001 was used to derive the number of infected flowers in 2000.

We hypothesize that it takes, on average, a certain number of ascospores landing on a pistillate flower to cause an infection. However, the total number of ascospores landing on a flower can be much greater, and so a single flower can experience many potential infection events. Whether each such potential event results in the establishment of an actual mycelium or whether one or a few of the earlier events occupy the available host tissue and prevent establishment of further infections is immaterial to our argument.

For the sake of this argument we assume that all the pistillate flowers of a single *A. americanum* plant are exposed to the same ascospore load, and hence experience the same number of potential infection events. We argue that the ascospore load consists of a stand-level background that is the same for all *A. americanum* plants in the study, and the local ascospores produced by mature perithecia present on individual *A. americanum* plants. Thus,

$$[4] \qquad \mu = a + bn$$

where μ is the average number of potential infection events per flower on a given *A. americanum* plant, a is the number of such events attributable to stand-level ascospores, b is the number of such events attributable to local ascospores produced by a single perithecia-bearing immature fruit on that *A. americanum* plant, and n is the number of *Caliciopsis* infected immature fruits. Given these assumptions and definitions, and considering that the maximum number of potential infection events per flower is very large and much greater than the number actually experienced, it is evident that

$$[5] \qquad p_x = \frac{(a+bn)^x . e^{-(a+bn)}}{x!}$$

where p_x is the probability of x potential infection events per flower given a and bn.

However, the experimental observations can only distinguish between $x = 0$ (no infection) and $x > 0$ (the flower is infected). For $x = 0$, model [5] reduces to

$$[6] \quad p_0 = e^{-(a+bn)}$$

and hence for $x > 0$ meaning the flower is infected,

$$[7] \quad p_{>0} = 1 - e^{-(a+bn)}$$

Model [6] was assessed using Gauss–Newton weighted nonlinear regression in SAS to determine the best fit of the data to distinguish between the contribution of local and stand-level inoculum, to the overall amount of infection on a single *A. americanum* plant.

Results

Infection of *A. americanum* by *C. arceuthobii* was obvious by August in the year of infection. In August, it was possible to observe two different age classes of *C. arceuthobii* infection, as well as mature, uninfected fruit and healthy immature fruit (Fig. 1). The lifecycle of *C. arceuthobii* and assessment dates are displayed diagrammatically in Fig. 2 and the timing of *A. americanum* fruit production is outlined in Fig. 3. When the impact of *C. arceuthobii* on *A. americanum* was quantified, it was found that the average percent fruit reduction induced by *C. arceuthobii* infection was high, but not consistent between years (Table 1). Parasitism by *C. arceuthobii* ranged from 0% to 100% on individual *A. americanum* plants, and there was variability in the number of *C. arceuthobii*-infected flowers on individual *A. americanum* plants from year to year. Over the course of the experiment, the average fruit reduction per year caused by *C. arceuthobii*, not including the predicted fruit reduction for 2002, was 58%. Of the eight replicates that were *C. arceuthobii*-free at the beginning of the experiment, only two were *C. arceuthobii*-free at the end.

Fruit production on individual *A. americanum* plants was variable throughout the course of the experiment; large crops of fruit were not sustained on single plants over the course of the experiment and different plants had maximum fruit production in different years. Average fruit production per year is recorded in Table 1.

When the contribution of local versus stand-level inoculum was analyzed using weighted nonlinear regression analysis (SAS; Gauss–Newton), it was found that the best fit of [6] to the data yielded

$$[8] \quad P_0 = e^{-(0.1189+0.0324n)}$$

The model represented by eq. 6 yielded a significantly better fit ($r^2 = 0.794$) than eliminating a ($r^2 = 0.7508$) or a nonweighted analyses ($r^2 = 0.5595$). Both the slope (b) and the intercept (a) were significantly different from zero. Biologically speaking, [8] can be interpreted as follows: the probability of a flower being infected by stand-level inoculum was 0.1189, and the probability of being infected by local inoculum was 0.0324. These results suggest that for pistillate *A. americanum* plants with $n \geq 4$ (>0.1189/0.0324)] spore-producing immature fruit, new infection of

Fig. 2. Timing of ascospore release and new perithecia production by *Caliciopsis arceuthobii* infecting *Arceuthobium americanum*. The spiral line is the timeline of one individual *C. arceuthobii*-infected *A. americanum*.

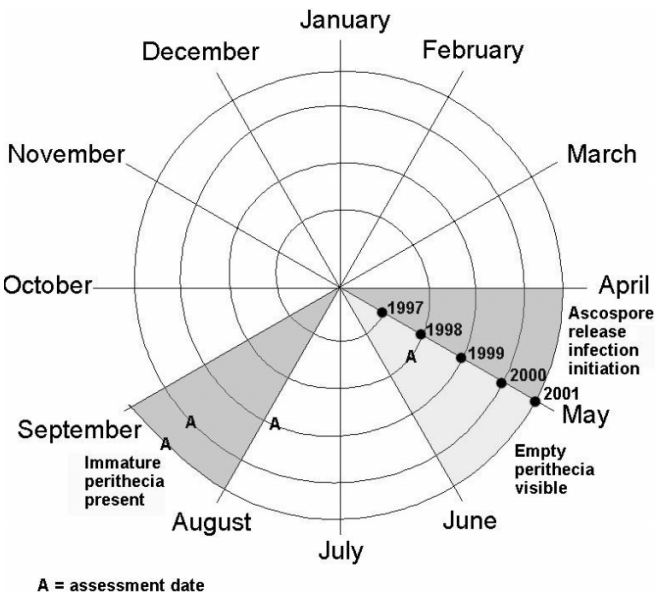


Fig. 3. Fruit production on *Arceuthobium americanum*. The spiral line is a timeline.

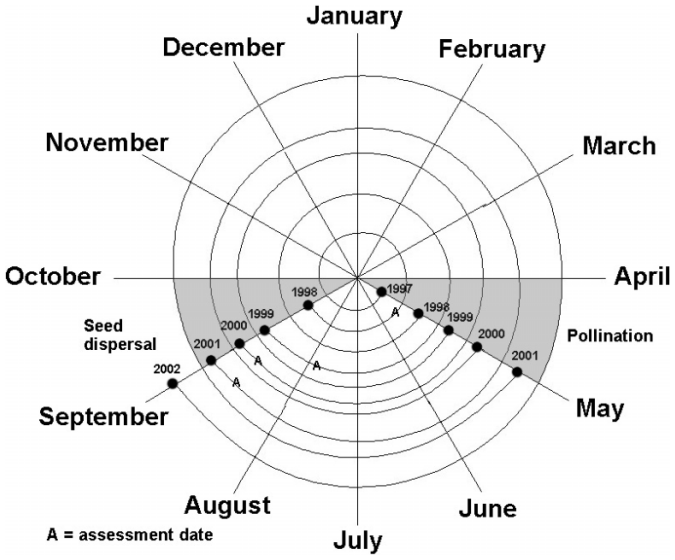


Table 1. Average reduction in fruit production caused by *Caliciopsis arceuthobii* infection of *Arceuthobium americanum* and average number of fruit produced each year.

Year	Average % reduction ± SEM	Average fruit production ± SEM
1998	46±7.4	58±10.7
1999	Cannot calculate	21±8.3
2000	72±10.8	10±6.5
2001	53±13.1	20±9.6
2002*	39±11.0	Was not counted

*Predicted.

that pistillate plant would be more likely to develop from local inoculum than from stand-level inoculum. In this study the number of such infected fruit per *A. americanum* infection ranged from zero to 423 (mean 41.1; median 4.5).

Discussion

Quantification of the impact of *C. arceuthobii* on *A. americanum* fruit production validated the anecdotal evidence that has been proposed in the past; this fungus has the potential to substantially reduce seed production on infected dwarf mistletoes, presumably resulting in a dramatic reduction in the rates of spread and intensification. When it is considered that only a small portion of dwarf mistletoe seeds from a healthy plant actually result in new dwarf mistletoe plants (13% was estimated for *Arceuthobium tsugense* (Rosendahl) G.N. Jones by Smith 1977), the effect of this fungus on the reproductive capability of the plant is significant. The results also indicate that the fungus persists on infected *A. americanum* individuals over time, and that it has the ability to spread to infect healthy plants. Parasitism of *A. americanum* by *C. arceuthobii* resulted in an average annual fruit reduction of 58% over the 4 years that the stand was studied.

The mechanism of spread and the environmental conditions favourable for infection by *C. arceuthobii* have not been well studied. Short distance splash dispersal during rain may be important to ascospore dispersal (Weir 1915; Dowding 1931). Dowding (1931) observed passive ascospore release; no spores were discharged explosively. Initially the fungus was thought to be limited to moist areas (Weir 1915; Dowding 1931), but subsequent studies indicated this was not the case (Wicker and Shaw 1968). Insects are likely also important vectors of the disease (Dowding 1931; Kuijt 1969) and evidence to support this includes visual observations of insects travelling from flower to flower and the coincidental timing of infection and pollination. Knutson and Hutchins (1979), however, hypothesize that insects may prevent infection of the pistillate flowers by removing spores as they feed on exudates produced by the pistillate flowers in the spring. Wind dispersal has also been implicated as a mechanism of spore dispersal (Weir 1915). The model developed in this study suggests that stand-level inoculum loading is a significant source of *C. arceuthobii* ascospores. As the stand-level contribution is higher than predicted by passive dispersal, insects likely play an important role in the long distance infection events. Pollinating insects, as well as other insects moving about on the dwarf mistletoe shoots, also likely play an important role in the local dispersal of ascospores. These insects spend time at each dwarf mistletoe plant, moving from flower to flower, and in the process distribute locally produced ascospores. Based on the assumptions of the study, the results suggest that local inoculum abundance is important in maintaining infection levels but that long distance dispersal of the pathogen must also occur and that long distance dispersal is likely aided by insect vectors.

The greatest challenge that must be overcome for this pathogen to be used as a biological control agent for spring flowering dwarf mistletoes is the difficulty of growing it in axenic culture to provide inoculum for inoculation in the field. Parker (1969) and Knutson and Hutchins (1979) were able to grow the fungus in culture, but it grows extremely slowly and does not produce ascospores in culture. No asexual stage has been observed on field material or in culture that would provide an abundance of conidia for inoculation. As this fungus has such a limited host range and has such a specific habitat, it is not surprising that it is difficult to grow in culture.

Clearly, this fungus has the potential to reduce the spread and intensification of spring flowering dwarf mistletoes by reducing seed production and, as it does not eliminate its host during the pathogenesis process, it would likely persist in the stand after successful establishment. Before the fungus could be used as a biological control agent for *A. americanum* an efficient method of producing inoculum must be developed.

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