FEEDBACK OF WATER STRESS ON WOOD PROPERTIES OF TREES: EXPERIMENTS WITH MISTLETOES ON *RHODODENDRON ARBOREUM* SM.

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ABSTRACT

Effects of infection by the mistletoe *Scurrula elata* (Edgew.) Danser, on wood properties of its common host *Rhododendron arboreum* Sm., were studied in the Annapurna Conservation Area of Central Nepal Himalaya. Heavy infection by mistletoes invariably causes decline of the host. Infested branches show inhibition of growth, defoliation and eventual death of branch parts distal to the site of infection. Anatomical properties of wood were compared in samples of branches proximal to the infection and in uninfected branches. The hypothesis that infection induces changes in basic wood anatomy could not be proven. Vessel density, vessel area, percentage lumen area and mean vessel diameter of the wood of infested and uninfected branches did not show any significant differences. The studied anatomical parameters were not correlated to the diameter of the host branch. These results show that infection by *S. elata* did not cause any changes in basic wood anatomy of its host *R. arboreum*. It appears that the studied anatomical parameters of *Rhododendron* wood are fairly stable and are not changed by stress due to infection by mistletoes. The damage to the host distal to the infected area most likely results from an insufficiency of total conductive area to supply both mistletoe and host. Unfortunately we could not determine annual conductive area increment, because *R arboreum* does not develop usable annual tree rings in the climate of the study area.

Key words: Himalayas, mistletoe. *Rhododendron arboreum, Scurrula elata*, water stress, wood anatomy.

INTRODUCTION

The host branches distal to a mistletoe infection directly compete with the mistletoe for water, inorganic nutrients, and organic compounds, if the mistletoe is partly or fully holoprarasitic. Competition becomes more severe as the mistletoe grows and diverts more water from the host branch, leading to decline and eventually death of the distal parts of the supporting host branch. Mistletoes do not only affect the physiology but also the morphology of their host plants. The morphological affects may be undetectable to very spectacular by forming witches broom, stem canker, swelling or hypertrophy of the host tissue. After establishment of mistletoe a certain amount of necrosis and hypertrophy of the host tissue is certain but it always varies by a combined effect of mistletoe species and host plants. The effect of mistletoes on the host may depend and vary with the amount of resources abstracted and also the supply available to the host, which could be

constrained by environmental conditions (Graves, 1995). When part of a host is heavily or systematically attacked by a parasite reproductive potential of the former drops or disappears and most commonly death of the host branch occur, which extends beyond the part of infection (Kuijt 1969).

Mistletoes of the families Loranthaceae and Viscaceae are well known in the world and most intensively studied in the nutritional relationship with their host. Despite the information on the nutritional relationship, very little is known on their effect on the properties and structures of host wood proximal to the haustorial region. In the context of plantation forestry (Yang *et al.* 2001) and forest decline by air pollution (Smith 1990) it is often argued that stress impacts on wood properties. Wilpert (1991) and Lecoeur *et al.* (1995) demonstrated that cell production, expansion and size can easily get affected by water stress.

The question to be investigated in this study was, whether severe stress as a consequence of excessive water loss by the transpiration of mistletoe and host leaves on the supporting branch would have a feedback on wood formation basal to the attachment point of the mistletoe. The model system in this study was *S. elata* on *R. arboreum*, a very common parasitic association at mid elevations (2000 to 2400 masl) of the Annapuma Conservation Area (ACA) in Nepal. In this association the mistletoe *S. elata* invariably causes inhibition of growth, defoliation and eventual death of the host branch parts distal to the mistletoe, which often stays alive, even when all distal branches of the host trees have died and withered.

MATERIALS AND METHODS

Study area

The study was conducted in the southern parts of the ACA located at the Central Himalayan region of Nepal, between $28^0 15'$ to $29^0 31'$ latitude and $83^0 31'$ to $84^0 24'$ longitude, 235 km north west of capital Kathmandu. The study area is in the vicinity of Ghandruk village (1,950 masl). The climate is upper temperate to sub-alpine Himalayan type. The area receives 3000 mm of annual precipitation because of advection of humid air masses in front of the Massive Annapurna. Monsoon starts from middle of June and lasts till middle of September. The winters are quite mild with infrequent snow and no severe frosts.

Study site and study species

Wood samples from Rhododendron branches were collected from the Bhalanche area between 2100-2350 m near Ghandruk Village. The site is at the margin of a broad-leaved semi deciduous forest at the sunny east-facing slope dominated by various sized trees of *R. arboreum, Lyonia ovalifolia, Daphniphyllum himalense, Lindera pulcherrima* and *Viburnum erubescens*. The most common mistletoe species in the area is *S. elata,* infecting a variety of host trees. Nearly all Rhododendrons have multiple infections by *S. elata.*

The study was conducted to investigate the effects *of S. elata* infection on the wood properties of *R. arboreum* proximal to the attachment of the parasite. In the Nepal Himalayas *Rhododendron* species are major elements of vegetation and *R arboreum is* a dominant tree species from the upper temperate zone to sub-alpine zone. It is a large evergreen tree (sometime reaching to 100 cm in diameter and 30 m in height) in the lower altitude and it becomes a small tree in the sub-alpine zone (Suzuki and Noshiro 1988). *R. arboreum* has diffuse-porous wood with abundant and evenly distributed of small pores without having any specific pattern. Growth rings are present but indistinct (Suzuki and Noshiro 1988). Wood structure of Nepalese *Rhododendron* is rather homogenous in qualitative characters (Suzuki and Noshiro 1988), but quantitative characters vary in relation to altitude and habit (Noshiro *et al.* 1995). Noshiro *et al.* (1995) reported the pore density between 300 and

500/mm^2 in Nepalese Rhododendron tree and sub-tree species. *S. elata* is a large bushy shrub measuring up to 2 m in height found commonly parasitizing *R. arboreum* Sm. in the Annapurna Area.

We harvested sections of about 10 cm length and about 1.0 to 2.5 cm diameter from the internodal region from infected and uninfected branches in the same canopy position from individual *R. arboreum* trees. The samples within one tree were selected for as close as possible equal diameter of infested and uninfested branches. The samples of infested branches were taken from the first internode proximal to the mistletoe attachment and were free of mistletoe haustorial tissue. A total of 20 trees were sampled in April 2001. Host wood branch diameter was measured using a Vernier Caliper. Prior to analysis the samples were kept in a mixture of water, 96% Ethanol and Glycerine (1:1:1) for four months. Cross sections of 25 μm thickness from the internodal region were made with a sliding microtome and stained with Methylene Blue. We studied four quantitative wood parameters: (1) total vessel area, (2) mean vessel perimeter, (3) major and minor vessel axes and (4) total number of vessels in a 426 X 284 μm area in the sap wood region, in wood of both infected and uninfected branches. Quantification was made by use of image analysis software NGH (National Institute of Health, US). Details of image analysis systems are described by Jagels and Telewski (1990). We calculated vessel density as the number of vessels per square millimetre. Mean vessel diameter was calculated as the mean of corresponding circular diameter from the lumen area, and the percentage of vessel lumen area by dividing the total area of vessels by the window size.

The normality of data for vessel number, area, and vessel size were checked using histograms and one-sample Kolmogorov-Smimov tests. Differences in mean between infected and uninfected branches for the vessel characteristics such as vessel density, mean vessel diameter and percentage of vessel lumen area were calculated by one-way ANOVA. The relationship between host branch wood diameter and the vessel variables in infected and uninfected host branches was presented with scatter plots.

RESULTS AND DISCUSSION

Table 1 shows the mean and standard deviations of wood anatomical variables of uninfected and infected host branches. No significant differences of vessel density, mean vessel diameter and percentage of vessel lumen area could be detected in the wood of uninfected and infected host branches of *R. arboreum*.
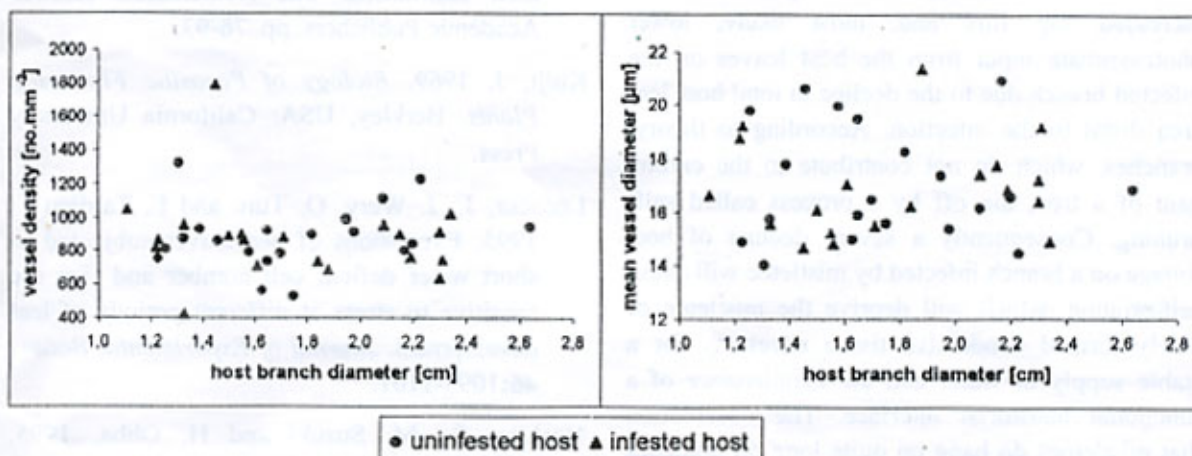

Fig. 1. Relationship between branch diameter and vessel density in host branches of *R. arboreum*.

Table 1. Wood anatomical variables in branches of *R. arboreum*, uninfected and infected by *S. elata*.

Host Branch	HBD (cm)	VD (no./mm²)	MVD (μm)	% LA
Uninfected	1.75±0.38	881.9 ± 190.6	17.1 ± 2.1	0.23±0.064
Infected	1.75±0.41	876.6 ± 260.8	16.8 ± 1.7	0.21±0.056

Mean ± standard deviation. Differences were not significant for all variables (P >0.05). HBD: Host branch diameter, VD: Vessel density, MVD: Mean vessel diameter, LA: % lumen area (vessel area/total area).

None of the wood anatomical variables showed any correlation with the host branch diameter. Fig. 1. shows scatter plots of host branch diameter vs. vessel density of uninfected and infected host branches with an extremely small correlation coefficient.

The results of our investigation indicates that infection by *S. elata* is not capable of inducing any changes in the basic wood properties of branches of its host *R arboreum* proximal to the point of attachment. This is a clear indication that there is no feedback of water stress on wood anatomy in stressed branches below (proximal) to the area where water is diverted by the mistletoe. As both host and mistletoe are evergreen, effects of differences in water use by leaves on wood formation can be excluded. The signals at the zone of wood formations in the sampled region of the host branches are lower water potentials and increased sap flux and, most likely, lower photosynthate input from the host leaves on the infected branch due to the decline in total host leaf area distal to the infection. According to theory, branches, which do not contribute to the carbon gain of a tree, die off by a process called self-pruning. Consequently a severe decline of host foliage on a branch infected by mistletoe will cause self-pruning, which will deprive the mistletoe of newly formed conductive tissue necessary for a stable supply of water and the maintenance of a functional haustorial interface. The observation that mistletoes do hang on quite long on branches with very few or no host leaves in *R. arboreum* may be interpreted as indication that self pruning is not pronounced in this open forest tree species and that repair mechanisms for embolism in conductive elements are not challenged under the climatic conditions of the research area, which experiences neither extreme draught nor harsh frosts. The small diameter vessels and the insurmountable difficulties encountered in distinguishing tree rings of *R. arboreum* in the samples could be interpreted in support of this idea.

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