Causal agents responsible for the die-back of *Dalbergia sissoo* in Nepal's eastern Tarai

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A preliminary investigation on the causal agents responsible for the die-back of *Dalbergia sissoo* in the Eastern Tarai districts (Saptari, Shiraha and Udaypur) was carried out in December 1998. Several species of insect borers (of the families Scolytidae, Buprestidae, Cerambycidae of the order Coleoptera, Beetles) were most prominent. Termites (white ants) were seen at places but did not seem to have caused death of trees. The main fungal species causing serious damage and death of sissoo were *Ganoderma lucidum* and *Fusarium* sp. Bacteria were also isolated from the infected samples. Some recommendations in line with the sanitation, control measures and research need are given. Also a well equipped wing to tackle problems of pests and diseases should be established at the Department of Forest Research and Survey.

Keywords: *Dalbergia sissoo*, die-back, root rot, causal agents, borer insects, pathogens, Terai, Nepal

Dalbergia sissoo Roxb. ex DC. (commonly known as sissoo) is distributed throughout the riverain forests of Nepal from east to west from 72 m to 1500 m above mean sea level. Since time immemorial, the species have been utilised for timber and Ayurvedic medicines (Dwibedi in Bhavaprakash Nighantu, 1971; Sharma in Drabyaguna Vigyan, 1994). It is a most valuable species and is preferred for plantation for its fast growth and multi-purpose use in the Tarai, inner Tarai and Bhabar regions. It is grown in private land, community land and forest land, along the bunds, river canals, roadsides, and even in the agriculture land as an agro-forestry crop.

During the implementation of the Tarai Community Forestry Project, which distributed free seedling, more than ninety percent of plantations done in the private and public land of the Tarai were that of sissoo. Therefore, since 1981, sissoo plantation area increased considerably.

Unfortunately, the plantations have been suffering from a serious die-back disease since past few years (Thapa, 1990). The damage caused by the disease now seems to be an epidemic. The disease is spreading very fast and sissoo are dying at all kinds of localities/habitats. The disease has been infecting the crops of all ages. There were top dying, half-dying and entirely dead plants. The impact is so serious that people are afraid to plant sissoo. The small-scale sissoo entrepreneurs, community forest user groups, and district forest office staff though were quite aware of the disease, could not control the disease in due time. It is also reported that similar disease has also started to infect and kill mango, eucalyptus, khair and other leguminous trees in the nearby plantations. People are bound to sell the trees as soon as the symptoms of the disease become visible on it. The price of the trees has gone down so much that even the timber grade trees are being sold at much lower rates. This has negatively affected the local people and national economy, whose impact is still to be assessed.

The field survey conducted at five districts of the mid-and eastern Tarai has indicated that the death of sissoo is due to root infection and decay. However, fungus could not be identified, but roots present especially in stiff clayey soils were reported of having such infections (FORESC, 1998). The study also reported borer insects in tree trunk and root nematodes.

The phytopathogenic fungal elements recorded by earlier authors on *Dalbergia sissoo* include *Phyllactinia dalberiae* Pyr. (Ivory, 1986); *Uredo sissoo* Syd. and Syd. (Lama, 1977; Durrieu, 1980; Ono, Adhikari and Rajbhandari, 1990; Adhikari, 1990, 1996 ab; Ono, Adhikari and Kaneko, 1996; Pawsey, 1989; Thapa, 1990). Two species of *Ganoderma* (*G. applanatum and G. lucidum*) have been reported from tropical to temperate belts of Nepal. *Ganoderma applanatum* (Pers.) Pat. has been found

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on rotten trees of the Tarai forest in Dharan (Balfour-Browne, 1968); Bakhri Kharka (north of Pokhara); Taglung, Kaligandaki (Balfour-Browne, 1968); Hetauda (Pandey, 1976). Similarly, it was also found on *Quercus* sp. in Daman (Singh, 1976) and Phulchoki (Singh and Nisha, 1976). This was also found on the tree trunk in Namjung of Gorkha District at an altitude of 2460 m (Adhikari, 1988) and on wood of *Betula utilis in* Nilgatti Odar of Bajhang District at an altitude of 3,500 m (Adhikari, 1988; 1996).

G. lucidum (Fr.) Karst. (Dadu chyau in Nepali) has been recorded on rotten trunks from Bakhri Kharka, north of Pokhara (Balfour-Browne, 1968) and Kenja, Likhu Khola (Ryv, 1979). It is also recorded on tree trunks from Lele of Kathmandu valley (Singh and Nisha, 1976) and on trunk of *Rhododendron arboreum* and *Quercus* sp. at Manichur (Adhikari, 1988). Similarly, it is recorded on stump in the area between Seti Khola Bagar and Agra Gaun of Bajhang District at an altitude of 1,700 m (Adhikari, 1988); in root crevices of stump in Phulchoki at an altitude of 1,800 m and Surya-vinayak at an altitude of 1,540 m (Adhikari, 1996; Adhikari et al., 1996).

Recently Adhikari (1996) has reported *Colletotrichum* sp., *Fusarium solani f. dalbergiae, Ganoderma lucidum, Maravalia achroa, Phyllochora dalbergiae, Phyllactinia dalbergiae* and *Uredo sissoo* parasitic on *Dalbergia sissoo.*

The death of *D. sissoo* due to *Ganoderma lucidum* had been reported as early as 1989 in Teekapur, Kailali District (Thapa, 1990). In another report, it was reported that this fungus caused 75% mortality of a ten-year-old *D. sissoo* plantations in Patabar-Khotena, Kailali District (Karki, 1992). It was also reported that the species was attacked by *Fusarium oxysporium* at a waterlogged site in Sagarnath.

Tuladhar (1996) published a monograph with the details of insect pests of *D. sissoo* from Nepal and the neighboring countries. White (1988), Thapa (1992), Amatya (1994), and Luitel (1995) have mentioned different insect pests related to sissoo trees. So far, no authentic control measures based on experimental trial have been reported for the control of borer insect pests of sissoo trees in Nepal.

Top-dying of sissoo trees which is attributed to different causes, have also been reported from different neighboring countries. However, no one has been able to work out the authentic practical solution so far. In India, this disease has been reported from the neighboring state of Bihar as well. The causal agent was to be a fungus (*Fusarium* sp.) (Personal communication with Dr. M. N. Jha, team member of the investigation team from ICFRE, Dehra Dun, India, and a report in "The Times of India, dated Jan. 13, 1999). Several reports from India also indicated the severe damage caused by *Ganoderma lucidum*, mainly in the northwestern region of the country (Bakshi *et al.*, 1976; Khara, 1993).

With this background, and also no earlier studies have identified the causal agents responsible for the die-back of *Dalbergia sissoo* in the Tarai, the present study aims to find out the same.

Methodology

Site selection

The study sites on eastern Tarai were selected on the basis of heavy infestation of disease. Geographical location (such as north, south, east or west) of the Churia Forestry Development Project was the another criteria for the stratifying the study sites. Age of the crop, site of plantation (such as on bunds/alley, agriculture field, riverside, canal bank, etc.) were also taken into consideration. Different sites from each stratum were visited. They were at Saptari and Siraha Districts. The age of the sissoo tree ranged from 1 to 35 years (and above).

Study sites

District	Study site
Saptari	Khojpur, Bhangaha, Banauli, Mahuli, Kanchanpur, Sakhada, Rampur Jamua, Kararbona, Birendra bazaar
Siraha	Gamharia Maheshpur, Jiba, Lahan, Bhagwanpur, Chandra-Udayapur, Lalpur, Mirchaiya, Badharamal
Udaypur	Gaighat, Hadiya
Dhanusha	Dharapani, Portaha
Sunsari	Ineruwa, Jhumka, Kushaha
Morang	Shishauli
Jhapa	Surunga, Ratuamai

Sample collection

The infected trees were externally examined on leaves, branches, stem and roots for insect damages or symptoms of fungal mass or growth. For insect and/or fungal damages, internal examination was done by tearing the branches, scrapping the bark, and uprooting the trees. Insects and larvae were collected in plastic bottles and preserved in 70% alcohol. The infested parts of the trees were also preserved. Some healthy trees were also cut and uprooted and wood samples were collected for comparative study. The insects were identified, and

attempt to study the life cycle of some insects in captivity is being done. Others were preserved as museum specimens. The plant tissues were collected for further study on fungi. Transverse and longitudinal sections of infected parts of the tree were made to see the extent of penetration of the fungal mycelia in active tissues and the cambial region. Small pieces of tissue from the diseased trees were inoculated in the sterilised PDA media to grow and isolate pathogens from the tissue of the trees. The growth of such pathogen mass in the media were later studied under microscope and identified.

The local people were interviewed about the situation, their knowledge, extent of damage and the control measures they have tried so far if any.

Results

Diagnostic visible features of diseased trees

The primary visual symptom for diagnosis of this disease is oozing of dark red to reddish brown (turning black after drying) sap from the infected trees. The local people reported that once this symptom is visible, the tree starts dying from the top. It may take from one month to one year for the tree to die. However, in some plantations diseased trees did not ooze. From a distance the diseased trees show the top dying, half-dying or entirely dead crown. The tree starts drying from the top, which progressively goes downwards. In many cases the drying of leaves are so fast that that all leaves appears drying at the same time. The wilting is very prominent and diagnostic in small plants and nursery seedlings. However, in case of grown-up trees it is not so noticeable.

In many cases, the symptoms of insects' damage were most prevalent in the diseased trees. Such symptoms included small bore holes with varying degree of depth (from 1 to 12 cm) and frequency (1 to 4 per square cm) in the infected area of the tree trunk, with wood dust thrown out of the hole. In several infected trees, a zigzag passage underneath the bark (in the cambial region and sapwood) is visible. In some young plantations, tunnels in the upper stem and branches are visible.

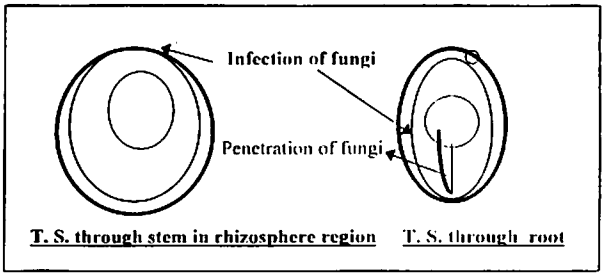
One or several or all of the above symptoms could be visible on diseased trees. This disease can infect trees of any age or size, whether in natural forest or in plantation or in private farmlands.

Age of infestation

The die-back disease has infected all ages of sissoo trees ranging from 1 to 35 years (and above). However the infestation was found severe between the age of 5 and 15 years. Infestation is at all types of plantations. However, the severity of disease is higher in the bund (alley) plantation as compared to other types.

Mycological findings

The fungus *Phyllactinia dalbergiae* and *Uredo sissoo* parasitic on the leaves of the *Dalbergia sissoo*, were found wide spread in Khojpur, Banauli, Gamharia-Maheshpur and Jiba plantations. To a varying degree, these fungi were observed at all the study areas. The logs collected from Banauli, Gamharia-Maheshpur and Jiba Community Forest showed the infection of fungal mycelium in transverse and longitudinal sections of the root and stem (Fig). The infection in the upper region was diffused and concentrated mainly in the cortical region.



At Gamharia-Maheshpur *Schizophyllum commune* was found on dead twigs. In Jiba Community Forest *Xylaria* sp. was found growing on the decayed bark of tree trunk while *Ganoderma lucidum* were found parasitic on trees and stumps at the rhizosphere region. The fungi was found to be very frequent - 3 to 4 per plant. *Xylaria* sp., *Coriolus versicolor*, *Coriolus tephroleucus* and *Polyporus* sp. were also recorded from the region.

The roots of dead trees or infected part or bark were yellow and juicy, emitting alcoholic odor, which was due to bacterial rotting and fermentation.

Culture studies

Fusarium sp., Bacteria and the mycelium of Basidiomycetes were isolated from the cultures. The isolates made from the roots and stems of those area revealed *Dalbergia sissoo* has been seriously attacked by *Fusarium* sp. (probably *F. solani*) and *Ganoderma lucidum*. Bacteria were also isolated in the cultures.

Entomological findings

The observation on entomological aspects can be categorised into following headings:

Nature of damage by insect

There could be one or more or all of the following symptoms of damage by insects:

1. Pinholes in bark, sapwood and heartwood.
2. Zigzag passages in cambial region and sapwood.
3. Sawdust on the stem.
4. Red sticky sap oozing out of stem.
5. Mud galleries covering stem.
6. Holes in branches.

The Khojpur plantations, (age 5 to 30 years), were infested by borers (3 to 4 holes per sq. cm) showing severe oozing of dark sap from stems. In some plantations, insect larvae were found to be the cause of one type of apical die-back especially in the younger plants.

At the Banauli area, 40 percent of the trees (plantations age, 3 to 20 years) were infected with borers. The density of insect holes was found to be 2 to 4 per sq. cm. In case of infected trees, the uprooting revealed that some portions of the roots were black and emitting the fermented odor. The use of Thimet (@ 50 gm per tree) by local people did not save the life of such infected trees.

At Gamharia-Maheshpur, a larva was found protruding through the hole a diseased tree.

In the Jiba Community Forest, the diseased trees did not show the oozing of sap. However, several holes made by insect were observed. The flow of red sap was found in some of the insect damaged specimens. The roots of trees were infested with larvae of borers.

Soil

The report on soil analysis showed that, all the parameters analysed are well within the tolerable limits of sissoo. However, limitation is that, as only some of the physical and chemical properties such as texture, electrical conductivity, pH, exchangeable sodium and potassium, organic carbon, total nitrogen and CEC were analysed. The analysis of some other macro and micro nutrients are missing together with some physical properties such as bulk density and porosity.

Discussion

Mycological

Excepting *Fusarium* sp. *Ganoderma lucidum and Polyporus* sp. rest of fungal species like *Xylaria, Uredo sissoo, Phyllactinia dalbergiae, Maravalia achroa* and *Phyllactinia coryle* do not cause any serious problem to the growing plantations, though they affect physiological activities of the host. The species like *Schizophyllum commune, Coriolus versicolor* and *Coriolus tephroleucus* grow only on the dead wood and branches of the host.

Ganoderma lucidum, Polyporus sp. and *Fusarium* sp. (probably *F. solani)* were among the main fungal elements, which were responsible for the death of the sissoo trees in the Eastern Tarai. These pathogens were not confined only to sissoo but also to other species in the locality. *Ganoderma lucidum* is found to attack several species including *Acacia* sp., *Acrocarpus* sp., *Albizia* sp., *Cassia* sp., *Dalbergia* sp. (*D. sissoo* and *D. latifolia*), *Eucalyptus* sp., *Melia azedarach, Morus* sp., *Shorea robusta, Terminalia* sp., etc.

Ganoderma lucidum is widely spread in natural forests in the region where its population is balanced by the presence of mixed species with varying degree of resistance. It usually spreads by the root contact and can infect through the intact as well as the damaged roots. Hosts show varying degree of susceptibility to its attack, the weaker ones may be infected easily and killed very quickly. On contrary, the strong ones show a great resistance to the infection and may remain unaffected until they are cut or wounded, allowing the fungus to infect and weaken the tree and ultimately kill the it. If the spores of the fungus fall on the freshly cut portion of the trees or stump, the fungus grows very fast and acts as the infection centre for other trees and stumps. If plantation is carried in such area the infection may occur after 3 to 5 years when the new plantation starts sending longer lateral roots which comes in contact with those of older infected stumps. This fungus can not survive in soil but only live only on the substrate such as old stumps, roots, etc. It attacks and kills the bark and causes white spongy rot in the sapwood (Bakshi, 1976). It can attack the host trees of any age. However, after the infection, the young trees are killed soon whereas the older trees takes longer. *Polyporus spongiosum* also shows similar affect on the host.

Fusarium sp. is usually found in waterlogged and poorly drained soil and causes wilting of the plant. This disease is more rampant during the rainy season. The leaves of the diseased trees turn yellow to brown, and ultimately die from top to bottom and slowly the leaves fall off. The tree may die within a few months of infection. As the pathogen can inhabit soil, this disease can also be soil borne.

The oozing of sap may also be related to the

production of toxin by the pathogen. Sometimes the sap oozes out from the insect bore-holes, but it also comes out by cracking the bark at certain locations.

Several causes such as shown below, of the spread of causal agents in the Tarai belt of Nepal, are possible

- Close plantations nearing the Indian border where severity of such disease has been reported.
- Introduction of diseased saplings from India.
- Selection of diseased seeds for germination of seedlings.
- Wide spread vector carrying fungal elements.
- Wide spread of fungal spores through wind and water.
- No pesticides being applied to the cuts and wounds of the host, which serves as the easy infection point.

There is a progression in this disease in past few years. This species was selected as a very good, multi-purpose, fast growing, native, nitrogen fixing tree species (after the bad experience of Psyllid attack on exotic Ipil Ipil a few years back). F-FRED (a Multi-Purpose Tree Species network) was involved in promoting this. However, in actual practice a few basics in forestry practices were missing, such as collection of seeds from a good source (though mentioned, very little was translated into reality) and matching site with species. Another important point was large scale monoculture was being practiced without a good preparedness for the expected pests and diseases. The responsible national institutions were not equipped to tackle it. Even after the disease was taking a serious toll, very little was being done by the concerned agencies to mitigate it. They might have their own problems, but this is a serious national issue and crisis which will keep on repeating in future, needs special attention.

The possibility that the out-break of this disease followed a natural cycle of several years, is not known yet. The relationship between the last year's extended period of cold waves in the Tarai region with the out-break this year should also be explored.

Entomological

The interviews with the local people revealed that the spread of disease is from the Indian border close to the area. This could either through the vectors or is air borne. Undoubtedly, insects are responsible for certain damage to the sissoo trees, however, the extent and the actual mode of their attack are yet to be studied and established. Four major types of insects are responsible for the serious damage of the sissoo trees directly or indirectly.

The insect larvae and adults of the family Scolytidae make pinholes and sawdust, and the larvae of the family Buprestidae make zigzag passages in the sapwood and feed on it. Heavy infestations by these borers destroy the phloem and cambium by making criss-cross holes in the sapwood. Such damage destroys the translocation system resulting insufficient flow of food (sap) to the crown simultaneously the loss of nutrients (sap) from the stem. This may injure the tree severely leading to its ultimately death. Borers of the family Cerambycidae and Bostrichidae, living on branches, main stems and roots, might have caused die-back and death of the tree by eating its xylem system. However, in some cases, if the drying branch is cut a few inches below, the branch survives and proliferate another shoot. The nesting of the termites and borers throughout the stem and the fungi causing foliage diseases have definitely reduced the physiological activities of the plant causing its slow growth and ultimate death. However, the death of the trees even after treated with Thimet (a systemic insecticide) suggests that there are some other causes of its death.

As mentioned earlier, the study is not a complete one. It has a number of limitations such as: the study was conducted at a limited area and time (in December); it is conclusive on the visual causal agents found in the study areas and the growth of fungus and bacteria in the culture media. However, for the areas where this could not be done, needs careful attention in generalising the findings. Not much information could be obtained about their mode of damage to the host, host-pest relationship, intermediary hosts (if any), etc.

Conclusion

Many species of fungi such as *Xylaria* sp., *Schizophyllum commune, Uredo sissoo,, Phyllactinia dalbergiae, Ganoderma lucidum, Coriolus versicolor, Coriolus tephroleucus* and *Polyporus* sp. *Maravalia achroa* and *Phyllactinia coryle* are parasitic to the same host also do occur in the same region which needs further investigation.

The cultures made from the roots and stems revealed that *Dalbergia sissoo* has been seriously attacked by *Ganoderma lucidum, Fusarium* sp. and Bacteria. The root rots were due to bacteria while the whitish patch in rings and scattered patches were due to the fungus. The fermentation in the roots and some portion of the trunk were due to bacteria, which appeared to be a secondary infection. However, it is

necessary to ascertain the role of bacteria also.

There are many species of *Ganoderma* that are similar to *Ganoderma lucidum* prevailing in the tropical region, which extend from Japan to Indian subcontinent. It is therefore, necessary to have intensive collections and studies to find out their presence, virulence and susceptibility.

Several fungal pathogens as well as insect pests including bacteria were recorded during this study. However, only a few of them are of serious concern as only these seriously threaten the sissoo trees and other important plantation tree species. The species of main concern are *Ganoderma lucidum*, *Fusarium* sp., and *Polyporus spongiosum*. The insects borers belonging to the families Scolytidae, Buprestidae, Cerambycidae of the order Coleoptera (Beetles) are most prominent and significant among the insects. However to some extent the role of termites has also to be considered. The role of insects and fungi in damaging the sissoo trees has to be studied to ascertain the primary and the secondary causes of infection.

Recommendations

The recommendation especially related to the use of systemic fungicide and insecticide is not a lab or field tested one, so needs to be administered cautiously. All the necessary precautions for the use of such chemical should be followed.

Control measures

- Trees, and any parts infested with borers and fungus, should be removed. Fungal basidiocarp prevailing in the surrounding areas should be burnt.
- Mixed plantation should be encouraged.
- Resistant strains of the plant should be selected for breeding.
- Growth and dominance of vectors and fungal elements should be discouraged by practicing distant planting of non-susceptible plant species.
- Use prescribed doses of fungicide at regular interval. Expose roots of the infected trees by making a trench around the tree and apply 0.2% solution of systemic fungicide such as Carbendazim 50% at the rate of 10 to 20 lt of solution per tree, depending on the size and age of the tree. Three applications are recommended for three months.
- Apply 0.1% systemic insecticide solution (such as Metasystox, Roger, etc) in the roots of the infested tree at the rate of 5 lt per tree for trees up to 15 ft high and 10 lt for trees over 15 ft high. If the systemic insecticide is in the form of granules (such as Furadon, Thimet, etc) the recommended dose is 15 gm per tree for trees up to 15 ft high and 20 gm for trees above 15 ft high. After applying these granules at the roots, the roots should be irrigated with 10 lt of water. Such applications should be followed once in 30 days and for three months.
- The application of systemic insecticide and fungicide should alternate every 15 days.
- Bordeaux paste (50% lime plus 50% copper sulfate plus water) should be painted on the fresh cut/pruned surfaces of the trees.
- Use seedlings of *Ailanthus* sp, Simal and teak for plantation in areas where sissoo is infected with *Ganoderma lucidum*.
- Site - species matching should be done before plantation.
- Tree hygiene, pruning, thinning, weeding, mixed plantation, irrigation of trees, etc. must be practiced to protect from insects.
- Dead and dying trees and branches must be cut and burnt to prevent further multiplication and spread of insect pests.

Other areas of study

- It is necessary to find out the distribution pattern and dominance of the fungi through out the Tarai. The percentage and/or frequency of disease has to be documented.
- It is necessary to find out the host range of *Ganoderma lucidum* and *Fusarium* sp. occurring in the region along with the presence and effect of other *Ganoderma* species which are similar to *G. lucidum*.
- The extent of damage of sissoo in relation to wood quality and uses of logs.
- It is necessary to carry out some experiments in relation to the control and management of diseases of sissoo. This is especially important to prescribe the dose of insecticide/fungicide
- Intensive soil analysis for some physical

properties, some macro and micronutrients should be done.

- A detail study on mode of infection should be carried.
- There is a need to collect the available information and share the experience prevailing in India. For this, the research team should visit Dehra Dun as soon as possible.
- A well equipped wing should be established at the Department of Forest Research and Survey to tackle problems of pests and diseases of forest trees.

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