SURVEILLANCE ON *Phytophthora ramorum*, THE CAUSE OF SUDDEN OAK DEATH: A POTENTIAL THREAT TO RUBBER AND UPLAND FLORA IN SRI LANKA

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Summary

Sudden Oak Death (SOD) is a Phytophthora *disease of a diverse range of tree and shrub species in America and many European countries. Although not yet recorded, there is a significant threat of this pathogen to Sri Lanka, since environmental conditions in the hill country suit the pathogen requirements. Economically important plant species belong to families susceptible to the pathogen are available in those areas and a threat to the Sri Lankan flora is possible. Rubber tree is also a susceptible host for some* Phytophthora *species and a considerable part of rubber plantations are located in the hill country. Therefore, rubber can also be infected by* P. ramorum *causing die back of shoots and branches or tree death. This pathogen could easily be transmitted to our land via trade links with American continent and European countries when importing live planting materials. To prevent entry of this pathogen to Sri Lanka, phytosanitory measures should strictly be followed on importation of such commodities.*

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INTRODUCTION

A pathogen causing Sudden Oak Death (SOD) of pine was first found in Germany and the Netherlands in 1993, which was also found to be responsible for killing both nursery and mature *Rhododendron* species. Later in 1998, it was recorded in *Viburnum* affecting similarly, and thereafter the causative agent was named as *Phytophthora ramorum* sp. nov. (Werres *et al.*, 2001). The devastation to the Oak (*Quercus agrifolia*, *Q. kelloggi*, and *Q. parvula* var. *shrevei*) forest ecosystems in USA, mainly in central coastal areas of California, by this pathogen has been remarkable. Since its initial observation in mid-1990s, it has been found in twelve countries including the central and northern coastal region of California (Moralejo & Descals, 2002).

The pathogen has been reported in the UK including Channel Islands and Republic of Ireland, Belgium, Denmark, France, Slovenia, Spain and Sweden (Appiah *et al.*, 2004). Presently, about 31 susceptible host trees and ornamental plants and more than 80 hybrids of *Rhododendron* have been reported in the UK and Republic of Ireland, and 9 host species have been reported in the other European countries (Sanford *et al.*, 2004). It has also been reported to cause oak mortality in the south-western part of the Iberian Peninsula (Brasier, 1992) and threatens important endemic plants in Cape Town, South Africa (Moralejo & Descals, 2002). The finding of *P. ramorum* in many places had been associated with the occurrence of less virulent *P. nemorosa* and *P. pseudosyringae* causing stem cankers on species of Fagaceae and leaves of various hosts in California and Oregon (Hansen *et al.*, 2003; Martin & Tooley, 2003), the host range and the symptoms of which are similar to *P. ramorum.* This could be a dangerous situation since identification problems may arise in new localities.

The eradication of the disease has been carried out by clear cutting and burning in the infested areas (Kanaskie *et al.*, 2002), although practical problems are associated with disease management and removal of dead trees causing significant regulatory problems (Martin & Tooley, 2003). Ornamental plant nursery diseases of *P. ramorum* are indeed manageable and can be prevented from establishing in nurseries. *P. ramorum* in nurseries can be controlled using common fungicides (Annex 1), which are used to control other *Phytophthora* species. (Jeffers, 2002). Intensified control measures will increase operational costs, but a fully geared nursery industry will respond positively to this situation. The success of these strategies comes at great cost to the nursery industry in affected areas, but the cost is borne because there is no alternative. It remains to be seen, the high cost of controlling *P. ramorum* forces some of these nurseries out of business.

Biology of the pathogen

Comparisons made by a variety of molecular and other methods revealed that, *P. ramorum* arises through interspecific hybridization as it forms a phylogenetically distinct species (Martin & Tooley, 2003). *P. ramorum* is identical to other *Phytophthora* species producing typical structures and disseminating through water. It is a cool temperature organism, which has an optimum growth at 20° C (Henricot & Prior, 2004), but, it can grow in temperatures up to 30° C as well (Werres *et al.*, 2001). The sporangia germinate in water to produce zoospores, which are responsible for penetrating leaves and young stems directly without wounding (Henricot & Prior, 2004). This is a unique character of this particular pathogen and other species such as *P. meadii* or *P. palmivora* on rubber, as they do not particularly need a wound to enter the host tissue.

In dead tissues, it produces resting spores (chlamydospores), which are tough, persist longer and may be spread in soil. Sporangia and chlamydospores are produced abundantly on foliar hosts than in woody materials (Swain, 2002), suggesting the importance of foliar hosts for the completion of its life cycle. Dissemination to distal places may be by humans *via* infected foliage plants or soil.

As *P. ramorum* is heterothallic organism, it requires the presence of two mating types to produce sexual spores (oospores). In Europe, only the mating type A1 was present until recently, while A2 type has been discovered on an imported *Viburnum* in Belgium (Werres & De Merlier, 2003) and subsequently the A1 type was also discovered on imported *Viburnum* and *Pieris* in Origon, USA (Hansen *et al.*, 2003). However, A1xA2 out-crossing system does not appear to be rather efficient as their successful partners are reported to be different for both types including *P. cinnamomi* (Werres & Zielke, 2003). Therefore, sexual reproduction has not been observed in nature yet, and if mating occurs it would therefore be possible to produce aggressive strains and cause diseases on certain other hosts (Brasier, 2003) including rubber.

The damage caused by this pathogen is immense, and can be described as bark cankers or leaf wilting and die back on oak species (Fig. 1a, Fig. 1b) or other types of damages such as lesions, die backs on many forest or ornamental plant species. These damages subsequently cause impacts on the environment since they lead to the death of infected trees. In addition, high costs are associated with removing infected or dead trees, and the respective government authorities will have to bare the cost. There is also the potential danger of development of aggressive hybrids through sexual recombination between European isolates (A1 mating type) and the American isolates (A2 mating type), if both are introduced into the same area (Appiah *et al.*, 2004).

Disease transmission pathway

Natural infection and transmission of *P. ramorum*, as in all oomycetes species, depend on the rainfall and high humidity over a prolonged period. At first, the pathogen produces asexual spores in sporangia on or within living plant tissues, such as leaves, green stem and woody stems. At temperatures of 15-20 ° C during high humidity period, sporangia release zoospores, and rain splashes or water streams transport live zoospores to distal hosts making new infections. In addition, *P. ramorum* prolifically produces chlamidospores in culture and on some foliar hosts indicating a possible mechanism for dormancy and survival in adverse conditions (Davidson & Shaw, 2002). Disease transmission through movement of contaminated materials such as soil, logs, wood, and ornamental plants by human and animal activities has also been reported (Tjosvold *et al.*, 2002).

A final step in the transmission cycle involves finding a succeptible host and successful infection on a wet tissue covered with a film of water at an optimum temperature of 20 ^{0}C. Disease cycle or infection transmission pathway in the ecosystem can be completed easily, if all the requirements are met with. There are many factors that are involved in these functions such as human, animal or natural factors. In woodland forests, animals such as deer can also carry viable spores in soil stuck to their feet possibly to short distances. There is also a great possibility of carrying live *P. ramorum* spores and chlamidospores by migrating birds from the infected forests of the Europe to South East Asian countries during the winter season, and this should also be investigated.

Forest hikers have been shown to carry spores of *P. ramorum* on their shoes or on tires of cars or mountain bikes as they travel through contaminated muddy or wet roads and leave infested tanoak-redwood forests (Tjosvold *et al.*, 2002). These tourists may subsequently visit other areas or countries with same shoes or infested material endangering the visiting site. In addition, leaves and branches of hosts such as bay laurel, Douglas-fir, and redwood are used in wreaths and garlands. Douglas-fir is framed for Christmas trees and they are transported to long distances. Although cones of redwood and Douglas-fir do not appear to be infected in the field, live zoospores may land on cones, and finally, it may be a source of a new infection (Davidson & Shaw, 2002). Likewise bark and wood products of infested trees, nursery stocks from infected sites may also serve as sources of inocula.

Heavy machineries which are used for forestry operations are another means of the disease transmission for long distances. Organic compost fertilizer made out of infested leaves can carry chlamidospores, which will be proliferated when added to wet soil.

Host range and their occurrence in Sri Lanka

The pathogen has a wide range of hosts including about 32 different genera belonging to 18 families (Henricot & Prior, 2004). Some tree species which are belonging to the families of recorded hosts of *P. ramorum* are available in Sri Lanka.

According to Table 1, some trees of the families Anacardiaceae, Aceraceae, Caprifoliaceae, Ericaceae, Fagaceae, Lauraceae, Oleaceae, Pittosporaceae, Rhamnaceae, Rosaceae and Theaceae are susceptible to this fungus. In Sri Lanka, there are some tree species, belonging to the affected genera of above families such as *Castenea*, *Fagus*, *Lithocarpus*, *Laurus*, *Syringae*, *Pittosporum*, *Rhamnus*, *Rhododendron*, *Camellia*, *Viburnum* and varieties of roses. Roses are widely grown in the hill country as an ornamental flowering plant for sale. *Rhododendron arboreum* var. *zeylanicum* (Maharathmal) is an endemic species in Sri Lanka.

Many of the related species are grown on high elevations in Sri Lanka such as Nuwara Eliya, Badulla and Bandarawela, where the temperature varies around 20-25 °C (Fig. 2), and this is the ideal climatic condition for the pathogen.

Although rubber tree does not belong to any of the above families in Table 1, it is also affected by *Phytophthora meadii* McRae, *P. palmivora* (Butl.) Butl. (Danthanarayane *et al.*, 1984), and *P. citricola* Sawada (Liyanage, 1989) rarely. It is also grown on higher elevations where the average temperature may be 2° C lower than the normal average in the wet zone (28°±2° C). Therefore, there is a possibility that *P. ramorum* may harbor on rubber too and become a new pathogen if conditions will be conducive, producing new strains that would be able to invade rubber or other host species in Sri Lanka. Biotic or abiotic factors may cause formation of new pathogenic strains (Jayasuriya, 2004) and therefore, precautions should be taken to minimize the risk of pathogen migration to our land. Since the pathogen has already arrived in Europe within a very short period, further spread may be expectable in ideal situations such as importation of infected plant material or alternate hosts, visitors from risk areas etc.

Figure 1a : Bleeding and tree die-back caused by *Phytophthora ramorum* on bark of oak tree. Source: Anon. (2002).

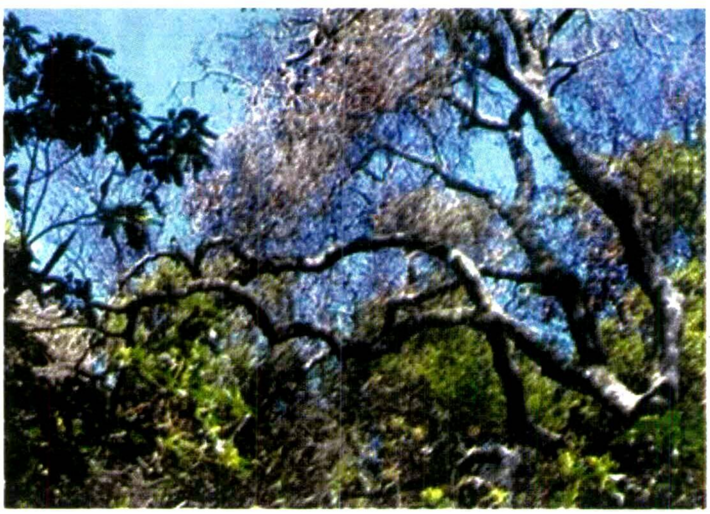
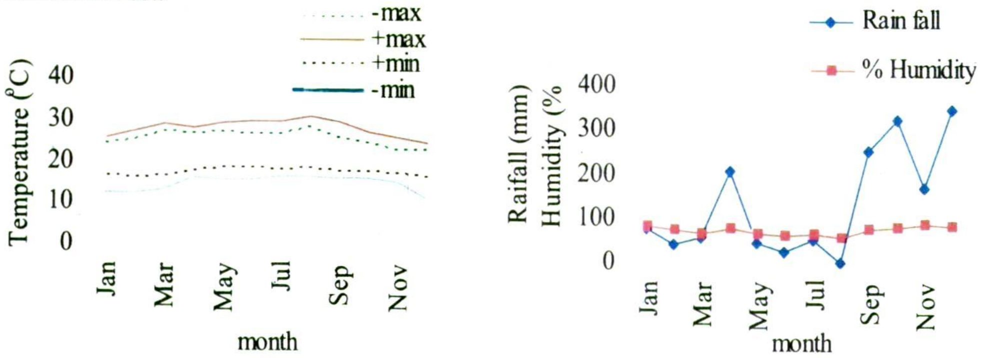
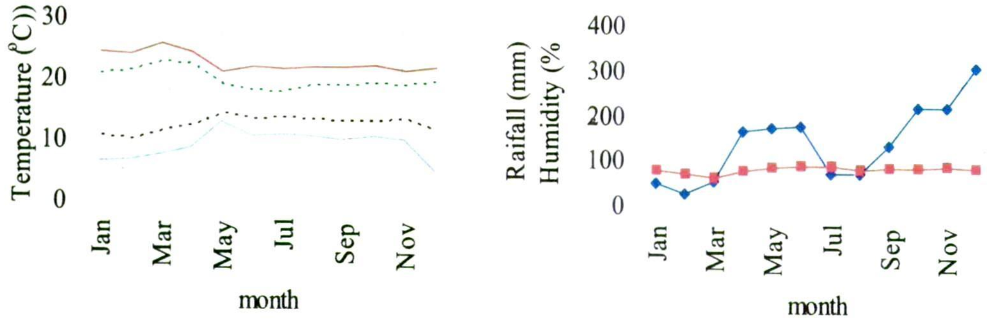


Figure 1b : Bleeding and tree die-back caused by *Phytophthora ramorum* on canopy of oak tree. Source: Anon. (2002).

Bandarawela



Nuwara Eliya



Badulla

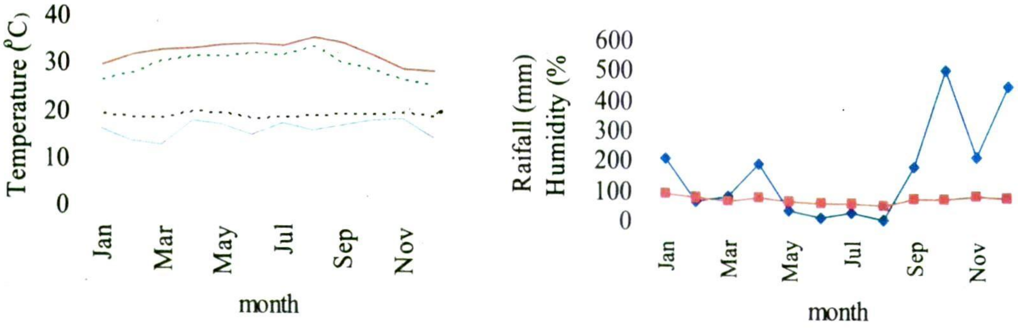


Figure 2: Temperature variations, rain fall and humidity patterns occurred in the year 2000 in three upland areas in Sri Lanka.

Table 1 : Host species succeptible to *Phytophthora ramorum*

Family	Latin name	Common name	Disease	Reference	Species available in Sri Lanka
Anacardiaceae	*Toxicodendron diversilobum*	Poison Oak	Stem cankers	Anon. (2004)	-
Aceraceae	*Acer macrophyllum*	Big leaf maple	Foliar lesions; long-term impact on individual plants unknown	Anon. (2004)	-
Ericaceae	*Rhododendron spp.*	Ornamental rhododendron	Twig wilt, dieback, stem lesions, leaf mid-rib or tip necrosis	Anon. (2004)	*Rhododendron* spp.
	Arbutus menziesii	Madrone	Branch cankers, foliar lesions; death of regeneration	Anon. (2004)	-
	Vaccinium ovatum	Evergreen huckleberry	Stem, branch cankers, foliar lesions; dieback of canes, death of plants	Anon. (2004)	-
Fagaceae	*Castanea sativa*	Sweet chestnut	Foliar/shoot blight	Anon. (2004)	*Castenea* spp.
	Fagus sylvatica	Beech	Bleeding trunk canker	Anon. (2004)	*Fagus* spp.
	Lithocarpus densiflorus	Tanoak	Bleeding trunk canker, shoot dieback, leaf blight	Goheen et al. (2002)	*Lithocarpus* spp.
	Quercus spp	Oak spp	Stem cankers, tree death	Anon. (2004)	-

Lauraceae	*Laurus nobilis*	Bay laurel	Leaf spot, twig dieback	Anon. (2004)	*Laurus* spp.
	Umbellularia californica	Bay laurel, Oregon myrtle	Foliar lesions; long-term impact on individual plants unknown	Anon. (2003)	
Oleaceae	*Syringa vulgaris*	Lilac	Leaf blight	Beales et al. (2004)	*Syringae* spp.
Pittosporaceae	*Pittosporum undulatum*	Victorian box	Leaf spot	Anon. (2001)	*Pittosporu-m* spp. (Kaputu)
Rhamnaceae	*Rhamnus californica,*	Californian coffee	Leaf spot	Anon. (2001)	*Rhamnus* spp.
	R. purshiana	Berry cascara			
Rosaceae	*Rubus spectabilis,*	Salmon berry		Hüberei et al. (2004)	14 spp. mostly wild berries
	Rosa gymnocarpa	Wood rose	leaf spot		
	Heteromeles arbutifolia	Toyon	Branch cankers, foliar lesions; branch dieback	Anon. (2003)	
Theacea	*Camellia* spp.	Camellia	Leaf blight & twig die-back	Anon. (2001) Beals et al. (2004)	*Camellia sinensis (tea)*
Caprifoliaceae	*Viburnum* spp.	viburnum	Basal stem lesions, wilting and death of entire plants		2 montane spp.

Disease Management

Identification of host species resistant against *P. ramorum* is a challenging task for scientists. Several cultivars of Rhododendron and the species *Rhododendron* 'P.J.M.', reported to have a low incidence of *Phytophthora* dieback in nurseries caused by species other than *P. ramorum* (Benson *et al.*, 1982). Leaves of *Rhododendron* 'P.J.M.', *R. maximum*, and *R. carolinianum* dipped in sporangia suspensions of *P. ramorum* developed lesions on less than 10% of the leaf area compared to a highly susceptible cultivar like Cunningham's White that developed up to 50% leaf lesion area with the most virulent isolate tested (Tooley *et al.*, 2002). Considering the host range of *P. ramorum* in California and Oregon forests, and the potential host range determined experimentally on nursery crops (Tooley *et al.* 2002), a cultivar-by-cultivar screening will no doubt identify resistant cultivars that can be exploited for the nursery trade.

Management of *P. ramorum* in nurseries through integrated pest management practices including cultural practices, fungicides (Annex 1), and host resistance will be paramount to producing disease-free plants as well as protecting the native plant environment around nurseries. As we learn more about the biology and ecology of the pathogen and the epidemiology of diseases caused by *P. ramorum*, management strategies will continue to be improved.

DISCUSSION

Forecasting about a possible establishment of a new wide-host range pathogen in a new area has been explored in this article. Similarly, the damage or devastation to plant species in affected countries has also been discussed and there is no doubt that a similar damage could be expected in Sri Lanka, since importing and exporting ornamental plants or plant parts has become a profitable trade in Sri Lanka and the majority of ornamental plant firms are established in the upland of Sri Lanka. It has been emphasized the possibility of *P. ramorum* to occur and develop optimally in the upland forest areas or rubber plantations in Sri Lanka, when a combination of three factors *viz.* an infective virulent pathogen, a susceptible host and a favorable environment similar to that in affected areas are available. It may not only be developed but also transmitted to other areas which are free from occurrence. Although the pathogen is still not detected in Sri Lanka, there is a possibility that it has already been landed but possibly hibernating until a susceptible host is found or formation of a virulent form. However, the favorable environment is available only in the mid country and chances of bypassing the peripheral area and landing the pathogen on the mid country is less. But it is worth enough to consider the possible avenues by which this dangerous pathogen might easily reach our country and subsequently favorable sites *via* internal transport of plant material or other modes of transmission.

The most possible passage through to Sri Lanka may be crossing over the south Pacific pathway through Australian sub continent or its surrounding islands, where climatic conditions may be more conducive to the pathogen than that of the Mediterranean basin, where *P. ramorum* has already been found in several localities (Moralejo & Descals, 2002). However, there are no reports of the pathogen in Australia or surrounding countries at this moment.

The situation would be more dangerous if the pathogen would land in Chinese or Mongolian territories which are closer to the Asian territories. Therefore, strict biosecurity or quarantine regulations should be imposed in importation of related plant material (Annex 2). Especially, those who are involved in horticultural industry should be well aware of this dangerous threat to the country. Quarantine Officers should also be alerted and be educated about the symptoms of the disease and possible susceptible hosts.

The importation or exchange of planting material between these countries and the affected ones would facilitate the pathogens passage through to unaffected area. Therefore, import restrictions on commodities deemed to be extreme risk and originating from areas of the U.S. and Europe known to have the disease. These included all propagative and non-propagative material including live plants from stock nurseries, logs with attached bark, mulch, pulpwood, of all species of oak (*Quercus* spp.), tanoak (*Lithocarpus* spp.) and rhododendron (*Rhododendron* spp.). The tourist industry would directly be related to this situation and hence, the authorities should be aware about this danger. Foreigners visiting our country from the risk areas should be educated not to bring contaminated material or there belongings especially the shoes and clothes should be disinfected at the port of entry. Strict regulatory measures should be imposed to make this success. On implementing regulatory restrictions against possible migration of *P. ramorum*, following areas should be investigated.

(1) Identify the difference of *P. ramorum* from other *Phytophthora* species which are currently available in Sri Lanka, and determine classical and molecular methods best confirm the identity of *P. ramorum.* The ITS-based PCR technique is specific for *P. ramorum* at low DNA concentrations and has proven useful for detecting it in infected host tissues. Other DNA-based methods for the detection of *P. ramorum* include assays based on the sequences for other nuclear genes (ß-tubulin), or sequences for organellar or mitochondrial genes (cox2). A promising new technique called single strand conformation polymorphism (SSCP) analysis may eventually allow for the detection and unambiguous identification of most, if not all *Phytophthora* spp. (Blomquist & Kubisiak, 2002).

(2) Methods should be proved and optimized to detect and survey the pathogen in the forest ecosystem and ornamental nurseries in Sri Lanka which are actually susceptible to the pathogen. This has to be done by training relevant personal for the particular task, and identifying potential carriers of the pathogen within the country.

To protect our flora from this potential pathogen it will be important to learn from Europe where it has been subjected to control (Annex 2) under emergency legislation under the EC Plant Health Directive since 2002 (Henricot & Prior, 2004). All the field officers who are directly involved in the forestry, ornamental plants, tea and rubber cultivation should be vigilant on infections or symptoms on the above plant varieties.

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Annex 1 : Fungicides registered for use against *Phytophthora* spp. in ornamental crops in nurseries.

Fungicide category	Active ingredient	Mech-anism of action	Movem-ent in plants	Risk of resista-nce	Labeled users[z]		Relative efficacy[y]	
					Foliar spray for aerial blight	Soil application for root rot	Aerial blight	Root rot
Oomycet specific	Mefenoxam, Metalaxyl	P+C	US	H		✓		+++
	Fosethyl -AL, Phosphorus acid, phophonate	P+C	S	M	✓	✓	+++	+++
	Dimethomorph	P+C	US	M	✓	✓	+++	++
	Etridiazole	P	NS	L		✓		++
	Propamocarb	P	US	L		✓		+
Strobilurin	Azoxystrobin	P+C	US	M	✓		++	
Broad spectrum protectants	Mancozeb, Maneb	P	NS	L	✓	✓	++	
	Chlorothalonil	P	NS	L	✓		++	
	Copper compounds	P	NS	L	✓	✓	++	+

[z] Recommended uses appearing on labels of registered products; [y]Relative efficacy; + = marginally effective; ++ = moderately effective; +++ = very effective.

[x]Mechanism of action; P = protectant; C = curative; [w]Systemic movement in plants; NS = non-systemic; US = upwardly systemic; S = systemic; [v] Relative risk of resistance developing; H = high; M = moderate; L = low; [u] Very few copper products are labeled for soil drench applications to control root rot.
(After: Jeffers, 2002)

Annex 2 : Articles and actions listed in the APHIS

Sudden Oak Death interim rule

Article	Action	Criteria
Areas	Quarantine	12 California county and 1 Oregon (partial) county
Nursery Stock	Regulated	Annual inspection & testing of hosts; Shipment inspection and subject to testing if symptoms observed
Forest Stock	Prohibited	All plants & parts not nursery grown
Wood	Regulated	Must be bark free
Bark	Prohibited	Cannot be commercially shipped
Soil	Prohibited	Nursery stock must be free of duff, Contaminated soil must be treated
Wreaths, Garlands & Greenery	Regulated	Hot water dip
Redwood & Douglas fir	Prohibited	Sprouts, twigs, needles (other parts ok)

After: Cohen & Jones (2002)