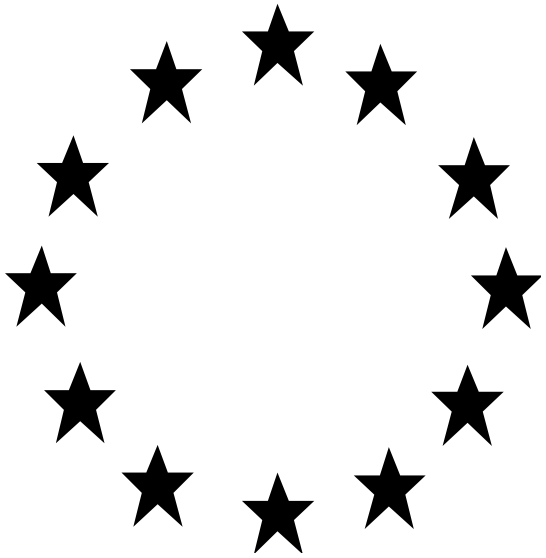

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Estonia for the existing active substance

PHLEBIOPSIS GIGANTEA

of the fourth stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, part 4, B.8

September 2008

Draft Assessment Report

Phlebiopsis gigantea

Volume 3

Annex B.8

Fate and behaviour in the environment

Rapporteur Member State: Estonia

April 2007

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B.8 Fate and behaviour in the environment (Annex IIB 7 and IIIB 9)

Phlebiopsis gigantea is found throughout the temperate Northern Hemisphere and has also been recorded in southern Europe, East Africa, Central America, Australia and New Zealand. There is some degree of variation within populations of *P. gigantea*. However, studies of sexual compatibility indicate that all European populations are interfertile, demonstrating that *P. gigantea* is a single species and there are no distinct geographic eco-types. (For further details see Volume 3 Annex B.1). Therefore it is acceptable to consider all the isolates supported in this dossier together.

P. gigantea is a saprophytic wood-rotting fungus, whose main host is moribund wood. Airborne spore numbers can naturally be very high in coniferous forests and, as a common saprotrophic decay fungus it can cause significant degrading of produce left for too long in the forest. Rates of spore deposition have been measured in managed forests in the UK and in Finland. In one UK forest over 80% of spore traps had one or more viable spores deposited per hour, with a mean rate from five sites of around 14 spores per $100cm^{-2}\,hr^{-1}$. These rates were recorded between March 1957 and August 1958, approximately 10 years after first thinning of the newly-planted pine forest began, and, incidentally, before any treatments with *P. gigantea* had begun in that area. (Rishbeth 1959). In a comparison with these early findings, Pratt (pers comm) measured more current ambient spore loads of both *H. annosum* and *P. gigantea* in the same general area at a time when stump treatment was being used in the forest. Spore traps were exposed in an area of pine forest not far from sites where stump treatment with *P. gigantea* was being done in harvesting operations. Viable spore deposition rates are shown below, along with rainfall (mm) during the previous month.

Table B.8.1: Monthly mean spore deposition (per 100 cm^2 per hour), and rainfall (mm) during previous month (Pratt, pers. comm.)

	Month (1996-1997)											
	Feb	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Pg	**7.9**	**2.3**	**0.4**	**0**	**0**	**0.4**	**0**	**0.4**	**1.5**	**4.8**	**16.0**	**0.2**
Ha	**0.4**	**0.3**	**0.1**	**0**	**0**	**0**	**0**	**0.1**	**0.8**	**0.5**	**2.1**	**0**
Rain	**21**	**46**	**19**	**5**	**22**	**15**	**59**	**98**	**17**	**55**	**100**	**N/A**

The results indicate wide variability in rates of spore deposition, a feature also noted by Rishbeth (1959). An arithmetic mean hourly deposition rate of *circa* 2.8×10^6 viable *P. gigantea* spores per ha (2.8 spores $100cm^{-2}\,hr^{-1}$) can be extrapolated from these data, and these levels do not exceed the levels that Rishbeth recorded close to forests. This indicates that there was no significant increase in spore levels in the forest after the introduction of stump treatment. Total spore loads applied to stumps during treatment operations are in the order of 3×10^8 viable spores per ha for manual and mechanical treatments (~1 spore $100cm^{-2}\,hr^{-1}$), the former over a period of a week or so, the latter within two days. Such calculations emphasise that natural deposition rates are comparable to those

applied artificially and stump treatment does not raise the levels of spores found naturally in woodland significantly. It's also indicated by Rishbeth (1959) that the deposition rates of *P. gigantea* are often low after periods of cold weather and observation suggest that this is due to drying out of sporophores rather than freezing. Apart from such temporary effects of weather, there is no evidence from section exposures of any definite periodicity in *P. gigantea* spore discharge throughout the year.

Bearing in mind these ambient spore loads, freshly cut stumps are often naturally colonised by *P. gigantea* regardless of the application of any stump treatment agent. On such stumps it is one of the earliest colonisers, and is able to compete successfully with the pathogen *H. annosum*, and other member of the *H. annosum* complex (Meredith, 1960; Gremmen 1963; Capretti & Mugnai, 1989). Although *P. gigantea* may utilise a degree of hyphal interference against *H. annosum* (Ikediugwu et al, 1970, Ikediugwu, 1976), there is no evidence in the available literature that *P. gigantea* controls *H. annosum* by antibiotic or toxic means. (See volume 3 Annex B.2 for more detailed discussion on mode of action). It is used in the manufacture of 3 biological control products in the Europe (see volume 3 Annex B.1) and is applied directly to stumps during manual or mechanical felling where it colonises the wood and bark of the stump.

P. gigantea is relatively quick-growing basidiomycete, although growth is affected by temperature and humidity. Meredith found growth to be slower in wood with lower moisture content and in relatively warm and dry season the growth rate of *P. gigantea* in stumps naturally infected at the cut surface was significantly lower than those in stumps produced during the cooler and wetter autumn (Meredith 1960) and field reports indicate *P. gigantea* grows more slowly in the cold winter months than in the summer (Rishbeth 1963). *In vitro* studies also demonstrate the influence of temperature on *P. gigantea* growth. At 10^{o}C on malt agar Cartwright and Findlay (1958) reported daily increments of 6mm and Thorpe (2001) reported 5.4mm. This increased to 16mm at 27.5-28^{o}C. Isolates grown on pine and spruce sawdust agar showed lower growth rates - 8mm at 28^{o}C (Niemi 1992a). Reports vary slightly as to the lethal upper limit for *P. gigantea*. Thorpe found growth ceased at 35^{o}C, although this temperature did not prove to be a lethal limit as samples re-incubated at lower temperatures recovered. Cartwright & Findlay (1958) found temperatures exceeding 38^{o}C were lethal whilst Niemi subjected working solutions of Rotstop to temperatures between 30^{o}C and 80^{o}C for 10 minutes and found reduced viability at 30, 35 and 40^{o}C. At 45^{o}C the temperature effect was more pronounced and all spores were killed at 50^{o}C and higher temperatures (Niemi 1992b).

The references that were used to summarise the above information on the ecology and life history of *P. gigantea* are listed in further detail below, grouped according to the general topic discussed.

<u>*Spore deposition*</u>

Reference: Rishbeth, J. (1959) Dispersal of *Fomes annosus* Fr and *Peniophora gigantea* (Fr.) Massee. Trans. Brit. mycol. Soc. Vol. 42 (2), pp. 243 – 260.

Not GLP. Published.

Summary: Dispersal and deposition of *P. gigantea* and *H. annosum* spores was measured by exposing freshly cut pine discs or sterilised muslin sheets in an East Anglian forest in the UK. Mean spore deposition levels of 5-23

viable spores per 100 cm^2 per hour were recorded over a year (1957-58). Deposition was highest in still air, and in the vicinity of fruit bodies, and was decreased in periods of dry weather. There was no pronounced seasonal variation in spore levels. Spore deposition levels were found to be similar in Swedish forests visited by Rishbeth. Muslin traps identified *P. gigantea* and *H. annosum* spores in areas up to 200 miles from any potential sources of inoculum, indicating their considerable ability to travel on air currents.

Reference: Rishbeth, J. (1963) Stump protection against *Fomes annosus*. III. Inoculation with *Peniophora gigantea.* Ann. Appl. Biol. Vol. 52 (1), pp. 63 – 77.

Not GLP. Published.

Summary: Field trials in southeastern England demonstrated the strong colonising ability of *P. gigantea* in UK pine forests, and Rishbeth observed the fungus out-competing and, to a limited extent, replacing *H. annosum* in the stump. Stump colonisation was slower in the winter than in the summer, and spore deposition rates were reduced during periods of very dry weather.

Mode of action

Reference: Capretti & Mugnai (1989) *In vitro* test of antagonism against *Heterobasidion annosum* (Fr.) Bref. Phytopath. Medit. Vol. 28, pp. 155 – 157.

Not GLP. Published.

Summary: In *in vitro* pairing tests between *H. annosum* P, S and F-type (now more commonly considered to be *H. annosum* s. stricto, *H. parviporum* and *H. abietinum*) and *P. gigantea*, the latter quickly overgrew colonies of *Heterobasidion* at 20°C and also at 12°C at a slightly slower rate. No mycelium-free inhibition zones were observed. The fact that there was no inhibition zone can be considered further evidence for simple competition rather than interference based on diffusible secondary metabolites.

Reference: Gremmen (1963) Biological control of the root-rot fungus *Fomes annosus* (Fr.) Cke by *Peniophora gigantea* (Fr.) Masse. Med Barbouw. Ned. Bosb. Tijdschr. Vol. 35(9), pp. 356-367.

Not GLP. Published.

Summary: *In vitro* tests showed that *P. gigantea* was able to suppress *H. annosum in vitro* through nutrient competition. Confluency and overgrowth occurred with no apparent zone of mutual antagonism.

References: Ikediugwu, F.E.O., Dennis, C., Webster, J. (1970) Hyphal interference by *Peniophora gigantea* and *Heterobasidion annosum*. Trans. Br. Mycol. Soc., Vol. 54 (2), pp. 307 – 309.

Not GLP. Published.

and

Ikediugwu, F.E.O. (1976) The interface in hyphal interference by *Peniophora gigantea* against *Heterobasidion annosum*. Trans. Br. Mycol. Soc. Vol. 66, pp. 291 - 296.

Not GLP. Published.

Summary: In *in vitro* tests where *H. annosum* and *P. gigantea* were allowed to grow towards each other at 25°C on 2% malt agar there was no evidence of a hyphal growth reduction as the colonies approached each other. In further tests *P. gigantea* colonised a cellophane membrane overlying malt agar (MA). On removing the membrane

and *P. gigantea* mycelium, colonies of *H. annosum* inoculated onto the underlying MA also showed no signs of growth reduction. These results suggest *P. gigantea* does not produce any readily diffusable antagonistic compounds in advance of the hyphae.

Further tests indicated that *P. gigantea* hyphae in close proximity to *H. annosum* (incubation at 25°C on 2% malt agar) caused major changes in the structure (and presumably also the functioning) of *H. annosum* hyphae, to the extent that some of the latter die. This hyphal interference occurs at temperatures tested (5-30°C), is not uncommon in inter-specific competitive fungi. However, the precise mechanism of interference has not been elucidated. Hyphal antagonism has only been demonstrated *in vitro* and its role or even existence in nature is not known.

Reference: Meredith, D.S. (1960) Further observations on fungi inhabiting pine stumps. Ann. Bot. Lond.(n.s), Vol. 24 (93), pp. 63-78.
Not GLP. Published.
Summary: In experiments conducted in pine forests in East England Meredith demonstrated *P. gigantea* was a vigorous competitor of *H. annosum* for initial colonisation of stumps. Stumps were sprayed with basidiospore solutions of both fungi, either mixed together, or as separate suspensions of one or the other species. In stumps inoculated with equal proportions of both, *P. gigantea* had a decisive advantage after 12 weeks (95% coverage on stumps) that was only decreased (60% and 2%) when the balance of spores was greatly in favour of *H. annosum* (10:1 and 100:1, respectively). In addition he compared rates of growth of *P. gigantea* and other fungi (including *H. annosum*) in stumps and on artificial media. *P. gigantea* and *H. annosum* have similar growth rates in wood and both were found to have penetrated into the roots of stumps within 6 months. Low temperatures and low moisture content of stumps reduced growth rates.

Growth rate

Reference: Cartwright, K,ST.G., Findlay, W.P.K. (1958) Decay of timber and its prevention. Forest Products Research Laboratory. 2nd Edition. Her Majesty's Stationery Office, London, pp.178-179.
Not GLP. Published.
Summary: In their book 'The Decay of Timber and its Prevention' Cartwright and Findlay provide growth rates of *P. gigantea* on malt agar. At 10°C on malt agar they reported daily increments of 6mm and this increased to 16 mm at 28°C. They also found temperatures exceeding 38°C to be lethal.

Reference: Niemi, M. (1992a) Effect of temperature on the growth of *Peniophora gigantea* and *Heterobasidion annosum*. Kemira Agro Oy, Espoo Research Centre. Test report 9241, 2 pp.
Not GLP. Unpublished.
Summary: In this experiment one strain of *P. gigantea* (the Rotstop isolate), *H. annosum* (P-type) and *H. annosum* (S-type) respectively, were grown on agar plates with pine or spruce sawdust as the sole carbon source. The plates were incubated at temperatures ranging from 4°C to 28°C, and the daily mycelial growth rate was measured. Growth of all strains was negligible at 4°C. From 8°C upwards the growth rate of *P. gigantea* increased

with increasing temperature, reaching a maximum of 8 mm/day at 28°C. Both strains of *H. annosum* had optimum growth (5-6 mm/day) at 22°C. The three strains grew equally well on both pine and spruce substrate.

Reference: Niemi, M. (1992b) Effect of high temperature on the viability of the spores of *Phlebiopsis gigantea.* Test report 9252, 1 p.

Not GLP. Unpublished.

Summary: The effect of high temperature on the viability of *P. gigantea* spores was assessed by keeping a working solution of Rotstop, containing $1.6x10^7$ cfu/L, for 10 minutes at temperatures between 30°C and 80°C and thereafter assessing the viability by plate counting. Spore counts were somewhat reduced at 30, 35 and 40°C. At 45°C the temperature effect was more pronounced and the spore count had dropped to $5.9x10^5$ cfu/L. All spores were killed at 50°C and higher temperatures.

Reference: Thorpe, K. (2001) Linear growth rates of *Phlebiopsis gigantea* on artificial media. Forest Research, Alice Holt Lodge. Report PPP01020, 3 pp.

Not GLP. Unpublished.

Summary: Thorpe compared the linear growth rates on 1.5% malt agar of a selection of isolates of *P. gigantea* from the UK and Scandinavia, at temperatures ranging from 5°C to 35°C. Mean growth at 10 and 27.5°C compared well with those described by Cartwright and Findlay (1958). No growth was observed in any isolates at 35°C, but all isolates recovered when sub-cultured and then incubated at 25°C, indicating that the higher temperature had not proved to be lethal.

B.8.1 Persistence and multiplication (Annex IIB 7.1.1)

B.8.1.1 Soil (Annex IIB 7.1.1)

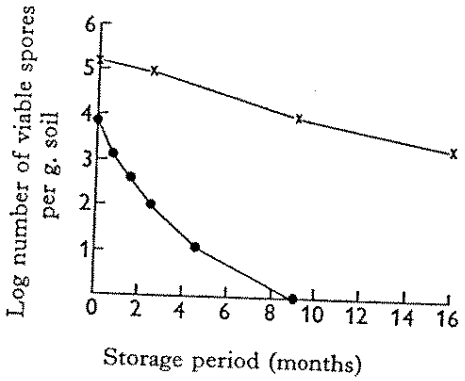
The natural habitat of *P. gigantea* is moribund wood rather than soil and the fungus is not thought to be able to persist or multiply within this medium. One study suggests basidiospores can survive in soil for a period of several months, but their viability decreases steadily over time (Rishbeth 1963). Oidia - the asexual spores used in stump treatment preparations - are much less robust structures and likely to survive for even shorter periods of time.

It is important to bear in mind that *P. gigantea* is a natural component of forest ecosystems and its spores will be present in the air and on most exposed surfaces within a forest environment. These ambient spore loads of *P. gigantea* tend naturally to be higher than the overall quantities of spores applied during treatment. In conclusion, *P. gigantea* is not thought to persist or grow in soil, and the ability of inoculated spores to survive and multiply in soil is considered to be insignificant in real terms due to the ubiquitous nature of this fungus in the forest environment.

Reference: Rishbeth, J. (1963) Stump protection against *Fomes annosus*. III. Inoculation with *Peniophora gigantea.* Ann. Appl. Biol. Vol. 52 (1), pp. 63 – 77.

Not GLP. Published.

Summary: Rishbeth found that colonisation of stumps by *P. gigantea* was much lower if pine stumps were covered by soil after felling rather than being left exposed. This implies that spores naturally present in the air could wash down on to the stump through the soil, although this appeared significantly to affect their viability. This observation was followed up with trials charting a pronounced decline in spore viability during storage in dry unsterile soil. Numbers of viable spores dropped exponentially over time, and none were found after 9 months.
Test system was following: A concentrated suspension of *Phlebiopsis gigantea* basidiospores was well mixed with unsterile soil (three acid and three alkaline soils) from a plantation and the initial population estimated by plating dilutions of a suspension on to pine sections. The soil was dried at 10°C and stored at this tempereature in the dark. The viable basidiospore population was then determined at intervals.



Text-fig. 1. The decline in viability of *Fomes annosus* and *Peniophora gigantea* basidiospores during storage in dry, unsterile soil at 10° C. ×—×, *F. annosus*; ●—●, *P. gigantea*.

B.8.1.2 Water (Annex IIB 7.1.2)

P. gigantea is a fungus adapted to live in moribund wood and it does not persist for long, or proliferate within water.

Two studies provide evidence for this: Thor *et al*. (1997a) found no appreciable decline in viability of spores maintained in solution at 20°C for 8 hours, followed by a slight decline after 72 hours. The spore germination was optimal at 30°C. At temperatures 30 and 40°C, viability dropped off sharply after 8 and 4 hours respectively. Pratt (pers. comm.) also conducted an exploratory study that suggested *P. gigantea* spores would survive for some days in water, although longevity depends on the ambient temperature. Suspensions of oidia in sterile distilled water were stored at 10°C and 20°C and viability examined after 4, 20, 24, 28 hrs, and 5, 14, and 22 days. Viability was retained for 22 days at 10°C but at 20°C, viability reduced after 14 days. The survival of spores in unsterile water out in the field was not examined but is likely to be influenced by the presence of competing microbes in water. This decline in spore viability in water is why instructions associated with products based on *P. gigantea* spores emphasise the importance of making up fresh spore solutions on a daily basis to ensure viability is not compromised.

Another report also suggests that *P. gigantea* has a limited persistence in water, or at least in wood with extremely high moisture content. Gibbs *et al*. 1996 conducted a study of fungal colonisation of logs (pine and spruce) kept in wet storage following the gales and subsequent forest loss in the UK in 1987. Amongst many other findings, *P. gigantea* was found not to be as abundant as expected in an area of forest where ambient spore levels are often extremely high. After 12 months of water storage of pine logs, *P. gigantea* was isolated from 2 boards and after 24 months of water storage from 1 board. Before water storage there were 12 logs out of 15 infected by *P. gigantea* or *H. annosum*. After 1 year of storage it was estimated that about 1% of the visible logs showed signs of decay fungi (*P. gigantea, H. annosum* and *S. sanguinolentum*).

It has to be remembered that ambient spore loads of *P. gigantea* tend naturally to be high in coniferous woodland, and in contrast the numbers of spores added through artificial stump treatment are very small. In conclusion, *P. gigantea* is not thought to persist or grow in water, and the ability of inoculated spores to survive and multiply in this medium is actually insignificant in real terms due to the ubiquitous nature of this fungus in the forest environment.

B.8.1.3 Air (Annex IIB 7.1.3 and IIIB 9)

Natural spore levels

The air naturally contains large numbers of viable fungal spores, including those of *P. gigantea*. These basidiospores are liberated from *P. gigantea* sporophores and are adapted for aerial dispersal. They have been trapped 250 miles from the nearest likely source. More spores are liberated at night than during the day. The rate of sporulation is reduced by extremes of temperature, and is inhibited during periods of hot, dry weather. Kallio (1970) measured deposition in Finland, and found it restricted from around late April to October, ceasing during the winter. Although sporophores are capable of spore release in temperatures ranging from 0-22.5^{o}C, they cease below zero when they are frozen. Trials in which stumps were covered to prevent infection via spores carried from foliage in raindrops but left open to aerial infection, suggest that most *P. gigantea* colonisation originates from aerial spores. However, a factor that may affect the incidence of stump infection is the resin content of stumps. Immediately, after a healthy tree is felled, resin begins to exude from the stump surface. The high resin content can effectively seal the stump against fungal invasion. The 'crust' from exuded resin covers the surface completely after 3-4 weeks. In temperate climates such as Great Britain, sporulation occurs in all months of the year, and in any one location it may be effectively continuous, albeit from a number of changing sources. Although there was considerable variation from season to season, with lower deposition during the summer months, (Meredith, 1959) mean hourly spore deposition rates of around 2.8 x 10^{6} viable *P. gigantea* spores per hectare have been recorded in UK forests.

Applied spore levels

The spores applied during stump treatment are asexual spores called oidia. The spore loads applied during treatment may be in the order of 3 x 10^{8} per ha, over a period of between two days (mechanical application) and a

week (manual application). This suggests that natural deposition rates far exceed those applied artificially. Artificial applications of spores are necessary simply to ensure the spores are put onto the stump surface before *H. annosum* can gain a hold, to enable *P. gigantea* to 'capture' the resource.

The role of oidia in the life cycle of *P. gigantea* is not fully understood. Although such spores are capable of infecting timber, their viability and mode of spread has not been studied, although there are indications that oidia do not travel far. For example, molecular studies of *P. gigantea* persistence in treated stumps confirm that oidia will spread small distances to nearby untreated stumps, presumably in air currents during harvesting operations , Later insect-mediated transfer could also be possible (Vainio *et al.*, 2001). The same study indicated no further transfer of oidia to stumps cut during later operations where no treatment was used. Many untreated stumps become naturally colonised as shown in studies by, for example Roy *et al* (2003) and Vainio *et al.* (2001).

In summary, the fate of artificially inoculated spores in air has to be considered in the context of the already high ambient levels of *P. gigantea* spores present in the atmosphere. *P. gigantea* is part of the forest ecosystem and levels of spores applied during treatments are minimal when compared to those already present.

The references used to generate the above summary are listed below in relation to country of origin:

Canada

Reference: Roy, G., Laflamme, G., Bussieres, G., Dessureault, M. (2003) Field tests on biological control of *Heterobasidion annosum* by *Phaeotheca dimorphospora* in comparison with *Phlebiopsis gigantea*. For. Path. 33, pp. 127-140.

Not GLP. Published.

Summary: In Eastern Canada Roy and colleagues set up a trial to compare the efficacy of two fungi, *Phaeotheca dimorphospora* and *P. gigantea* against *H. annosum* using red pine (*Pinus resinosa*) logs to represent stumps. Efficacy of *P. dimorphospora* was variable and depended on the formulation whilst *P. gigantea* was 100% effective against the pathogen. In addition to high levels of *P. gigantea* colonisation in treated logs, natural levels of colonisation from airborne spores were also extremely high, with 100% of logs in all but 1 of the 5 treatments colonised by *P. gigantea* after 2 months in one of the sites.

Scandinavia

Reference: Kallio, T. (1970) Aerial distribution of the root-rot fungus *Fomes annosus* (Fr.) Cooke in Finland. Acta Forest. Fenn. Vol 107, 55 pp.

Not GLP. Published.

Summary: Spore deposition rates of *H. annosum*, *P. gigantea* and *Trichoderma viridae* in Finland were measured with the aid of spruce discs and agar plates. Sites were situated from Helsinki to as far north as Ivalo. Spore deposition of *P. gigantea* almost coincided with that of *H. annosum*, occurring from April to late October, peaking in July. Highest deposition occurred at night.

Reference: Vainio, E., Lipponen, K., Hantula, J. (2001) Persistence of a biological strain of *Phlebiopsis gigantea* in conifer stumps and its effects on within-species genetic diversity. For. Path. Vol. 31, pp. 285-295.

Not GLP. Published.

Summary: In a Finnish study on persistence of a biological stump treatment agent, many wild-type genotypes of *P. gigantea* isolates could be isolated from pine stumps after 1 year. These had originated from natural airborne infection.

UK

Reference: Meredith, D.S. (1959) The infection of pine stumps by *Fomes annosus* and other fungi. Ann. Bot. Lond. (n.s), Vol. 23 (91) pp. 455 – 476.

Not GLP. Published.

Summary: Meredith investigated the infection biology of stump-colonizing fungi in managed pine forests in Eastern England. He showed that *P. gigantea* was one of the first naturally occurring fungi to initiate infection, mainly through air-borne spores, and was also a vigorous competitor, often replacing other fungi. Colonisation of stumps was dependent on spore availability and infections peaked in the winter and early spring.

B.8.1.4 Other/special studies

Persistence of P. gigantea *within stumps*

P. gigantea is a natural part of the forest ecosystem and is often found fruiting on stumps and timber lying in close contact with the ground. In addition to such natural spore infection, *P. gigantea* is applied directly to stumps during manual or mechanical felling and the oidia colonise the wood and bark of the stump. Several studies have examined how persistent *P. gigantea* is on such material, both in treated and untreated timber. In Scandinavia it is commonly found fruiting on untreated pine and spruce stumps, especially 2-3 years after cutting (Käärik & Rennerfelt, 1957). *P. gigantea* is also extremely common on treated stumps, and molecular and cultural work shows that the specific isolate of *P. gigantea* applied during treatment can be successfully re-isolated from spruce and pine stumps for a number of years (Roy *et al.*, 1997; Vasiliauskas, 2005). The degree of persistence depends on the species of the stump. For example Vainio *et al.* (2001 & 2005) found *P. gigantea* in 6 year-old Norway spruce, but only in younger, 1 year-old Scots pine stumps. No *P. gigantea* was isolated from the older pine stumps. In addition, they confirmed the actual Rotstop isolate had persisted in treated spruce, and yet untreated stumps contained different genotypes, originating from natural airborne spores.

The consensus is that regardless of whether the *P. gigantea* originates from natural or artificially inoculated spores; the fungus commonly affects stumps. After around 6 years *P. gigantea* is replaced, as a process of natural succession, by fungi such as *Resinicium bicolor*, *Sistotrema brinkmannii* and *Hypholoma capnoides* and this fungal succession process appears to be more rapid in pine than spruce stumps (Vainio *et al.*, 2001 & 2005).

The following papers, arranged geographically, describe in more detail the persistence of *P. gigantea* in stump tissue.

Canada

Reference: Roy, G., Cormier, M., Hamelin, R. C., Dessureault, M. (1997) Comparison of RAPD technique and somatic incompatibility tests for the identification of *Phlebiopsis gigantea* strains. Can. J. Bot. Vol. 75, 2097-2104

Not GLP; Published

Summary: In Eastern Canada somatic incompatibility tests and PCR-RAPD analysis was used to characterise *P. gigantea* isolates taken from stumps of red pine (*Pinus resinosa*) 1 year after treatment with *P. gigantea*. The two tests produced similar results in that inoculated isolates of *P. gigantea* could still be identified from stumps after a year. In addition many stumps were colonised by naturally occurring *P. gigantea*.

Scandinavia

Reference: Käärik, A., Rennerfelt, E. (1957) Investigation of the fungal flora of spruce and pine stumps. Meddelanden från Statens Skogsforskningsinstitut, Vol. 47, 88 pp.

Not GLP. Published.

Summary: Pine and spruce stumps were sampled over time in forests in Sweden, and details are given of all the significant fungi isolated. *P. gigantea* was one of the most frequently isolated fungi, especially after 2-3 years. Large fruit bodies were more commonly found on pine than spruce, and were present on 90% of 1-year-old pine stumps. The growth was noted to be moderate to rapid, 9-14cm in 10 days.

Reference: Vasiliauskas, R., Juska, E., Vasiliauskas, A., Stenlid, J. (2002) Community of aphyllophorales and root rot in stumps of Picea abies on clear-felled forest sites in Lithuania. Scand. J. For. Res. Vol. l17, 398-407

Not GLP; Published

Summary: In a Lithuanian study populations of *Aphyllophorales* fungi (which include *P. gigantea* and *H. annosum*) on Norway spruce stumps were examined by looking at fruit body production 1.5-4.5 years after cutting. *P. gigantea* was one of the fastest species to produce fruit bodies, being quite abundant after 1 year, with fruit body numbers peaking at around 3-4 years, but was then seen less frequently in 4.5 year old stumps. Greater numbers of fruit-bodies were found on stumps cut in summer than those cut in winter.

Reference: Vasiliauskas, R., Lygis, V., Thor, M., Stenlid, J. (2004) Impact of biological (Rotstop) and chemical (urea) treatments on fungal community structure. Biological Control Vol. 31 (3), 405-413

Not GLP; Published

Summary: Stumps of Norway spruce in Swedish forest stands were treated with chemical (urea) and biological (Rotstop) stump treatments. The impact of both on fungal community structure was studied using cultural methods and vegetative compatibility tests to check relatedness of isolated *P. gigantea* to the isolate originally applied to the stumps. Although there was a slight decline, after 7 weeks it appeared that neither treatment significantly affected species richness in the stumps and the Rotstop treatment did not significantly change species composition. Vegetative compatibility tests confirmed that all *P. gigantea* cultures isolated from treated stumps matched the original Rotstop isolate, and all *P. gigantea* isolated from untreated stumps originated from wild strains.

Reference: Vasiliauskas, R., Larsson, E., Larsson K. H., Stenlid, J. (2005) Persistence and long-term impact of Rotstop biological control agent on mycodiversity in *Picea abies* stumps.
Biological Control Vol. 32, 295-304
Not GLP; Published

Summary: To gauge the persistence, long-term impact of Rotstop treatment and mycodiversity of treated stumps in Sweden, Vasiliauskas *et al*., sampled Norway spruce stumps, where 60 of were treated with Rotstop and 70 not, 4 and 6 years after felling. Fungi were identified using morphological and molecular means. Rotstop treatment seemed to have a negative impact on the colonisation of stumps by certain species after 4 and 6 years, namely causing a decrease in *H. annosum*, but also reducing the presence of 3 asco- and deuteromycetes. Species richness was lower in Rotstop-treated stumps. The *P. gigantea* isolate from the Rotstop product (and this isolate alone) could frequently be isolated from 4-year-old treated stumps and also produced abundant fruiting bodies, but in untreated stumps this isolate was absent and wild-type *P. gigantea* was less common than other stump colonising fungi. After 6 years the Rotstop isolate ceased to be dominant in treated stumps and the fruiting bodies were never observed. In untreated stumps *P. gigantea* had been replaced by other species.

Reference: Vainio, E., Lipponen, K., Hantula, J. (2001) Persistence of a biological strain of *Phlebiopsis gigantea* in conifer stumps and its effects on within-species genetic diversity. For. Path. Vol. 31, pp. 285-295.
Not GLP. Published.

Summary: Fungal isolations and genetic fingerprinting were used to determine whether stump treatment with a single genotype of *P. gigantea* (Rotstop) had affected the genetic diversity of *P. gigantea* populations in Scots pine and Norway spruce stumps. Rotstop could be isolated for up to 6 years from Norway spruce stumps. The usage of Rotstop did not seem to increase the occurrence of the fungus 5 years after the treatment in fresh (1-year-old) untreated stumps within the same forest stands. All the isolates from the 6-year-old treated spruce stumps were identical in genotype with the Rotstop strain, whereas all isolates from the fresh untreated spruce and pine stumps differed from it. Within the treated pine stand, the biocontrol usage seemed to have caused a slight reduction in genetic markers not related to Rotstop. However, there were no statistically significant differences in the frequencies of the non-Rotstop markers and the local natural population. It was concluded that stump treatment with Rotstop is not likely to pose any immediate threat to the genetic diversity of *P. gigantea*. The results cannot be applied to possible future situations if practically all forest stands are treated with Rotstop or if the treatments will be continued over several tree generations. Therefore it would be important to continually monitor possible changes in the genetic diversity of *P. gigantea* in managed forests.

Reference: Vainio, E., Hallaksela, A-M., Lipponen, K., Hantula, J. (2005) Direct analysis of ribosomal DNA in denaturing gradients: application on the effects of *Phlebiopsis gigantea* treatment on fungal communities of conifer stumps. Mycol. Res. Vol. 109 (1), 103-114
Not GLP; Published

Summary: Direct PCR was used to investigate the effects of *P. gigantea* treatment of stumps on fungal diversity on Norway spruce and Scots pine stumps in Finland. *P. gigantea* was very common in fresh pine stumps, and fairly common in spruce. The Rotstop isolate did not seem to have migrated to untreated stumps cut at a slightly

later date in the same area of forest. *P. gigantea* was not found during sampling of 6 year old treated spruce stumps, but was present as wild-type isolates, in stumps which had not received any treatment. *P. gigantea* had been completely replaced by other fungi in pine stumps after 4 and 6 years. Treated stumps of both species tended to differ qualitatively in terms of species composition but overall diversity was not significantly affected.

B.8.2 Mobility (Annex IIB 7.2 and IIIB 9)

P. gigantea is a natural component of forest ecosystems and its spores will be present in the air and on most exposed surfaces within a forest environment. These ambient spore loads of *P. gigantea* tend naturally to be higher than the overall quantities of spores applied during treatment. In conclusion, *P. gigantea* is not thought to persist or grow in soil or water, and the ability of inoculated spores to survive and multiply in these compartments is considered to be insignificant in real terms due to the ubiquitous nature of this fungus in the forest environment. As the active substance is typical aerobic organism that mainly colonise moribund wood, the number of *Phlebiopsis gigantea* transported to lower soil layers through water percolation are expected to die before reaching groundwater due to the unfavourable conditions that exist for their survival. If the cells do reach groundwater, they are not expected to grow and are likely to die very quickly.

The air naturally contains large numbers of viable fungal spores, including those of *P. gigantea*. These basidiospores are liberated from *P. gigantea* sporophores and are adapted for aerial dispersal. They have been trapped 250 miles from the nearest likely source. More spores are liberated at night than during the day. The rate of sporulation is reduced by extremes of temperature, and is inhibited during periods of hot, dry weather.

The role of oidia in the life cycle of *P. gigantea* is not fully understood. Although such spores are capable of infecting timber, their viability and mode of spread has not been studied, although there are indications that oidia do not travel far. For example, molecular studies of *P. gigantea* persistence in treated stumps confirm that oidia will spread small distances to nearby untreated stumps, presumably in air currents during harvesting operations. Later insect-mediated transfer could also be possible (Vainio *et al.*, 2001). The same study indicated no further transfer of oidia to stumps cut during later operations where no treatment was used. Many untreated stumps become naturally colonised as shown in studies by, for example Roy *et al* (2003) and Vainio *et al.* (2001).

Therefore in conclusion it can be said that *P. gigantea* spores are non-mobile and to achieve any degree of mobility must rely on passive transport in air currents (basidiospores), possibly arthropod vectors (oidia) or man (through stump treatment).

B.8.3 References relied on

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner **
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**: Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted any of the Member States in support of an application for authorization, or (though the study report has been submitted) has not been used any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization, of a plant protection product before the date of submission of the dossier to Rapporteur Member State.*

***: Owners' code identifications and names (Code identification: VRA, Name: Verdera Oy) (Code identification: FOC Name: Forestry Commission)*