*Phytopathologia Mediterranea* (2012) 51, 2, 374–382

Short Notes

# Occurrence of *Ganoderma adspersum* on *Pinus pinea*

Daniele DE SIMONE and Tiziana ANNESI

CRA - Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy

**Summary.** During a survey in a pine stand in Rome, six basidiomes of *Ganoderma adspersum* were collected from declining 50-60 year-old *Pinus pinea*. Species identification was accomplished through observation of macroscopic and microscopic morphological traits. Furthermore, ITS sequences of the collected specimens, 28 samples collected elsewhere, one isolate from the German collection of Microorganisms and Cell Cultures (DSMZ), and 21 sequences from GenBank were analyzed using MP and UPGMA. Both analyses showed that isolates fell into four well-delineated clades, namely *G. adspersum*, *G. applanatum*, *G. resinaceum* and *G. lucidum*. All the isolates collected from *P. pinea* were confirmed as *G. adspersum*. Previously, *G. adspersum* had been reported on hardwood trees and infrequently on a few conifers. Only sporocarps identified on the basis of morphological characters as *G. applanatum* have sporadically been recorded on *P. pinea*; there is no previous record of *G. adspersum* on this host. Somatic incompatibility tests showed that infections, probably via basidiospores, and symptoms apparently associated with wood colonization by *G. adspersum*, worsened during the 3-year course of the study.

**Key words:** ITS region, wood decay, conifer.

## Introduction

Several species within the genus *Ganoderma* are agents of wood decay and root rot, and cause decreased structural strength of diseased trees. These pathogens are evident mainly in urban forestry where anthropogenic factors and adverse environmental conditions favour the infection of ornamental trees. *Ganoderma adspersum* (Schulzer) Donk, *G. applanatum* (Pers.) Pat (synonym *G. lipsiense* [Batsch] G.F. Atk.)], *G. resinaceum* Boud., *G. lucidum* (Curtis) P. Karst and *G. valesiacum* Boud. are some of the most frequently reported species on ornamental or forest trees in Italy (Intini, 1986; Nicolotti *et al.*,1992; Nicolotti *et al.*, 2004; Bernicchia, 2005).

*Ganoderma applanatum* has been reported on conifers such as *Abies*, *Picea* and *Pinus*, while *G. adspersum* has been recorded almost exclusively on hardwoods (Nicolotti *et al.*, 1992; Gottlieb *et al.*, 1998; Schwarze and Ferner, 2003; Bernicchia, 2005; Lasserre *et al.*, 2010). *Ganoderma adspersum* and *G. applanatum* differ in their ability to break through the reaction zones formed in infected trees. *Ganoderma adspersum* can penetrate intact reaction zones of infected wood blocks, while *G. applanatum* seems unable to do so. In the absence of reaction zones, however, *G. applanatum* causes more extensive and intense decay (Schwarze and Ferner, 2003). As a consequence, the correct identification of the causal agent is important for a reliable assessment of the potential risks caused by infected trees. It is not easy to distinguish *G. adspersum* and *G. applanatum* from each other on the basis of morphological characters of sporocarp or mycelial cultures, and the two species can often be confused (Peterson, 1987; Leonard, 1998; Moncalvo, 2000; Terho *et al.*, 2007; Kaliyaperumal and Pudupalayam, 2008). Molecular methods have therefore been useful to separate *Ganoderma* species (Moncalvo, 2000; Guglielmo *et al.*, 2008).

During a survey carried out in a pine stand in a public park in Rome, Italy, sporocarps of *Ganoderma* sp. were observed on two declining *Pinus pinea* L. trees (Scirè *et al.*, 2008). The present study was investigated with two main objectives: 1) to identify *Gano-*

Corresponding author: T. Annesi
Fax: +39 06 86802296
E-mail: tiziana.annesi@entecra.it


ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm

*derma* samples from *P. pinea* and from other hosts in Italy, using both morphological and DNA sequence information, and 2) to determine the mode and extent of spread of this pathogen, using somatic incompatibility tests.

## Materials and methods

### Study sites and fungal isolates

In 2008, during a survey carried out within a public park in Rome, several pines with decline symptoms were detected. In particular, two of them had sporocarps typical of the genus *Ganoderma* at their trunk bases (Scirè *et al*., 2008). In follow up observations, carried out until 2010, 70 *P. pinea* trees (50–60 years old), distributed in an area of approximately 4000 $m^2$, were carefully examined for external symptoms and for the possible presence of *Ganoderma* sporocarps. This site is part of a pine stand where pines had been planted at 8 × 8 m spacings, which was rather irregular due to the presence of paths, hedges and small buildings (such as a water tank), and where unfavourable abiotic factors and high human activity could have negatively affected the growth of the trees over the years. In 2010, two heavily declining pines were felled.

All the detected basidiocarps, occurring on the trunks or butts, were collected and firstly identified on the basis of macro and micro-morphological characters according to Bernicchia (1990, 2005). Isolation was attempted by plating small fragments of the basidioma on potato dextrose agar (PDA, Oxoid, Basingstoke, UK) or on selective substrate (Kuhlman and Hendrix, 1962).

All isolates collected from the disease centre were included in a molecular analysis, together with 22 additional Italian *Ganoderma* spp. isolates. These were obtained during previous surveys from sporocarps collected from conifers and hardwoods, and had been identified on the basis of the macro- and micro-morphological characteristics only, and stored at 5°C on PDA. One isolate from the German Culture Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) (DSM 8525) was also analyzed for comparison (Table 1).

### rDNA ITS sequences

All isolates analyzed in this study are listed in Table 1. They were grown on PDA, and fungal DNA was extracted according to Cenis (1992). Primers ITS4 and ITS5 were used to amplify the ITS regions by PCR (White *et al*., 1990). Amplification was performed under the following conditions: 5 min at 95°C; 33 cycles of 40 sec at 95°C, 45 sec at 53°C, 1 min at 72°C, and a final extension at 72°C for 7 min. PCR products were separated on 1% agarose gel, stained with ethidium bromide, and observed under low intensity NUV light (360 nm). DNA fragments were recovered using a Gel Extraction kit (Qiagen, Hilden, Germany) and sequenced. All the obtained sequences were deposited in the GenBank database (Table 1).

Twenty-one additional ITS sequences of *Ganoderma* spp. downloaded from the GenBank database were included in the analyses for comparison (Table 2), and a further sequence of *Amauroderma subrugosum* (AJ537386) was included as an outgroup (Moncalvo *et al*., 1995; Gottlieb *et al*., 2000). Sequences were aligned using Clustal W (EMBL-EBI) and analyzed using the Maximum Parsimony (MP) and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithms in MEGA 4 (Tamura *et al*., 2007). MP trees were obtained using the close-neighbour-interchange algorithm, with initial trees determined by the random addition of sequences for ten replicates (Eck and Dayhoff, 1966; Nei and Kumar, 2000). UPGMA analysis was performed using evolutionary distances computed by the maximum composite likelihood method (Sneath and Sokal, 1973; Tamura *et al*., 2004). Robustness of internal branches was assayed by bootstrap analysis (1000 runs) for both methods (Felsenstein, 1985).

### Somatic compatibility

All the heterokaryotic isolates collected within the studied pine stand were paired in every possible combination, including against themselves as experimental controls. Mycelial disks (4 mm diam.) were placed 1.5 cm apart on 1.5% of malt extract agar in Petri plates, in four replications. Isolates were incubated in the dark at 25°C and observations were made weekly for up to 21 d. Reactions between paired isolates were assessed as incompatible when sparse mycelium or barrage formation with dark pigment were present, indicating different genotypes. Pairings were considered compatible when mycelia coalesced, and in these cases the isolates were considered as identical genotypes (Miller *et al*., 1999).

**Table 1.** List of fungal isolates analyzed in this study. Hosts, provenance, collection numbers and Genbank accession numbers are indicated.

| Species[a] | Host | Provenance[d] | Stock No. | Accession No. |
|---|---|---|---|---|
| *Ganoderma adspersum* | *Cercis siliquastrum* L. | Rome | PF275 | JN176903 |
| *G. adspersum* | *Prunus avium* L. | Rome | PF268 | JN176887 |
| *G. adspersum* | *Robinia* sp. | Rome | PF264 | JN176879 |
| *G. adspersum* | *Fagus sylvatica* L. | Pescocostanzo, AQ | PF267 | JN176886 |
| *G. adspersum* | *Laurus nobilis* L. | Rome | PF266 | JN176892 |
| *G. adspersum* | *Quercus pubescens* Willd | Rome | PF273 | JN176884 |
| *G. adspersum* | *Quercus ilex* L. | Rome | PF272 | JN176898 |
| *G. adspersum*[b] | *Pinus pinea* L. | Rome | PF284 | JN176907 |
| *G. adspersum*[b] | *Pinus pinea* L. | Rome | PF285 | JN176901 |
| *G. adspersum*[b] | *Pinus pinea* L. | Rome | PF286 | JN176902 |
| *G. adspersum*[b] | *Pinus pinea* L. | Rome | PF287 | JN176906 |
| *G. adspersum*[b] | *Pinus pinea* L. | Rome | PF288 | JN176905 |
| *G. adspersum*[b] | *Pinus pinea* L. | Rome | PF289 | JN176904 |
| *G. adspersum* | *Pinus pinea* L. | Anzio, RM | PF261 | JN176883 |
| *G. adspersum* | *Pinus pinea* L. | Ostia, RM | PF270 | JN176893 |
| *G. adspersum* | *Pinus* sp. | Mentana, RM | PF263 | JN176908 |
| *G. adspersum* | *Abies* sp. | Pieve S. Stefano, AR | PF262 | JN176889 |
| *G. adspersum* | *Abies* sp. | Pieve S. Stefano, AR | PF269 | JN176888 |
| *G.* applanatum[c] | *Laurus nobilis* L. | Rome | PF271 | JN176890 |
| *G. applanatum*[c] | *Fagus sylvatica* L. | Soriano nel Cimino, VT | PF265 | JN176891 |
| *G. applanatum* | *Fagus sylvatica* L. | Germany | DSM 8525 | JN176900 |
| *G. lucidum* | *Quercus ilex* L. | Catania | PF276 | JN176899 |
| *G. lucidum* | *Quercus ilex* L. | Rome | PF277 | JN176895 |
| *G. lucidum* | *Quercus ilex* L. | Rome | PF278 | JN176897 |
| *G. resinaceum* | *Platanus* × *acerifolia* (Aiton) Willd. | Rome | PF282 | JN176882 |
| *G. resinaceum* | *Quercus cerris* L. | Rome | PF281 | JN176885 |
| *G. resinaceum* | *Quercus suber* L. | Rome | PF279 | JN176880 |
| *G. resinaceum* | *Platanus* × *acerifolia* (Aiton) Willd. | Rome | PF280 | JN176881 |
| *G. resinaceum* | *Quercus robur* L. | Rome | PF283 | JN176896 |

[a] Identified as *Ganoderma adspersum* by molecular analysis.
[b] Identification based on morphological characteristics of sporocarps.
[c] Sporocarps collected within the pine stand disease centre examined in this study.
[d] AQ, L'Aquila; AR, Arezzo; RM, Rome; VT, Viterbo.

## Results

### Investigated site and fungal isolates

Investigations carried out over 3 years in the pine stand showed that four trees had sporocarps of *Ganoderma* at the bases of their trunks (Figure 1). The four infected trees were not adjacent to each other, ranging from 12 to 32 m apart. In particular, the first two infected trees observed in 2008 had completely wilted branches 2 years later and were felled. Stump surfaces clearly showed the presence of deep white rots affecting the whole sapwood. Six months after felling, one further sporocarp was observed on each stump. One isolate was obtained from each collect-

**Figure 1.** Sporocarps of *Ganoderma adspersum* at the base of a trunk of *Pinus pinea*.

ed sporocarp (PF 284-289) (Table 1). On the basis of macro- and micro-morphological characters, all the six basidioma collected in the pine stand were identified as *G. adspersum*.

#### rDNA ITS sequences

The MP phylogenetic tree constructed for the ITS1, ITS2 and 5.8S ribosomal RNA regions of the *Ganoderma* isolates is shown in Figure 2.

The MP analysis of all the *Ganoderma* sequences produced 151 most parsimonious trees (length = 298). The consistency index was 0.823529, the retention index was 0.979885 for parsimony-informative sites, and the composite index was 0.910833 for all sites and 0.806964 for the parsimony-informative sites. There were a total of 505 positions in the final dataset, of which 68 were parsimony informative.

The MP phylogenetic tree clearly grouped the 28 Italian *Ganoderma* isolates (Table 1) into three distinct clades: *G. adspersum*, *G. lucidum* and *G. resinaceum*, with 99%, 99% and 100% bootstrap support, respectively. Phylogenetic analysis confirmed the morphological identification of all the *G. adspersum* sporocarps collected, both from the pine stand and the other sites. The isolates also formed a single clade with the *G. adspersum* sequences from GenBank (Table 2). The twelve *G. applanatum* sequences from Finland, Norway, Estonia, UK, and Germany formed a distinct clade with 100% bootstrap support. On the other hand, molecular analysis on the Italian isolates derived from two sporocarps previously identified as *G. applanatum* (PF 265 and 271) on the basis of morphological characters also clustered with *G. adspersum*, indicating they had been previously misidentified.

When the sequences were analyzed by UPGMA methods, the topology of the phylogenetic tree was identical to the MP tree, and in the UPGMA tree the cluster containing *G. adspersum* and *G. applanatum* showed 100% bootstrap support.

#### Somatic compatibility

After 3 weeks, an incompatible reaction between isolates was visible, with zones of both sparse mycelium and dark barrages, between all the paired colonies (Figure 3B, C). Only self-paired isolates always showed fully compatible reactions (Figure 3A). Thus, the six tested isolates were different genotypes. Replications always gave the same compatible or incompatible reactions.

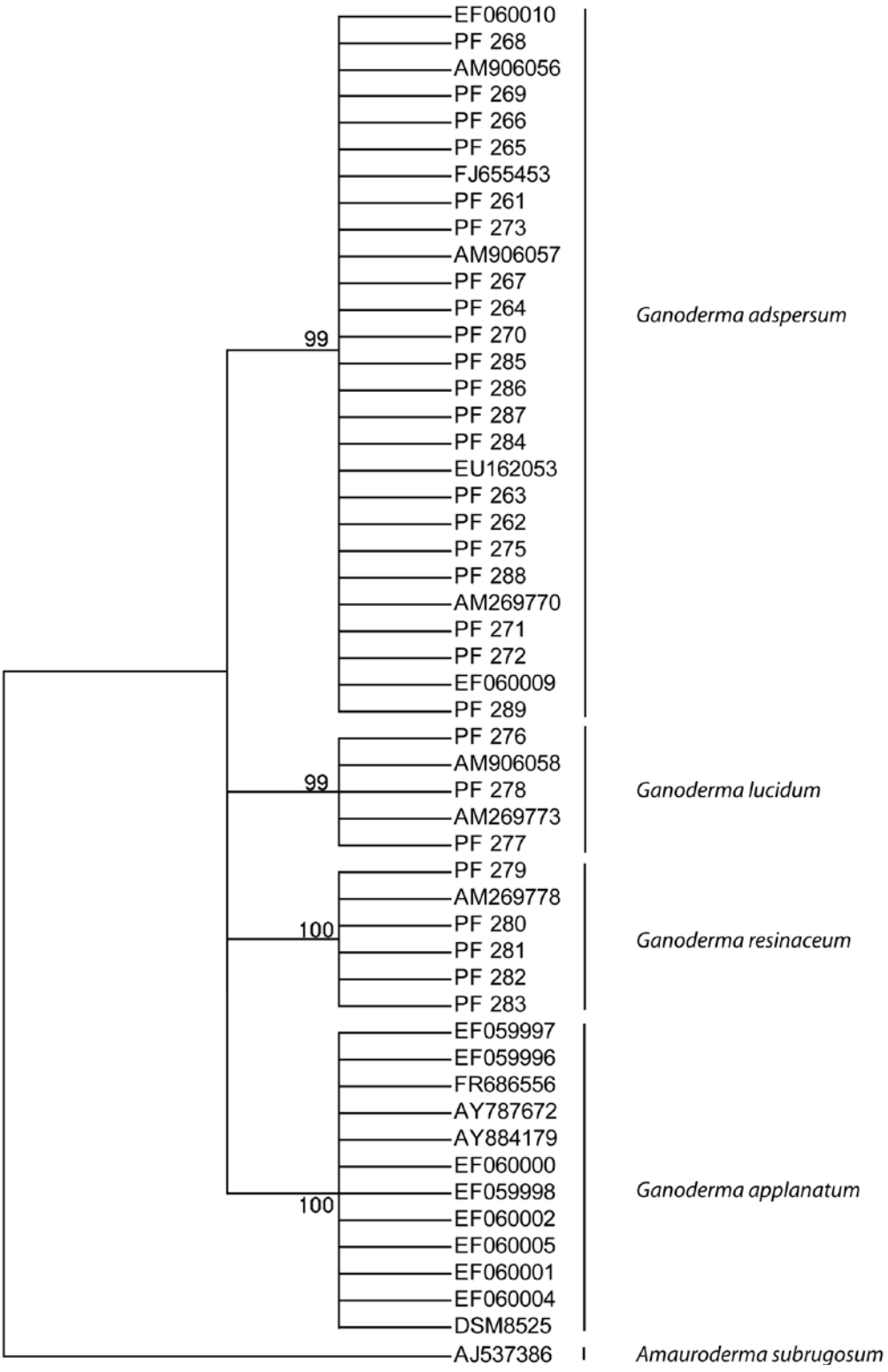


**Figure 2.** Phylogenetic tree, obtained by maximum parsimony analysis (MP), based on comparison of ITS region sequences of *Ganoderma* spp. (Tables 1 and 2). Values at nodes are confidence levels (1000 bootstraps).

**Table 2.** Sequences of *Ganoderma* spp. from GenBank included in the analysis. Provenance and accession numbers are indicated.

| Species | Provenance | Accession No. |
|---|---|---|
| *Ganoderma adspersum* | Italy | AM269770 |
| *G. adspersum* | India | FJ655453 |
| *G. adspersum* | Italy | AM906057 |
| *G. adspersum* | Italy | EF060010 |
| *G. adspersum* | Belgium | EU162053 |
| *G. adspersum* | Italy | AM906056 |
| *G. adspersum* | Italy | EF060009 |
| *G. lucidum* | Italy | AM269773 |
| *G. lucidum* | Italy | AM906058 |
| *G. resinaceum* | Italy | AM269778 |
| *G. applanatum* | Finland | EF060001 |
| *G. applanatum* | Finland | EF059998 |
| *G. applanatum* | Finland | EF059997 |
| *G. applanatum* | Finland | EF060000 |
| *G. applanatum* | Finland | EF060004 |
| *G. applanatum* | Estonia | EF059996 |
| *G. applanatum* | Germany | FR686556 |
| *G. applanatum* | UK | AY884179 |
| *G. applanatum* | Lithuania | AY787672 |
| *G. applanatum* | Norway | EF060002 |
| *G. applanatum* | Norway | EF060005 |

## Discussion

The examination of ITS sequences of the 28 Italian isolates, which had initially been performed on the basis of morphological characters of their sporocarps, confirmed the identification of *G. adspersum*, *G. lucidum* and *G. resinaceum*. Two sporocarps from pines previously identified as *G. applanatum* were reclassified as *G. adspersum* in this study. It is reported in the literature that *G. applanatum* and *G. adspersum* can be confused, especially when the morphological observations are carried out on young sporocarps, where it is more difficult to appreciate the specific characteristics considered distinctive between the two species (Leonard, 1998). To validate our study, a large number of *Ganoderma* spp. sequences downloaded from the Genbank database were included in the analysis for comparative purposes. In particular, *G. applanatum* sequences were from isolates collected in Northern Europe, the majority from Finland, where this species is reported as the dominant species of *Ganoderma* (Terho and Hallaksela, 2005; Terho *et al.*, 2007). No sequence of *G. applanatum* was available from Italy or from other Mediterranean areas. On the basis of the MP phylogenetic tree, *G. adspersum* and G. *applanatum* sequences formed two clearly distinct clades, with 99% and 100% bootstrap support, respectively.

All the pine isolates previously collected from the four sites in Central Italy (Lazio) (Table 1) were referred to as *G. adspersum*. These results highlight that the presence of the pathogen on *P. pinea* is not an occasional finding, and indicate that *G. adspersum* can infect both deciduous trees and conifers. This

contrasts with the fact that previously only *G. applanatum* sporocarps, identified on the basis of morphological characters, had been sporadically reported in Italy on *P. pinea* (Nicolotti *et al*.,1992; Bernicchia, 2005; Bernicchia *et al*., 2007).

Wood decay fungi show great variability in their ability to infect conifers. Some species, such as *Phaeolus schweinitzii* or *Heterobasidion annosum*, can be significant primary pathogens, while many others are known to act more like secondary agents, thriving in trees that are physiologically weakened by other factors. Although our study did not include evaluation of *G. adspersum* pathogenicity, on the basis of careful observations over 3 years, we believe that progression of this fungus at the study site may have been favoured by some predisposing conditions, such as wounds, excessive stem density, or water stress. Notwithstanding the primary reason for such weakening, we observed a significant amount of wood decay near the root collars, indicating that this fungus

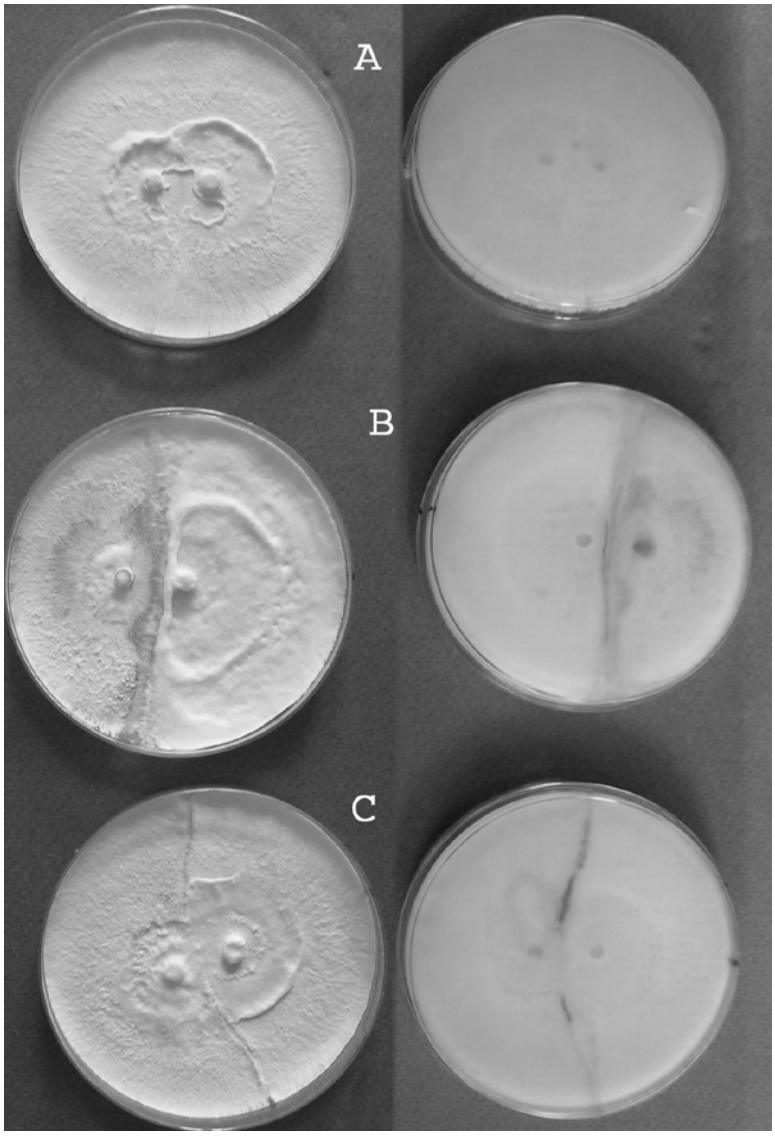


**Figure 3.** Examples of somatically compatible (self-pairing) (A) and incompatible (B and C) reactions between paired isolates of *Ganoderma adspersum*.

can act as an active wood decay agent on susceptible *P. pinea* individuals. It is possible that the wilting of branches we observed may also have been caused by *G. adspersum*. The fact that the reported occurrence of this pathogen is low on conifer hosts further indicates that this fungus may not be an active primary pathogen of pines in Italy, but that in specific conditions its effects on standing trees may be considerable. Sporocarps were observed on stumps a few months after trees were felled. Their presence indicates that the fungus continued to grow saprotrophically and highlights the importance of the prompt removal of heavily declining trees in order to avoid the risk of stem breakage and to reduce inoculum load and fungal spread. Although applied on six isolates only, our somatic incompatibility tests indicate that this species is infecting trees primarily through airborne basidiospores and not through secondary somatic growth through root contact or root grafts.

In conclusion, the analysis of the ITS region, recently reported in the literature as useful for distinguishing isolates of *Ganoderma* spp. (Moncalvo, 2000; Smith and Sivasithamparam, 2000), enabled us to correctly identify the *Ganoderma* spp. isolates included in the study. In the literature on conifers, *G. adspersum* has been recorded on *Pinus taeda* L. in Argentina (Gottlieb *et al*., 1998), and on *Cedrus deodara* (Roxb. ex D. Don) G. Don and on *Abies alba* Mill. in Italy (Nicolotti *et al*., 1992; Scirè *et al*., 2008). On the basis of our results, and to the best of our knowledge, this is the first time that *G. adspersum* is reported on *P. pinea*.

## Acknowledgements

This study was supported by Environmental Department of the Municipality of Rome and by Italian Ministry of Agriculture, Food and Forestry. E. Motta and M. Pilotti are acknowledged for providing isolates of *Ganoderma* spp.

## Literature cited

Bernicchia A., 1990. Polyporaceae s.l. in Italia. Istituto Patologia Vegetale, Università degli Studi di Bologna, Italy, 596 pp.

Bernicchia A., 2005. Polyporaceae s.l. Edizioni Candusso, Savona, Italy, 808 pp.

Bernicchia A., E. Savino and S. Pérez Gorjón, 2007. Aphyllophoraceous wood-inhabiting fungi on *Pinus* spp. in Italy. *Mycotaxon* 101, 5–8.

Cenis J.L., 1992. Rapid extraction of fungal DNA for PCR amplification. *Nucleic Acid Research* 20, 23–80.

Eck R.V. and M.O. Dayhoff, 1966. *Atlas of Protein Sequence and Structure*. National Biomedical Research Foundation, Silver Springs, Maryland, USA.

Felsenstein J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.

Gottlieb A.M., B.O Saidman and J.E. Wright, 1998. Isoenzymes of *Ganoderma* species from southern South America. *Mycological Research* 102, 415–426.

Gottlieb A.M., E. Ferrer and J.E. Wright, 2000. rDNA analyses as an aid to taxonomy of species of *Ganoderma*. *Mycological Research* 104, 1033–1045.

Guglielmo F., P. Gonthier, M. Garbelotto and G. Nicolotti, 2008. A PCR-based method for the identification of important wood rotting fungal taxa within *Ganoderma*, *Inonotus* s.l. and *Phellinus* s.l. *FEMS Microbiology Letters* 282, 228–237.

Intini M., 1986. Ricorrenza di marciumi radicali e processi di carie causati da funghi del genere *Ganoderma* in alberature di medio ed alto fusto di differenti età. *Monti e Boschi* 37(3), 25–28.

Kaliyaperumal M. and T.K. Pudupalayam, 2008. *Ganoderma australe* from southern India. *Microbiological Research* 163, 286–292.

Kuhlman E.G. and F.F. Hendrix, 1962. A selective medium for the isolation of *Fomes annosus*. *Phytopathology* 52, 1310–1312.

Lasserre B., E. Motta, L. D'Amico, M. Scirè and M. Marchetti, 2010. Utilizzo del Resistograph® per l'individuazione di diverse tipologie di alterazione del fusto di abete bianco in Alto Molise. *Forest@* 7, 190-198 [online: 2010-10-13] URL: http://www.sisef.it/forest@/?doi=10.3832/efor0638-007

Leonard A.C., 1998. Two *Ganoderma* species compared. *Mycologist* 12, 65–68.

Miller R.N.G., M. Holderness, P.D. Bridge, G.F. Chung and M.H. Zakaria, 1999. Genetic diversity of *Ganoderma* in oil palm plantings. *Plant Pathology* 48, 595–603

Moncalvo J.M., 2000. Systematic of *Ganoderma*. In: Ganoderma *Disease of Periennal Crops*. (J. Flood, P.D. Bridge, M. Holderness, ed.), CABI Publishing, CAB International, Wallingford, UK, 23–45.

Moncalvo J.M., H.H. Wang and R.S. Hseu, 1995. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and ribosomal DNA sequences. *Mycologia* 87, 223–238.

Nei M. and S. Kumar, 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, NY, USA, 333 pp.

Nicolotti G., M. Negri and N. Anselmi, 1992. Incidence and biology of *Ganoderma* species causing decline of trees in northern Italy. *Petria* 2, 159–170.

Nicolotti G., P. Gounthier, D. Pecollo, 2004. Ecologia e grado di preferenza d'ospite dei funghi agenti di carie (II parte). *Acer* 20(2), 60–66.

Peterson J.E., 1987. *Ganoderma* in Northern Europe. *Mycologist* 1, 62–67.

Schwarze F.W.M.R. and D. Ferner, 2003. *Ganoderma* on trees - Differentiation of species and studies of invasiveness. *Arboricoltural Journal* 27, 59–77.

Scirè M., L. D'Amico, E. Motta and T. Annesi, 2008. Alcuni

aspetti fitosanitari della "foresta" nella città di Roma. *Atti del terzo Congresso Nazionale di Selvicoltura*, Taormina (ME), 16–19 ottobre 2008. Accademia Italiana di Scienze Forestali, Firenze, 1424–1428.

Smith B.J. and K. Sivasithamparam, 2000. Internal transcribed spacer ribosomal DNA sequence of five species of *Ganoderma* from Australia. *Mycological Research* 104, 943–951.

Sneath P.H.A. and R.R. Sokal, 1973. The estimation of taxonomic resemblance. In: *Numerical taxonomy*. W.H. Freeman and Co, San Francisco, CA, USA, 114–187.

Tamura K., Nei M. and S. Kumar, 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (USA) 101, 11030–11035.

Tamura K., J. Dudley, N. Masatoshi and S. Kumar, 2007. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Molecular Biology and Evolution* 24, 1596–1599.

Terho M. and A.M. Hallaksela, 2005. Potential hazard characteristics of *Tilia*, *Betula* and *Acer* trees removed in the Helsinki city area during 2001–2003. *Urban Forestry and Urban Greening* 3, 113–120.

Terho M., J. Hantula and A.M. Hallaksela, 2007. Occurrence and decay patterns of common wood-decay fungi in hazardous trees felled in the Helsinki city. *Forest Pathology* 37, 420–432.

White T.J., T. Bruns, S. Lee and J.W. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. (M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White, ed.), Academic Press, Inc., New York, NY, USA, 315–322.

*Accepted for publication: October 24, 2011*