UNIVERSITÀ degli STUDI di CATANIA
Dipartimento di Agricoltura, Alimentazione e Ambiente
Di3A

International PhD

PLANT HEALTH TECHNOLOGIES AND PROTECTION OF AGROECOSYSTEMS

XXVII CYCLE

2012-2014

Molecular characterization of *Rhizoctonia* spp. isolates and sustainable approaches to control Rhizoctonia diseases in ornamental nursery

This thesis is presented for the degree of
Doctor of Philosophy by

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This work was supported by MIUR project PON01_01611 (SO.PRO.ME.: Sustainable production of Potted Plants in Mediterranea Environment)

Contents

1. The genus *Rhizoctonia*

1.1. Introduction

Rhizoctonia species are soil-borne pathogens causing root and foliar diseases on a wide range of agronomic crops, turf grasses, ornamental plants, and fruit and forest trees worldwide (Adams, 1988; Sneh *et al*., 1991; Couch, 1995). *Rhizoctonia solani* is the most widespread species, with a host range that includes over 500 plant species (Farr *et al*., 1995). The genus concept of *Rhizoctonia* spp. was established by de Candolle (1815) (Sneh *et al*., 1998). However, the lack of specific characters led to the classification of a mixture of unrelated fungi as *Rhizoctonia* spp. (Parmeter and Whitney, 1970; Moore, 1987). Ogoshi (1975) enhanced the specificity of the genus concept for *Rhizoctonia* by elevating the following characteristics of *R. solani* to the genus level. Based on this revised genus concept, species of *Rhizoctonia* can be differentiated by mycelia colour (Fig. 1), number of nuclei per young vegetative hyphal cell (fig. 2) and the morphology of their teleomorph.

Figure 1 – Radial colony growth of different isolates of *Rhizoctonia* spp. on Potato Dextrose Agar (PDA)

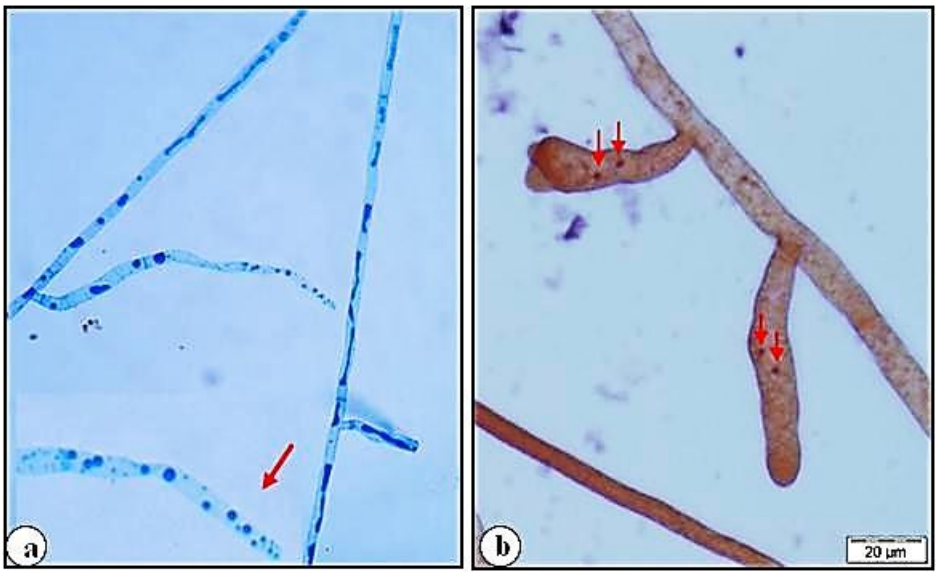


Figure 2 – Nuclei in *Rhizoctonia solani* (a) and *Rhizoctonia* BNR (b) hyphae

The teleomorph of *Rhizoctonia* spp. belongs to the sub-division *Basidiomycota*, class *Hymenomycetes*.

The anamorphs of *Rhizoctonia* are heterogeneous. Moore (1987) placed the anamorphs of *Thanatephorus* spp. in *Moniliopsis* and reserved the genus *Rhizoctonia* for anamorph of ustomycetous fungi which have septa with simple pores. *Moniliopsis* species have smooth, broad hyphae with brown walls, multinucleate cells, dolipore septa with perforate parenthesomas and teleomorphs in the genera *Thanatephorus* and *Waitea*. Related on the binucleate *Rhizoctonia* spp., the anamorphs of the *R. repens* group (teleomorph *Tulasnella*) were assigned to the new genus *Epulorhiza*. Anamorph of *Ceratobasidium* was assigned to the new genus Ceratorhiza (Moore, 1987). Moore's system is taxonomically correct and justified. At present, the concept of genus *Rhizoctonia* has become clear from these taxonomical studies at the molecular level (Gonzalez *et al*., 2001). However, many researchers (Sneh *et al*., 1998) in the world still retain the name *Rhizoctonia* for *Moniliopsis* spp., *Ceratorhiza* spp. and *Epulorhiza* spp.

1.2. Anastomosis group (AG) of *Rhizoctonia solani* and BNR and molecular analysis

Genetic diversity is broad in *Rhizoctonia* genus, to the extent that the species is segregated into anastomosis groups (AGs) based on hyphal reactions of individuals.

In 1969, J. R. Parmeter and his colleagues reintroduced the concept of "hyphal anastomosis" which implies that isolates of *Rhizoctonia* spp. that have the ability to recognize and fuse (i.e. "anastomose") with each other are genetically related, whereas isolates of *Rhizoctonia* spp. that do not have this ability are genetically unrelated. Anderson (1982) defined the anastomosis as a manifestation of somatic, or vegetative, incompatibility.

Anastomosis reactions between hyphae of paired isolates of *R. solani* consist of several types; such as perfect fusion (C3), imperfect fusion (C2), contact fusion (C1) and no reaction (C0) (Matsumoto *et al*., 1932; Carling *et al*. 1996). These classes have been accepted by many researchers and are useful for a better understanding of the genetic diversity of *R. solani* populations, because of the background genetically supported by vegetative or somatic compatibility (VC or SC) of confronted isolates (MacNish *et al*, 1997). The four classes of reactions can be distinguished according to the degree of interaction between hyphae from interacting isolates.

The category that represents the total fusion is the C3. It occurs for the same anastomosis group, same vegetative compatibility population (VCP) and the same isolate. The merger is realized by the fusion of walls and membranes accompanied with protoplasm connection. The anastomosis point frequently is not obvious with a diameter equal or nearly equal hyphal diameter. Anastomosing cells and adjacent cells may die, but generally do not.

The C2 category occurs in same AG, but not between different VCPs. Evidences are a wall connection and an uncertain membrane contact, besides the death of anastomosing and adjacent cells.

C0 and C1 are characterized by a little or no hyphal fusion between isolates. An apparent wall contact between hyphae without a wall penetration and membrane-membrane contact is typical for the C1 category; occasionally one or both

anastomosing cells and adjacent cells die. These categories occur between different AGs or in the same AG.

Affinity for hyphal fusion (anastomosis) (Parmeter *et al*., 1969; Parmeter and Whitney, 1970; Ogoshi *et al*., 1983a, b; Burpee *et al*., 1980) has been used to characterize isolates among *R. solani*, *R. zeae*, *R. oryzae*, *R. repens* and binucleate *Rhizoctonia* spp. with *Ceratobasidium* teleomorphs.

To date, isolates of multinucleate *R. solani* have been assigned to 13 anastomosis groups (AG-1 to AG-13), which may possess similar characteristics, such as host preference, pathogenicity, and type of disease symptom caused (Carling *et al*. 2002a). Isolates of *R. zeae* and *R. oryzae* have been assigned to WAG-Z and WAG-O, respectively (Sneh *et al*., 1998; Carling *et al*., 1999, 2002a). Isolates of binucleate *Rhizoctonia* spp. with Ceratobasidium teleomorphs have been reported. A system developed in Japan (Ogoshi and Uti,1979, Ogoshi *et al*., 1983 a,b; Sneh *et al*., 1998; Hyakumachi *et al*., 2005) includes 21 anastomosis groups designated AG-A to AG-U, in which at present AG-J and AG-M still are in question as members of binucleate *Rhizoctonia*. Another system developed in the USA (Burpee *et al*., 1980) includes 7 anastomosis groups designed as CAG-1 to CAG -7. CAG-1 corresponds to AG-D, CAG-2 to AG-A, CAG-3 and CAG-6 to AG-E, CAG-4 to AG-F, CAG-5 to AG-R, and CAG-7 to AG-S (Sneh *et al*., 1998; Ogoshi *et al*., 1983a, b). At present, the anastomosis system based on AG-A through AG-U is widely accepted by many researchers.

Although the anastomosis method is accurate, valid, and largely used, it is sometimes impossible to determine to which AG an isolate belongs by anastomosis, because certain isolates do not anastomose with representatives of any known AG while some isolates have lost their capability to self-anastomose (Hyakumachi and Ui 1988). On the other hand, isolates of certain AGs anastomose also with isolates of more than one AG (Sneh *et al*. 1991; Carling 1996). In addition, determination of AGs by hyphal anastomosis requires meticulous microscopic experience, and it is a time-consuming procedure.

As suggested by Sharon *et al*. (2006, 2008) the introduction of various molecular and biochemical tools have confirmed the genetic relatedness validity of the AGs and

greatly advanced the accuracy of its classification. On the other hand the variety of these methods has been used to develop rapid PCR-based diagnostic tools for accurate identification of the isolates to AGs and their subgroups.

Sharon *et al*. in 2006 described the advances in various molecular techniques for classification of multinucleate *Rhizoctonia* (MNR); later, in 2008, continued the studies on binucleate and uninucleate *Rhizoctonia* (BNR and UNR). All the various molecular methods used for classification of *Rhizoctonia* spp., including isozyme analysis, total cellular fatty acids analysis, electrophoretic karyotyping, DNA–DNA hybridization, RAPD, AFLP, repetitive probe, AT-rich DNA RFLP, single-copy nuclear RFLP, rDNA RFLP, and rDNA sequence analysis have been explored (Tab. 1).

It was assessed that DNA sequences encoding ribosomal RNA genes, especially the internal transcribed spacer regions (ITS1 and ITS2) flanking the 5.8S subunit (rDNA-ITS sequence analysis), among the various molecular classification methods used for classification of *Rhizoctonia* spp., seems to be the most appropriate one.

This technique is based on rDNA-ITS sequence alignment analysis (by which the genetic relatedness of the isolates is exhibited by clustering of isolate sequences in a tree), complemented with detailed percent sequence similarity within and among AGs and subgroups; these are compared with the anastomosis grouping method (Sharon *et al*. 2006, 2008).

Despite the fact that new methods and techniques have been developed in fungal systematics, classification of *Rhizoctonia* species still is considered to be in developmental stage. In general terms, systematic approaches for this group of fungi need both the study either the determination of the characterization of anastomosis groups, either the rDNA-ITS sequence analysis. MacNish *et al*. (1996) deemed appropriate for study of population biology, that molecular techniques might be complemented with hyphal anastomosis behaviour. For the characterization of AG8 isolates, a pool of methods, including hyphal anastomosis, pectin isozymes, RAPD and DNA fingerprinting, was used.

Table 1 - Relative efficacies of the various molecular methods used for classification of *Rhizoctonia* spp. (Sharon *et al*. 2006)

Method	Different AG	Same AG	Subgroups within AG	Individuals
Nucleic acids				
a. DNA–DNA hybridization		+++	+++	
b. RFLP (restriction fragment length polymorphism)				
- 18S, 28S rDNA	+++	+	+	
- ITS (internal transcribed spacer) rDNA		+++	+	
- AT-rich DNA		+	+	+++
- Single-copy nuclear DNA		+	+	+++
c. DNA fingerprinting				
- RAPD (random amplified polymorphic DNA)				+++
- AFLP (amplified fragment length polymorphism)				+++
d. DNA sequencing				
- 18S, 28S rDNA	+++	+++	+	
- ITS (internal transcribed spacer) rDNA	+++	+++	+++	
e. Electrophoretic karyotyping		+	+	+
Protein				
a. Isozymes				
b. Zymograms		+	+	+++
Cellular fatty acids	+++	+	+	

+++, range that can be expected to yield better results; +, range that might be expected to yield less suitable results

1.3. Systematics of *Rhizoctonia* species

Members of the form genus *Rhizoctonia* D.C. are considered as a complex mixture of filamentous fungi that differ in many significant features, including their sexual stages (teleomorph), asexual stages (anamorph), having in common the possession of a non-spored imperfect state, usually referred to as the *Rhizoctonia* anamorph (Sneh *et al*. 1991, Talbot, 1970, Tu and Kimbrough 1978).

The group includes several of the most devastating crop pathogens like *Thanatephorus cucumeris* (Frank) Donk (anamorph = *Rhizoctonia solani* Kühn), the majority of orchid mycorrhizal symbionts (mainly belonging to genus *Ceratobasidium* D.P. Rogers) and a collection of saprotrophic organisms of different systematic placement. The *Rhizoctonia* anamorph is characterized by several common features present among members of the entire *Rhizoctonia* species complex. Taxa from the group have been rearranged into several groups of higher fungi, including both Ascomycota and Basidiomycota, and split into several genera, employing criteria such as the analysis and ultrastructural comparison of septal apparatus. Until very recently, classification for some of the groups within the complex has been exclusively based on criteria such as hyphal anastomosis, since other types of diagnostic features are usually scarce in these fungi. Phytopathological studies in the complex have represented the major contingent of contributions in the group, especially in the case of *R. solani*. Some members of the complex have been reported to be protective isolates against pathogenic members of *Rhizoctonia* and some other fungal pathogens. (Gonzales Garcia *et al*., 2006)

Genus Thanatephorus [*Rhizoctonia* s. str.]

The genus *Thanatephorus* (*Ceratobasidiaceae*, *Ceratobasidiales*, *Basidiomycota*), was initially proposed by Donk (1956) to designate teleomorphic phases of the *Rhizoctonia solani* multinucleate anamorph. It is commonly accepted that *Thanatephorus* applies to most parasitic fungi (as in the case of *R. solani*) which are characterized by hypochnoid and cymose hyphae just above basal hyphae (Roberts, 1999). Somatic hyphae in *Thanatephorus* are constantly wider (more than 10 μm in diameter) (Roberts, 1999) than in *Ceratobasidium*, a closely related genus in the family *Ceratobasidiaceae*.

The morphology of *Botryobasidium* Donk (Donk, 1956) differs from *Thanatephorus* owing to the presence of short-sterigmate basidia, no repetitive basidiospores and the absence in culture of monilioid cells or sclerotia. Furthermore, Langer (1994) has provided evidence in Botryobasidium of septal pores with continuous parenthesomas, in contrast with discontinuous parenthesomas in both *Thanatephorus* and *Ceratobasidium*. Donk (1956) simultaneously established the genera *Thanatephorus* and *Uthatobasidium*, reserving the later epithet for taxa similar to Thanatephorus, but saprophytic and not producing sclerotia. Furthermore, Talbot and Keane (1971) proposed the name *Oncobasidium* P.H.B. Talbot and Keane for plant pathogenic taxa similar to *Thanatephorus* but not producing sclerotia. Finally, authors like Roberts (1999), considered these two genera to be synonyms of *Thanatephorus*, assuming that parasitism in this genus is considered to be facultative (*T. cucumeris* isolates are commonly reported to occur as saprotrophs), and generic distinction could be considered as weak for these three names. Other genera close to *Thanatephorus* have been recently synonymized by Roberts (1999), including *Ypsilonidium* Donk, *Cejpomyces* Svrcek and Pouzr., *Aquathanatephorus* C.C. Tu and Kimbr. (=*R. solani* AG1) and *Tofispora* G. Langer. Evolutive relationships between *Thanatephorus* and its close relative *Ceratobasidium* remain controversial. Employing classical taxonomic approaches, some authors (Stalpers and Andersen, 1996; Roberts, 1999) have considered both genera to be part of a generic complex, where delimitation among them presents some difficulties, and differences in morphometrical features and ecological behaviour are gradual along the several taxa within both genera. Roberts (1999) considered, after studying the type material for all accepted taxa of both genera, in combination with a preliminary ITS-based molecular phylogeny of selected species, that the two genera should be considered as synonyms. Recently, González *et al*. (unpublished), in an ITS-based phylogeny of family *Ceratobasidiaceae* (including taxa from *Ceratobasidium*, most accepted *R. solani* anastomosis groups and sequences from genus Waitea), suggested that both genera, although closely related, must be retained as independent entities within *Ceratobasidiaceae*. The phylogenetic reconstruction carried out also demonstrated the convenience of segregation, in accordance with some authors (Boidin *et al*.,

1998; González *et al*., 2001), of *Rhizoctonia solani* (*Thanatephorus cucumeris*) into at least four different biological species. Thus, molecular analyses suggested that *Thanatephorus praticola* Kotila and Flentje could be assigned to define, at a specific level, AG 4 isolates (and their subgroups); *T. sasakii* (Shirai) C.C. Tu and Kimbr. could represent the valid epithet to name isolates from AG 1-IA and AG 1-IC; *T. microsclerotium* (G.F. Weber) Boidin could represent AG1-IB strains, while the rest of AGs actually defined, should be confined to *Thanatephorus cucumeris* s. str.

Genus Ceratobasidium [binucleate *Rhizoctonia* (BNR)]

The genus *Ceratobasidium* (*Ceratobasidiaceae*, *Ceratobasidiales*, and *Basidiomycota*) was initially proposed by Rogers (1935) to accommodate four taxa (*C. calosporum* Rogers, the type species of the genus designated by him, *C. cornigerum* (Bourd.) Rogers, *C. sterigmaticum* (Bourd.) Rogers and *C. obscurum* Rogers), some of them usually included as part of a complex mixture of genera and species arranged in the several groups and sections recognized for wide ancient genera like Corticium or Hypochnum. Thus, two of the above mentioned taxa, C. cornigerum and C. sterigmaticum formed part of section Botryoidea of Corticium. Donk (1931) was the first author to segregate part of the *Ceratobasidiales*, erecting the genus *Botryobasidium* Donk within the family *Tulasnellaceae*, due to the presence in members of the new genus of basidiospores capable of germinating by repetition, a diagnostic feature in phragmobasidiate fungi. Later, Rogers (1935) suggested such a taxonomical concept by adding *Ceratobasidium* to this group of fungi. Martin (1948) equally defined the family *Ceratobasidiaceae* to accommodate the genus. Donk (1956), being conscious that *Botryobasidium* still contained certain heterogeneity of "holo-" and "heterobasidiate" taxa, segregated these last elements (species with autoreplicative spores and large sterigmata) into two genera, *Thanatephorus* and *Uthatobasidium* Donk, which were subsequently included by Jülich (1981) within his concept of *Ceratobasidiales*. Both *C. sterigmaticus* and *C. obscurum* are considered to belong to *Thanatephorus* in the modern concept of the genus, and are excluded from *Ceratobasidium* (Roberts, 1999). Donk (1958) erected the genus *Koleroga* to accommodate *K. noxia* Donk, a species morphologically close to *Ceratobasidium*, except for the absence of autoreplicative spores. Talbot (1965)

demonstrated the presence of this type of spore in some collections of *K. noxia*. Subsequently, some authors (Roberts, 1999) considered the genus *Koleroga* as a nomenclatural synonym of *Ceratobasidium* (*C. noxium*). Currently, there are between 10 (Roberts, 1999) and 11 (Kirk *et al*., 2001) species accepted for the genus. Species of *Ceratobasidium* are characterized for the presence of a non-sporulating, *Rhizoctonia*-like (genus *Ceratorhiza*) anamorphic phase, binucleate somatic hyphae (uninucleate in *Ceratobasidium bicorne*) and saprophytic, mycorrhizal or parasitic teleomorphic phases. Ceratobasidium taxa produce effuse fruitbodies of ceraceous consistency, with globose to sphaeropedunculate basidia, produced directly from basal hyphae or in raceme-like groups, usually showing a division between hypo- and epibasidium, producing basidiospores with high rates of repetitive germination (Rogers, 1935). This set of diagnostic characters (mostly referred to hyphal cytology and nutritional behaviour), differentiate *Ceratobasidium* from *Thanatephorus*. Concerning the nomenclature of anamorphic stages of the genus, only the above mentioned genus *Ceratorhiza* is considered to date, to be the correct name to designate anamorphs with *Ceratobasidium* teleomorph (Roberts, 1999). From an ecological point of view, the genus includes saprophytic, symbiotic and even parasitic taxa. Thus, most species have been described as saprophytic on soil or plant debris (wood and leaf litter from both angiosperm and gymnosperm hosts), or as forming part of the fungal component of orchid mycorrhizae. A small number of taxa have been reported as parasites of herbaceous plants (some of them of economic interest) or bryophytes. Regarding its evolutive relationships, the genus could represent for several authors a transitional evolutive line to modern basidiomycetes, from typically heterobasidiate (with clearly segmented basidia, possessing hipo- and epibasidia), resembling *Tulasnella*-like forms (probably the closest heterobasidious relative to *Ceratobasidium*), towards typically holobasidiate forms with non-segmented basidia and true sterigmata. In this sense and in accordance with its current systematic position (Roberts, 1999; Kirk *et al*., 2001), the family *Ceratobasidiaceae* could represent the most primitive group of holobasidiomycetes, with hymenial structures showing morphologies where segmentation of the basidia is still easily observed, and partition on sterigmata occurs. Studies on the ultrastructure

of septal apparatus in *Ceratobasidium* and *Thanatephorus* have revealed their affinities with the remaining holobasidiate basidiomycetes. In this sense, Binder *et al*. (2005) have recently reported phylogenetic evidence of the relationships of the Ceratobasidiales with the remaining holobasidiomycetes.

1.4. Biology

Rhizoctonia solani causes different types of diseases to a wide variety of plants, all over the world and under different environmental conditions (Adams, 1988; Sneh *et al*., 1991; Couch, 1995). In order to understand the *Rhizoctonia* disease occurrences should be considered the host, the pathogen and the environment (fig. 3)

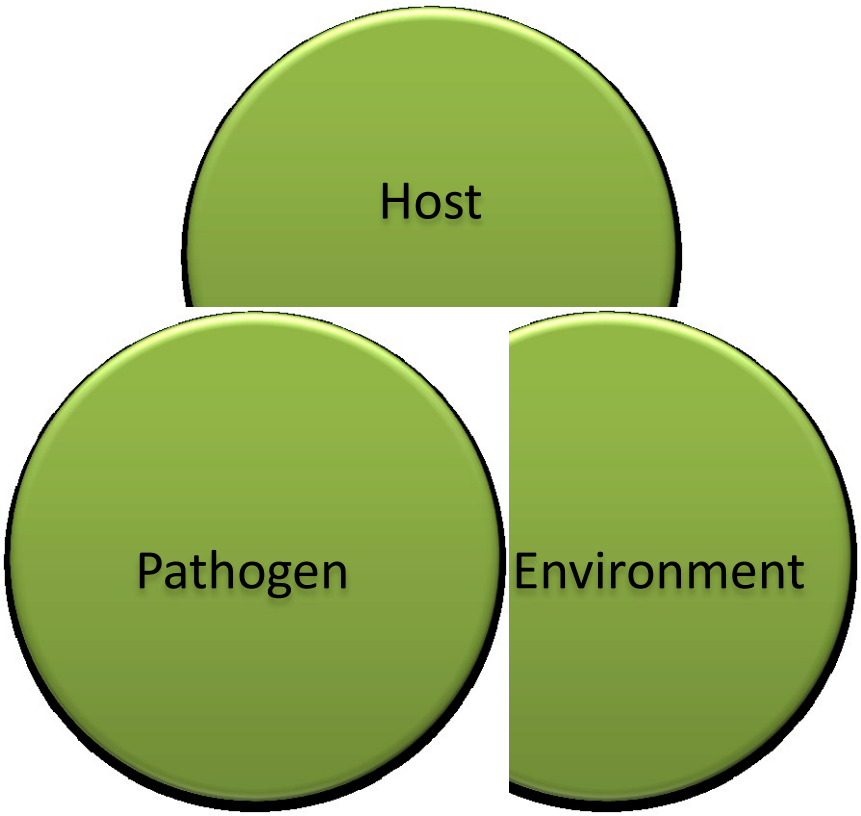


Figure 3 - Disease triangle

Rhizoctonia spp. attack its **hosts** during their juvenile stage of development such as seed, seedlings and cuttings (figure 4) and diseases can occur in cases of severe epidemics under specific conditions. Rhizoctonia species are responsible of root and stem rot, leaf spot, seedlings damping-off and foliar web blight (Chase, 1991; Benson and Cartwright, 1996; Hyakumachi *et al*., 2005; Rinehart *et al*., 2007). Besides the type of germination is the most critical phase, because it influences the length of exposure of the seedling to the invasion by pathogen (Singh, 1955; Ruppel *et al*. 1964). In hypogeal germination the cotyledons remain underground and susceptible to attack whereas in epigeal germination the cotyledons are carried above

the soil, and may thereby escape decay. Factors that delay the germination may increase seed decay and preemergence damping-off. The susceptibility of the host to the fungus attack declines with maturation and lignification of tissues; thus the pathogen attacks the aerial parts, under very moist conditions (Baker, 1970).

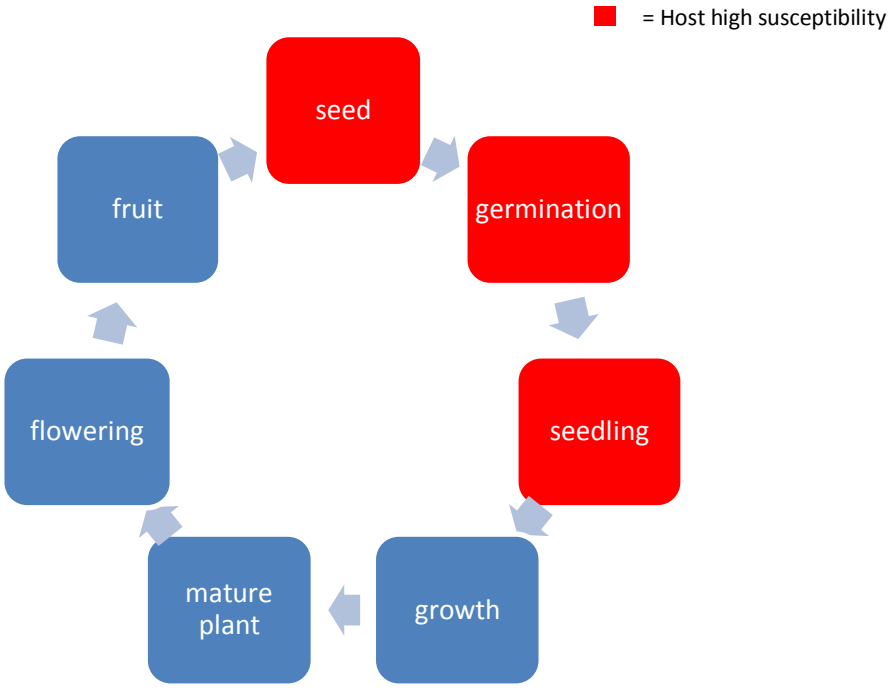


Figure 4 - Host life cycle

Rhizoctonia species remain for all the year in more or less continuous vegetative growth even if it forms resting structures that allows to avoid unfavourable condition. Once the environmental conditions come back closely to the optimum range, the pathogen starts again to differentiate fresh mycelium to infect hosts newly. The resting structure, sclerotia, not only are necessary to face the unfavourable conditions (i.e. dry periods) but are also a means of dissemination by wind or water (Echandi, 1965; Baker, 1970).

Environmental factors are very important in influencing the development of *Rhizoctonia* diseases. The temperature at which infection occurs, the ability to develop in lower soil levels, the ability to form sclerotia, the growth rate and the survival in a certain area may or may not show up given the environment and host.

Due to the phylogenetic heterogeneity, these environmental conditions differ among

taxa, and even among isolates belonging to the same species. The optimal relative humidity, essential for the production of teleomorphs, could range between 40-100%. For sexual sporulation the pathogen needs the intake of O_2 and an efficient removal of CO_2. The optimum temperature range for sexual fruiting is from 20 to 30°C. Moreover, variations in day/night temperatures also seem to play an important role in fruiting. Uchida *et al*. (1986) reported in *Rhizoctonia* species that light stimulates hymenial formation but inhibits the ripening of basidia. This intuition is confirmed by the fact the higher rates of sporulation are usually found by night, followed by a drop in diurnal temperatures. Plants are more prone to attack by pathogens when stressed by an inhospitable environment and usually caused by a lack of nutrients or related to the existence of a strong hydrophobicity gradient in the colonizing substrates (Adams and Butler, 1983; Kotila, 1947; Flentje, 1956).

1.5. Pathogenicity and hosts

Rhizoctonia diseases of ornamental plants can occur in cases of severe epidemics. A wide range of disease symptoms have been recorded, including root and stem rot, leaf spot, seedlings damping-off and foliar web blight (Chase, 1991; Benson and Cartwright, 1996; Hyakumachi *et al*., 2005; Rinehart *et al*., 2007).

BNR typically are weakly virulent, less destructive pathogens or not pathogenic considered as mycorrhizal or biocontrol agents (Harris *et al*., 1994; Andersen and Rasmussen, 1996; Hwang and Benson, 2002; Burns and Benson 2000). However, several studies reported BNR as pathogenic on economically important agricultural and horticultural crops (Priyatmojo *et al*., 2001; Kuramae *et al*., 2007; Aiello *et al*., 2012).

1.5.1. Seed decay

Rhizoctonia may invade the seed while still in the fruit, decaying in there or merely infecting it. The decay process is then resumed after the seed is planted and before germination (Baker, 1947; Neergaard, 1958).

The seed may also be invaded by growth of the fungus from infested soil in which it is planted; the grater the amount of inoculum in the soil, the more certainly and the more rapidly this occurs. In either case, the invaded seed serves as a food base, enabling the pathogen to reach adjacent seedling. It is thus in common to find seedlings with pre-emergence damping-off in the vicinity of a rotted seed. The greater the distance seed are separated, the less the probability of such spread (Singh and Singh, 1955). Thus, losses are usually greater in seed flats and nursery beds than in the wild.

1.5.2. Damping-off of seedling

Rhizoctonia may attack newly emerged plants or cuttings at the base of stem causing extensive water-soaked lesions. As a consequence, the attack may followed by wilt and collapse of the plant. The damping-off can occur in pre- and post-emergence phase. The first one is an extension of seed decay; the two together are often designated as “poor stand”. The damping-off may be considered as a delayed attack,

due to unfavourable environment (temperature or moisture) or insufficient inoculum for faster action, of the seed decay. The longer that seedling emergence in delayed, the greater the opportunity for invasion and the greater is the preemergence damping-off. The emergence is tightly related on the seed depth, seed vitality, type of germination (hypogeal or epigeal) and the physical soil conditions (temperature, moisture or pH) (Singh, 1955; Hartley *et al*., 1918; Germ, 1960; Sinclair, 1965; Leach, 1947; Beach, 1949; Peace, 1962; Baker, 1970). The post-emergence damping-off represents still a further delay in attack and/or expression of symptoms. Symptoms may develop any time after emergence through the soil surface, until the seedling is still in the juvenile stage. The susceptibility of the seedling declines with maturation and lignification of tissues. This increased resistance may be due to conversion of pectin to calcium pectate, rendering the tissues resistant to the polygalacturonase of the fungus (Bateman and Lumsden, 1965).

Symptoms appear on the stem near soil level, but may later advance downward into the roots. Small bits of soil or organic matter dangle from the coarse mycelium of *Rhizoctonia* attached to the infected seedlings when removed from the soil (Duggar, 1916). Thus by examination, with a hand lens, it is possible to distinguish the coarse, hyaline to brown mycelium of *Rhizoctonia*.

The disease may spread into two directions: in circular or irregular patches, when seed is randomly sown and in turf grass; in linear strips if it is sown in rows. These represent either a dispersion ways of inoculum either a via for the pathogen introduction. In disinfested soil, commonly used in nursery, the introduction of the pathogen will success with seed or transplants, soil fragments or infected tissues splashed in by water, carried in by wind or workers, or survival on the pots. The more nearly sterile the soil the more rapid will be the spread, and the larger the area of spread (Baker, 1957, 1962)

1.5.3. Crown and root rot

Crown rot is typified by extensive water-soaked, dark brown lesions at the crown level that girdled entire stem and an internal brown discoloration of cortical tissue (Fig. 6). Crown rot of sugar beet is one of the historic diseases caused by *R. solani*.

Infection apparently occurs in the young leaflets or in leaf bases, and causes petiole decay. As the crown leaves die out, lateral young ones appear. The crown of the plant may eventually be killed and the fungus advance into the top of the fleshy root, causing a dry brown decay (Edson, 1915; Walker, 1957).

Root rot is not generally the most important type of disease caused by this fungus, but severe losses can be produced on some crops. Dark, circular to oblong, sunken cankers with brown borders develop at the point of origin of secondary roots (Fig. 5). Secondary roots may be invaded directly, or may be cut off by the basal lesions.

Figure 5 - Crown and root rot on *Catharanthus roseus*

Underground fleshy roots are often affected by cankers, as well as being attacked at the crown. Plants died a few days later due to the disruption of translocation of water and nutrients. As reported by Durbin (1957) there are some subterranean type of *R. solani* (such as the alfalfa isolates) that are more tolerant of CO_2 than the surface or aerial strains. Among all the species susceptible to root rot there are several agronomical crops including sweet pea, alfalfa, cereals, sugar beet and ornamentals as well (Weber and Foster, 1928; Samuel and Garrett, 1932; Blair, 1942; Moore, 1959). On turnips concentric light and dark bands are produced on the side of the root in early season; later, the lesions may be large, rough, dry and pithy. Attack may occur in the field, as well as in storage (Dana, 1925; Lauritzen, 1929). Sclerotia may develop on the surface of the cankers on either host. Tulip bulbs may be russetted by *R. solani*

(Moore, 1959).

R. solani has been shown to attack the roots of conifers (Hartley, 1921) and coffee trees (Crandall and Arillaga, 1955), but the importance of this trouble under field condition has not been demonstrated.

1.5.4. Hypocotyl and stem cankers

Stem lesions may develop in fully mature plants with well-developed secondary tissue. The stem rot of carnation is very destructive where this plant is grown in soil infested by *R. solani* or where infected cutting are used. A soft moist rot starts at the soil surface and advances into the stem; the decayed cortex easily rubs off, leaving the stele beneath. Strands of mycelium, and sometimes sclerotia, appear on the surface. Roots remain intact until late in the disease, but the tops wilt, turn brown, and die (Baker and Sciaroni, 1952). The fungus causes a destructive "neck rot" of gladiolus, infecting at soil level through the leaf bases, and producing brown shredded lesions from decay of parenchymatous tissue. Cankers may occur on mature tomato plants at the base of stem branches; these often have alternate light and dark bands. Infection may occur through a leaf in contact with the soil and spread to the cortex and stele (Conover, 1949). Basal cankers or "foot rot" may also be produced on tomato plants in glasshouses (Small, 1927).

A more unusual type of stem rot was produced on aquatic plants in the coastal waters of Virginia and North Carolina following the opening of a canal. Dark lesions occurred at the soil surface, regardless of the depth and concentration of sea water, producing great destruction to four genera of plants. This strain of *R. solani* was able to attack potatoes and that from potatoes to attack the aquatic plants (Bourn and Jenkins, 1928).

1.5.5. Bud rot

The flower buds, which are the first to arise from the crown, are killed. Lateral dormant buds may later grow out sometimes giving a witches'-broom effect (Wilhelm, 1957). A preplanting soak of the plant in gibberellin (10 ppm) accelerates the rate of emergence of shoots from the soil and thus decreases *Rhizoctonia* bud rot.

1.5.6. Aerial (web, leaf, thread) blight

Under condition of high relative humidity, warm temperature and reduced sunlight, aerial blight may develop on the canopy of bedding plants and other plants in the greenhouse and landscape (Weber and Roberts, 1951; Wehlburg and Cox, 1966; Frisina and Benson, 1987). *R. solani*, that lead an aerial existence independent of the soil, may spread through the tops of plants.

Foliar blight initially develops as water-soaked lesions on stem and leaves. In succulent plants it rapidly develops in complete plant collapse; while in less succulent ones foliage concentric rings may result from diurnal expansion of lesion (Chase, 1987).

Web blight symptoms occur in the interior portion of the canopy, while the outer canopy leaves remain healthy, typically (Frisina and Benson, 1989).

Stems also were infected and turned dark, reddish, brown or black as disease progressed. If new shoots come out in dry weather they remain healthy and presumably resistant. The small brown spots are concentrically brown-ringed.

1.5.7. Turf grasses patch

The strains that cause brown patch of turf grasses appear to act in every way as the truly aerial forms just discussed, though growing so close to the ground that soil-surface types might be expected. Leaves are infected through stomata and through mowing wounds. Initial leaf symptoms observed are small, tan lesions that enlarged and become surrounded by reddish brown margins over time. Eventually grass leaves become necrotic and brown in colour. The diseased areas of grass are usually 30-90 cm in diameter, but may reach 6-15 m. A dark purplish-green advancing margin 1,3-5,0 cm wide, in which the mycelium is webbed, is visible in the mornings, but soon dries, and the central leaves die and turn light brown.

The crown and the roots are only rarely invaded. Sclerotia are formed near the base of the plant. Because grass is close to the moist soil, there may be a good deal of dew and guttation fluid on the leaves at night; the environment is therefore similar to those trees in the humid tropics.

Table 2 - Binucleate *Rhizoctonia* groups and hosts

Group	**AG-A**: (Mazzola, 1997; Sneh et al., 1998; Yang et al., 2007; Polizzi et al., 2009c, 2010b; Wang and Wu, 2012; Miles *et al*. 2013; Yang and Wu 2013, Li *et al*. 2014)
Symptoms	root rot, damping-off, browning, tortoise shell, crown rot, stem rot and stem canker
Host	strawberry, sugar beet, bean, pea, sunflower (*Helianthus annuus* Linn.), tomato, melon, cucumbear (*Cucumis sativas* Linn.), leaf lettuce, spinach, peanut, potato, *Solanum tuberosum*, apple, swiss chard, *Dodonea viscosa*, *Thryptomene saxicola* and foxtail millet (*Setaria italica*)
Note	Some isolates in this group form mycorrhizal associations with orchids
Group	**AG-Ba** (Sneh *et al*., 1998)
Symptoms	grey sclerotium disease, sclerotium disease, gray southern blight
Host	rice, *Echinochloa crugalli* subsp. *submitica* var. typica, and foxtail millet
Note	-----
Group	**AG-Bb** (Sneh *et al*., 1998)
Symptoms	brown sclerotium disease, grey sclerotium disease, and sheath spot
Host	fox tail, millet, and rice
Note	-----
Group	**AG-C** (Sneh *et al*., 1998; Hayakawa *et al*., 1999)
Symptoms	symbiosis (orchids)
Host	orchids, sugar beet seedlings, subterranean clover, and wheat
Note	No important pathogens have been reported
Group	**AG-D: I, II, III** (Sneh *et al*., 1998; Toda *et al*.,1999; Hayakawa *et al*., 2006)
Symptoms	sharp eye spot, yellow patch, foot rot, Sclerotium disease, snow mold, root rot, damping-off, lesions on stems, winter stem rot and sheath rot
Host	cereals, turf grass, wheat, barley, sugar beet, clove, pea, onions (*Allium cepa* Linn.), potato, cotton, bean, soybean, mat rush, foxtail millet, subterranean clover and *Zoysia japonica*
Note	Recently this group is classified into subgroup AG-D (I) that causes *Rhizoctonia* patch and winter patch diseases. AG-D (II) causes elephant footprint disease
Group	**AG-E** (Sneh *et al*., 1998)
Symptoms	web-blight, damping-off, seedlings, and symbiosis (orchids)
Host	bean, pea, radish, onion, leaf lettuce, tomato lima bean, snap bean, soybean, peanut, cowpea (*Vigna savi*), flax, sugar beet, *Rhododendron* L., long leaf pine (*Pinus palustris* Mill.), slash, lobolly pine (*Pinus taeda* L.), and rye (*Secale cereale* L.)
Note	-----
Group	**AG-F** (Sneh *et al*., 1998; Eken and Demirci, 2004, Yin *et al*., 2011, Aiello *et al*., 2012; Saroj *et al*. 2013; Meza-Moller *et al*. 2014; Harveson and Bolton 2013)
Symptoms	roor rot, watermelon vine decline, damping-off and dry rot canker
Host	bean, pea, radish, onion, peanut, leaf lettuce, tomato, subterranean clover radish, tomato, cotton, taro, strawberry (source: DDJB), *Fragaria x ananassa*, *Musa* spp., watermelon (*Citrullus lanatus*), *Tagetes erecta* and sugar beet
Note	-----
Group	**AG-G** (Mazzola, 1997; Sneh *et al*., 1998; Leclerc *et al*., 1999; Martin, 2000; Botha *et al*.,

	2003; Fenille *et al*., 2005; Polizzi *et al*., 2009°; Tuncer and Eken 2013)
Symptoms	damping-off, root rot, and browning
Host	strawberry, sugar beet, bean, pea, tomato, melon, sunflower, peanut, yacoon, apple, *Rhododendron* Linn., *Fragaria x ananassa*, *Viburnum tinus* and pepper (*Capsicum annuum*)
Note	Non-pathogenic binucleate *Rhizoctonia* spp. provide effective protection to young bean seedlings against root rot caused by *R. solani* AG-4 (Leclerc *et al*., 1999). On pepper is non-pathogenic
Group	**AG-H** (Hayakawa *et al*., 1999)
Symptoms	symbiosis (orchids)
Host	Dactylorhiza aristata (Orchidaceae)
Note	-----
Group	**AG-I** (Mazzola, 1997; Sneh *et al*., 1998; Ravanlou and Banihashemi, 2002)
Symptoms	root rot and symbiosis (orchids)
Host	strawberry, sugar beet, wheat, apple, orchids, and *Fragaria x ananassa*
Note	-----
Group	**AG-J**: (Sneh *et al.,* 1998)
Symptoms	none
Host	apple
Note	-----
Group	**AG-K** (Demirci, 1998; Li *et al*., 1998; Sneh *et al*., 1998; Ravanlou and Banihashemi, 2002; Tuncer and Eken 2013)
Symptoms	Root rot
Host	sugar beet, radish, tomato, carrot, onion, wheat, maize, *Allium cepa* (source: DDJB), Pyrus communis (pear) (source: DDJB), Fragaria x ananassa and pepper (*Capsicum annuum*)
Note	On pepper is non-pathogenic
Group	**AG-L**: No special diseases have been reported (Sneh *et al*., 1991)
Symptoms	-----
Host	-----
Note	-----
Group	**AG-N:** No special diseases have been reported (Sneh *et al.,* 1991)
Symptoms	-----
Host	-----
Note	-----
Group	**AG-O:** No special diseases have been reported (Sneh *et al.,* 1991)
Symptoms	-----
Host	-----
Note	-----
Group	**AG-P:** (Sneh *et al*., 1998; Yang *et al*., 2006)
Symptoms	black rot and wirestem
Host	tea (*Camellia* Linn.), red birch
Note	-----

Group	**AG-Q:** (Sneh *et al*., 1998)
Symptoms	none
Host	Bentgrass
Note	-----
Group	**AG-R:** (Sneh *et al*., 1998;Yang *et al*., 2006, 2008)
Symptoms	wire stem and rhizome blight
Host	bean, pea, radish, onion, leaf lettuce, tomato, lima bean, snap bean, soybean, cowpea, peanuts, red birch, azalea and ginger
Note	-----
Group	**AG-S** (Demirci, 1998; Sneh *et al*., 1998)
Symptoms	no specific diseases
Host	azalea, wheat, barley, and azalea
Note	-----
Group	**AG-T:** (Hyakumachi *et al.,* 2005)
Symptoms	stem rot and root rot
Host	miniature roses
Note	-----
Group	**AG-U:** (Hyakumachi *et al*., 2005)
Symptoms	stem rot and root rot
Host	miniature roses (*Rosa rugosa* Thunb.)
Note	-----

Table 3 - Multinucleate *Rhizoctonia* isolates and hosts

Group	**AG-1 IA** (Li and Yan, 1990; Sneh *et al.*, 1998; Fenille *et al*., 2002; Naito, 2004; Williamson *et al*., 2006; Garibaldi *et al.*, 2007, 2009a, 2010, 2012; 2013 Yan *et al*. 2013; Frake and Spurlock 2013)
Symptoms	sheath blight, foliar blight, leaf blight, web-blight, head rot, bottom rot, brown patch, leaf rot and aerial blight
Host	rice (*Oryza sativa* L.), corn (*Zea mays* L.), barley (*Hordeum vulgare* L.), sorghum (*Sorghum vulgare* Pes.), potato (*Solanum tuberosum* L.), barnyard millet, common millet, soybean, peanut (*Arachis hypogaea* L.), lima bean, cabbage, leaf lettuce, stevia, orchard grass, crimson clover, tall fescue (*Festuca arundiacea* Schreb), turfgrass, creeping bentgrass, perennial ryegrass, gentian (*Gentiana scabra*), camphor, soybean, coral bells (*Heuchera sanguinea*), foxglove (*Digitalis purpurea*), woodland sage, winter savory (*Satureja Montana*) rosemary (*Rosmarinus officinalis*)
Note	This group has a tendency to attack aerial parts of the plants. Basidiospore infection of rice has been reported, but sclerotia are more important as an infection source. The optimum growth temperature is higher than those of AG-1 IB
Group	**AG-1 IB** (Sneh *et al*., 1998; Naito, 2004;Yang *et al*., 2005b, Williamson *et al*., 2006; Garibaldi *et al.*, 2006b, 2013a,b,c; Bai *et al.*, 2010 2014; Baiswar *et al.*, 2012, 2013; Nitzan *et al.*, 2012; Miranda *et al.* 2014
Symptoms	sheath blight, leaf blight, foliar blight, web-blight, summer blight, root rot, damping-off, head rot, bottom rot and seedling blight
Host	corn, sugar beet, gay feather (*Liatris* spp.), common bean, fig (*Ficus* L.), adzuki bean, soybean, cabbage, leaf lettuce, redtop, bentgrass, orchard grass, leaf lettuce, apple (*Malus pumila* Mill), Japanese pear, European pear, lion'ear (*Leonotis leonurus*), hortensia (*Hydrangea* spp.), *Larix* spp., gazania (*Gazania* spp.) *Cotoneaster* spp., Egyptian atar-cluster (*Pentas lanceolata*), Chinese lantern plant (*Physalis alkekeng* var. franchetii), *Hypericum patulum*, marigold, *Acacia* spp., rosemary, *Eucalyptus* spp., pine (*Pinus* L.), cypress (*Cupressus* spp.), soybean, elephant foot (*Amorphophallus Konjac*), Madagascar periwinkle (*Catharanthus roseus*), *Dioscorea nipponica*, *Basella alba*, mint (*Mentha longifolia*), white clover (*Trifolium repens*), *Rumex acetosa*, *Mucuna pruriens*, *Origanum vulgare*, *Lavandula officinalis* and *Nigella damascena*.
Note	-----
Group	**AG-1 IC** (Sneh *et al.,* 1998; Naito, 2004)
Symptoms	damping-off, summer blight, foot rot, crown rot canker and root rot
Host	sugar beet, carrot (*Daucus carota* L.), buckwheat (*Eriogonum* Michx), flax (*Linum usitatissimum* L.), soybean, bean (*Phaseolus* L.), cabbage, pineapple (*Ananas comosus* (Linn.) Merr.), panicum (*Panicum* spp.), spinach (*Spinacia oleracea* L.), and radish (*Raphanus sativus* Linn).
Note	-----
Group	**AG-1 ID** (Priyatmojo *et al.*, 2001; Thuan *et al.*, 2008)
Symptoms	leaf spot and leaf blight
Host	coffee (*Coffea* Linn) and durian (*Durio zibethinus*)

Note	this subgroup was recently reported in the Philippines (Priyatmojo *et al.*, 2001) Undetermined subgroup: buckwheat, flax, spinach, and radish
Group	**AG-2-1** (Satoh *et al*., 1997; Camporota and Perrin, 1998; Sneh *et al*., 1998; Rollins *et al*., 1999; Khan and Kolte, 2000; Naito, 2004; Paulitz *et al.*, 2006; Zhang *et al.*, 2009; Baiswar *et al.*, 2010; Bai *et al.*, 2010; Mercado Càrdenas *et al.*, 2012; Caesar *et al.* 2014; Misawa and Kuningana 2013)
Symptoms	damping-off, leaf rot, leaf blight, root rot, foot rot, bottom rot, bud rot, head rot, seedling blight and leaf margin necrosis
Host	sugar beet, wheat (*Triticum aestivum* Linn.), potato, cowpea (*Vigna unguiculata* (Linn.) Walp), canola, rape (*Brassica napus* Linn.), cauliflower (*Brassica oleracea* var. *botrytis* Linn.), mustard (*Sinapis* Linn.), turnip (*Brassica rapa* Linn.), pepper (Piper Linn.), *Silene armeria,* spinach, leaf lettuce, strawberry (*Fragaria ananassa* Duchesne), tulip (*Tulipa gesneriana* Linn.), tobacco (*Nicotiana* Linn.), clover (*Medicago* Linn.), table beet, canola, cabbage, *Ctenanthe oppenheimiana*, *Dioscorea nipponica*, tobacco, *Lepidium draba* and chinese chives (*Allium tuberosum* Rottler ex Sprengel)
Note	This group includes the AG-2-1 tulip strain (former AG-2t) and the AG-2-1 tobacco strain (former homogenous Nt-isolates) (Kuninaga *et al.*, 2000)
Group	**AG-2-2 III B** (Sneh *et al.*, 1998; Priyatmojo *et al.*, 2001; Naito, 2004; Caesar *et al.*, 2009; Woodhall *et al.*, 2012a; Garibaldi *et al.* 2014; Zhao and Wu 2014)
Symptoms	brown sheath blight, dry root rot, root rot, brown patch, large patch, black scurf, stem rot, stem blight, web blight, *Rhizoctonia* rot, damping-off, stem canker, collar rot, and crown brace rot
Host	rice, soybean, corn, sugar beet, edible burdock (*Arctium lappa*), taro (*Colocasia esculenta*), *Dryopteris* spp., elephant foot, crocus, saffron (*Crocus sativus* Linn.), redtop, bentgrass, St. Augustine grass, turf, balloon flower (*Platycodon grandiflorum*), Christmas-bells (*Sandersonia aurantiaca*), *Hedera rhombea*, mat rash, *gladiolus*, ginger, *Iris* Linn, *Centaurea stoebe*, potato and *Rebutia perplexa*
Note	-----
Group	**AG-2-2 IV:** (Sneh *et al*., 1998; Naito, 2004)
Symptoms	leaf blight, foliage rot, root rot, and stem rot
Host	sugar beet, carrot, eggplant (*Solanum* Linn), pepper, spinach, stevenia (*Stevenia* Adams et Fisch), and turfgrass
Note	-----
Group	**AG-2-2 LP:** (Aoyagi *et al*., 1998)
Symptoms	large patch
Host	Zoysia grass
Note	-----
Group	**AG 2-3:** (Naito and Kanematsu, 1994; Sumner *et al*., 2003)
Symptoms	leaf blight and root rot
Host	soybean
Note	-----
Group	**AG-2-4:** (Sumner, 1985)

Symptoms	crown rot, brace rot, and damping-off
Host	corn and carrot
Note	-----
Group	**AG-2-BI:** (Carling *et al*., 2002b)
Symptoms	nonpathogenic
Host	isolates, obtained only from soils and plants in forests
Note	former name is AG-BI; Undetermined subgroup: sesame (Sesamum Linn.), white mustard (Sinapsis alba), primrose (Primula spp.), white lace flower (Ammi majus), carnation, baby's-breath (Gypsophila paniculata), rusell prairie gentian (Eustoma grandiflorum), snap bean, lima bean, and Chinese radish
Group	**AG 3: PT, TB** (Sneh *et al*., 1998; Kuninaga *et al*., 2000; LaMondia and Vossbrinck, 2011, 2012; Wu *et al*., 2012; Muzhinji *et al.* 2014a; McCormack *et al.* 2013; Tuncer and Eken 2013)
Symptoms	black scurf, leaf spot, target leaf spot, damping-off and root rot
Host	PT: potato with black scurf symptoms; maize with stem bases and roots rot TB: tobacco with target leaf spot symptoms Pepper (*Capsicum annuum*)
Note	Undetermined subgroup: eggplant, sugar beet, tomato, and wheat. Their pathological and ecological information is less
Group	**AG-4: HG-I, HG-II, HG-III** (Baird, 1996; Holtz *et al*., 1996; Sneh *et al.*, 1998; Fenille *et al*.,2002; Ravanlou and Banihashemi, 2002; EI Hussieni, 2003; Kuramae *et al*., 2002, 2003; Naito, 2004; Yang *et al*., 2005c, Garibaldi *et al*., 2006a; Yang *et al*., 2007; O' Brien *et al*., 2008; Aiello *et al*., 2008a, b; Hsiao *et al*., 2008; Aiello *et al*., 2009; Garibaldi *et al*., 2009b, c, d, Polizzi *et al*., 2009b; Polizzi *et al*., 2010a, c; Srinivasan and Visalakchi, 2010; Polizzi *et al*., 2011a, b; Bai *et al*., 2012; Liao *et al*., 2012; Mathew *et al*., 2012; Nitzan *et al*., 2012; Woodhall *et al*., 2012b; Muzhinji *et al.* 2014b; Yang and Wu 2013)
Symptoms	damping-off, root rot, stem canker, black scurf, fruit rot, stem rot, leaf blight, web blight, crown rot, basal rot, wilt and leaf yellowing and wilting
Host	pea, sugar beet, melon, soybean, adzuki bean, common bean, snap bean, lima bean, carrot, spinach, taro, tomato (*Lycopersicon esculentum* Mill.), potato, alfalfa (*Medicago sativa* Linn.), elephant foot, arrowleaf clover, beans, barley, buckwheat, cabbage, canola, turnip, carnation, cauliflower, Chinese chive, chrysanthemum, corn, cotton (*Gossypium* Linn.), table beet, tobacco, turfgrass, wheat, white lupine, parsley (*Petroselinum* Hill), *Cineraria* Linn., stock, poinsettia, primrose, hybrid bouvardia, *Citrus* Linn., cauliflower, *Euphorbia* spp., geranium (*Pelargonium* spp.), Russel prairie gentian, statice (*Limonium* spp.), baby's-breath, *Astragalus membranaceus*, lamb's lettuce, swiss chard, mung bean (*Vigna radiata*), *Lagunaria patersonii*, redwood, african daisy (*Osteospermum)*, orange jessamine (*Murraya paniculata*), Washington lupine (*Lupinus polyphyllus*), *Hosta fortune*, fan columbine (*Aquilegia flabellata*), *Coprosma repens* and *C. lucida*, *Chamaerops humilis*, *Streptosolen jamesonii*, sunflower, *Passiflora mollissima*, *Tabebuia impetiginosa*, Rhodiola secholiensis, Besella rubra, Pisum sativum, mint (*Mentha longifolia*)
Note	-----

Group	**AG-5** (Li, *et al*., 1998; Demirci, 1998; Sneh *et al*., 1998; Ravanlou and Banihashemi, 2002; Eken and Demirci, 2004; Naito, 2004; Mattew *et al*.,2012; Yang and Wu, 2012)
Symptoms	root rot, damping-off, black scurf, brown patch, stem cacker and symbiosis (orchids)
Host	soybean, adzuki bean, apple, barley, chickpea, common bean, lima bean, potato, strawberry, sugar beet, table beet, tobacco, turfgrass, wheat, white lupine and *Pisum sativum*
Note	-----
Group	**AG-6: HG-I, GV** (Mazzola, 1997; Meyer *et al*., 1998; Sneh *et al*., 1998; Carling *et al*., 1999; Pope and Carter, 2001; Naito, 2004; Tuncer and Eken 2013)
Symptoms	root rot, crater rot, and symbiosis (orchids)
Host	apple, wheat, carrot, carnation and pepper (*Capsicum annuum*)
Note	all isolates from forests are non-pathogenic
Group	**AG-7:** (Naito, *et al*., 1993; Baird and Carling, 1995; Carling, 1997, 2000; Carling *et al*.,1998; Rani *et al*. 2013; Kamel *et al*. 2010)
Symptoms	damping-off, root rot, and black scurf
Host	carnation, cotton, soybean, watermelon (*Citrullus lanatus* (Thunb.) Mansfeld), Raphanus Linn., and potato
Note	-----
Group	**AG-8:** (Sneh *et al*., 1998; Naito, 2004)
Symptoms	bare patch
Host	barley, cereals, green pepper, potato, and wheat
Note	-----
Group	**AG-9:** (Sneh *et al*., 1998; Naito, 2004).
Symptoms	black scurf
Host	potato, crucifers, wheat, and barley.
Note	-----
Group	**AG-10:** (Sneh *et al*., 1998; Schroeder and Paulitz, 2012)
Symptoms	(weak pathogenic) root rot
Host	barley, wheat and canola
Note	-----
Group	**AG-11:** (Kumar *et al*., 2002).
Symptoms	damping-off and hypocotyls rot
Host	barley, lupine, soybean, and wheat
Note	-----
Group	**AG-12:** (Kumar *et al*., 2002)
Symptoms	symbiosis (orchids)
Host	Dactylorhiza aristata (Orchidaceae)
Note	-----
Group	**AG-13:** (Carling *et al*., 2002a).
Symptoms	none
Host	cotton
Note	-----

2. *Rhizoctonia* diseases on ornamental plants in Europe and Italy

Several studies of last twenty years reported a broad plant-host group susceptible to *Rhizoctonia* diseases. Different symptoms such as root and stem rot, leaf spot, seedling damping-off and foliar leaf blight are caused by *Rhizoctonia* spp. in different countries of Mediterranean basin.

In Europe 7 different species of *Rhizoctonia* are reported as pathogen on ornamental plants. In detail: *R. crocorum* is reported to be pathogen on *Dianthus* sp., *Humulus lupulus* and *Morus* sp., in Bulgaria (Bobev, 2009). *Rhizoctonia endophytica* var. *endophytica* and *Rhizoctonia endophytica* var. *filicata* are reported on *Picea abies* in Norwey (Roll-Hansen and Roll-Hansen, 1968). Roberts (1999) have found out *Rhizoctonia rubiae* on *Rubia* sp. in France. *Rhizoctonia tuliparum* and *Rhizoctonia violacea* are pathogen on *Tulipa* sp. in Poland and *Citrus* sp. in Italy, respectively (Mulenko, 2008; Greuter, 1991). The main specie that has more than 500 host and has been reported in 8 European country (Bulgaria, Denmark, Germany, Greece, Italy, Madeira Islands and Spain) is *R. solani* (Aiello 2008 a, b; Aiello 2009; Bobev 2009; Braun 1930; Crous *et al*. 2004; Garibaldi 2006a, b; Garibaldi 2009 a, b, c, d; Garibaldi 2007; Garibaldi 2003 a, b; Gonzalez *et al*. 2011; Holevas *et al*. 2000; Kowalski and Andruch, 2012; Mulenko *et al*. 2004; Mulenko *et al*. 2008; Polizzi *et al*. 2009 a; Polizzi 2010 *et al*. a, b; Polizzi 2011 *et al*. a, b; Richardson 1990, Garibaldi *et al*. 2013 a, b, c, d, Garibaldi *et al*. 2010, Garibaldi *et al*. 2012, Garibaldi *et al*. 2014). In Italy intensive researches have been conducted on vegetable crops and ornamental plants.

These studies allowed to identify *R. solani* as causal agent of aerial blights on *Anubias heterophylla*, *Aquilegia flabellata*, *Catharanthus roseus*, *Digitalis purpurea*, *Heuchera sanguinea*, *Hosta fortunei*, *Lantana camara* (Garibaldi *et al*. 2003a; Garibaldi *et al*. 2009a; Garibaldi *et al*. 2006a; Garibaldi *et al*. 2009b,c,d; Garibaldi *et al*. 2007; Garibaldi *et al*. 2003a,b). Most of these reports concern on surveys conducted in Sicilian nursery, especially located in the Eastern side of the Island. Damping-off caused by *R. solani* has been reported on *Chamaerops humilis*, *Lagunaria patersonii*, *Osteospermum* sp., *Tabebuia impetiginosa* (Polizzi *et al*.

2010a; Aiello *et al*. 2008a, b; Polizzi *et al*. 2011a). Polizzi *et al*. 2009a; Aiello *et al*. 2009; Polizzi *et al*. 2011b reported crown and root rot on *Coprosma lucida* and *C. repens*, *Murraya paniculata*, *Passiflora mollissima*. Greuter *et al*. (1991) wrote a checklist of Sicilian fungi in which is reported *R. violacea* as causal agent on *Citrus* sp.

2.1. *Rhizoctonia* diseases management

Since the earliest days of agriculture, humans have had to protect their crops against yield loss from weeds, insect pests and diseases (Lamberth *et al*., 2013).
Understanding the life history of pathogenic fungi is critical for developing appropriate strategies for disease management (Vilgalys and Cubeta, 1994).
Plants are protected from infections by a "skin", a waxy cuticular layer atop the cell wall. Would-be pathogens breaching this barrier encounter an active plant immune system that specifically recognize pathogen and altered-self molecules generated during infection. Consequent regulation of a network of inducible defences can halt pathogen proliferation and signal distal plant organs to become nonspecifically primed against further infection (Dangl *et al*., 2013).
Although *R. solani* and binucleate *Rhizoctonia* spp. attack numerous ornamentals, control strategies are similar for both (Chase, 1987, 1991; Frisina and Benson, 1988). The most important consideration is the judicious use of preventative measures, regardless of venue.
An effective program can encompass primarily, or in combination, cultural practices, fungicides, biological control and eradicants (Benson and Cartwright, 1996).

2.1.1. Chemical control

Ever since the destructive potential of *Rhizoctonia* pathogens on plants was realized, application of synthetic fungicides has been the most used disease control measure employed by the farmers. Even today thus disease-prone and high-yielding varieties have been developed, fungicides treatments are needed to maintain crop health. Chemical fungicides have been largely successful used against *Rhizoctonia* species pathogens on many different crops and in different areas.
Chemical control of *Rhizoctonia* has been the subject of hundreds of studies conducted since 1913 when stem-formalin treatment was first recommended to control potato black scurf (Winston, 1913).
Rhizoctonia diseases are controlled by seed, soil treatments or foliar applications using fungicides of diverse chemical groups. From 1935 to 1965 quintozene was the

most popular chemical fungicide and had had monopoly for *Rhizoctonia* control. Later, in 1966, the first systemic fungicide, carboxin, was commercially launched and provided highly effective against *R. solani* damping-off and root rot (Kataria and Gisi, 1996). Nowadays two chemicals are constantly used for managing Rhizoctonia disease: tolclofos-methyl, included in AH-fungicides (Aromatic Hydrocarbons) and pencycuron, a phenylureas following the FRAC Code List© 2014. Tolclofos-methyl has been used for its excellence in controlling almost all types of Rhizoctonia diseases on a high number of crop species under diverse environmental conditions. Whereas, pencycuron has been developed specifically to control rice sheath blight and potato scurf (Kataria and Gisi, 1996).

In 2013, Haralson *et al*. conducted two trials for managing Cylindrocladium and Rhizoctonia root rot on blueberry. In each trial were tested fludioxonil, azoxystrobin and flutolanil. These fungicides significantly reduced lesion incidence and lesion length in both trials except flutolanil that reduced lesion length only in the second trial. For controlling Rhizoctonia root rot azoxystrobin may be used in a rotation, because it may be subject to resistance development. Haralson *et al*. (2013) concluded that azoxystrobin may provide control in the short to medium term, but other fungicides are needed for use as rotation partners. Azoxystrobin, flutolanil and fludioxonil should be considered as part of a rotation for the control of Rhizoctonia root rot.

2.1.2. Integrated pest management

The concept of disease control has evolved over the years. In the early days, the goal was to eradicate the pathogens as effectively as possible, using drastic chemicals while ignoring the consequences to the environment. It was only later realized that in addition to the pathogen, there are other components in disease development, such as, the host and the biotic and abiotic components of the environment, which can be manipulated to suppress the disease. Consequently, the integrated-management approach, as detailed below, became a major pillar in disease suppression, and the term “disease management” replaced that of “disease control”. IPM gradually replaced pest, pathogen and weed control, not only in terminology but also in

concept. The concept of IPM was first developed and disseminated by entomologists in the 1950s; it was only later adopted by plant pathologists, initially for foliar diseases. The further adoption of IPM for soil-borne diseases, which is more complex understanding, occurred later still. In 1998, Kogan defined the IPM such a "decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated in a management strategy, based on cost/benefit analyses that take into account the interest of and the impact on producers, society and the environment". Later, in 2010 Chellemi stated that the IPM involves the coordinated use of multiple tactics to maintain damage from specific pests below an economic threshold and to conserve beneficial organism. Thus, the integration of management tools is a basic principle of IPM, regardless of its many definitions (Kendrick, 1988; Kogan, 1998; Katan, 2006; Gray *et al*., 2009; Chellemi, 2010; Katan 2012, 2014).
Katan (2006, 2012) has described the basic principles of IPM related to soil disinfestation as follows:

1. Combining and integrating methods for pest management, or alternating them, should be at the heart of IPM. Integration means the harmonious use of multiple methods (Gray *et al*., 2009)
2. Any methods for disease suppression, even if it is only partially effective, should be examined and considered since it might be effective when combined with other methods
3. As with any group of pests, IPM for soil-borne pathogens has to be holistic. All sources of inoculum, at all sites, have to be managed, during the entire life cycle of the pathogen, especially those inocula that reinfest the soil after disinfestation
4. The impact of disease management on the crop, the agricultural and non-agricultural environments, natural resources and human health should be considered
5. The tool selected to manage one pest should be compatible with those used to manage others pests of particular crop

Disease monitoring integrated with economic injury thresholds and a detailed knowledge of the pathosystem should be used to plan the rational application of

management strategies. Papaviza and Lewis (1979) commented that the literature on integrated management of *Rhizoctonia* in the field was practically non-existent. Fidanza and Dernoeden (1996) reported success in controlling Rhizoctonia blight in ryegrass with nitrogen source and fungicide applications. In 2001, the combination of azoxystrobin and the *Bacillus* isolate MSU-127 was showed to be effective in Rhizoctonia crown and root rot of sugar beet reduction and greatest root and sucrose yield increase (Kiewnick *et al*. 2001).

2.1.3. Biological control

Since 80 years, biological control of soil-borne pathogens by introduced microorganism has been studied, but only since last decades researches have focused their attentions on biological control and the companies have developed programs for the production of organism, the **biological control agent** (**BCA**), as commercial products.

In plant pathology, the term biological control, or its abbreviated synonymous "biocontrol", has been applied to the use of microbial antagonists to control diseases (Pal and McSpadden Gardener, 2006).

Biological control, in general, is defined as the reduction of pest populations by natural enemies and typically involves an active human role. Thus, could be considered as the suppression of damaging activities of one organism by more other organisms. With regards to plant disease, biological control includes the cultural practices such as the rotation and planting of disease resistant cultivars. Moreover, the inducing host resistance could be considered a form of biological control. However, biological control *sensu strictu* refers to the utilization of microbial inoculants in order to suppress a single pathogen by a single antagonist, or by a complex of antagonists, and a single type or several classes of plant disease (Pal and McSpadden Gardener 2006).

Garrett (1965) described the biological control as a set of conditions or practices useful to reduce survival or activity of a pathogen through the activity of any other living organism. This means that the biocontrol agents (BCAs) were evaluated as a substitute of chemicals. Today the definition of biological control is replaced by the

"biological management" of plant disease, so to cover all aspects referring to suppression rather that real control of plant diseases accomplished by BCAs (Tjamos *et al*. 2010). So the BCAs are organisms that interact with the components of disease triangle to manage the disease (host, pathogen and environment). BCAs may contrast pathogen by physical contact and/or a high-degree of selectivity for the pathogen (direct antagonism) and/or by deplete the food base and by physical occupation of site, or by producing compounds that have a direct effect on the pathogen (indirect antagonism). All the mechanisms are probably never mutually exclusive.

A typical example of direct antagonism is represented by the **parasitism**, a state in which one organism, the parasite, lives on or inside another organism, the host, and derives its nourishment and other needs from it. Microorganisms that are parasitic on other fungi are usually referred to as mycoparasites (Baker and Cook, 1974). Many mycoparasites occur on a wide range of fungi and some of them have been proposed to play an important role in disease control (Adams, 1990; Lo, 1997; Maloy, 1993). Mycoparasitism is a potential mode of action and relies on the production of fungal cell-wall-degrading enzymes (Elad 1995). In literature there are several examples of mycoparasites. For example, Weindling (1932) suggested the inoculation of soil with *Trichoderma* spores to control damping off of citrus seedling caused by *Rhizoctonia solani* (92). Besides, *T. viride* and other *Trichoderma* species were observed to parasitize also *Rhizoctonia bataticola* (Baker and Cook, 1974). More recently *T. harzianum* and *T. hamatum* have been marketed as wound dressings for ornamental and forest trees and decay inhibitors for utility poles (Adams, 1990; Maloy, 1993). Mycoparasitism is performed in a four-step process (Chet, 1987; Handelsman and Parke, 1989; Harman and Nelson, 1994; Lam and Gaffney, 1993; Lo, 1997;Lorito *et al*. 1994;Tunlid *et al*. 1992):

1- The biocontrol fungi grow tropistically toward the target fungi that produce chemical stimuli (chemotropic growth)

2 and 3- Recognition and cell wall degradation. Mycoparasites can usually either coil around host hyphae or grow alongside it and produce cell wall degrading enzymes to attack the target fungus (Chet, 1987; Harman and Nelson, 1994).

4- Penetration. The biocontrol agent produces appressoria-like structures to penetrate

the target fungus cell wall (Chet, 1987; Deacon and Berry, 1992; Lo C-T, 1998). However, the fact that *Trichoderma* isolates produced cell-wall-degrading enzymes does not necessarily guarantee good biocontrol activity (Elad, 2000).

Typical process of indirect antagonism is the **competition** between pathogen and BCA, for **nutrients** or **space**. Pathogen is excluded by depletion of a food base or by physical occupation of the site to get established in the environment. Nelson (1990) assessed the competition exists because BCAs have more efficient utilizing uptake system for the **nutrients** than the pathogens even though no method is available to determine the significance of each component on disease suppression (Janisiewicz *et al*., 2000). The competition concerns not only nutrients but also specific substances or stimulants for germination of microorganisms, when microbes need specific stimulants due to soil fungistasis to germinate. Nevertheless, BCAs may be able to grow in the same ecological site of the pathogen. This characteristic allows the biocontrol agent to interact with the pathogen e to develop a successful mechanism of control. This is the competition for physical **space** (site).

Plants possess a range of active defence apparatuses that can be actively expressed in response to biotic or abiotic stresses. **Induced resistance** is a state of enhanced defensive capacity developed by a plant when appropriately stimulated. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance included in mechanism of indirect antagonism and can protect plants against soil and foliar pathogens (Paulitz and Matta, 2000). In both SAR and ISR, plant defences are preconditioned by prior infection or treatment that results in resistance (or tolerance) against subsequent challenge by a pathogen or parasite. The first one is mediated by salicylic acid (SA), frequently produced after the pathogen infection and typically expression of pathogenesis-related (PR) proteins. ISR is a response to jasmonic acid (JA) and/or ethylene, produced in response to some not pathogenic rhizobacteria applications. Elad (2000) demonstrated the ISR by applying a BCA at a location separate from the plant organ that is challenged by a pathogen. Poromarto *et al*. in 1998 studied the association of BNR *Rhizoctonia* with soybean and determined the induced resistance in the mechanism of biocontrol showed as inhibition of hyphal growth of *R. solani* on the soybean surface tissue.

Studies with many different bacterial biocontrol agents, including PGPR, have demonstrated that one mechanism by which effective strains reduce plant disease is elicitation of induced systemic resistance (ISR) in various model systems. Applications of these findings in practical disease management strategies for commercial agriculture are now possible using seed treatment with aerobic spore-forming PGPR (the bacilli). Strain INR-7 is registered by the EPA as a biological fungicide and which is currently marketed as Yield Shield™ by Bayer Crop Sciences for managing *Fusarium* spp. and *Rhizoctonia solani* on soybean and beans.

2.1.3.1. *Trichoderma* spp.

Trichoderma is a genus of filamentous deuteromycetes. Its members are generally found in all soils including forest humus layer (Wardle, Parkinson and Waller, 1993) as well as in agricultural and orchard soils (Chet, 1987; Roiger *et al*., 1991).

Danielson and Davey (1973) reported that individual species showed preference for soil temperature and moisture content of forest soils. Widden and Aribtol (1980) found seasonality to species distribution, *Trichoderma* viride Pers. does seem to be adapted to cooler climates (Roiger *et al*., 1991; Samuels, 1996).

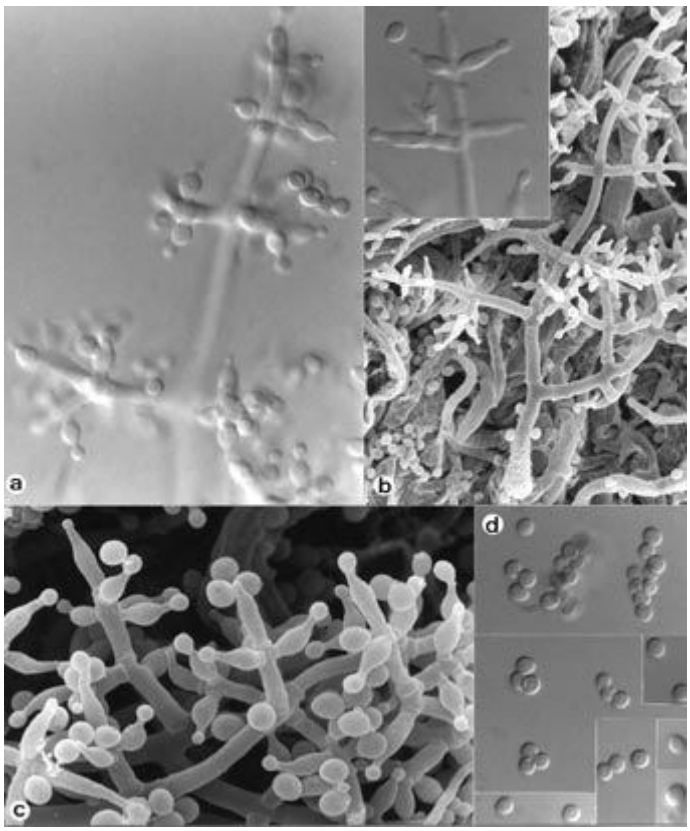


Figure 6 - *Trichoderma harzianum*
a, b. Pyramidal conidiophores; c, d. detail of phialides and conidia. a, d. x1600; b. x1300; c. x3300. ©MycoBank

Past research indicated that *Trichoderma* can parasitize fungal pathogens and produce antibiotics (Tran, 2010). More recent research

indicated that certain strains of *Trichoderma* can induce systemic and localized resistance to several plant pathogens. Biocontrol activity may serve as an alternative to some chemicals.

Biocontrol of fungal pathogens involves various models of action, the most studied include mycoparasitism, competition and antibiosis (Elad, 1996). In general these modes of action have been described above.

Plants treated with *Trichoderma* in the root zone can produce higher levels of peroxidase, of chitinase activity, of deposition of callose-enriched wall appositions on the inner surface of cell walls and pathogenesis-related proteins. Moreover, some strains may enhance plant growth and development. For example was observed by several researchers who treated plants with *T. harzianum* resulting in large increases in root area and cumulative root length, as well as significant increases in dry weight, shoot length, and leaf area over that of the untreated control (Howell, 2003).

The ability of some species to produce enzymes and/or to attack or inhibit other fungi has prompted researchers to investigate various aspects, including biological control of plant disease, and enzyme production, as well as in studies of genetic control and manipulation in filamentous fungi.

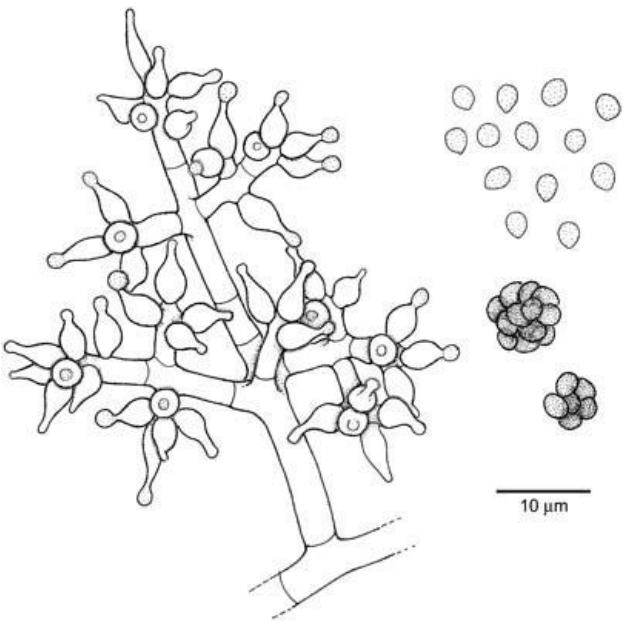


Figure 7 - *Trichoderma harzianum* - Conidiophore and conidia ©MycoBank

T. harzianum and *T. virens* (mostly under the name *Gliocladium virens*) are the most commonly utilized and studied species in biological control (Papavizas, 1985; Chet, 1987). Control of *Rhizoctonia solani* and *Pythium ultimum* by *Trichoderma* species, including *T. harzianum*, may be affected through direct penetration of host hyphae (Dennis and Webster, 1971; Benhamou and Chet, 1993). Inbar and Chet (1992) have

found that mycelium of *T. harzianum* is preferentially attached to nylon fibres that had been coated with a plant lectin (concanavalin A) or a lectin derived from the cell wall of *Rhizoctonia solani*.

T. harzianum, alone or in combination with other *Trichoderma* species or chemical adjuvants, has been used in control of several diseases. Some of these include: Rhizoctonia damping-off in radish (Lifshitz *et al*., 1985), corn and soybean (Kommedahl *et al* 1981); cucumber fruit rot caused by *R. solani* J. G. Kuhn (Lewis and Papavizas, 1980), *R. solani* and *Fusarium oxysporum* Schldl. in lentil and chickpeas (Mukhopadhyay, 1995). Soils amended with *T. harzianum* can be disease suppressive, have greener turf, probably by enhanced root growth, and reduced brown patch caused by *R. solani*.

T. virens and *T. longibrachiatum* Rifai were useful in control of groundnut root and stem rot diseases caused by *R. solani* in India (Sreenivasaprasad and Manibushanrao, 1993).

Bari *et al*. (2007) have been also successfully studied the action role of *Trichoderma* spp. on organic solid waste for bioconversion. *T. harzianum* was able to completely break down the organic waste and result in no bad smell (Sultana 2006). Furthermore in the bioconversion the temperature of the process is very important because increasing the temperature, for the first 2-3 weeks) increases the process speed (Moqsud 2003; Tchnobanoglous 1997). Beside Zhend and Shetty (1998) showed the *T. harzianum* ability to produce diverse polysaccharide degrading enzymes, which help the long chain carbon compounds degradation.

2.2. Soil disinfestation (fumigation)

Soil is an extremely conductive habitat for a variety of microorganisms, which relative numbers may fluctuate because affected by seasons, cropping history and type of amendments (James, 1989).

Soil hygiene is a key pillar for successful and sustainable crop production, particularly in high-value and intensive crops, where limited options for pest and disease control are available.

Soil-borne pathogens cause heavy losses to all major crops. Therefore, effective control measures need to be developed that are economically, environmentally and technologically effective and acceptable. Soil disinfestation in one of the potential tools for ensuring crop production and prolonging the use of lands for this purpose. Appropriate management of soil-borne pathogens aims to reduce disease levels effectively and economically with minimal disturbance to the environment and natural resources. Effective control can be achieved by manipulating one or more of the biotic components involved in disease caused by a soil-borne pathogen, namely, the pathogen, he host and the soil microbial community, as well as components of the abiotic components. The basic concept is to treat all the inoculum source that include soil-borne pathogens resting structures, infected plant residues in the soil, infected propagation material, other hosts infected by the pathogen, aerial propagules, to name a few.

The basic principle of soil disinfestation is the elimination of a wide spectrum of harmful organisms in the soil before planting. In the last few decades we have seen remarkable changes in agriculture, in society's and policy-makers' attitude, and in soil-disinfestation technology, and the drastic chemical or physical means have been replaced even more from less drastic means such as soil solarization or soil biofumigation or from the use of sub-label rates of different fumigants in order to minimize the environmental impact.

Soil fumigation with broad-spectrum biocides is a non-selective means of killing not only soil-borne pathogens, but also antagonists, competitors, pathogen parasites and mycorrhizal fungi.

2.2.1. Metham sodium and dazomet

A universal control agent such as methyl bromide does not exist, so the efficacy of a combined use of chemicals and biological is needed. Fumigantion is a common practice to control soil pathogen; on the other hand they have an eradicant activity on microorganism naturally present into soil (Ślusarski and Pietr, 2009).

Since the methyl bromide was banned the multinationals have carried out experiments to find out other chemicals to control soil-borne pathogens. An example is represented by metham sodium (MS) and dazomet (DZ), two chemical molecules that in soil undergo a process of chemical degradation which produces methyl isothiocyanate (MITC), a volatile substance which is responsible of the disinfection effect of their application. The main difference between these two substances is in their physical status of the respective formulations: metham sodium is generally produced as liquid formulations while dazomet is included in a granular or microcrystalline formulation. Metham sodium and dazomet are broad spectrum soil fumigants that can be used to control pathogens and pests affecting a wide array of economically important fruit and vegetable crops. Unlike methyl bromide, they have no effect on the stratospheric ozone layer, and when utilized with a correct application methodology, there are no residues left on crops (Ślusarski *et al*., 2012).

Fumigants, in which the active constituents are MS and DZ, are registered for use in controlling weeds of monocots and dycots species (e.g., annual bluegrass, bermuda grass, chickweed, dandelion, ragweed, etc.), nematodes (e.g. root knot, cysts, etc.), and several species of soil fungi (e.g. *Rhizoctonia*, *Fusarium*, *Pythium*, *Phytopthora*, *Verticillium*, *Sclerotinia*).

This two molecules are included into Annex I of Dir. 91/414 (repealed by Reg. 1107/2009) and are available to growers in different EU member States and can be applied within Integrated Pest Management systems, as they can be used in conjunction with resistant varieties/rootstocks, improved sanitation techniques, biological control agents, and soil pasteurization (by solarization, hot water or steam injection).

These substances have been used for several decades as a cost effective, technically viable method of soil disinfection on different crops and with different commercial

applications. However, the results obtained after their application can be hampered if the application technique is not carefully applied. Indeed, correct application procedures and awareness of the soil conditions at the time of application are critical to insure the success in the control of pests, especially of nematodes and fungi (Ślusarski *et al*., 2012).

There is a need to reduce the loss of the product due to emission into air. Practices found to reduce fumigant emissions include sub-labels rates of application, use of plastic films: virtually impermeable film (VIF) o better totally impermeable film (TIF), compaction of the soil, drip application and co-application of organic amendments. The effectiveness in controlling different soil-borne pathogens and soil pest is another key feature for these products. Trials with metham sodium greatly reduced Pythium and Fusarium soil levels and root infection in tomatoes. The control of these common soil fungi along with nematode control induced an overall plant growth and yield increase that occurred in most experiments with dazomet. Dazomet and metham sodium are also effective in reducing symptom severity of soil-borne disease induced by pathogens which management is known to be very difficult, for example *Phomopsis sclerotioides*.

A characteristic feature of MITC generator substances is the relative long time of active substance persistence in the soil at a concentration that is phytotoxic. The time that shall elapse from the moment of application of the product till the planting of seedlings or sowing of seeds depends mainly on the soil temperature. The lower the soil temperature, the longer the time of persistence.

2.2.1.1. Metham sodium, dazomet and *Trichoderma* spp.

Rhizoctonia solani is a soil-borne pathogen that means is well adapted to soil environment having the ability to colonize organic matter before others and being rather resistant to microbial competitors. Some experiments reported *Rhizoctonia* as one of the most sensitive pathogen to fumigants (James, 1989). On the other hand fumigation my cause a “microbial vacuum” by killing soil organisms that play two pivotal roles in maintain soil health. First, they are essential for soil processes such as decomposition of organic matter and nutrient cycling. Second, suppress soil-borne

pathogen and reduce root diseases. This ecologically disruptive practice can set the stage for rapid reinvasion of soil by pathogen. So it is important to rebuild or restore the function of the soil ecosystem to create an environment that inhibits soil disease (Elad *et al*. 1982; Eo and Park, 2014; Fravel and Lewis 2004). Two via are possible to avoid this risk: the introduction of BCAs, such as *Trichoderma* spp., artificially or the application of fumigants at a sub-label rates (Elad, 1982; Fravel and Lewis, 2004). Both the possibilities have been investigated with satisfactory results.

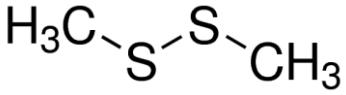
As argued by Garrett (1956) the application at label or sub label rates is of great interest because it may favour selective survival and recolonization of fumigated soil by indigenous microorganisms antagonistic to soil-borne plant pathogens. Especially for the low rates the behaviour of *Trichoderma* is really interesting while they result to be the most resistant to dazomet (Eo and Park, 2014) and to be combined with metham sodium giving great result, (Fravel and Lewis, 2004).

An interesting future challenge is the study of the possible integrated use of fumigants and antagonists, such as *Trichoderma* spp. Early experiments were conducted in 1984 (Lewis and Papavizas) due to report and understand any possible interaction between fumigants and different strains of *Trichoderma*. They conducted trials *in vitro* and *in vivo* as well. With the *in vitro* experiment they understood the *Trichoderma* conidial germination was more susceptible that the fungus growth to the toxic volatiles produced by the fumigants. With the soil trial it was confirmed that populations of isolates introduced 4 days before or at time of the treatment with fumigants were significantly less than those in not fumigated soil whereas populations of isolates introduced 4 days after the fumigation were similar than those in not fumigated ones.

Thus a combined use of low rates of fumigants and BCAs could be possible as fungal propagules treated with sub-label rates are viable but weakened and easily attacked by antagonists. Fravel and Lewis, in 2004, demonstrated that combining label rates of metham sodium with *Trichoderma hamatum*, *T. harzianum*, *T. virens* and *T. viride* is possible with great results. They explain this success with the *Trichoderma* ability to survive to the fumigations and its activity against pathogen propagules that may have been weakened during the treatments. Also Eo concluded that the combination

of dazomet and *Trichoderma* is a promising strategy to improve fumigation efficacy and recover the suppressiveness of soil.

2.2.2. New experimental fumigant: dimethyl disulfide (DMDS)



Another alternative soil fumigant to methyl bromide (MB) is dimethyl disulfide (DMDS), a soil fumigant for pre-plant use on agricultural fields. Methanesulfonic acid (MSA), the main degradation product of DMDS, is naturally produced in the environment as part of the sulfur cycle and generally exhibits low to moderate toxicity.

DMDS can occur naturally in soil, wetlands, oceans and outdoor air, and plays a role in the global sulfur cycle; it is also naturally produced by some plants, including certain food crops, and can be present in certain dairy products. There are several experiences where the end-use is to control pre-emergent weeds, soil-borne plant pathogens, and nematodes in soils used to grow vegetables, cucurbits, strawberries, blueberries, field-grown ornamentals and forest nursery stock (Zanón, 2014a,b; Sasanelli, 2014; Curto, 2014; Leocata, 2014; Fritsch, 2014; Abou Zeid and Noher 2014).

Because DMDS can damage crops, it must be applied 21 days before sowing, planting or transplanting so DMDS levels in the soil can decrease to a safe level. Since dimethyl disulfide is injected beneath the soil surface and immediately covered with a Totally Impermeable Film (TIF), high concentrations are unlikely to come into contact with wildlife. The application of TIF is also useful to evaluate a stepwise decrease in fumigant rates. Fumigant in which the active constituent is DMDS is already used as nematicide. Several experiments have been carried out to control nematodes on tobacco, melon, tomato, carrot, lettuce and early potatoes, in greenhouses and open field conditions, in Italy, Spain, and France (Zanón, 2014a,b; Sasanelli, 2014; Curto, 2014; Leocata, 2014; Fritsch, 2014). Abou Zeid and Noher (2014) have conducted a two-year greenhouse experiment carried out with the aim to

test the efficacy of DMDS against the major soil-borne pathogens, root-knot nematode and weeds on pepper, cucumber and tomato, with positive results.

3. Thesis aim

The ornamental-plants industry is highly dynamic and constantly evolving. In recent decades it has been particularly developed the production of outdoor ornamental plants both in Italy and in the rest of the world and research played a key role in the introduction of new species and varieties to meet new market demands. This has been reflected in the introduction and subsequent adaptation of exotic species. The intense flow of plant material, however, has also meant the emergence of new diseases with serious consequences for the entire sector.

The species belonging to the genus *Rhizoctonia* include a high number of fungi that are responsible of significant economic losses of agronomic crops (cereals, cotton, sugar beet, potato, trees fruit, vegetables and tobacco), turf grasses, ornamentals and fruit and forest trees worldwide (Adams, 1988; Sneh, 1991; Couch, 1995; Benson and Cartwrigt, 1996). *Rhizoctonia* spp. can be grouped based on the number of nuclei per hyphal cell, with multinucleate species complex represented by *Rhizoctonia solani* that comprised 13 anastomosis groups (AGs) (Farr *et al*., 1995) and several others *Rhizoctonia* binucleates (BNR) divided into 21 AGs (Ogoshi, 1975; Ogoshi *et al*., 1983a, b; Sneh *et al*., 1991; Hyakumachi *et al*., 2005).

Rhizoctonia spp. may cause different symptom patterns such as seedlings damping-off, collar, root and stem rot, leaf spots, turf brown patches on several ornamental hosts (Farr *et al*., 1989). In Italy, *Rhizoctonia* spp. are the causal agents of significant losses, especially in the early stages of rearing young cuttings, seedlings and young plants and it has also been ascertained their spread and permanent settlement in the nursery of ornamental plants (Aiello *et al*., 2008a,b; 2009; Garibaldi *et al*., 2003a,b; 2006a; 2007; 2009a,b,c; 2010; 2014; Polizzi *et al*., 2009a,b,c; 2010a,b,c; 2011a,b).

BNR typically are weakly virulent, less destructive pathogens or not pathogenic considered as mycorrhizal or biocontrol agents (Harris *et al*., 1994; Andersen and Rasmussen, 1996; Hwang and Benson, 2002). However, several studies reported BNR as pathogenic on economically important agricultural and horticultural crops (Priyatmojo *et al*. 2001; Kuramae *et al*. 2007; Aiello *et al*. 2012).

Considering the importance of Rhizoctonia diseases and high economic losses caused by these fungi, the first aim of the present study was to determine the impact

of Rhizoctonia infections in commercial ornamental nurseries located in Catania province (eastern Sicily, Italy). Thus, a survey was conducted over a 3-year period and 88 *Rhizoctonia* isolates were recovered from 30 ornamental species and identified. AGs and subgroups of *Rhizoctonia* spp. were determined using the ITS sequence and hyphal interaction. In addition, was evaluated the pathogenicity of representative *Rhizoctonia* isolates on hosts of origin.

Primary inoculum of *Rhizoctonia* species consist of sclerotia that can survive in the soil or potting medium on cultural debris without the presence of a susceptible host and can reinfest the potting substrate and infect and kill an entire stock bed or flat in just a little time. The production of plants in greenhouse heated and irrigated overhead could have a role in promoting Rhizoctonia infections, such as the use of non-disinfected soil that represent a possible source of pathogen inoculum.

Moreover, the recent trend of "sustainable nursery production", i.e. obtaining potting media from recycled materials, could increase infection risks in ornamental nursery.

Due to *Rhizoctonia* ability to survive in soil, a key-role in diseases management is played by the soil disinfestation. According to the directive 2009/128/EC on the "Sustainable Use of Pesticides" the management of pathogens should be directed toward a high use of biological control agents, reducing at the same time, the use and the rates of chemical compounds. Recently, newly studies were conducted on the effects of label and sub-label rates of fumigants in combination with different strains of *Trichoderma* spp. (Fravel and Lewis, 2004; Ślusarski and Pietr, 2009). Moreover, the use of Metam sodium (MS) and Dazomet (DZ) has been extended until 30 June 2022 and 31 May 2021, respectively, although with restrictions. From 01-01-2015, for example, MS is allowed to be used once every three years on the same field and the maximum admissible rate is 153 kg/ha (corresponding to 86.3 kg/ha of MITC) for open field applications. Furthermore application of innovative impermeable film, such as, for example, totally impermeable film (TIF) whose structure induces a significant dissipation of fumigant and VOC emissions and creates a significant potential for fumigant rate reduction opportunities are required.

Therefore, taking in consideration the current regulations and the little or no information on efficacy of fumigants against *Trichoderma* species, the second

objective of this work was to evaluate the effects of label and sub-label rates of DZ and MS on sclerotia of *R. solani*. Besides, the effects of these fumigants were also tested on four *Trichoderma* species (*T. harzianum*, *T. atroviride*, *T. koningii* and *T. reesei*). Five experiments were performed in open field in a commercial nursery located in Catania province (Sicily, Italy). In two experiments the fumigants were applied at label and sub-label rates under virtually impermeable film (VIF). Three open field trials were conducted using reduced rates and the VIF was compared to a totally impermeable film (TIF). Another soil fumigant, for pre-plant use on agricultural fields, is dimethyl disulfide (DMDS). Methanesulfonic acid (MSA), the main degradation product of DMDS, is naturally produced in the environment as part of the sulphur cycle and generally exhibits low to moderate toxicity. This fumigant is already used as nematicide (Curto *et al*. 2014; Fritsch *et al*. 2014; Leocata *et al*. 2014; Sasanelli *et al*. 2014 and Zanón *et al*. 2014a, b) but a few studies were performed on the efficacy against the major soil-borne pathogens (Abou Zeid and Noher, 2014). Thus, other two experiments were carried out in semi-field conditions for evaluated the effects of another soil fumigant (dimethyl disulfide) on *Rhizoctonia solani* AG-4 and binucleate *Rhizoctonia* AG-R. Treatment efficacy was evaluated under VIF barriers compared to a DMDS assessed film (DAF).

Management of Rhizoctonia diseases in the nursery cannot rely on a single control measure. Thus, besides the reduction of primary inoculum by soil disinfestation and the use of uncontaminated potting medium, an integrated program, including chemical and biological control agents as well as good nursery practices could be adopted. Application of synthetic fungicides has been the most used disease control measure employed by the farmers. Nowadays only tolclofos-methyl, belonging to Aromatic Hydrocarbons fungicides, is constantly used for managing Rhizoctonia disease. Azoxystrobin, flutolanil and fludioxonil were also effective in rotation for controlling Rhizoctonia root rot on blueberry (Haralson, 2013). However, it is known that azoxystrobin shows cross resistance between all members of the QoI group also with *R. solani* (www.frac.info). Among the alternatives used in nurseries it would be desirable to evaluate the use of antagonistic microorganisms to limit the environmental impact and other negative effects of fungicides (resistance, broad

spectrum of action, etc.). The application of BCAs would be evaluated not only to contain infections but also to improve the young plants growth and, therefore, to reduce the amount of fertilizer used in the nursery. In literature there are several examples of *Trichoderma* spp. activity in controlling Rhizoctonia diseases (Dennis and Webster, 1971; Inbar and Chet, 1992; Benhamou and Chet, 1993). Indeed, for the development and use of antagonistic microorganisms is necessary, however, to consider several factors, which may affect the success of their use, such as, for example, the environment (physical, biological, chemical and nutritional conditions) and the interaction pathogen-microorganism (surviving and ability to colonize the rhizosphere, the substrate and the foliage).

Thus, considering the directive 2009/128/EC, the incidence and severity of Rhizoctonia diseases in nurseries and the few fungicides labelled on ornamental plants, the third objective of the thesis was to evaluate alternative chemical active ingredients and bioformulates for controlling Rhizoctonia diseases in ornamental nurseries. The first step was the *in vitro* evaluation of the antagonistic activity of several BCAs against *R. solani* (AG-4) and BNR (AG-A, AG-G and AG-F). In detail, were evaluated the activity of mycoparasitism, and the production of diffusible, non-volatile metabolites. This first evaluation was followed by three growth chamber assays in which 8 biological compounds were compared to 6 fungicides in managing crown and root rot on *Dodonaea viscosa*. Treatments efficacy was evaluated by calculating disease incidence, representing the number of plants showing disease symptoms out of the total number.

4. Occurrence and characterization of *Rhizoctonia* species causing diseases on ornamental nurseries in southern Italy

4.1. Materials and methods

Field survey and isolation

Surveys were conducted during 2010-2012 in 10 nurseries located in eastern Sicily. The disease incidence on each host species was recorded. Plants showing symptoms referable to *Rhizoctonia* sp. were randomly collected for analysis.

Small sections from the edge of symptomatic tissues were surface disinfected with 1.5% sodium hypochlorite for 1 min, rinsed in sterile water (SDW) and placed on potato dextrose agar (PDA) plates amended with 100 ppm streptomycin sulphate (PDA+A). Plates were incubated at 25±1°C under continuous dark conditions for 7 days. Hyphal tips of the representative *Rhizoctonia*-like colonies were placed on PDA plates.

Molecular characterization

Genomic DNA from each of the 88 isolates was extracted using a conventional method (Izumitsu *et al*., 2012). PCR amplification of the rDNA, including regions of ITS1, 5.8S rDNA and ITS2 for each isolate was performed with the primer set of ITS1-F and ITS4-B (Gardes and Bruns, 1993). The nucleotide sequences generated by the sequencing were edited and assembled using the combination of Sequencher 5.0.1 (Gene Codes Corp., USA) and Indelligent (Dmitriev and Rakitov, 2008) with manual adjustment. Sequences from all isolates were compared with those in the GenBank nucleotide database provided by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) using BLAST algorithm to determine sequence identity and find the closest match based on maximal percent identity.

Pathogenicity

Pathogenicity tests were performed with 38 representative isolates on potted, healthy, seedlings or cuttings grown in a growth chamber. From 20 to 50 plants were

inoculated for each host species per replicate (3). The same number of plants served as negative control. All plants were inoculated at the base of each stem plants with two mycelial plugs obtained from cultures grown on PDA plates. Not-inoculated plants served as a control, for all the hosts. Plants were covered with a plastic bag for 48 h and maintained at 25±1°C and 95% relative humidity (RH) under a 12-h fluorescent light/dark regimen. All plants were irrigated 2-3 times per week and examined weekly for disease symptoms.

4.2. Results

Field survey and isolation

Symptoms referable to *Rhizoctonia* spp. were detected in all nurseries and on 30 ornamental species with a diseases incidence varied from 10 to 50%, according to the host species and isolate. The symptoms consisted to crown and root rot, stem rot, damping-off and web blight (Table 4). Rhizoctonia diseases were observed during propagation stage on unrooted and rooted cuttings and on established plants (Fig. 8,9).

Early in the disease development, crown and stem rot was characterized by water-soaked lesions at the soil line that turned light reddish brown to dark brown and expanded to girdle the stem and internal brown discolouration of cortical tissues; root rot sometimes occurs in association with these symptoms. The infected roots become dark brown or black and were partially or completely destroyed. As a consequence of root and stem rot, basal leaves initially turned chlorotic and gradually became necrotic and sometimes infected plants wilted and died. Damping-off consisted to the decay of the stem at soil level, causing it to fall over because it has not yet thickened supporting tissue. Symptoms of web blight o aerial blight included interveinal and marginal irregular necrotic lesions that progress to total leaf necrosis and leaf-drop. During nursery production, especially under the hot humid conditions, the web-like brown mycelium of the pathogen covered portions of the infected plants resulting in brown patch disease. Web blight symptom was associated with crown and root rot. Unrooted cuttings and cuttings during rooting stage were susceptible to infection.

A total of 88 monosporic isolates of *Rhizoctonia* spp. were obtained from ornamental species during the three years of investigation (Tab. 4).

Figure 8 - Field symptoms referable to *Rhizoctonia* spp. detected on rooted cuttings and seedlings. 1, 2- *Cistus salvifolius*; 3- *Polygala myrtifolia*; 4- *Streptosolen jamesonii*; 5- *Tabebuia impetiginosa*; 6- *Trachycarpus fortunei*; 7- *Chamaerops humilis*

Figure 9 - Field symptoms referable to *Rhizoctonia* spp. detected on potted plants. 1, 2- *Dodonaea viscosa*; 3- *Viburnum tinus* 4- *Catharanthus roseus*; 5- *Thryptomene saxicola*; 6- *Carissa grandiflora*; 7- *Citrus aurantium*

Table 4 - List of hosts showing symptoms of *Rhizoctonia* diseases collected in Sicily (Southern Italy) from ornamental nurseries

Host	Geographical location	Collection year	Symptoms[x]
***Carissa macrocarpa* (Eckl.) A. DC.**	Giarre, Nursery 1	2011	CRR-SR
***Carissa grandiflora* (E. Mey.) A. DC.**	Giarre, Nursery 1	2011	CRR-SR
***Cistus salvifolius* L.**	Giarre, Nursery 1	2011	CRR-SR
***Catharanthus roseus* (L.) G. Don**	Giarre, Nursery 2	2012	CRR-SR
***Butia capitata* (Mart.) Becc.**	Giarre, Nursery 1	2012	DO
***Trachycarpus fortunei* (Hook.) H. Wendl.**	Giarre, Nursery 1	2012	DO
***Phormium* spp. J.R. Forst.** and **G. Forst.**	Mascali, Nursery 1	2011	CRR
***Citrus aurantium* L.**	Giarre, Nursery 4	2010	DO-CRR
***Citrus volkameriana* Tan. and Pasq.**	Giarre, Nursery 4	2010	DO-CRR
***Citrus sinensis* Osbeck x *Poncirus trifoliata* Raf.**	Giarre, Nursery 4	2012	DO-CRR
***Chamaerops humilis* L.**	Giarre, Nursery 1	2010	DO
***Arbutus unedo* L.**	Giarre, Nursery 1	2010	CRR-DO
***Dodonaea viscosa* (L.) Jacq.**	Riposto, Nursery 1	2010	CRR
***Eugenia myrtifolia* Sims**	Giarre, Nursery 3	2010	CRR
Phillyrea angustifolia L.	Giarre, Nursery 3	2010	CRR
***Grevillea* sp. R. Br. ex Knight**	Riposto, Nursery 2	2010	CRR
***Lagunaria patersonii* (Andrews) G. Don**	Giarre, Nursery 1	2010	DO
***Osteospermum* spp. L.**	Giarre, Nursery 1	2010	DO
***Laurus nobilis* L.**	Giarre, Nursery 1	2010	DO-CRR
***Murraya paniculata* L.**	Giarre, Nursery 1	2010	CRR
***Quercus ilex* L.**	Giarre, Nursery 6	2010	CRR
***Thevetia peruviana* (Pers.) K. Schum.**	Giarre, Nursery 6	2010	CRR
***Thryptomene saxicola* (A. Cunn. ex Hook.) Schauer**	Riposto, Nursery 2	2010	CRR
***Viburnum tinus* L.**	Mascali, Nursery 2	2010	CRR-SR
***Passiflora mollissima* (Kunth) L.H. Bailey**	Giarre, Nursery 5	2011	CRR
***Tabebuia impetiginosa* (Mart. ex DC.) Standl.**	Giarre, Nursery 5	2011	DO-CRR
***Pittosporum tobira* (Thunb.) W.T. Aiton**	Giarre, Nursery 1	2012	CRR
***Streptosolen jamesonii* (Benth.) Miers**	Giarre, Nursery 1	2011	CRR-SR
***Bignonia* sp. L.**	Giarre, Nursery 5	2011	CRR
Polygala myrtifolia L.	Giarre, Nursery 1	2012	WB-CRR

[x]CRR crown and root rot; SR stem rot; DO damping-off; WB web blight

Molecular characterization

PCR amplification of the rDNA was conducted using ITS1-F and ITS4-B primers and the restriction enzymes described by Gardes and Bruns (1993). This technique enabled the characterization of *Rhizoctonia* isolates. Eighty-eight *Rhizoctonia* isolates, recovered from 30 ornamental species, were studied for their identification to specific anastomosis groups (AGs) and subgroups. According to sequence analysis, 56 isolates were binucleate *Rhizoctonia* spp. and 32 were multinucleate and identified as *R. solani*. Of these, 31 belonged to AG-4-III, 24 to AG-R, 18 to AG-A, 11 to AG-G, 1 to AG-2-2, AG-F and AG-B. The most prevalent anastomosis group was AG-4 HG-III (35.2%).

Cluster analysis was performed with the rDNA-ITS retrieved from the GenBank (www.ncbi.nlm.nih.gov). To this purpose the BLAST algorithm was used to determine sequence identity and find the closest match based on maximal percent identity (Tab. 5).

The pairwise percent similarities of the rDNA ITS sequences of isolates obtained to that present in GenBank was evaluated. The similarity range was from 85% to 100%, although for most AGs or subgroups (88%) the range was narrower (98–100%).

Table 5 - Isolates of *Rhizoctonia* spp. collected from ornamental plants

Hosts	English Name	Isolates		Sample Code	Genotypic AG	Identity
***Carissa macrocarpa* (Eckl.) A. DC.**	Natal Plum	1-7	CMA	01_CMA	AG-R	450/459 (98%)
				02_CMA	AG-R	450/458 (98%)
				03_CMA	AG-R	449/458 (98%)
				04_CMA	AG-R	449/458 (98%)
				05_CMA	AG-R	687/695 (99%)
				06_CMA	AG-R	470/482 (98%)
				07_CMA	AG-R	431/464 (93%)
***Carissa grandiflora* (E. Mey.) A. DC.**	Natal plum	8-13	CGR	08_CGR	AG-R	686/694 (99%)
				09_CGR	AG-A	478/497 (96%)
				10_CGR	AG-R	686/694 (99%)
				11_CGR	AG-R	686/694 (99%)
				12_CGR	AG-A	671/674 (99%)
				13_CGR	AG-A	477/495 (96%)
***Cistus salvifolius* L.**	Sage-leaved Cistus	14-17	CSA	14_CSA	AG-A	735/744 (99%)
				15_CSA	AG-A	722/725 (99%)
				16_CSA	AG-R	333/349 (95%)
				17_CSA	AG-R	423/447 (95%)
***Catharanthus roseus* (L.) G. Don**	Madagascar periwinkle	18-26	CRO	18_CRO	AG-A	726/733 (99%)
				19_CRO	AG-A	728/738 (99%)
				20_CRO	AG-A	409/422 (97%)
				21_CRO	AG-A	376/424 (89%)
				22_CRO	AG-A	560/657 (85%)
				23_CRO	AG-G	753/759 (99%)
				24_CRO	AG-A	424/432 (98%)
				25_CRO	AG-A	726/733 (99%)
				26_CRO	AG-G	745/746 (99%)
***Butia capitata* (Mart.) Becc.**	South American jelly palm	27-28	BCA	27_BCA	AG-G	727/745 (98%)
				28_BCA	AG-R	702/707 (99%)

Tab 5 - Continued

***Trachycarpus fortunei* (Hook.) H. Wendl.**	Chusan Palm	29-34	TFO	29_TFO	AG-4 HG-I	698/707 (99%)
				30_TFO	AG-4 HG-I	728/736 (99%)
				31_TFO	AG-4 HG-I	698/709 (98%)
				32_TFO	AG-4 HG-I	698/711 (98%)
				33_TFO	AG-4 HG-I	725/733 (99%)
				34_TFO	AG-4 HG-I	594/602 (99%)
***Phormium* spp. J.R. Forst.** and **G. Forst.**	New Zealand flax	35-39	PSP	35_PSP	AG-G	636/668 (95%)
				36_PSP	AG-G	747/755 (99%)
				37_PSP	AG-G	726/729 (99%)
				38_PSP	AG-Fb	682/690 (99%)
				39_PSP	AG-2-2	512/512 (100%)
***Citrus aurantium* L.**	Bitter orange	40-41	CAU	40_CAU	AG-4 HG-I	528/529 (99%)
				41_CAU	AG-4 HG-I	525/525 (100%)
***Citrus volkameriana* Tan. and Pasq.**	Volkamer lemon	42-45	CVO	42_CVO	AG-4 HG-I	708/709 (99%)
				43_CVO	AG-4 HG-I	505/505 (100%)
				44_CVO	AG-4 HG-I	512/512 (100%)
				45_CVO	AG-4 HG-I	724/735 (99%)
***Citrange Carrizo* (*Citrus sinensis* Osbeck x *Poncirus trifoliata* Raf.)**	*???Hardy orange???*	46-47	PTR	46_PTR	AG-4 HG-I	526/527 (99%)
				47_PTR	AG-4 HG-I	528/529 (99%)
***Chamaerops humilis* L.**	European fan palm	48-49	CHU	48_CHU	AG-4 HG-I	706/707 (99%)
				49_CHU	AG-4 HG-I	707/712 (99%)
***Arbutus unedo* L.**	Strawberry tree	50	AUN	50_AUN	AG-4 HG-I	695/697 (99%)
***Dodonaea viscosa* (L.) Jacq.**	Florida hopbush	51	DVI	51_DVI	AG-A	677/701 (97%)
***Eugenia myrtifolia* Sims**	Australian brush cherry	52	EMY	52_EMY	AG-A	725/729 (99%)
Phillyrea angustifolia L.	Narrow-leaved vetch	53	PAN	53_PAN	AG-G	743/745 (99%)
***Grevillea* sp. R. Br. ex Knight**	Silky-oak	54	GSP	54_GSP	AG-A	560/657 (85%)
***Lagunaria patersonii* (Andrews) G. Don [excluded]**	Pyramid tree	55-57	LPA	55_LPA	AG-4 HG-I	514/521 (99%)
				56_LPA	AG-4 HG-I	703/707 (99%)
				57_LPA	AG-4 HG-I	527/527 (100%)
***Osteospermum* sp. L.**	African daisy	58-60	OSP	58_OSP	AG-4 HG-I	519/521 (99%)

Tab 5 - Continued

				59_OSP	AG-4 HG-I	531/531 (100%)
				60_OSP	AG-4 HG-I	527/527 (100%)
***Laurus nobilis* L.**	Bay laurel	61	LNO	61_LNO	UNID*	560/562 (99%)
***Murraya paniculata* L.**	Orange jasmine	62-64	MPA	62_MPA	AG-4 HG-I	691/694 (99%)
				63_MPA	AG-4 HG-I	527/527 (100%)
				64_MPA	AG-4 HG-I	695/702 (99%)
***Quercus ilex* L.**	Holm oak	65-66	QIL	65_QIL	AG-A	605/611 (99%)
				66_QIL	AG-G	581/583 (99%)
***Thevetia peruviana* (Pers.) K. Schum.**	Yellow oleander	67	TPE	67_TPE	AG-4 HG-I	529/529 (100%)
***Thryptomene saxicola* (A. Cunn. ex Hook.) Schauer**	Rock Thryptomene	68	TSA	68_TSA	AG-A	605/606 (99%)
***Viburnum tinus* L.**	Laurustinus	69-70	VTI	69_VTI	AG-G	744/746 (99%)
				70_VTI	AG-B	601/613 (98%)
***Passiflora mollissima* (Kunth) L.H. Bailey**	Passion flower	71-73	PMO	71_PMO	AG-R	670/682 (98%)
				72_PMO	AG-R	667/682 (98%)
				73_PMO	AG-A	609/610 (99%)
***Tabebuia impetiginosa* (Mart. ex DC.) Standl.**	Pink lapacho	74-75	TIM	74_TIM	AG-4 HG-I	626/632 (99%)
				75_TIM	AG-4 HG-I	686/702 (98%)
***Pittosporum tobira* (Thunb.) W.T. Aiton**	Japanese Pittosprum	76-77	PTO	76_PTO	AG-G	751/754 (99%)
				77_PTO	AG-G	750/754 (99%)
***Streptosolen jamesonii* (Benth.) Miers**	Marmalade Bush	78	SJA	78_SJA	AG-4 HG-I	695/697 (99%)
***Bignonia* sp. L.**	Bignonia	79	BSP	79_BSP	AG-4 HG-I	696/698 (99%)
Polygala myrtifolia L.	Myrtle-leaf milkwort	80-88	PMY	80_PMY	AG-R	637/642 (99%)
				81_PMY	AG-R	637/641 (99%)
				82_PMY	AG-R	641/646 (99%)
				83_PMY	AG-R	615/619 (99%)
				84_PMY	AG-R	635/641 (99%)
				85_PMY	AG-R	637/641 (99%)
				86_PMY	AG-R	639/643 (99%)
				87_PMY	AG-R	635/641 (99%)
				88_PMY	AG-R	637/645 (99%)

UNID*=unidentified AG

Pathogenicity

Thirty *Rhizoctonia* isolates tested were pathogenic to the different origin hosts inoculated (Table 6), produced symptoms identical to those observed in the nursery and caused mortality ranging from 13 to 100%, according to the host species and isolate. Eight isolates were not pathogenic.

In detail, all *R. solani* isolates and most of binucleate AG-R, AG-A and AG-G isolates were pathogenic. The 61LNO isolate belonging to unidentified anastomosis group was also pathogenic on original host (*Laurus nobilis*). The 35PSP (AG-G), 38PSP (AG-F) and 39PSP (AG-2-2) isolates from *Phormium* spp., 70VTI (AG-B) from *Viburnum tinus* and 54GSP (AG-A) from *Grevillea* sp. were not pathogenic.

On some ornamental species only one binucleate AG recognized was pathogenic (AG-A from *Carissa grandiflora* and *Quercus ilex*, AG-R from *Butia capitata* and AG-G from *Viburnum tinus*).

On other species the different AGs detected were both pathogenic (AG-A and AG-R on *Cistus salvifolius* and *Passiflora mollissima*, AG-A and AG-G on *Catharanthus roseus*).

The symptoms appeared from 7 days to 3 months after pathogen inoculation, depending on the host species (Fig. 10). The pathogen was reisolated from the artificially inoculated plants and identified as previously described, completing Koch's postulates. No disease was observed on control plants.

Figure 10 – Symptoms caused by *Rhizoctonia* isolates on artificial inoculated host species. 1- CRR on *Bignonia* sp.; 2- CRR and SR on *Carissa grandiflora*; 3- WB and CRR on *Polygala myrtifolia*; 4- CRR and SR on *Cistus salvifolius*; 5, 6- DO and CRR on *Citrus sinensis* × *Poncirus trifoliate* and *Citrus volkameriana*

Table 6 - Pathogenicity of *Rhizoctonia* isolates collected from ornamental plants

Isolates (no.)	Hosts	Genotypic AG	Pathogenicity
08_CGR	*Carissa macrocarpa*	AG-R	-
08_CGR	*Carissa grandiflora*	AG-R	-
09_CGR	*Carissa grandiflora*	AG-A	+
14_CSA	*Cistus salvifolius*	AG-A	+
16_CSA	*Cistus salvifolius*	AG-R	+
18_CRO	*Catharanthus roseus*	AG-A	+
26_CRO	*Catharanthus roseus*	AG-G	+
27_BCA	*Butia capitata*	AG-G	-
28_BCA	*Butia capitata*	AG-R	+
29_TFO	*Trachycarpus fortunei*	AG-4 HG-I	+
35_PSP	*Phormium* spp.	AG-G	-
38_PSP	*Phormium* spp.	AG-Fb	-
39_PSP	*Phormium* spp.	AG-2-2	-
40_CAU	*Citrus aurantium*	AG-4 HG-I	+
42_CVO	*Citrus volkameriana*	AG-4 HG-I	+
46_PTR	*Citrus sinensis* x *Poncirus trifoliata*	AG-4 HG-I	+
48_CHU	*Chamaerops humilis*	AG-4 HG-I	+
50_AUN	*Arbutus unedo*	AG-4 HG-I	+
51_DVI	*Dodonaea viscosa*	AG-A	+
52_EMY	*Eugenia myrtifolia*	AG-A	+
53_PAN	*Phillyrea angustifolia*	AG-G	+
54_GSP	*Grevillea* sp.	AG-A	-
55_LPA	*Lagunaria patersonii*	AG-4 HG-I	+
58_OSP	*Osteospermum* sp.	AG-4 HG-I	+
61_LNO	*Laurus nobilis*	UNID*	+
62_MPA	*Murraya paniculata*	AG-4 HG-I	+
65_QIL	*Quercus ilex*	AG-A	+
66_QIL	*Quercus ilex*	AG-G	-
67_TPE	*Thevetia peruviana*	AG-4 HG-I	+
68_TSA	*Thryptomene saxicola*	AG-A	+
69_VTI	*Viburnum tinus*	AG-G	+
70_VTI	*Viburnum tinus*	AG-B	-
72_PMO	*Passiflora mollissima*	AG-R	+
73_PMO	*Passiflora mollissima*	AG-A	+
74_TIM	*Tabebuia impetiginosa*	AG-4 HG-I	+
76_PTO	*Pittosporum tobira*	AG-G	+
78_SJA	*Streptosolen jamesonii*	AG-4 HG-I	+
79_BSP	*Bignonia* sp.	AG-4 HG-I	+
80_PMY	*Polygala myrtifolia*	AG-R	+

*=unidentified AG

4.3. Discussion

In this study, 88 *Rhizoctonia* isolates were recovered from 30 ornamental species and their AG and subgroups determined. Fifty-six isolates were binucleate *Rhizoctonia* spp. and 32 were multinucleate and identified as *R. solani*. The most prevalent anastomosis group of *R. solani* was AG-4 HG-III (97%) followed by AG-2-2 (3%). Of the 56 isolates binucleate, 42.8% were AG-R, followed by AG-A (32.1%), AG-G (19.6%), while AG-F and AG-B were recognized in low frequency.

Disease symptoms were observed in 10 ornamental nurseries and included damping-off, crown, root and stem rot and web blight thought damping-off was mainly associated with *R. solani* AG-4 while crown and stem rot was caused primarily from BNR.

All *R. solani* AG-4 isolates in this study and the majority of the AG-R, AG-A and AG-G isolates were pathogenic to original host. Moreover, the results showed the presence in the same host species of isolates belonging to different AGs and with different behaviour pathogenic. In some cases, several BNR isolates from the same original host were all capable to cause disease while in other case only one AG recognized appeared pathogenic.

In literature several studies reported the presence of some not pathogenic binucleate *Rhizoctonia* isolates, which colonize plant roots and can have an antagonistic activity (Harris *et al*., 1994; Sneh and Ichielevich-Auster 1998; Sharon *et al*., 2007) or considered as mycorrhizal species (Andersen and Rasmussen, 1996).

R. solani is widespread in ornamental nurseries in Italy (Aiello *et al*, 2008a, b, 2009; Polizzi *et al*., 2009a, 2010a,b, 2011a, b; Garibaldi *et al*., 2003a,b, 2006a, 2009a,b,c, 2013d, 2014) where represents a very limiting factor for ornamental plants cultivated in Sicily (Southern Italy). The pathogen has a very broad host range worldwide (Farr at al., 1995) and among these several ornamental species have been reported in the literature (Chase, 1991; Sneh *et al*., 1991; Priyatmojo *et al*., 2001). However, no data has been founded of diseases caused by *Rhizoctonia* spp. and *R. solani* AG-4 on *Citrus volkameriana*, Citrange, *Trachycarpus fortunei*, *Arbutus unedo*, *Thevetia peruviana* and *Bignonia* sp.

The results of these surveys from ornamental nurseries showed that, overall, BNR were the most prevalent *Rhizoctonia* species isolated in Sicily (South Italy). BNR AG-A and AG-G are the two most common groups associated with root rot on strawberry in the world (Martin, 2000; Botha *et al*., 2003) and widespread in strawberry-growing areas in Nouthern Italy (Manici and Bonora, 2007). Recently, have been also reported on ornamentals in Southern Italy (Polizzi *et al*., 2009a; Polizzi *et al*., 2009c; Polizzi *et al*., 2010b).

No data has been reported of diseases caused by binucleate *Rhizoctonia* AG-A on *Carissa* spp., *Catharanthus roseus*, *Eugenia* sp. and *Quercus ilex*. On *C. grandiflora*, *Carissa* spp. and *Eugenia* sp. was reported *R. solani* in Florida (Alfieri *et al*., 1972; 1984), and on *Quercus* spp. was reported *R. solani* and *Rhizoctonia* sp. (Collado *et al*., 1996; Mulenko *et al*., 2008).

No data resulted of disease caused by *Rhizoctonia* spp. and binucleate AG-G on *Phyllirea angustifolia*, *Pittosporum tobira* and *Catharanthus roseus*. Chase (1991) reported a binucleate *Rhizoctonia* sp. causing aerial blight and root rot of *P. tobira* while Alfieri *et al*. (1984) reported *R. solani* in Florida, while AG-G in this work.

In literature, on *C. roseus* have been reported binucleate *Rhizoctonia* sp. and *R. solani* in United States (Alfieri *et al*., 1984; Chase, 1991; Holcomb and Carling, 2002) and *R. solani* AG-1B in Italy (Garibaldi *et al*., 2006).

AG-R, the binucleate AG most frequently found in this survey, has only been reported from the USA, Australia, Brasil and China (Burpee *et al*., 1980; Sumner, 1985; Rinehart *et al*., 2007; Yang *et al*., 2006, 2008), and does not seem to be present in Italy (Europe). This study is the first report of this disease on *C. salvifolius*, *B. capitata*, *Polygala myrtifolia* caused by AG-R.

5. *In vitro* antagonism of BCAs against *Rhizoctonia* spp.

5.1. Materials and methods

Fungal cultures

Five isolates of *Trichoderma*, one of *Clonostachys* and one of *Penicillium* were used for these studies. These include *T. harzianum* (T22), *T. harzianum* (T67), *T. reesei* (T34), *T. atroviride* (P1), *T. koningii* (8009), *C. rosea* (CR) and *P. oxalicum* (PO). The activity was tested against 4 isolates of *Rhizoctonia* belonging to *R. solani* (AG-4) and BNR (AG-A, AG-F and AG-G). The isolates were maintained on PDA medium.

Dual culture technique

The interaction between different BCAs and *Rhizoctonia* spp. were verified through the dual-culture technique (Sanchez *et al*., 2006) that enable to investigate the efficacy of the mycoparasitism developed by these antagonists against *Rhizoctonia* isolates.

One 5-mm diameter mycelial disc of biocontrol agents and pathogen, cut with a cork-borer from the margin of 7 days-old-culture, were inoculated at the same time on the opposite side of plates containing PDA+A and incubated at a temperature of 25±1°C. In control plates a single 5-mm diameter mycelial disc of pathogen was placed in the centre plate. Three replicates were used in each experiment.

The antagonistic activity of each BCAs was evaluated by recording radial growth reduction of pathogens (R%). These percentage reductions were measured by applying the formula:

$$\text{Percent reduction (R\%)} = \frac{C_2 - C_1}{C_2}$$

where, C_2 means growth of pathogen and C_1 means growth of pathogen in treatments.

The antagonistic potentialities of the *Trichoderma* spp. isolates were also evaluated through Bell's scale (Bell *et al*., 1982) after 4 and 7 dd, after inoculation.

Table 7 - Bell *et al*. (1982) 0-to-4 scale for 4th day relief

	4th day (inhibition of mycelial growth)
0	Antagonist colonizes completely the pathogen and covers the whole surface of the medium
1	Antagonist colonizes at least the 2/3 parts of the media surface
2	Antagonist and pathogen colonized each half of the media surface
3	Antagonist colonizes at least the 1/3 part of the media surface
4	Antagonist completely covered by the pathogen which has colonized the whole surface of the medium

Table 8 - Bell *et al*. (1982) 0-to-7 scale for 7th day relief

	7th day (mycoparasitism)
0	Pathogen overgrowth > 1 cm
1	Pathogen overgrowth upon the antagonist mycelium 0,5 cm < > 1 cm
2	Slight overgrowth
3	No pathogen overgrowth
4	Antagonist trace on the pathogen mycelium
5	Antagonist colonizes half of the pathogen mycelium
6	Antagonist colonizes at least the 2/3 parts of the pathogen mycelium
7	Antagonist overgrows completely to pathogen and covers the whole surface of the medium

Cellophane technique

Since Weindlin (1934) reported that some metabolites produced by *T. lignorum* were toxic against fungal pathogen, an intensive research was performed about it. The effect of diffusible, non-volatile metabolites produced by BCAs on mycelial growth of *Rhizoctonia* isolates was evaluated using the cellophane method proposed by Dennis and Webster (1971). A single sterile membrane (9 cm-diam) was placed on the surface of PDA in plates contained 20 ml of medium. A 5-mm mycelial plug was cut with a cork-borer from the margin of a 7-days-old culture of each BCAs and placed in the centre of each cellophane membrane. The plates were incubated for two days at a temperature of 25±1°C. Following this period the cellophane membrane was removed and a mycelial plug of *Rhizoctonia* was placed at the centre position previously occupied by antagonists. In control plates a single 5-mm diameter mycelial disc of pathogen was placed in the plate centre. Three replicates were used in each experiment. For this experiment the radial growth was recorded once after 5 days and the R% evaluated as for dual culture experiment.

5.2. Results

Dual culture

All antagonists reduced the mycelial radial growth of all *Rhizoctonia* isolates except for *C. rosea*. After 4 days, it was just recorded an appreciable percentage reduction between 28 and 48% for all *Trichoderma* strains. Following other 3 days the reduction was still recorded and there were a few cases in which the reduction was less that the first record (i.e. AG-A). After 4 days, AG-showed a least mycelial inhibition; these results were confirmed 3 days later (26.7%). While AG-G was the isolate that showed the highest sensitivity degree average (35.8%) after 4 days from the inoculation. In the second relief (at 7 dd) AG-A showed the highest value of mycelial radial growth reduction (39%). In general, among all BNR isolates, AG.A showed the highest reduction with a value of 37.4%. *Rhizoctonia* isolate with the lowest sensibility was *R. solani* AG-4. Related on the antagonists, the most effective was *T. koningii* 8009 with a suppression of pathogen between 36 and 47% after 4 days, and 33 and 52% after a week, from the inoculation. The weakest was CR that showed no activity (Tab. 9; Fig. 11).

Table 9 – Percentage inhibition of *Rhizoctonia* isolates mycelial growth due to the effect of isolates of *Trichoderma* spp. in dual culture

Reduction (%)	4th day						7th day					
	T22	T34	P1	8009	T67	CR	T22	T34	P1	8009	T67	CR
AG-4	36	32	28	36	32	0	36	31	28	33	32	0
AG-A	40	31	34	43	41	0	50	40	43	52	49	0
AG-F	48	33	41	43	39	0	48	31	38	43	37	0
AG-G	36	38	47	47	47	0	30	32	43	46	45	0

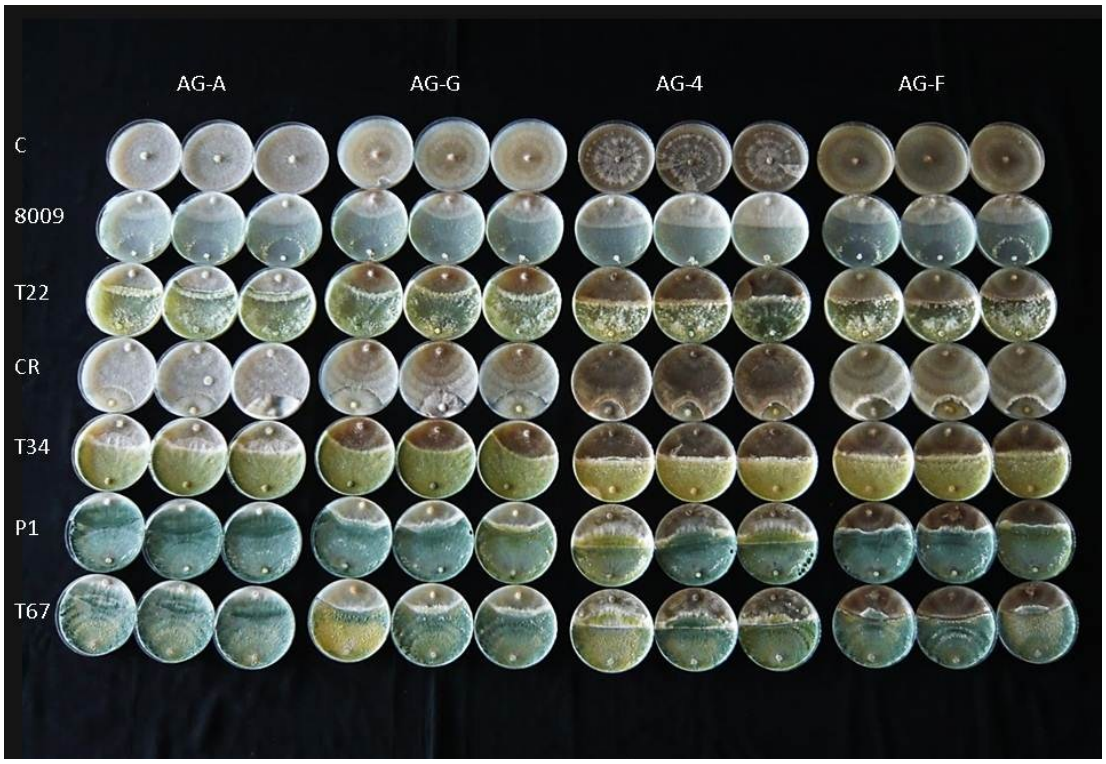


Figure 11 - Effect of isolates of *Trichoderma* spp. in mycoparasiting the mycelial growth of *Rhizoctonia* isolates in dual culture. C: untreated control; 8009: *Trichoderma koningii*; T22: *Trichoderma harzianum*; CR: *Clonostachys rosea*; T34: *Trichoderma reesei*; P1: *Trichoderma atroviride*; T 67: *Trichoderma harzianum*

Data of mycoparasitism evaluated by the 0-4 and a 0-7 scale (Tab. 10) are reported in table 10. After 4 days, all *Trichoderma* strains were able to colonize between 50 and 66% of the media surface, whereas CR colonized at least 1/3 part of the media surface. Following other 3 days it was recorded a more heterogeneous antagonist-pathogen interaction. Indeed, all BCAs were able to show overgrowth to different pathogen isolates, with different percentages, except for CR that was slightly covered by all pathogens mycelia. Besides, AG-G showed a slight overgrowth to *T. harzianum* T22.

The best mycoparasitism activity was recorded with P1, 8009 and T67. Indeed these BCAs overgrow completely to AG-F, AG-G and AG-f, respectively, and cover the whole surface of the media.

Table 10 - Percentage inhibition of *Rhizoctonia* isolates mycelial growth due to the effect of isolates of *Trichoderma* spp. in dual culture evaluated by Bell's scale

Classes	Mycoparasitism rating (0-to-4)						Mycoparasitism rating (0-to-7)					
	T22	T34	P1	8009	T67	CR	T22	T34	P1	8009	T67	CR
AG-4	2	1	2	1	2	3	4	4	6	4	6	2
AG-A	1	2	1	1	1	3	4	4	5	5	4	2
AG-F	1	2	1	1	1	3	4	6	7	5	7	2
AG-G	1	2	2	1	1	3	2	4	5	7	4	2

Cellophane

The results of non-volatile metabolites experiment are showed in tab 11.

T. koningii 8009 was the only one to inhibit significantly the growth of all *Rhizoctonia* isolates although in some case to a lesser extent (Fig. 12).

Table 11 - Percentage inhibition of *Rhizoctonia* isolates mycelial growth due to the effect of isolates of *Trichoderma* spp. in production of non-volatile compounds

Reduction (%)	5th day						
	T22	T34	P1	8009	T67	PO	CR
AG-4	0	0	0	9	0	0	0
AG-A	2	5	12	20	37	4	4
AG-F	0	0	2	38	13	0	0
AG-G	0	0	10	40	4	0	0

Figure 12 - Percentage reduction of mycelial radial growth induced by 8009 on *Rhizoctonia* isolates tested. C: untreated control; 8009: *Trichoderma koningii*

5.3.Discussion

The best results have been recorded with *T. koningii* 8009 in both experiment and against all pathogen isolates.

The ability of antagonistic microorganisms to inhibit the growth of *Rhizoctonia* isolates in dual culture varied among all strains. Although all isolates showed inhibition percentage values between 38 and 52, only *T. koningii* 8009 and *T. harzianum* T22 were able to reduce to least half the mycelial radial growth of AG-A isolate. Besides, little differences have been observed from first and second relief, underlining that the time of incubation is an important factor that affects the ability of the antagonist to inhibit the mycelial radial growth of the pathogen.

Concerning the non-volatile metabolites, only BNR *Rhizoctonia* AG-A showed sensitivity to all the biocontrol agents, and *T. koningii* 8009 was the best and unique antagonist to induce a mycelial radial growth reduction of all *Rhizoctonia* isolates tested.

The results of these studies indicate that the antagonistic activity of different BCAs varies when these are performed with different pathogens making necessary a specific selection of biocontrol agents for each plant pathogen isolate.

6. Efficacy of different fungicides and BCAs in controlling of Rhizoctonia root rot on *Dodonaea viscosa* in growth chamber assays

6.1. Introduction

In the preliminary experiment carried out *in vitro* to evaluate the mycoparasitism activity of different biological control agents, all *Trichoderma* isolates tested showed an excellent antagonistic activity against *Rhizoctonia solani* and *Rhizoctonia* BNR isolates. In detail, *T. koningii* 8009 showed the best performances.

However, the growth chamber assays, performed on a host plant-pathogen model, had the aim to evaluate the efficacy of fungicides and BCAs available as commercial formulates in order to set up of valid control of Rhizoctonia root rot on ornamental plants.

6.2. Materials and methods

To perform a growth chamber trial, *condicio sine qua non* is the availability of a model plant that is really susceptible to pathogen and able to germinate in a short time to produce quickly data. For the individuation of this plant a search among the susceptible species to *Rhizoctonia* spp. was conducted in ornamental nurseries located in the Eastern Sicily. At the end of the search the choice fell on Florida hopbush [*D. viscosa* (L.) Jacq.], an evergreen bush or small tree native to Australia and a member of *Sapindaceae*. In 2009, Polizzi *et al*. reported *Rhizoctonia* AG-A as causal agent of crown and root rot on *D. viscosa* and subsequent laboratory trials demonstrated that *Dodonaea* was susceptible also to *R. solani* AG-4.

Three trials were carried out on *D. viscosa* plants to assess the efficacy of chemical and biological treatments in controlling crown and root rot. All treatments, 9, 12 and 9, respectively for experiment I, II and III, were compared with untreated control plots.

Fungal cultures

Fresh culture of *R. solani* AG-4 was obtained by transferring agar plugs from stock culture onto PDA plates and let it to grow at a temperature of 25±1°C. After 7 days a

5-mm mycelial plug was cut with a cork-borer from hedge of actively growing mycelial culture and placed in the centre of Petri dishes containing PDA. The plates were incubated for one week at a temperature of 25±1°C.

Fungicides

Commercial formulations of fourteen fungicides including six chemicals, representing five chemical groups, and eight bioformulates (Tab. 12) were evaluated for their efficacy at the standard use rates in reducing Rhizoctonia crown and root rot on *D. viscosa*.

The treatments with both biological and chemical compounds were performed, by spraying using a hand sprayer, applying approximately 30 ml of suspension for each replicate, the day before inoculation. The lay-outs of the trials are reported in table 12.

Trial conditions

In the first experiment, 1 fungicide and 8 bioformulates, containing different BCAs alone or in mixture, were tested to control Rhizoctonia crown and root rot. Second experiment was carried out applying 4 fungicides and 8 bioformulates, whereas in third experiment, the efficacy of 4 bioformulates was compared to 5 fungicides. All experiments included relative not-inoculated and untreated controls.

In each treatment, 28 seedlings per replicate, in seed alveolar trays, were used for the inoculation following a scheme at randomized complete blocks with three replicates.

Sixty-three plants for each treatment were inoculated by placing two 5-mm mycelial plugs of PDA from 5-day-old actively growing mycelial culture near the base of the stem. The same number of plants was treated with two 5-mm plugs of PDA as controls. Plants were kept at 25±1°C and 95% RH on a 12-h fluorescent light/dark regimen.

Once symptoms were observed in untreated plots, fungicides efficacy was evaluated by calculating disease incidence (DI), representing the number of plants showing symptoms out of the total number.

Statistical analysis

All data, obtained from the inoculation trials, were subjected to analysis of variance according to parametric approaches (Statistica 10; StatSoft Inc., Analytical Software for Windows) with factorial treatment structure and interactions.

Data from all experiments were separately analysed and the mean values of DI for each treatment were calculated, averaging corresponding values determined for each replicate. All DI percentage data were previously transformed using arcsine (sine^{-1} square root x) prior to statistical analysis. The untransformed values of DI (%) are presented.

Mean separation of the percentage recovery data in each experiment was conducted using Duncan test at p=0.01.

Table 12 - Design and lay-out of the growth chamber trial carried out to evaluate the efficacy of different fungicides and BCAs in controlling of Rhizoctonia root rot on *Dodonaea viscosa*

Product	Group Name (FRAC Code List© 2014)	Rate	Experiment		
			I	II	III
Tolclofos-methyl	AH-fungicides (Aromatic Hydrocarbons)	250 g/hl	√	√	√
Boscalid+pyraclostrobin	SDHI (Succinate dehydrogenase inhibitors)+ QoI-fungicides (Quinone outside Inhibitors)	150 g/hl		√	√
Trifloxystrobin	QoI-fungicides (Quinone outside Inhibitors)	30 g/hl		√	√
Boscalid	SDHI (Succinate dehydrogenase inhibitors)	120 g/hl		√	
Fluopyram	SDHI (Succinate dehydrogenase inhibitors)	500 ml/hl			√
Propamocarb+fosetyl-Al	Carbamates + phosphonate	250 g/hl			√
***T. harzianum* T-22**		100 g/hl	√	√	√
***T. asperellum* TV1**		400 g/hl	√	√	√
***T. harzianum* ICC 012+ *T. viride* ICC 080**		250 g/hl	√	√	√
***T. atroviride* T11+ *T. asperellum* T25**		250 g/hl	√	√	√
***B. amyloliquefaciens* spp. *plantarum* D747**		250 g/hl	√	√	
***P. chlororaphis* MA342**		500 g/hl	√	√	
***S. griseoviridis* strain K61**		25 g/hl	√	√	
***B. subtilis* strain QST713**		400 g/hl	√	√	

6.3. Results

Symptoms of root and crown rots appeared 7 days after inoculation while control plants remained healthy. In the first experiment, tolclofos-methyl was the most and unique effective treatment in reducing DI of Rhizoctonia infections. Otherwise, remaining bioformulates applications were ineffective. In the second experiment, tolclofos-methyl was the highest effective among tested treatments including other fungicides, showing the most significant DI value, whereas all bioformulates were ineffective. Boscalid was the least effective treatment in reducing Rhizoctonia DI (data not significant if compared to the most BCAs). In third experiment, tolclofos-methyl showed the best performances confirming previous results, followed by boscalid+pyraclostrobin and fluopyram that significantly reduced Rhizoctonia crown and root rot on *D. viscosa*. However, no significant differences were found among fluopyram, propamocarb+fosetyl-Al, bioformulates and control (Tab. 13). In detail, tolclofos-methyl showed the lowest percentage of infected seedlings (1.6%), followed by boscalid+pyraclostrobin (23%). Although with a significant reduction of Rhizoctonia disease, trifloxystrobin was the least effective treatment with a DI of 70.6%, that was not significantly different from values reported for *T. harzianum* T22, *T. asperellum* TV1 and *T. atroviride* T11+*T. asperellum* T25.

Table 13 – Effects of fungicides and bioformulates on disease incidence of crown and root rot caused by *Rhizoctonia solani* on *Dodonaea viscosa* under growth chamber conditions

Treatment	Experiment		
	First[x, y]	Second[x, y]	Third[x, y]
Tolclofos-methyl	11.7 b	5.4 d	1.6 d
Boscalid+pyraclostrobin	-	48.9 c	23.0 c
Trifloxystrobin	-	66.3 c	70.6 b
Boscalid	-	90.6 b	-
Fluopyram	-	-	96.0 a
Propamocarb + fosetyl-Al	-	-	96.0 a
***T. harzianum* T22**	88.4 a	94.6 ab	87.4 ab
***T. asperellum* TV1**	85.6 a	98.4 ab	91.9 ab
***T. atroviride* T11+*T. asperellum* T25**	89.7 a	98.5 ab	93.1 ab
***T. harzianum* ICC012+*T. viride* ICC080**	93.0 a	93.7 ab	96.0 a
***B. amyloliquefaciens* ssp. *plantarum* D747**	84.3 a	96.9 ab	-
***P. chlororaphis* MA342**	86.4 a	96.3 ab	-
***B. subtilis* QST713**	93.7 a	99.2 a	-
***S. griseoviridis* K61**	95.3 a	100 a	-
Control	91.4 a	100 a	91.4 a

[x] Since treatment x trial interactions are significant, data are presented as single experiment. Same letters within each column denote not significant differences among treatments.
[y] Data are means of three replicates each containing 28 seedlings. Arcsine ($\sin^{-1}$ square root x) transformation was used on percentage data prior to analysis; untransformed data are presented. p = significant probability values associated with Duncan test. ns = not significant data.

6.4. Discussion

Comprehensively to the light of these findings, the application of tolclofos-methyl in controlling Rhizoctonia crown and root rot on *D. viscosa* can be strongly recommended since it showed an excellent activity through the three experiments performed. These results fully confirmed what is reported in literature, where tolclofos-methyl is the unique effective active ingredient labelled on ornamentals, until now (Coley-Smith *et al*., 1991; Hobo *et al*., 2010). Further studies should be performed for boscalid+pyraclostrobin mixture since it provided encouraging results in two experiments where it was tested. SDHIs and propamocarb+fosetyl-Al failed in controlling Rhizoctonia infections, even trifloxystrobin, belonging to QoI-fungicides, like azoxystrobin, resulted ineffective. Despite, Haralson (2013) reported azoxystrobin effective in containing Rhizoctonia root rot on blueberry.

Otherwise no BCAs showed certain activity compared to control unlike what reported in literature (Lewis *et al*. 1990; Gary *et al*., 1994; van der Boogert and Luttikholt, 2004). Indeed, for the development and use of antagonistic microorganisms is necessary, to consider several factors, which may affect the success of their use, such as, for example, the environment (physical, biological, chemical and nutritional conditions), the interaction pathogen-microorganism-hosts (surviving and ability to colonize the rhizosphere, the substrate and the foliage) or the application techniques of BCAs. Indeed further studies on these should be performed, used different mode of antagonistic application. The preliminary application of BCAs, could improve the rhizosphere and soil colonization by antagonist microorganism allowing the best results in controlling Rhizoctonia disease.

7. Effects of fumigation on survival of *Rhizoctonia* spp. and *Trichoderma* spp.

7.1. Introduction

Rhizoctonia spp. are soil-borne pathogens causing root and foliar diseases on a wide range of agronomic crops, ornamental plants and fruit and forest trees worldwide. Fumigation is a common practice to control soil pathogens, but little is known about the impact of fumigation on other soil biota group (Eo). Early studies reported several combinations between fumigants and *Trichoderma* spp. but only a few focuses on the possible use of fumigants at reduced rates.

7.2. Materials and methods

Fungi isolates

A representative isolate of *Rhizoctonia solani* and four isolates of *Trichoderma* spp. were used in field experiments. These include *R. solani* AG-4, *T. harzianum* T22, *T. harzianum* T67, *T. reesei* T34, *T. atroviride* P1 and *T. koningii* 8009. For the trials, vegetal debris were prepared by the use of rice kernels and carnation leaf for *Rhizoctonia* and *Trichoderma*, respectively. Briefly, rice kernels and carnation leaves were autoclaved at 121 °C twice, each time for 20 minutes, at a distance of two days to allow any surviving resting structures to germinate after the first cycle of autoclaving and then to be killed with the second cycle. Thus the kernels were placed on PDA and inoculated, with four 5-mm mycelium plug for each plate, at the same time. The plates were incubated for 3 weeks at 25±1°C for sclerotia production. The carnation leaves, cut in ~ 6 cm segments, were dried in an oven at ~ 55°C for ~ 2 days. Then, they were autoclaved as described for the rice kernels. Subsequently 10 sterilized carnation leaves were placed on Petri dishes containing water agar (CLA) and inoculated with four 5-mm mycelium plugs (Fisher *et al*., 1982). The plates were incubated for 2 weeks at 25±1°C for conidia and clamydospore production.

Effects of label and sub-label rates of fumigants on *Rhizoctonia* survival and viability of biocontrol agents (BCAs)

Two fumigants were tested: Divapan® [Metham sodium (MS) 42.8% = 510 g/L, registration holder is Taminco Italia s.r.l., Milan, Italy] and Basamid Granulat® [Dazomet (DZ) 99%, registration holder is Kanesho Soil Treatment SPRL/BVB, Brussels, Belgium]. For each fumigant three different rates were applied: the highest, the lowest and a sub-label rate. MS was applied as drench directly to the soil at the rates of 1000, 700 and 400 l/ha whereas DZ was mixed to the soil at the rates of 500, 400, 200 kg/ha. Two open field trials (October and November 2012) were carried out to assess the efficacy of the fumigants on survival of *Rhizoctonia* sclerotia and *Trichoderma* conidia and clamydospores.

Three nylon bags, containing 20 colonized rice kernels or 6 carnation leaves colonized, for each isolate were buried (about 15 cm depth) into soil which would have been fumigated. To this aim, a layer of soil (about 40 cm height) was placed on a cement bed. After the fumigation the soil was immediately covered with a virtual impermeable film (VIF, Ecobrom®**,** AgriPlast S.r.l., Vittoria, Ragusa, Italy) for three weeks (Fig. 13). The experimental plots in all treatments including bare controls, each 2.5 m × 5.0 m, were arranged in a randomized complete block design with three replications.

Effects of reduced rates of fumigants on *Rhizoctonia* survival and viability of biocontrol agents (BCAs)

Three open field trials (July and November 2013 and May 2014) were carried out to assess the efficacy of the fumigants on survival of *Rhizoctonia* sclerotia and viability of *Trichoderma* conidia and clamydospore. For each fumigant three different reduced rates were tested: 400, 350 and 250 (l/ha) for MS, 200, 160 and 100 (kg/ha) for DZ.

Three nylon bags, each containing 20 colonized rice kernels or 6 carnation leaves colonized, for each isolate were buried into soil which would have been fumigated. To this purpose, a layer of soil (40 cm height) was arranged on a cement bed. This time two different plastic films were tested: the VIF and a totally impermeable film (TIF™, EVAL™, Kuraray, America INC: Pasadena Texas, USA). The plots were covered for three weeks (Fig. 13).

The experimental plots in all treatments, including bare controls, each 2.5 m × 5.0 m, were arranged in a randomized complete block design with three replications.

Comprehensively, for all the trials (5), the plastic films were cut after three weeks and the nylon bags retrieved. Nine fragments, obtained by cutting rice kernels and carnation leaves, for *Rhizoctonia* isolate and *Trichoderma* isolates, respectively, were washed with SDW and subsequently placed, onto Ko and Hora's medium (KoH) and PDA+A, respectively, and maintained for one week at 25±1°C to evaluate the efficacy of the treatment. The percentage of cultural debris pieces from which fungi colonies developed was used to determine the survival of single isolates after treatments.

The soil temperature was measured at 15 cm depth in each tray and plot continuously using a commercial temperature sensor (Fourtec-Fourier Technologies, model MicroLite).

Figure 13 - Experiment in open field before (on the top) and after (on the bottom) treatments

Statistical analysis

All data obtained from the inoculation trials were subjected to analysis of variance according to parametric approaches (Statistica 10; StatSoft Inc., Analytical Software for Windows) with factorial treatment structure and interactions.

Data from all experiments were separately analysed and the mean values of recovery percentage for each treatment were calculated, averaging corresponding values determined for each replicate. All recovery percentage data were previously transformed using arcsine ($sine^{-1}$ square root x) prior to statistical analysis. The untransformed values of recovery (%) are presented.

Mean separation of the percentage recovery data in each experiment was conducted using Fisher's least significance difference test at $p = 0.01$.

7.3. Results

Effects of label and sub-label rates of fumigants on *Rhizoctonia* survival and viability of biocontrol agents (BCAs)

In the trial performed using label and sub-label rates of MS and DZ against *R. solani* and *Trichoderma* spp., the soil temperatures recorded in treated plots were comprised between 25 and 31°C. The results for this experiment are reported in the tables 14 and 15 for *R. solani* and *Trichoderma* isolates, respectively.

Two fumigants in both trials at all rates induced a total suppression of sclerotia viability of *R. solani* retrieved from treated plots (Tab. 14).

Table 14 - Recovery (%) of viable *Rhizoctonia solani* colonies from infested rice kernels buried into soil after chemical fumigation in October (1st) and November (2nd) 2012

Treatment	*R. solani* (%)
Trial	First and second combined [x, y]
MS (400 l/ha)	0.0 b
MS (700 l/ha)	0.0 b
MS (1000 l/ha)	0.0 b
DZ (200 kg/ha)	0.0 b
DZ (400 kg/ha)	0.0 b
DZ (500 kg/ha)	0.0 b
Bare control	99.4 a
	$p < 0.001$ (trt); $p > 0.01$ (trt*trial)^{ns}

[x] Since treatment × trial interactions are not significant, data are the pooled results of the two experiments. Same letters within each column denote not significant differences among treatments according to Fischer Least significant different test.

[y] Data are means of three replicates, each containing 54 infected rice kernel fragments or single sclerotia of *R. solani*. Arcsine (sin^{-1} square root *x*) transformation was used on percentage data prior to analysis; untransformed data are presented. *p*=significant probability values associated with *F* test. Bare control=untreated and inoculated.

Figure 14 - Viability of *Rhizoctonia solani* on Ko and Hora's medium

Otherwise, the fumigation treatments were not able to totally suppress viability of *Trichoderma* isolates from colonized carnation debris buried into soil, although recovery percentages always differed significantly from the bare control (Tab. 15). However, significant differences were recorded among all treatments. Viability of *T. koningii* was strongly reduced by all DZ treatments and by label rates of MS, whereas it was equal to 44.8% at sub-label rate (400 l/ha) of MS. The viability of *T. atroviride* was strongly reduced by all MS treatments and by DZ applied at label rates, whereas a high recovery percentage of viable colonies was detected in the plots fumigated with DZ at sub-label rate (200 kg/ha) (significant data). All treatments reduced significantly *T. harzianum* viability if compared with bare control, showing similar data among all fumigated plots. Survival of *T. reesei* was strongly reduced by all MS treatments and by the highest rate of DZ, whereas DZ at 200 and 400 kg/ha reduced to half the viability of this antagonist.

Table 15 - Recovery (%) of viable *Trichoderma* species colonies from infested rice kernels buried into soil after chemical fumigation in October (1st) and November (2nd) 2012

Treatment	*T. koningii* 8009 [x,y]	*T. atroviride* P1 [x,y]	*T. harzianum* T22 [x,y]	*T. reesei* T34 [x,y]
Trial	I and II [x, y]	I and II [x, y]	I and II [x, y]	I and II [x, y]
MS (400 l/ha)	44.8 b	23.2 bc	0.6 b	10.8 c
MS (700 l/ha)	8.1 c	5.3 c	0.6 b	1.2 c
MS (1000 l/ha)	1.3 c	1.2 c	0.9 b	0.0 c
DZ (200 kg/ha)	16.1 bc	52.5 b	2.5 b	54.9 b
DZ (400 kg/ha)	6.8 c	21.0 bc	1.2 b	46.3 b
DZ (500 kg/ha)	0.9 c	3.7 c	0.3 b	8.0 c
Bare control	100.0 a	100.0 a	100.0 a	100.0 a
	$p < 0.001$ (trt) $p > 0.001$ (trt*trial)^{ns}	$p < 0.001$ (trt) $p > 0.001$ (trt*trial)^{ns}	$p < 0.001$ (trt) $p > 0.01$ (trt*trial)^{ns}	$p < 0.001$ (trt) $p > 0.01$ (trt*trial)^{ns}

[x] Since treatment × trial interactions are not significant, data are the pooled results of the two experiments. Same letters within each column denote not significant differences among treatments.
[y] Data are means of three replicates each containing 54 carnation leaf pieces. Arcsine ($\sin^{-1}$ square root x) transformation was used on percentage data prior to analysis; untransformed data are presented. p = significant probability values associated with F test. ns = not significant data. Bare control=untreated and inoculated.

Effects of reduced rates of fumigants on *Rhizoctonia* survival and viability of biocontrol agents (BCAs)

The effects of fumigants applied at reduced rates on survival of *R. solani* and *Trichoderma* spp. was assessed in three trials conducted in July (1st) and November (2nd) 2013, with soil temperatures ranging from 24.6 to 41.9°C and from 15.7 to 31.8°C, respectively, and May 2014 (3rd) with soil temperatures comprised between 20.2 and 33.2°C.

Single data, analysed for each trial and film, were reported below (Tab. 16-23).

In the trial conducted on July 2013, both fumigants, at tested rates totally inhibit the viability of *Rhizoctonia* isolate, under both films (*data not shown*). Otherwise, different results have been reported for BCAs depending on the film applied. Under VIF barrier, very low percentages of viable *Trichoderma* colonies were retrieved from all fumigated plots. In detail, all treatments strongly reduced and/or suppressed the viability of BCAs (highly significant data) (Tab. 16).

Table 16 - Recovery (%) of viable *Trichoderma* species colonies from infested rice kernels buried into soil after chemical fumigation performed under VIF barriers in the first trial (July 2013)

Treatment	*T. koningii* 8009 [x]	*T. atroviride* P1 [x]	*T. harzianum* T67 [x]	*T. harzianum* T22 [x]
MS (400 l/ha)	0.6 bcB	0.0 bB	0.0 bB	0.0 cB
MS (350 l/ha)	0.0 cB	0.0 bB	0.0 bB	0.0 cB
MS (250 l/ha)	2.5 bB	2.5 bB	0.0 bB	0.6 bcB
DZ (200 kg/ha)	0.6 bcB	2.5 bB	1.2 bB	1.9 bB
DZ (160 kg/ha)	0.0 cB	0.0 bB	0.0 bB	0.0 cB
DZ (100 kg/ha)	0.0 cB	0.0 bB	0.0 bB	0.0 cB
Bare control	100.0 aA	100.0 aA	100.0 aA	100.0 aA

[x] Same letters within each column denote not significant differences among treatments according to Fischer Least significant different test. Small letters indicate a significativity at $p=0.05$ whereas capital letters denote significativity for $p=0.01$.
[y] Data are means of three replicates each containing 54 sclerotia or infected debris (carnation leaf fragments or rice kernels) pieces. Arcsine ($\sin^{-1}$ square root x) transformation was used on percentage data prior to analysis; untransformed data are presented. Bare control=untreated and inoculated.

Under TIF film, viability of three *Trichoderma* isolates was totally inhibited in all treated plots (Tab. 17), thus showing its best performances to retain fumigants if compared to VIF (Tab. 17).

Table 17 - Recovery (%) of viable *Trichoderma* species colonies from infested rice kernels buried into soil after chemical fumigation performed under TIF barriers in the first trial (July 2013)

Treatment	*T. koningii* 8009 [x, y]	*T. atroviride* P1 [x, y]	*T. harzianum* T67 [x, y]	*T. harzianum* T22 [x, y]
MS (400 l/ha)	0.0	0.0	0.0	0.0
MS (350 l/ha)	0.0	0.0	0.0	0.0
MS (250 l/ha)	0.0	0.0	0.0	0.0
DZ (200 kg/ha)	0.0	0.0	0.0	0.0
DZ (160 kg/ha)	0.0	0.0	0.0	0.0
DZ (100 kg/ha)	0.0	0.0	0.0	0.0
Bare control	100.0	100.0	100.0	100.0

[x] Since error mean square is zero, homogeneous groups cannot be computed and, consequently letters within each column for post-hoc analysis of significant differences are omitted.
[y] Data are means of three replicates each containing 54 carnation leaf pieces. Arcsine ($\sin^{-1}$ square root x) transformation was used on percentage data prior to analysis; untransformed data are presented. p = significant probability values associated with F test. Bare control=untreated and inoculated.

In the second experiment, survival of *R. solani* was significantly reduced in all fumigated plots, when both VIF and TIF barriers were applied (Tab. 18), except for treatment with DZ applied at lowest rate (data not significant) under VIF barrier.

Table 18 - Recovery (%) of viable *Rhizoctonia solani* colonies from infested rice kernels buried into soil after chemical fumigation performed under VIF and TIF barriers in the second trial (November 2013)

Treatment	VIF [x, y]	TIF [x, y]
MS (400 l/ha)	0.0 cC	0.0 bB
MS (350 l/ha)	0.0 cC	0.0 bB
MS (250 l/ha)	0.0 cC	0.0 bB
DZ (200 kg/ha)	3.1 cC	0.0 bB
DZ (160 kg/ha)	17.9 bB	0.0 bB
DZ (100 kg/ha)	99.4 aA	90.0 bB
Bare control	100.0 aA	100.0 aA

[x] Same letters within each column denote not significant differences among treatments according to Fischer Least significant different test. Small letters indicate a significativity at *p=0.05* whereas capital letters denote significativity for *p=0.01*.
[y] Data are means of three replicates each containing 54 carnation leaf pieces. Arcsine ($\sin^{-1}$ square root *x*) transformation was used on percentage data prior to analysis; untransformed data are presented. Bare control=untreated and inoculated.

Otherwise, *Trichoderma* species were significantly reduced in all fumigated plots under VIF barrier, except for *T. atroviride* in plots treated with MS at 250 l/ha (Tab. 19). MS at 350 l/ha and DZ at the lowest rate showed a low activity in reducing viability of *T. atroviride* (Tab. 19).

Table 19 - Recovery (%) of viable *Trichoderma* species colonies from infested rice kernels buried into soil after chemical fumigation performed under VIF barriers in the second trial (November 2013)

Treatment	*T. koningii* 8009 [x, y]	*T. atroviride* P1 [x, y]	*T. harzianum* T67 [x, y]	*T. harzianum* T22 [x, y]
MS (400 l/ha)	0.0 cB	0.0 dC	0.0 cB	0.0 cC
MS (350 l/ha)	3.7 bcB	40.1 bcBC	6.2 bcB	0.0 cC
MS (250 l/ha)	22.8 bcB	76.5 abAB	18.5 bB	7.4 cBC
DZ (200 kg/ha)	0.0 cB	0.0 dC	0.0 cB	0.0 cC
DZ (160 kg/ha)	6.8 bcB	42.6 bcBC	13.6 bcB	5.6 cBC
DZ (100 kg/ha)	37.0 bB	14.8 cdBC	11.1 bcB	40.7 bB
Bare control	100.0 aA	100 aA	100.0 aA	100.0 aA

[x] Same letters within each column denote not significant differences among treatments according to Fischer Least significant different test. Small letters indicate a significativity at *p=0.05* whereas capital letters denote significativity for *p=0.01*.
[y] Data are means of three replicates each containing 54 sclerotia or infected debris (carnation leaf fragments or rice kernels) pieces. Arcsine ($\sin^{-1}$ square root *x*) transformation was used on percentage data prior to analysis; untransformed data are presented. Bare control=untreated and inoculated.

Related to TIF application, all treatments suppressed and/or reduced significantly the viability of four BCAs tested, thus showing once again best performances of this film if compared with VIF. However, the viability of four BCAs was averagely highest in the plots fumigated with MS at 250 l/ha (Tab. 20).

Table 20 - Recovery (%) of viable *Trichoderma* species colonies from infested rice kernels buried into soil after chemical fumigation performed under TIF barriers in the second trial (November 2013)

Treatment	*T. koningii* 8009 [x, y]	*T. atroviride* P1 [x, y]	*T. harzianum* T67 [x, y]	*T. harzianum* T22 [x, y]
MS (400 l/ha)	0.0 dC	0.0 cC	0.0 cC	0.0 cB
MS (350 l/ha)	0.0 dC	0.0 cC	0.0 cC	0.0 cB
MS (250 l/ha)	19.1 bB	19.8 bB	14.8 bB	3.7 bcB
DZ (200 kg/ha)	0.0 dC	0.0 cC	0.0 cC	0.0 cB
DZ (160 kg/ha)	5.0 cBC	3.1 cBC	0.0 cC	3.7 bB
DZ (100 kg/ha)	13.6 bcB	10.5 bcBC	0.0 cC	3.7 bcB
Bare control	100.0 aA	100.0 aA	100.0 aA	100.0 aA

[x] Same letters within each column denote not significant differences among treatments according to Fischer Least significant different test. Small letters indicate a significativity at *p=0.05* whereas capital letters denote significativity for *p=0.01*.
[y] Data are means of three replicates each containing 54 carnation leaf pieces. Arcsine ($\sin^{-1}$ square root *x*) transformation was used on percentage data prior to analysis; untransformed data are presented. Bare control=untreated and inoculated.

In the third experiment (May 2014) survival of *R. solani* was significantly reduced if compared to control, however performances lightly different between two films were recorded. Fumigation treatments under VIF provided variable results in reducing viability of pathogen (Tab. 21). However, the highest performances were detected in plots fumigated with MS at 400 and 350 l/ha and DZ al 200 kg/ha (total suppression). Otherwise, the lowest reductions of survival were detected with DZ at 100 kg/ha (significant data). Under TIF condition inoculum was totally inhibited except for the lowest DZ rate (25.3%). This result underlines once again how TIF increases the treatment efficacy if compared with VIF (Tab. 21).

Table 21 - Recovery (%) of viable *Rhizoctonia solani* colonies from infested rice kernels buried into soil after chemical fumigation performed under VIF and TIF barriers in the third trial (May 2013)

Treatment	VIF [x, y]	TIF [x, y]
MS (400 l/ha)	0.0 cC	0.0 cB
MS (350 l/ha)	0.0 cC	0.0 cB
MS (250 l/ha)	4.3 cBC	0.0 cB
DZ (200 kg/ha)	0.0 cC	0.0 cB
DZ (160 kg/ha)	1.2 cC	0.0 cB
DZ (100 kg/ha)	39.5 bB	25.3 bB
Bare control	100.0 aA	100.0 aA

[x] Same letters within each column denote not significant differences among treatments according to Fischer Least significant different test. Small letters indicate a significativity at *p=0.05* whereas capital letters denote significativity for *p=0.01*.
[y] Data are means of three replicates each containing 54 carnation leaf pieces. Arcsine ($\sin^{-1}$ square root *x*) transformation was used on percentage data prior to analysis; untransformed data are presented. ns = not significant data. Bare control=untreated and inoculated.

Variable results in reducing viability of BCAs tested were observed when fumigation treatments have been performed under VIF barriers (Tab. 22). However, the best performances were detected in plots fumigated with MS at 400 l/ha (total suppression) and 350 l/ha and in plots fumigated with DZ at 200 kg/ha. In the contrast, the lowest reductions of viability were detected with DZ at 100 kg/ha for *T. atroviride* P1 and *T. reesei* T34 (significant data) and *T. koningii* 8009, *T. harzianum* T22 and *T. harzianum* T67 (not significant data at p=0.01), as well as for MS at 250 l/ha for *T. koningii* 8009 and *T. reesei* T34 (not significant data).

Table 22 - Recovery (%) of viable *Trichoderma* species colonies from infested rice kernels buried into soil after chemical fumigation performed under VIF barriers in the third trial (May 2014)

Treatment	*T. koningii* 8009 [x, y]	*T. atroviride* P1 [x, y]	*T. harzianum* T67 [x, y]	*T. harzianum* T22 [x, y]	*T. reesei* T34 [x, y]
MS (400 l/ha)	0.0 bB	0.0 dC	0.0 cC	0.0 bB	0.0 dD
MS (350 l/ha)	22.2 bB	9.9 cC	1.9 cC	6.2 bB	0.0 dD
MS (250 l/ha)	86.4 aA	5.0 cdC	58.7 bB	0.0 bB	81.5 abAB
DZ (200 kg/ha)	0.6 bB	3.2 cdC	1.9 cC	0.0 bB	1.3 dD
DZ (160 kg/ha)	20.4 bB	3.7 cdC	62.3 bB	1.2 bB	20.4 cdCD
DZ (100 kg/ha)	76.5 aA	78.4 bB	71.6 bAB	42.0 aA	53.1 bcBC
Bare control	100.0 aA	100.0 aA	100.0 aA	52.5 aA	100.0 aA

[x] Same letters within each column denote not significant differences among treatments according to Fischer Least significant different test. Small letters indicate a significativity at $p=0.05$ whereas capital letters denote significativity for $p=0.01$.

[y] Data are means of three replicates each containing 54 sclerotia or infected debris (carnation leaf fragments or rice kernels) pieces. Arcsine ($\sin^{-1}$ square root x) transformation was used on percentage data prior to analysis; untransformed data are presented. ns = not significant data. Bare control=untreated and inoculated.

Under TIF barrier, the recovery percentages of viable colonies of *Trichoderma* spp. from fumigated plots were averagely lowest than ones detected under VIF barrier, thus underlying a higher capacity of fumigant retention for TIF (Tab. 23). However in these conditions, the lowest reduction was detected in plots fumigated with DZ 100 kg/ha for *T. atroviride*, although this reduction was significant if compared with untreated control.

Table 23 - Recovery (%) of different *Trichoderma* species from infested soil after chemical fumigation performed under TIF barriers in the third trial (May 2014)

Treatment	*T. koningii* 8009 [x, y]	*T. atroviride* P1 [x, y]	*T. harzianum* T67 [x, y]	*T. harzianum* T22 [x, y]	*T. reesei* T34 [x, y]
MS (400 l/ha)	32.1 bcB	0.0 dC	1.9 cdBC	2.5 bB	0.0 cC
MS (350 l/ha)	0.6 bcB	0.0 dC	1.2 cdC	2.5 bB	0.0 cC
MS (250 l/ha)	0.6 bcB	2.5 cC	9.9 bB	0.0 bB	0.0 cC
DZ (200 kg/ha)	0.0 cB	0.0 dC	0.0 cC	0.0 bB	0.0 cC
DZ (160 kg/ha)	3.1 bcB	0.0 dC	0.6 cC	0.0 bB	0.6 cC
DZ (100 kg/ha)	38.3 bB	61.7 bB	8.6 bcBC	29.6 aA	23.5 bB
Bare control	100.0 aA	100.0 aA	100.0 aA	35.8 aA	98.1 aA

[x] Since error mean square is zero, homogeneous groups cannot be computed and, consequently letters within each column for post-hoc analysis of significant differences are omitted according to Fischer Least significant different test. Small letters indicate a significativity at *p=0.05* whereas capital letters denote significativity for *p=0.01*.
[y] Data are means of three replicates each containing 54 carnation leaf pieces. Arcsine ($\sin^{-1}$ square root *x*) transformation was used on percentage data prior to analysis; untransformed data are presented. ns = not significant data. Bare control=untreated and inoculated.

Overall, for the full set of trials performed with reduced rates of fumigants a very highly significant effect of treatment was always observed both for *R. solani* and *Trichoderma* species recovery. Otherwise, interactions among different factors, treatment × trial × film, treatment × film and trial × film were not significant for *R. solani* and *T. harzianum* T22 (Table 24). Thus, the data were combined for all trials and for all film used and then reported in figures 18 and 19 for *R. solani* and *T. harzianum* T22, respectively.

Table 24 - Effects of treatment, treatment × trial, trial × film, treatment × film and treatment × trial × film interactions on recovery (%)of *Rhizoctonia solani* and *Trichoderma harzianum* T22 from infested soil after chemical fumigation in July (1st) and November (2nd) 2013, May (3rd) 2014

Recovery percentage[x]						
	R. solani			*T. harzianum* T22		
	df	*F* [x]	*p* value	Df	*F* [x]	*p* value
Treatment	6	323.6916	0.00^{****}	6	257	0.00^{****}
Trial	2	30.7429	0.00^{****}	2	10.4961	0.00^{****}
Film	1	6.4375	0.013^{*}	1	7.2895	0.008^{**}
Treatment × trial	12	20.8479	0.00^{****}	12	23.5	0.00^{****}
Trial × film	2	1.7870	0.173^{ns}	2	1.2	0.30^{ns}
Treatment × film	6	1.9173	0.087^{ns}	6	1.97	0.08^{ns}
Treatment × trial × film	12	0.6757	0.770^{ns}	12	1.6	0.09^{ns}

[x] *F* test of fixed effects, df = degree of freedom, *p* value associated to *F*, ns = not significant data
****= very highly significant interaction; ***= highly significant interaction; **= significant interaction; *= low significant interaction

Analysing these data all fumigants at different rates were highly effective in suppressing or reducing viability of *R. solani* resting structures. Nevertheless, a very little percentage of viable sclerotia (data not significant) were retrieved only from soil fumigated by MS at 250 l/ha and DZ at 100 kg/ha (Fig 15).

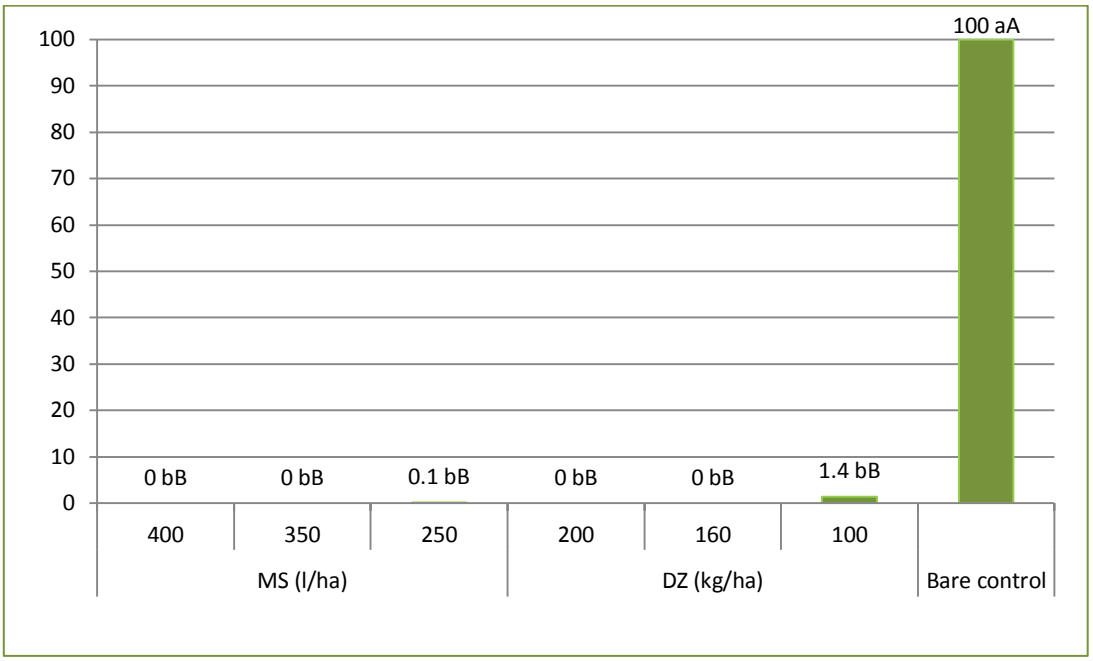


Figure 15 - Recovery (%) of viable *Rhizoctonia solani* colonies from infested rice kernels buried into soil after chemical fumigation performed under VIF and TIF barriers under all film used

Otherwise, viable colonies of *T. harzianum* T22 from colonized carnation debris were retrieved from all fumigated plots, including the highest rates of MS and DZ tested, although with very low recovery percentages. A certain viability of *T. harzianum* T22 was detected in the plots fumigated with DZ at 100 kg/ha (significant data) (Figg.16-17).

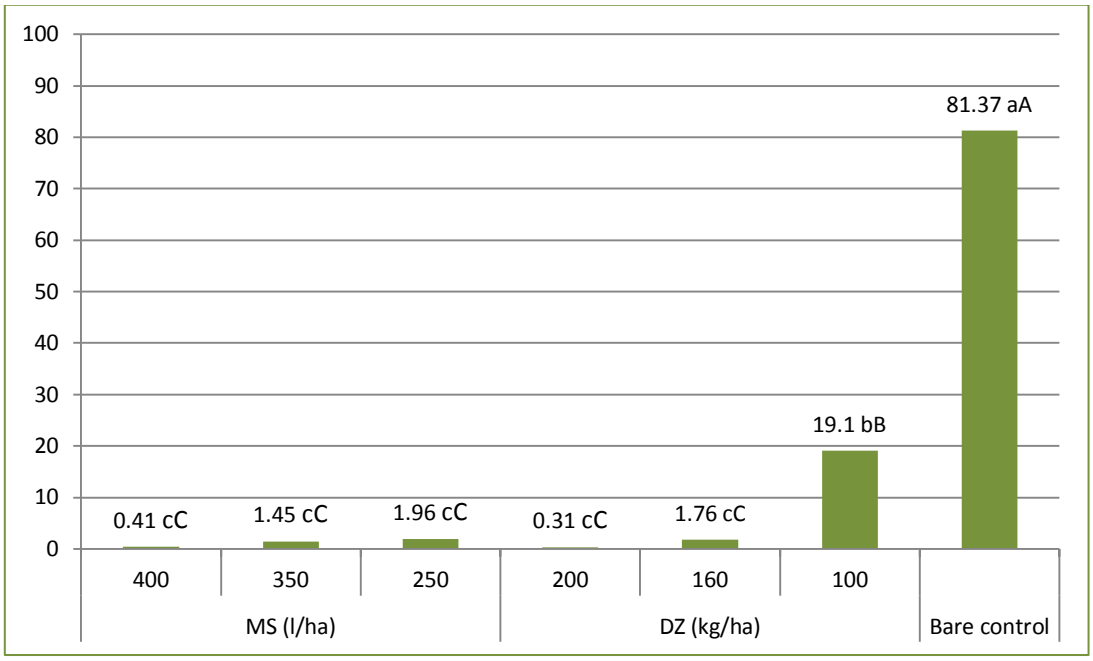


Figure 16 - Recovery (%) of viable *Trichoderma* species colonies from infested rice kernels buried into soil after chemical fumigation performed under all film used

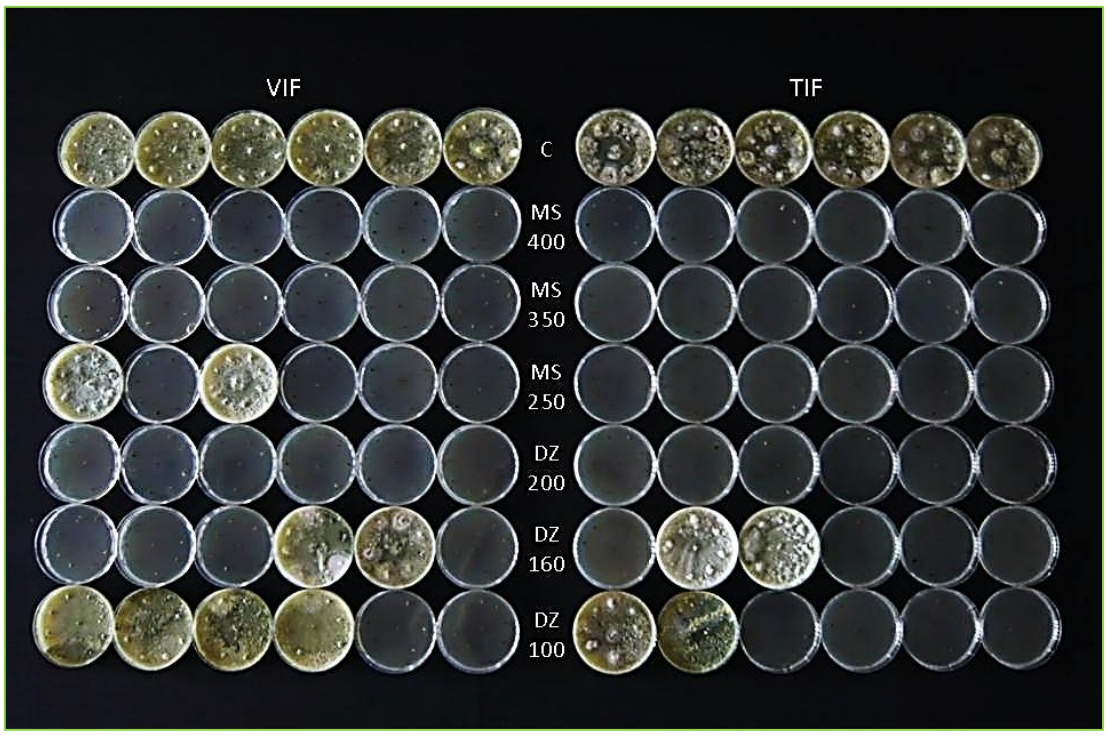


Figure 17 - Viability of *Trichoderma harzianum* T22 on PDA+A, after fumigation under VIF barriers and TIF barriers.

7.4. Discussion

Rhizoctonia diseases currently represent a heavy threat for ornamental plant production in south Italy. Potential use of soil disinfestation or BCAs for reduced structures survival of pathogen has been addressed in some scientific papers (Elad *et al*. 1982; Fravel and Lewis 2004; Eo and Park, 2014). Little information has been published on efficacy of MS and DZ fumigants tested in these experiments against Rhizoctonia diseases. Besides, little is known on the effects of fumigants on the *Trichoderma* spp. (Lewis and Papavizas, 1984; James, 1989; Eo and Park, 2014).

These trials provides new information on the efficacy of both fumigants applied at low rates for suppressing *R. solani* sclerotia in nursery and the side effect on four *Trichoderma* species.

In all experiments the fumigants reduced significantly resting structures survival of pathogen and antagonist viability in relation to the highest temperatures recorded. Moreover, in different experiments some little differences in levels of reduction and/or suppression could be due to the pathogen and antagonist isolates tested. Best performances of fumigants were always performed under TIF if compared to VIF.

According to the European legislation on the "Sustainable Use of Pesticide" the application of reduced rates may offer substantial benefits resulting efficacy in controlling plant pathogens and allowing the survival of *Trichoderma* (Elad *et al*. 1982; Lewis and Papavizas, 1984; James, 1989; Ślusarski and Pietr, 2009; Eo and Park, 2014). Moreover, these fumigants show a wide activity range and could represent a valid alternative for the disinfection of soil and substrates used in pot-grown plants production. Further investigation could be performed on the effects on beneficial microbial population naturally present in the soil and more the side effect against other soil-borne pathogens of ornamental plants, such as *Sclerotium rolfsii* or *Ilyonectria* spp.

8. Effect of a new fumigant against binucleate and multinucleate *Rhizoctonia* isolates

8.1. Materials and methods

Two experiments were performed to evaluate the efficacy of DMDS against *Rhizoctonia* spp. in perlite and vermiculite substrates. For both experiments two modes of artificial inoculation were used: 1) mycelial or resting structures suspension; 2) infected vegetal debris. The mycelial suspension was applied to the substrate surfaces using a hand sprayer. Inoculated rice kernels were used as vegetal debris and were prepared as previously described. Successively, rice kernels were put in nylon bags and buried in the pots. The first experiment was performed under semi-field conditions by placing 3-liter-pots in aluminium benchs under single-span greenhouse (Fig. 18). The efficacy of DMDS applied at two rates (400 and 600 l/ha) was evaluated against *Rhizoctonia* BNR AG-R and *R. solani* AG-4 isolates. The treatments were performed two weeks after the inoculation by pathogen suspension and immediately after burying the infected vegetal debris. The pots were covered with VIF film (VIF, Ecobrom®, AgriPlast S.r.l., Vittoria, Ragusa, Italy).

Figure 18 - Pots filled with perlite and vermiculite substrates before the fumigation treatment with DMDS

The second experiment was performed at the same conditions of the previous experiment and using only vermiculite as substrate where efficacy of DMDS against *R. solani* AG-4 was evaluated (Fig. 19). The inoculation modes were the same as above reported for the first experiment. For this experiment, the activity of DMDS was compared to MS one (used as standard treatment) applied at the lowest label rate (700 l/ha) and using two films as cover: VIF and DMDS assessed film (DAF).

The substrate temperature was measured at 15 cm depth in each tray and plot continuously using a commercial temperature sensor (Fourtec-Fourier Technologies, model MicroLite).

Figure 19 - Pots filled with vermiculite containing the infested vegetal debris before the fumigation treatments

The DMDS application was performed diluting, with a dropping glass bottle Ranvier type (Fig. 20), 0.8 ml and 1.2 ml of commercial product in 3.2 ml and 4.8 ml of water, respectively.

Figure 20 - DMDS application by dropping glass bottle Ranvier type

The evaluation of efficacy of fumigants was referred to percentage of cultural debris pieces or substrate fragments from which pathogen colonies developed. In detail, nine pieces were washed with SDW, placed onto PDA+A and, subsequently, maintained for one week at 25±1°C. This mode was also used to determine the survival of *Rhizoctonia* isolate following fumigation. For the first experiment, the retrieved cultural debris with pathogen resting structures were mixed to substrate in aluminium trays. In each tray 50 *Solanum lycopersicum* seeds were subsequently placed to germinate to evaluate pathogen infection ability. To this aim, an additional nylon bag was previously buried for each replicate. The retrieved cultural debris were cut into smaller pieces prior to mixing to cultivation substrate (sand) (Vitale *et al*., 2013). Thus obtained trays were placed in a growth chamber and brought to water field capacity before *S. lycopersicum* seeding. The percentage of seedlings showing crown and root rot symptoms of the total number of examined seedlings was recorded about 8–10 dd after seeding.

The efficacy of fumigation, in which inoculation by fungal suspension method was used, was evaluated by the percentage of vermiculite and perlite fragments from which pathogen colonies developed. For the first experiment, the infection ability of the inoculum was tested on *S. lycopersicum* seedlings. Therefore, 5 seedlings of *S. lycopersicum* were transplanted in each pot inoculated with *R. solani* 21 d after fumigation. Thus obtained pots were placed in a growth chamber and brought to water field capacity. The percentage of plants showing crown and root rot symptoms of the total number of examined plants was recorded 15 and 30 d after the transplant.

Data analysis

All data obtained from the inoculation trials were subjected to analysis of variance according to parametric approaches (Statistica 10; StatSoft Inc., Analytical Software for Windows) with factorial treatment structure and interactions.

Data from all experiments were separately analysed and the mean values of recovery percentage and DI for each treatment were calculated, averaging corresponding values determined for each replicate. All recovery and DI percentage data were

previously transformed using arcsine (sine^{-1} square root x) prior to statistical analysis. The untransformed values of recovery and DI (%) are presented.
Mean separation of the percentage recovery data in each experiment was conducted using Duncan at $p = 0.01$.

8.2. Results

In this first experiment viability of *R. solani* on cultural debris was totally suppressed by DMDS at two applied rates in both substrates. Similar data were found for *Rhizoctonia* BNR on pots fumigated with DMDS at 600 l/ha. Higher recovery percentages of viable colonies were obtained from infested cultural debris buried into pots fumigated with DMDS at 400 l/ha, although these reductions were significant if compared to control but not significant if compared to the higher rate of fumigant (Tab. 25).

Table 25 - Recovery (%) of *Rhizoctonia* spp. from infested rice kernels after DMDS fumigation

Treatment	*Rhizoctonia* BNR (%)		*Rhizoctonia solani* (%)	
Media	Perlite [x, y]	Vermiculite [x, y]	Perlite [x, y, z]	Vermiculite [x, y, z]
DMDS (400 l/ha)	18.1 b	34.3 b	0.0	0.0
DMDS (600 l/ha)	0.5 b	0.0 b	0.0	0.0
Bare control	71.8 a	81.5 a	100.0	100.0

[x] Same letters within each column denote not significant differences among treatments according to Duncan test at p=0.01.
[y] Data are means of four replicates each containing 54 rice kernels. Arcsine (sin^{-1} square root x) transformation was used on percentage data prior to analysis; untransformed data are presented. ns = not significant data.
[z] Since error mean square is zero, homogeneous groups cannot be computed and, consequently letters within each column for post-hoc analysis of significant differences are omitted. Bare control=untreated and inoculated.

Pathogen virulence on *S. lycopersicum* from treated and colonized rice kernels following DMDS fumigation are reported in table 26. The percentages of seedlings with Rhizoctonia symptoms (DI-disease incidence) were very variable between two *Rhizoctonia* species. In detail, it was not possible to reproduce *Rhizoctonia solani* symptoms inoculating colonized rice kernels retrieved from all pots fumigated with DMDS. These data clearly showed no virulence of inocula and fully confirmed previous data on viability (Tab. 25). Otherwise, *Rhizoctonia* BNR was able to infect tomato seedlings to a different extent (DI significantly different between two DMDS rates only in perlite substrate) when recovered from cultural debris retrieved from

pots fumigated at two DMDS rates. Once again these data confirmed previous viability data (Tab. 25).

Table 26 - Incidence (%) by *Rhizoctonia* spp. on tomato plants cultivated on media infested by colonized rice kernels recovered after DMDS fumigation

Treatment	*Rhizoctonia* BNR (%)		*Rhizoctonia solani* (%)	
Media	Perlite [x, y]	Vermiculite [x, y]	Perlite [x, y]	Vermiculite [x, y]
DMDS (400 l/ha)	49.2 a	41.4 ab	0.0 b	0.0 b
DMDS (600 l/ha)	4.8 b	12.6 bc	0.0 b	0.0 b
Bare control	71.3 a	72.7 a	46.2 a	35.4 a
Not-inoculated control	0.0 b	0.0 c	0.0 b	0.0 a

[x] Same letters within each column denote not significant differences among treatments according to Duncan test at p=0.01

[y] Data are means of four replicates each containing 26 up to 50 tomato seedlings. Arcsine ($\sin^{-1}$ square root x) transformation was used on percentage data prior to analysis; untransformed data are presented. Bare control=untreated and inoculated.

Regarding inoculation mode by pathogen suspension, efficacy of DMDS was evaluated on basis of recovery (%) of *Rhizoctonia* colonies from infected perlite and vermiculite pieces (Tab. 27). All DMDS rates suppressed viability of *Rhizoctonia* isolates except on perlite for *Rhizoctonia* BNR, of which few colonies were yielded from infected substrate pieces. However, both DMDS fumigations induced recovery percentage data significantly lower if compared to relative controls (only at *p=0.05*).

Table 27 - Recovery (%) of *Rhizoctonia* spp. from infected pieces of perlite and vermiculite media after inoculation by mycelial suspension and following DMDS fumigation

Treatment	*Rhizoctonia* BNR (%)		*Rhizoctonia solani* (%)	
Media	Perlite [x, y]	Vermiculite [x, y]	Perlite [x, y]	Vermiculite [x, y]
DMDS (400 l/ha)	1.0 bA	0.0 bB	0.0 bA	0.0 bA
DMDS (600 l/ha)	0.5 bA	0.0 bB	0.0 bA	0.0 bA
Bare control	4.7 aA	19.9 aA	9.7 aA	6.0 aA

[x] Same letters within each column denote not significant differences among treatments according to Duncan test. Small letters indicate a significativity at *p=0.05* whereas capital letters denote significativity at p=0.01

[y] Data are means of four replicates each containing 54 media (perlite or vermiculite) pieces. Arcsine ($\sin^{-1}$ square root x) transformation was used on percentage data prior to analysis; untransformed data are presented. Bare control=untreated and inoculated.

The data on *Rhizoctonia* BNR virulence from treated and colonized perlite pieces on *S. lycopersicum* following DMDS fumigations are reported in table 28. Although few fungal isolates were retrieved from perlite pots in previous experiment (Table 27), this little inoculum amount was not able to cause infections on tomato. Virulence of *Rhizoctonia* BNR from infected vermiculite pieces and virulence of *R. solani* from

both perlite and vermiculite pieces were not ascertained since inoculum viability in previous trial was equal to zero (Table 27).

Table 28 - Incidence (%) by *Rhizoctonia* BNR on tomato plants cultivated on perlite media previously inoculated by conidial suspension and following DMDS fumigations

Treatment	*Rhizoctonia* BNR (%)
Media	Perlite [x, y]
DMDS (400 l/ha)	0.0 a
DMDS (600 l/ha)	0.0 a
Bare control	15.0 a

[x] Same letters within each column denote not significant differences among treatments according to Duncan test at p=0.01.
[y] Data are means of four replicates each containing 5 tomato seedlings. Arcsine ($\sin^{-1}$ square root x) transformation was used on percentage data prior to analysis; untransformed data are presented. Bare control= untreated and inoculated pots.

In second experiment efficacy of fumigants was evaluated only against *R. solani*. In this trial also substrate temperature was estimated (values comprised between 10 and 27°C). All treatments were effective in reducing viability of pathogen in cultural debris both under VIF and DAF barriers (tab 29). Nevertheless, DAF provided best performances than VIF in reducing viability of *R. solani* [significant data at the same rate (400 l/ha) of DMDS].

Table 29 - Recovery (%) of *Rhizoctonia solani* from infested rice kernels following chemical fumigations

Treatment	*Rhizoctonia solani* (%)	
Film	VIF [x, y]	DAF [x, y]
DMDS (400 l/ha)	10.1 b	1.5 b
DMDS (600 l/ha)	0.0 b	0.0 b
Metham sodium (700 l/ha)	0.0 b	0.0 b
Bare control	29.7 a	32.0 a

[x] Same letters within each column denote not significant differences among treatments according to Duncan test at p=0.01; [y] Data are means of 4 replicates each containing 54 rice kernels. Arcsine ($\sin^{-1}$ square root x) transformation was used on percentage data prior to analysis; untransformed data are showed. Bare control=untreated and inoculated pots.

These data were further confirmed by results referred to the recovery (%) of *R. solani* from infected pieces of vermiculite media inoculated with the suspension. Although the pathogen showed a high ability in colonizing substrate, only 1% of viable inoculum was retrieved from perlite pots treated with low rate under VIF and with both rates of DMDS under DAF barriers (Tab. 30).

Table 30 - Recovery (%) of *Rhizoctonia solani* from infected pieces of perlite and vermiculite media following inoculation by conidial suspension and after DMDS fumigation

Treatment	*Rhizoctonia solani* (%)	
Film	VIF [x, y]	DAF [x, y]
DMDS (400 l/ha)	1.0 b	1.0 b
DMDS (600 l/ha)	0.0 b	1.0 b
Metham sodium (700 l/ha)	0.0 b	0.0 b
Bare control	80.0 a	80.0 a

[x] Same letters within each column denote not significant differences among treatments according to Duncan test. p=0.01; [y] Data are means of four replicates each containing 54 pieces of perlite and vermiculite. Arcsine ($\sin^{-1}$ square root x) transformation was used on percentage data prior to analysis; untransformed data are showed. Bare control=untreated and inoculated pots.

8.3. Discussion

These trials provide new information on the efficacy of new fumigant DMDS actually labelled against against *Meloydogine* spp., *Heterodera* spp., *Globodera* spp and *Pratylenchus* spp. nematodes (Zanón, 2014a,b; Sasanelli, 2014; Curto, 2014; Leocata, 2014; Fritsch, 2014). Although Abou Zeid N.M. and Noher A.M. (2014) started experimenting on the efficacy of DMDS against *R. solani*, no information are available on BNR *Rhizoctonia* until now.

The experiments allow evaluating the efficacy in suppressing *R. solani* and BNR sclerotia in nursery. All treatments almost totally inhibits the viability of *R. solani*, whereas little differences in levels of reduction have been recorded for *Rhizoctonia* BNR.

Although, these data showed an interesting effect in reducing or suppressing *R. solani* and BNR viability, further investigations should be performed against other isolate belonging to other AGs groups and under open field conditions.

9. Conclusion

The present PhD thesis provides new findings about Rhizoctonia diseases. During surveys performed in Sicily, infections caused by BNR *Rhizoctonia* (AG-A, AG-G, AG-R) and *R. solani* (AG-4) were frequently detected on new hosts, as *Citrus volkameriana*, *Citrus sinensis* × *Poncirus trifoliata* (Citrange), *Trachycarpus fortunei*, *Arbutus unedo*, *Thevetia peruviana*, *Bignonia* sp., *Carissa* spp., *Catharanthus roseus*, *Eugenia* spp., *Quercus ilex*, *Phyllirea angustifolia*, *Pittosporum tobira*, *Cistus salvifolius*, *Butia capitatis* and *Polygala myrtifolia*. BNR *Rhizoctonia* AG-R were detected for the first time in Italy and Europe, being pathogen previously reported from USA, Australia, Brazil and China. The high incidence of infections observed during the surveys in the last years, demonstrates that Rhizoctonia diseases represent a serious threat for ornamental plant production in nursery in Sicily.

Data regarding soil and substrate disinfection clearly demonstrated as good performances can be obtained by MS and DZ fumigation, besides at the label rates, also with 5-fold reduced dosages. In addition, TIF barrier application may be recommended in nursery since it shows best performances to retain fumigants than VIF, thus enhancing fumigation treatment. Interesting data reported for a new fumigant (DMDS) that opening a new way to soil disinfection, although further studies should be performed on a larger number of isolates or species and under different nursery conditions.

Since reduction of the primary inoculum may not be sufficient, fungicide treatments are often required during all growing stages to control Rhizoctonia diseases. Data reported in this PhD thesis confirmed once again the best performances of fungicide tolclofos-methyl, although other fungicides showed encouraging results. Further studies should be performed by using BCAs in nursery since lower performances were detected in the present studies.

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