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REVIEW ARTICLE

Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts

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Summary Medicinal plants have been used for thousands of years in folk medicines and still are used for their health benefits. In our days medicinal plants are exploited for the isolation of plant-derived drugs as they are very effective and have relatively less or no side effects. However, the natural resources of medicinal plants are gradually exhausted and access to plant bioactive compounds is challenged by the low levels at which these products accumulate in native medicinal plants. For instance, to meet the market demands of 3 Kg per year of vinca alkaloids, powerful plant-derived anticancer drugs, $1.5x10^6$ Kg dry leaves are required. In this regard, this review aims to highlight the fact that endophytic fungi residing in medicinal plants are capable to biosynthesize pharmacologically active secondary metabolites similar or identical to those produced by their host medicinal plant. Furthermore, the evolutionary origin of the genes involved in these metabolic pathways as well as the approaches designed to enhance the production of these metabolites by the isolated endophytic fungi are also discussed.

Additional key words: metabolites from endophytic bacteria and actinomycetes, chemical ecology

Introduction

Plant endophytes consist of bacterial and fungal communities that colonize and spend the whole or part of their life cycle inside the plant tissues, without instigating any noticeable symptoms of infection or visible manifestation of disease to their hosts (Petrini and Fisher, 1990). Evidence of plant-associated microorganisms found in the fossilized tissues of land plants stems and leaves suggests that endophyte–plant associations may have evolved along with the evolution of higher land plants (Krings *et al*., 2007). Nearly all vascular plant species studied were found to harbor endophytic bacteria and/or fungi (Rodriguez *et al*., 2009; Hardoim *et al*., 2015). They are found to be present in virtually all organs of a given plant host, and some are seed borne. Endophytes often confer considerable benefits to the host plant they inhabit, since they can promote the growth of host plants, enhance resistance to biotic and abiotic stresses (Rodriguez *et al*., 2009; Hardoim *et al*., 2015), and accumulate bioactive secondary metabolites (Kusari *et al*., 2012). The ecological role of secondary metabolites produced by endophytes is not clear. However, recent studies have shown that these metabolites are involved in deterrence of herbivory (Pannaccione *et al*., 2014), protection against fungal (Soliman *et al*., 2015) or bacterial pathogens (Mousa *et al*., 2017) and amelioration of plant abiotic stress (Hamayum *et al*., 2016).

Bioactive secondary metabolites derived from medicinal plants are gradually decreasing - Alternative approaches for their production

Medicinal plants, as a rich source of nat-

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ural products, have been used to treat various ailments and have been the foundation for discovery and development of modern therapeutics (Pan *et al*., 2013). Up to 80 % of people in developing countries are totally dependent on herbal drugs for their primary healthcare. More than 51% of small molecule drugs approved between 1981 and 2014 were based on natural products, the rest being synthetic (Chen *et al*., 2016). With the increasing demand for herbal drugs, natural health products and secondary metabolites, the use of medicinal plants is growing rapidly throughout the world (Chen *et al*., 2016). However, we are facing the accelerated loss of wild medicinal plant species; one third of the estimated 50.000-80.000 medicinal plant species are threatened with extinction from overharvesting and natural anthropogenic habitat destruction (Chen *et al*., 2016). Furthermore, the feasibility of access to plant bioactive compounds is challenged by the low levels at which these products accumulate in native medicinal plants, the long growth periods required for plant maturation, and the difficulty in their recovery from other plant-derived metabolites (Staniek *et al*., 2014). For example, the taxol concentration is about 0.001–0.05% in *Taxus brevifolia*, which is the most productive species. Thus, 15 kg of Taxus bark, three trees, are required for production of 1 g, while every cancer patient requires about 2.5 g (Malik *et al*., 2011).

Therefore, it is important to find alternative approaches to produce the medicinal plant-derived biologically active compounds, in particularly those derived from endangered or difficult-to-cultivate plant species, to meet the medical demand. This can be achieved by the application of plant cell and tissue culture, heterologous production, total chemical synthesis, semi-synthesis, or by starting with a microbially - produced or plant-extracted natural product occurring more abundantly in nature (Atanasof *et al*., 2015; Rai *et al*., 2016; Ramirez-Estrada *et al*., 2016) or by exploiting the ability of endophytic fungi residing in plants to produce the same or similar bioactive compounds as their hosts (Zhao *et al*., 2011). In this review, we aim to show that the large number of medicinal plants used for the isolation of medically important bioactive compounds harbor endophytic fungi capable of host-independent biosynthesis of the same or similar bioactive secondary metabolites as their hosts. This review will also discuss the evolution and origin of pathways involved in the biosynthesis of these bioactive compounds and potential approaches aiming to enhance their production.

Medicinal plants harbor endophytic fungi capable of mimicking their host plant secondary metabolite profile- Case studies on medicinal plants producing metabolites of known medical importance

Since the first report of endophyte *Taxomyces andreanae* that produces the same bioactive secondary metabolite taxol (paclitaxel) as its host *Taxus brevifolia* in 1993 (Stierle *et al*., 1993), several studies have shown that plant-derived secondary metabolites are produced by endophytes (Zhao *et al*., 2011). In this section, we will present a literature survey aiming to show that medicinal plants used for isolation of medically important secondary metabolites usually harbor endophytic fungi which are capable of host-independent biosynthesis of these metabolites. In each one of the presented case studies, emphasis will be placed on the plant species, the organ where the bioactive compound is accumulated and the organ from which the active compound-producing fungi were isolated.

Salvia sp. (Lamiaceae)

Salvia species have many important medicinal properties with proven pharmacological potential. Some of these properties may be mediated by biologically active polyphenols or terpenoids (Wu *et al*., 2012). Two kinds of bioactive compounds, tanshinones (tanshinone I, tanshinone IIA, tanshinone IIB, isotanshinone I, and cryptotanshinone) and salvianolic acids (salvianolic acid

and rosmarinic acid) have been found in the roots and leaves of *S. miltiorrhiza*, respectively. Tanshinones belong to diterpenoid quinones, and are considered as potent anti-carcinogenic, antiatherosclerosis, and antihypertensive, whereas salvianolic acids are phenolic acids, which are mainly responsible for beneficial effects on cardiovascular and cerebrovascular diseases (Chun-Yan *et al*., 2015). Several *Salvia* species produce the bioactive phenolic labdane-type diterpenes rosemarinic acid, carnosic acid and carnosol. These compounds show distinct anti-oxidant activity with carnosic acid carnosol being approved food additives (Wu *et al*., 2012). *Salvia divinorum* produces a novel diterpenoid, salvinorin A, which is a powerful hallucinogen in humans and shows a selective, high efficacy agonist activity (Butelman and Kreek, 2015).

Eighteen endophytic fungal strains have been isolated from the roots of *Salvia miltiorrhizae,* the site of tanshinones accumulation, and 58 fungal strains from the leaves, the main site of salvianolic acid accumulation. Liquid culture extracts of all the fungi were screened for the presence of tanshinones or salvianolic acid, respectively. One fungus in each case was proven to produce tanshinones or salvianolic acid compared with authentic standards. However, the yield was quite low; about 4µg/L for tanshinones and 47µg/L for salvianolic acid (Ming *et al*., 2013; Li *et al*., 2016).

Catharanthus roseus (L.) G.Don (Apocynaceae)

Catharanthus roseus is well known for the production of several anticancer vinca alkaloids such as vincristine, vindesine, vinorelbine, vinblastin and the recently discovered vinflunine (Kumar *et al*., 2014). The two major anticancer vinca alkaloids, vincristine and vinblastine, used in chemotherapy regimens, have been isolated from leaves (Kumar *et al*., 2014).

The different *C. roseus* plant organs harbour a plethora of endophytic fungi (Kharwar *et al*., 2008; Kumar *et al*., 2013; Palem *et al*., 2015; Kuriakose *et al*., 2016). Screening all the endophytes for the production of vinca alkaloids revealed that only endophytic fungi residing in the leaves of *C. roseus* were capable of producing vinblastine and vincristine. These endophytic fungi were identified as *Fusarium oxysporum, Talaromyces radicus* and *Eutypella* spp. The drugs were purified by TLC and HPLC and authenticated using UV-Vis spectroscopy, ESI-MS, MS/MS and ^{1}H NMR. Culture filtrates of the fungi yielded >55 µg/L of vinblastine or vincristine, respectively (Kumar *et al*., 2013; Palem *et al*., 2015; Kuriakose *et al*., 2016).

Coleus forskohlii (Willd.) Briq. (Lamiaceae)

Coleus forskohlii or Indian *Coleus* is a tropical perennial shrub of the *Lamiaceae* family and grows in the subtropical temperate climates of South-east Asia and India. The plant is extensively cultivated in southern India and the roots are used in Indian folk medicine for treating a broad range of human health disorders (Kavitha *et al*., 2010). The roots of the herb contain a pharmacologically active compound called forskolin that accumulates in the root cork (Pateraki *et al*., 2014). The approved and potential applications of forskolin range from alleviation of glaucoma, anti-HIV or antitumor activities, treatment of hypertension and heart failure to lipolysis and body weight control (Pateraki *et al*., 2017).

Screening of endophytic fungi isolated from inner tissues of root and stems of *C. forskohlii* for the production of forskolin revealed that one of the endophytic fungi identified as *Rhizoctonia bataticola* was able to stably synthesize forskolin and interestingly, release it into the broth (Mir *et al*., 2015).

Macleaya cordata (Willd.) R.Br. (Papaveraceae)

Sanguinarine (SA) is a benzophenanthridine alkaloid isolated from *Macleaya cordata* leaves, and is known to have a wide spectrum of biological activities, such as antibacterial, antihelmintic, antitumor and anti-inflammatory (Wang *et al*., 2014). SA is

used in feed additives for livestock production (Kantas *et al*., 2014). Most of the SA currently used in herbal supplements and medicines is extracted from *M. cordata*. Recently, SA has gained increasing attention as a potential agent in the treatment of cancer (Yu *et al*., 2014).

Screening of endophytic fungi isolated from leaves of *M. cordata* revealed that one of 55 isolates has the capacity to produce SA (Wang *et al*., 2014).

Cajanus cajan (L.) Millsp. (Fabaceae)

Cajanus cajan (pigeon pea) is a grain legume crop in semitropical and tropical areas of the world. The extract of pigeon pea leaves exhibit therapeutic effects on sickle cell anemia, plasmodiosis, and hepatic disorders. Moreover, pigeon pea roots are used as a sedative, a vulnerary preparation. The active constituents of pigeon pea are flavonoids and stilbenes. Cajaninstilbene acid (CSA) is one of the major stilbenes found in pigeon pea. Pharmacological studies have revealed that CSA exhibited anti-inflammatory and analgesic effects. In addition, CSA has an antioxidant activity similar to that of the natural antioxidant resveratrol (Liang *et al*., 2013). Cajanol is a isoflavone isolated from pigeon pea roots. Pharmacological studies have shown that cajanol has antiplasmodial, antifungal and antimicrobial activities. In addition, cajanol has been described as a novel anticancer agent, which induced apoptosis in human breast cancer cells (Luo *et al*., 2011).

A total of 245 endophytic fungi isolated from roots, stems and leaves of pigeon pea plants were screened for the production of cajaninstilbene acid or cajanol. Three fungal strains isolated from leaves were capable of producing CSA and one strain isolated from roots stably produced cajanol at a concentration of 500μg/L (Zhao *et al*., 2012; Zhao *et al*., 2013).

Cephalotaxus hainanensis H.L.Li (Cephalotaxaceae)

Cephalotaxus hainanensis H. L. Li is an indigenous conifer tree of China. The bark and leaves of *Cephalotaxus* have been used in Chinese folk medicine as anticancer agents, and its biological active constituents were proved to be alkaloids. Among these alkaloids, homoharringtonine (HHT) was shown effective against acute myeloid leukemia and has recently been approved by the Food and Drug Administration for the treatment of chronic myeloid leukemia (Hu *et al*., 2016).

A large number of endophytic fungi have been obtained from *Cephalotaxus* phloem. The bioactive compounds isolated from their culture extracts were characterized as sesquiterpenoids, anthraquinones and aromatic compounds, which exhibited cytotoxic and antibacterial activities (Lu et al., 2012; Xue *et al*. 2012; Zheng *et al*., 2011). The hunt for an HHT-producing endophytic fungus was eventually successful following the screening of 213 strains isolated from the bark of *Cephalotaxus* trees grown in China and Thailand. The fungus was identified as *Alternaria tenuissima* and stably produced 100 μg/L HHT (Hu *et al*., 2016).

Cinchona spp. (Rubiaceae)

The bark of the stem and roots of various trees of the genus *Cinchora* produce quinine alkaloids (quinine, quinidine, cinchonidine and cinchonine), which were the only effective treatment of malaria for more than four centuries. *Cinchona* bark and its alkaloids remained the most efficient treatment of malaria until the 1940s when chloroquine and other synthetic antimalarial compounds were developed (Kaufman and Ruveda, 2005). With the development of resistant malaria strains, the quest for new antimalarial compounds was successful with the discovery of artemisinin from a Chinese herbal medicine based on *Artemisia annua* L. (Tu, 2011).

Twenty-one endophytic fungi have been isolated from *Cinchona ledgeriana* young plant stems and screened for the presence of Cinchora alkaloids. These fungi comprised of *Phomopsis, Diaporthe*, *Schizophyllum*, *Penicillium*, *Fomitopsis* and *Arthrinium* species while fermentation studies demon-

strated that all produce quinine and quinidine, as well as cinchonidine and cinchonine (Maehara *et al*., 2011; Maehara *et al*., 2013).

***Passiflora incarnata* (Passifloraceae)**

Passiflora consists of 500 species that are found mostly in warm and tropical regions. *Passiflora incarnata* leaves were found to contain several active compounds, including alkaloids, phenols, glycosyl flavonoids, and cyanogenic compounds. The major compounds present in *P. incarnata* are C-glycosyl flavonoids (vitexin, isovitexin, orientin and chrysin) and b-carbolinic alkaloids (harman, harmin, harmalin, harmol, and harmalol). Among these natural products, chrysin has shown interesting biological activities, including antibacterial, anti-inflammatory, anti-diabetic, anxiolytic, hepatoprotective, anti-aging, anticonvulsant and anticancer effects (Seetharaman *et al*., 2017).

Three endophytic fungi identified as *Altenaria alternata*, *Colletotrichum capsici*, and *C. taiwanense* were isolated from leaves of *P. incarnata* and production of fungal chrysin was confirmed through UV-vis spectroscopy, FT-IR, LC-ESI-MS, and $^{1}H_{1}$ NMR analysis of their extracts. The quantitative HPLC analysis revealed that the yield of chrysin from *A. alternata* was higher when compared with previously reported bioresources (Seetharaman *et al*., 2017).

***Fritillaria cirrhosa* D.Don (Liliaceae)**

Bulbus *Fritillaria* have been used in traditional Chinese medicine for more than 2000 years, and at present, they are among the most widely used antitussive and expectorant drugs. The major biological active ingredients of Bulbus *Fritillaria cirrhosa* are steroidal alkaloids, such as peimisine, imperialine-3β-D-glucoside, and peimine (Wang *et al*., 2011).

Several dosens of endophytic fungi were isolated from fresh bulbus of *Fritillaria unibracteata* var. *wabensis*. One of these fungal endophytes, *Fusarium redolens* 6WBY3 was capable of producing and secreting in the culture medium peimisine and imperialine-3β-D-glucoside whereas a second endophytic fungus was found to secrete peimisine and peiminine. Interestingly, a large number of the remaining endophytes were able to produce large amounts of antioxidants, such rosemarinic acid (Pan *et al*., 2014; Pan *et al*., 2015; Pan *et al*., 2017).

***Huperzia serrata* (Thunb. ex Murray) Trevis (Huperziaceae)**

Huperzia serrata is a traditional Chinese herb medicine and has been extensively used for the treatment of a number of ailments, including contusions, strains, swellings, schizophrenia, myasthenia gavis and organophosphate poisoning. These pharmaceutical applications of *H. serrata* are mainly due to its biologically active lycopodium alkaloids. Among the lycopodium alkaloids, huperzine A (HupA) was found to possess potent acetylcholine esterase inhibition (AChEI) and is clinically used for the treatment of Alzheimer's disease (Zhao *et al*., 2013). The content of HupA in the leaf is richer than that in the stem and root of *H. serrata* (Gu *et al*., 2005).

Several groups have isolated endophytic fungi from leaves, stems and roots of *H. serrata*. Screening culture extracts of these fungi for HupA production revealed that most of HupA-producing fungi were isolated from leaf tissues (Su *et al*., 2017). The HupA-producing endophytic fungi belong to *Penicillium griseofulvum*, *Penicillium* sp., *Aspergillus flavus*, *Mycoleptodiscus terrestris*, *Trichoderma* sp., *Colletotrichum gloeosporioides* strain ES026 and *Shiraia* sp.. The productivity of these strains is less than 60-90 µg/L, with *Shiraia* sp. Slf14 being the best producer (327.8 µg/L) (Su *et al*., 2017). Interestingly, many *H. serrata* endophytic fungi with AChE inhibitory activity did not contain HupA in their extracts (Su *et al*., 2017; Wang *et al*., 2016) suggesting that some endophytic fungi produce new compounds with activity against AChE.

***Rhodiola* spp. (Crassulaceae)**

Rhodiola rosea is a perennial herbaceous plant that belongs to the family *Crassu-*

laceae. This species is mainly distributed in high altitudes of >2,000 m in the Arctic and mountainous regions throughout Asia and Europe. This typical alpine plant has been widely used as an important food crop and folk medicine since ancient times by many countries, such as Sweden, Russia, India, and China (Chiang *et al*., 2015). *Rhodiola* rhizome, as a traditional folk medicine, stimulates mental and physical endurance, counteracts depression, improves sleep quality, and prevents high-altitude sickness. Modern pharmacology research suggests that *Rhodiola* rhizome has received considerable attention because of its biological behavior, including antioxidant and anti-aging properties, anti-microwave radiation, antihypoxia and adaptogenic activities. Most of these effects are ascribed to phenolics, such as salidrosides and p-tyrosol, and glycosides like rosavins (Chiang *et al*., 2015).

Screening of 347 endophytic fungal strains isolated from rhizomes of *R. crenulata*, *R. angusta* and *R. sachalinensis* revealed that four endophytic fungi were capable of producing salidrosides and p-tyrosol (Cui *et al*., 2015). One of these endophytic fungi identified as *Phialocephala fortinii* was able to stably produce large amounts of salidrosides and p-tyrosol, 2.3 and 2 mg/ml of culture medium, respectively (Cui *et al*., 2016).

Solanum nigrum L. (Solanaceae)

Solanum nigrum L., family *Solanaceae* is a well-known medicinal plant which possesses several biological activities such as antioxidant, hepato-protective, antiinflammatory, antipyretic, diuretic, antimicrobial and anticancer activities due to its flavonoid and steroidal alkaloids content (Jain *et al*., 2011). Solamargine, one of the major steroidal alkaloids in *S. nigrum* has been demonstrated to exhibit potent anticancer activity against colon, prostate, breast, hepatic and lung cancer cell lines (Jain *et al*., 2011). Solamargine is always found in a complex mixture with other glycoalkaloids such as solasonine and solanine, which makes solamargine isolation from the plant quite difficult (Milner *et al*., 2011). Chemical synthesis of solamargine is possible, however it does not appear to be practical as the overall yield was only 10.5%, requiring 13 steps (Wei *et al*., 2011).

Three fungal endophytes have been isolated from *S. nigrum* stems, leaves and fruits. Their culture extracts were screened for the potential production of steroidal alkaloids. The stem derived endophytic fungal strain *A. flavus* was able to steadily produce solamargine with a titer of about 250–300 µg/ L which is higher than the plant callus culture method (El-Hawary *et al*., 2016).

Piper longum L. and *Piper nigrum* L. (Piperaceae)

Piperine is a major alkaloid present in the fruit of *Piper longum* and *Piper nigrum* and it is known to have a wide range of pharmaceutical properties including antibacterial, antifungal, hepato-protective, antipyretic, anti-inflammatory, anti-convulsant, insecticidal and antioxidant. The amount of piperine varies in plants belonging to the *Piperaceae* family; it constitutes 2% to 7.4% of both black pepper and white pepper (Corgani *et al*., 2017). Screening of endophytic fungi isolated from both plant species revealed the presence of piperine in culture extracts of endophytic *Periconia* strains isolated from leaves of *P. longum* (Verma *et al*., 2011) and *Colletotrichum gloeosporioides* from the stems of *P. nigrum* (Chithra *et al*., 2014).

Digitalis lanata Ehrh. (Plantaginaceae)

Glycosides from plants of the genus *Digitalis* have been reported to be cardiotonic and are widely used in the treatment of various heart conditions namely atrial fibrillation, atrial flutter and heart failure. The bioactive glycosides accumulate in the leaves and to a less extent in other organs of the plant (Alonso *et al*., 2009).

A total of 35 fungal endophytes were isolated from stems and leaves, and screened for the production of secondary metabolites. Crude extracts of fungal cultures revealed the production of glycoside digoxin from cultures of five endophytic strains (Kaul *et al*., 2013).

***Capsicum annuum* L. (Solanaceae)**

Capsaicin, the pungent alkaloid of red pepper (*Capsicum annuum*), is present in large quantities in the placental tissue, the internal membranes and, to a lesser extent, the other fleshy parts of the fruits of *Capsicum*. The pharmacological properties of capsaicin include cardio protective influence, anti-lithogenic effect, anti-inflammatory and analgesia, thermogenic influence, and beneficial effects on gastrointestinal system (Srinivasan *et al*., 2016).

An endophytic fungal strain identified as *Alternaria alternata* has been isolated from fruits of *C. annuum* and has been found to produce and secrete capsaicin up to three generations (Devari *et al*., 2014).

***Ginkgo biloba* L. (Ginkoaceae)**

Ginkgo tree contains in bark and leaves flavones and terpenoide lactones, among which, bilobalide and ginkgolides (terpenoide lactones) have been shown to be beneficial to human health (Usai *et al*., 2011). Ginkgolide B has revealed potent antagonistic effects on platelet activating factors involved in the development of a number of renal cardiovascular, respiratory and central nervous system disorders (Usai *et al*., 2011) while bilobalide was found to exert neuroprotective effects (Kiewert *et al.,* 2008).

Screening of 27 endophytic fungal strains isolated from the bark of *G. biloba* trees revealed that only one isolate *F. oxysporum* SY0056, was capable of producing Ginkgolide B (Cui *et al*., 2012). The search for bilobalide -producing endophytic fungi was far more copious; a total of 57 fungal strains were isolated from stem, root, leaf, and bark of the plant *G. biloba* and their extracts were evaluated for the presence of bilobalide. Only the isolate *Pestalotiopsis uvicola* GZUYX13 residing in leaves was proven to be a bilobalide-producing fungus (Qian *et al*., 2016).

***Silybum marianum* (L.) Gaertn. (Asteraceae)**

Silymarin is a bioactive extract of the fruits of *Silybum marianum* and contains seven flavolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, and silydianin) with reported chemoprevention and hepatoprotective properties (Feher and Lengyel, 2012).

Twenty one endophytic fungi were isolated from stems, leaves, roots, and seeds of *S. marianum* and were examined for production of flavolignans (El-Elimat *et al*., 2014). Two of these compounds, silybin A and silybin B, have been extracted as fermentation products of two strains of *Aspergillus iizukae* isolated from the leaves and stems of *S. marianum,* respectively. Subcultur of one flavonolignan-producing strain revealed an attenuation of the production of flavonolignans. However, when autoclaved leaves of the host plant were added to the growth medium, the production of flavonolignans could be resumed (El-Elimat *et al*., 2014).

***Vinca minor* L. (Apocynaceae) and *Nerium indicum* Mill. (Apocynaceae)**

Vincamine indole alkaloids (vincamine, tabersonine and catharanthine) are widely found in plants of the *Apocynaceae* family and show beneficial properties for human, such as prevention of cerebrovascular, precaution of chronic ischemic stroke, and reduction of vascular dementia or memory impairment (Saurabh and Kishor, 2013). Vincamine is a precursor compound for other medicinal alkaloids such as 11-bromovincamine, ethyl-vincamine and vinpocetine, which have shown potential clinical therapeutic effect (Manda *et al*., 2015). Vincamine is accumulated in the leaves and stems of *Vinca minor* and *Nerium indicum*. Though abundant chemical synthesis and semi-synthesis research results have been reported, the main sources of vincamine indole alkaloids are stems and leaves of *Vinca minor* L.

Eleven fungal strains have been isolated from the stems and roots of *Nerium indicum* and fungal culture extracts were screened for the presence of indole alkaloids (Yin and Sun, 2011). One fungal strain, CH1, produces vincamine alkaloids as its host plant as determined by TLC, HPLC and LC–MS analysis. The yield of vincamine, ethyl-vincamine,

and tabersonine was 1.279, 1.279 mg/L, 0.102 mg/L, respectively (Na *et al*., 2016). In a similar study, 10 endophytic fungal strains were isolated from the roots, stems and leaves of the plant *V. minor*. One fungal strain isolated from the stems was found to produce vincamine although with a relatively lower yield as compared to that of another fungal strain isolated from *N. indicum* (Yin and Sun, 2011).

***Rheum palmatum* L. (Polygonaceae)**

Rheum palmatum is a medicinal plant and its air-dried roots have been used in the traditional medicine. *R. palmatum* presents cathartic effect on the digestive movement of the colon, protects the damaged liver, and has antibacterial, anti-inflammation, and anti-aging properties. The most effective biologically active compounds in the roots of the genus *Rheum* are anthraquinones including emodin, rhein, physcion, aloe-emodin. Pharmacological tests revealed that rhein can alleviate pain and fever and inhibits inflammation (You *et al*., 2013).

Fourteen endophytic fungal strains have been isolated from *R. palmatum*: 12 strains were isolated from the root, 2 strains from the stem. The strain R13, isolated from the roots, was capable to produce the bioactive compounds rhein and emodin. The yield of rhein in R13 can reach 5.67 mg/L (You *et al*., 2013).

***Forsythia suspensa* (Thunb.) Vahl. (Oleaceae)**

The main chemical constituents of *F. suspensa* are composed of lignans including phillyrin and forsythiaside, triterpenic acids including oleanolic acid and ursolic acid. Phillyrin was reported to have various biological activities such as antioxidant, anti-inflammatory, anti-hyperlipidemia and antipyretic activities (Qu *et al*., 2008). Studies on phillyrin have shown its presence mainly in the leaves and fruits of the plant *F. suspensa* (Piao *et al*., 2008).

A total of 24 fungal strains were isolated from stems, leaves and fruits of *F. suspensa* and screened for phylirin production. One strain *Colletotrichum gloeosporioides* isolated from the fruits was found to produce the active constituent phillyrin as was judged by TLC, HPLC and HPLC-MS analysis (Zhang *et al*., 2012).

***Miquelia dentata* Bedd. (Icacinaceae), *Camptotheca acuminata* Decne. (Nyssaceae) and *Nothapodytes nimmoniana* (Graham) Mabb. (Icacinaceae)**

Camptothecine (CPT), a quinoline indole alkaloid and its analog, 10-hydroxy camptothecine (10-OH-CPT) are potent inhibitors of the eukaryotic topoisomerase I and are currently used as efficient anticancer drugs against a broad band of tumor types such as small lung and refractory ovarian cancers. (Kai *et al*., 2015). CPT and 10-OH-CPT are naturally produced by several plant species of the Asterid clade. Among them however, the major sources of commercial CPT in the world market are *Camptotheca acuminata* and *Nothapodytes nimmoniana* (Uma Shaanker *et al*., 2008). Exceptional high levels of CPT and 10-OH-CPT are also found in the fruits and seeds of *Miquelia dentata* (Ramesha *et al*., 2013).

Twenty-three fungal isolates were obtained from different fruit parts of *M. dentata*. All fungal isolates produced CPT though in varying quantities (Shweta *et al*., 2013). Three fungal species, *A. alternata*, *Phomopsis* sp. and *Fomitopsis* sp., were identified as CPT-producers with the highest yield of CPT being obtained from *A. alternata* (73.9 μg/g DW) (Shweta *et al*., 2013). CPT-producing endophytic fungi have also been isolated from *C. acuminata* (Pu *et al*., 2013) and *N. nimmoniana* (Bhalkar *et al*., 2016).

Biochemical convergence or horizontal gene transfer confer the ability to the endophytic fungi to produce the same bioactive compounds as their host

The discovery of endophytic fungi producing the same or similar bioactive compounds as their hosts raises the question as to whether parallel pathways evolved sim-

ply because each lineage has benefitted from making a given compound completely independently of the other or whether horizontal gene transfer (HGT) events took place between the fungi and the plant.

There is precedent for the independent development of the same biosynthetic pathway (biochemical convergence) in fungi or plants and other organisms. For instance, although higher plants and endophytic fungi produce structurally identical GAs, profound differences have been found in the GA pathways and enzymes of plants and fungi (Hamayum *et al*., 2016), e.g. 7-methyl-cyercene-1 found in both the fungus *Leptosphaeria maculans* (anamorph *Phoma lingam*) and the marine mollusk *Ercolania funereal* is produced by distinct enzymes (Cutignano *et al*., 2012). Cyanogenic glucosides linamarin and lotaustralin found in both the moth *Zygaena filipendulae* and their food plant *Lotus japonicus* are biosynthesized by distinct enzyme systems(Jensen *et al*., 2010). However, a horizontal gene transfer event between plants and fungi, although rare, should not be excluded (Richards *et al*., 2009).

Several studies have reported the presence of *Taxus* tree key genes (*ts*,*dbat* and *bapt*) which are involved in plant paclitaxel biosynthesis in taxol-producing endophytic fungi. These results stimulated the conjecture that the origin of this pathway in these two physically associated groups could have been facilitated by horizontal gene transfer (Kusari *et al*., 2014). Other studies, however, provided evidence that microbial taxol genes exist independent of the plant genes (Xiong *et al*., 2013). Recent data support the latter proposal; genome sequencing and analysis of the taxol-producing endophytic fungus *Penicillium aurantiogriseum* NRRL 62431 revealed that out of 13 known plant Taxol biosynthetic genes, only 7 showed low homology(>30%) with genes identified in *P. aurantiogriseum* (Yang *et al*., 2014). Furthermore, polyclonal antibodies against Yaxus TS strongly cross-reacted with a protein of the taxol-producing fungus *Paraconiothyrium* SSM001 grown in liquid culture, whereas PCR analysis did not reveal the presence of *Taxus ts* gene sequences in SSM001 (Soliman *et al*., 2013). Hence, the divergence of the two biosynthetic pathways is supported with conservation only in specific enzyme sites to be important for the activity rather than the whole protein structure. Similar findings have been reported in the case of huperzine A producing endophytes. Their fungal amine oxidase genes have been found to present low similarities to the corresponding plant genes, and only conserved consensus sequences were present by the fungal and plant functional amine oxidase proteins (Yang *et al*., 2014; Yang *et al*., 2016; Zhang et al., *2015*), which supports the co-evolution theory rather than the HGT theory. This has been well established in the case of gibberellin biosynthetic pathways in fungi and higher plants where differences in genes and enzymes indicated converged evolution of GA metabolic pathways (Bömke and Tudzynski, 2009).

The list of taxol producing endophytic fungi is large and encompasses numerous fungi belonging to diverse genera (Stierle and Stierle, 2015). A similar situation appears to hold for CPT-producing fungi (Pu *et al*., 2013) and HupA-producing endophytic fungi (Su *et al*., 2017) suggesting a horizontal transfer of large secondary metabolism gene clusters between fungi. Several studies offer support to this idea; the complete sterigmatocystin gene cluster in *Podospora anserine* was horizontally transferred from *Aspergillus* (Slot and Rokas, 2012). Furthermore, it has been shown that CTP is also produced by a diverse group of endophytic bacteria (Shweta *et al*., 2013; Pu *et al*., 2015) suggesting that bacterial CPT biosynthesis may represent an independently assembled pathway from that in fungi or plants. This may be surprising since converged evolution of the diterpene GA metabolic pathway in plants, fungi and bacteria is well established (Tudzynski *et al*., 2016). Therefore, extensive genome sequencing of the various endophytic fungi will provide an opportunity for a comprehensive study on the phylogenetic origin of fungal and bacterial metabolic pathways.

Exploring endophytes for sustainable and enhanced production of secondary metabolites

The discovery of endophytic fungi capable of producing the same bioactive compounds as their host medicinal plant has raised the expectation that these compounds could be produced in large scale through fermentation processes, thus meeting the growing demand of the market, while relieving the dependence on their respective endangered host plants for the metabolites. However, this expectation remains hampered primarily by the low yields as well as the attenuation of metabolites production after sub-culturing of fungi (Kusari *et al*., 2011; Kumara *et al*., 2014; El-Elimat *et al*., 2014). The reasons for the attenuation could be attributed to factors that stem from loss of presumed signals provided by the host or co-existing endophytes, resulting in the silencing of genes in axenic monocultures (Sachin *et al*., 2013).

Passage of attenuated CPT-producing endophytic fungi from the host plants restored CPT production in the re-isolated endophytic fungi (Vasanthakumari *et al*., 2015) suggesting that a certain critical signaling may be necessary for the fungus to maintain its endogenous production. Co-cultivation studies of taxol producing fungus *Paraconiothyrium* SSM001 with endophytic fungi isolated from *Taxus* tree revealed an eightfold increase in fungal Taxol production from SSM001 (Soliman and Raizada, 2013). Co-cultivation of the endophytic fungus *Fusarium tricinctum* with the bacterium *Bacillus subtilis*, led to an up to 78-fold enhancement in the accumulation of the constitutively present fungal metabolites (Ola *et al*., 2013). Co-cultivation (mixed fermentation) under optimized conditions of the two CPT-producing fungal species *Colletotrichum fruticola* and *Corynespora cassiicola* isolated from the same host tree *N. nimmoniana* enhanced the yield of produced CPT (Bhalkar *et al*., 2016).

Epigenetic modifications using chemical inhibitors have also been found to be effective in stimulating the transcription of attenuated biosynthetic gene clusters of endophytic fungi (Vasanthakumari *et al*., 2015; Magotra *et al*., 2017), thereby resulting in the enhancement of the production of desired secondary metabolites. Bioprocess engineering strategies such as manipulation of media and culture conditions, co-culture condition, epigenetic modulation, elicitor and or chemical induction, mixed fermentation, and fermentation technology, have been proven promising in alleviating to some extent these obstacles (Venugopalan and Srivastava, 2015).

Upon availability of the endophytic fungal genomes, the putative genes encoding the enzymes involved in the biosynthesis of bioactive compounds could be identified and their function could be verified through transcriptomic, proteomic and metabolomic, RNA interference, gene knockout, and gene over expression. Genome editing technologies implemented for metabolic engineering of filamentous fungi may be applied for triggering the biosynthesis of metabolites. Alternatively, the identified biosynthetic pathway of the corresponding bioactive compounds can be assembled, engineered and then introduced in other genetically tractable microorganisms to increase their yields (El-Sayed *et al*., 2017; Wakai *et al*., 2017).

Medicinal plant endophytes in Greece

Greece is endowed with a rich biodiversity of medicinal plant species with a long tradition in herbal medicines, and their complex endomicrobiome may be directly and indirectly responsible for the production of a wealth of explored and unexplored bioactive compounds. Thus, it is expected that many new or known products for medicine may emerge through the exploration of the endophytes of these medicinal plants. We are currently isolating fungal and bacterial endophytes from indigenous medicinal plant species in the genera such as *Fritillaria*, *Hypericum*, *Teucrium*, *Calendula*, *Salvia*

as well as *Olea europaea* and the exotic *Nigella sativa* aiming to identify such bioactive compounds.

Conclusions

Medicinal plants offer an extensive bioresource of new bioactive compounds that have significant potential as antiparasitics, antibiotics, antioxidants, and anticancer agents. During the last 10 years it became apparent that endophytes are capable to produce the same bioactive secondary metabolites as their hosts and therefore there is a tremendous interest of the scientific community towards isolation, characterization and exploitation of endophytic fungi from medicinal plants as was judged by the amount of publications and number of patents (Gokhale *et al*., 2017).

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ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Ενδοφυτικοί μύκητες που διαβιούν εντός των φαρμακευτικών φυτών έχουν την ιδιότητα να παράγουν τους ίδιους ή παρόμοιους δευτερογενείς μεταβολίτες με τους ξενιστές τους

Α. Βενιεράκη, Μ. Δήμου και Π. Κατινάκης

Περίληψη Τα φαρμακευτικά φυτά χρησιμοποιούνται εδώ και χιλιάδες χρόνια στην παραδοσιακή φαρμακολογία και ιατρική. Στις μέρες μας, τα φυτά αυτά αξιοποιούνται για την απομόνωση ιδιαίτερα αποτελεσματικών φυτικών φαρμακευτικών ουσιών, με καθόλου ή ελάχιστες παρενέργειες στο χρήστη. Οι φυσικές πηγές φαρμακευτικών φυτών εξαντλούνται σταδιακά με αποτέλεσμα πέραν της οικολογικής διατάραξης από την εξαφάνιση του φυτικού είδους, να κινδυνεύει δραματικά η απόκτηση του βιοδραστικού προϊόντος, το οποίο ούτως ή άλλως βρίσκεται σε χαμηλή συγκέντρωση στο φυτό. Επί παραδείγματι, η ποσότητα των αλκαλοειδών που προέρχονται από φυτά βίνκας και τα οποία χρησιμοποιούνται ως ισχυρά αντικαρκινικά φάρμακα, ανέρχεται στα 3 κιλά ανά έτος δηλαδή απαιτούνται $1.5x10^6$ κιλά ξηρού βάρους φύλλων. Από αυτήν την άποψη, η παρούσα βιβλιογραφική ανασκόπηση αποσκοπεί στο να τονίσει τη σημασία των ενδοφυτικών μυκήτων που διαβιούν εντός των φαρμακευτικών φυτών και οι οποίοι είναι ικανοί να βιοσυνθέτουν τους ίδιους ή παρόμοιους δευτερογενείς μεταβολίτες με τους ξενιστές τους. Επιπλέον, συζητείται η εξελικτική προέλευση των γονιδίων που εμπλέκονται σε αυτές τις μεταβολικές οδούς καθώς και οι προσεγγίσεις που αποσκοπούν στην ενίσχυση της παραγωγής αυτών των μεταβολιτών από ενδοφυτικούς μύκητες.

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SHORT COMMUNICATION

First record of *Aphis craccivora* Koch (Hemiptera: Aphididae) on aronia crop in Montenegro

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Summary The aphid *Aphis craccivora* was recorded on the crop of aronia, *Aronia melanocarpa*, in Montenegro, in June 2015 and 2016. This is the first record of *A. craccivora* in Montenegro on aronia.

Additional keywords: aphid, *Aphis craccivora*, *Aronia melanocarpa*, southeastern Europe

In recent years aronia, *Aronia melanocarpa* (Michx.) Elliott (Rosales: Rosaceae), has become a quite popular fruit crop in Montenegro. It is a woody perennial shrub, resistant to cold and can be successfully grown in conditions of severe continental climate (Nikolić and Milivojević, 2010), which dominates in the northern part of Montenegro. It is currently considered as a profitable crop due to a relatively high price of the fruit (black chokeberries) and its other uses, including processed products (i.e., syrup, juice, soft spreads, tea, food colors) (McKay, 2001) and as an ornamental plant (Yovkova *et al.,* 2013). For all these reasons and the fact that it is attacked by a small number of pests and diseases, aronia has earned a profound place in the organic production in Montenegro, where among the total number of 203 registered organic producers, 20 of them grow aronia berries at a surface area of approximately 10 ha.

In June 2015, at the locality of Bojna Njiva, Municipality of Mojkovac, aphids were observed to infest an aronia plantation at altitudes between 1063 m and 1077 m. They were spotted on two plants among a total of 1600 bushes. One year later, in June 2016, the presence of aphids was recorded on numerous bushes of aronia among a total of 3000 plants at the locality Stevanovac of the same Municipality at altitudes between 875 m and 905 m. Samples of aphids were collected in 2016 and were identified as *Aphis craccivora* Koch (Hemiptera: Aphididae). To our knowlegde, this is the first record of *A. craccivora* infesting aronia in Montenegro. Aphids have been previously reported as pests of aronia (infestation of shoot tips) but the consequent slow down effect on the plant growth is not considered serious because the plants are vigorous (McKay, 2001). Recently, *Aphis spiraecola* Patch (Hemiptera: Aphididae) and *Aulacorthum circumflexum* (Buckton) (Hemiptera: Aphididae) were identified as pests of *A. melanocarpa* from southeastern Europe (Bulgaria) (Yovkova *et al.,* 2013).

Aphis craccivora is a relatively small species. The apterous viviparous female individuals have a shiny black or dark brown body with a prominent cauda and brown to yellow legs. The immatures are slightly dusted with wax while adults appear without wax. The antennae have six segments. The distal part of femur, siphunculi and cauda are black. The length of apterae individuals ranges between 1.4 and 2.2 mm. The alate viviparous *A. craccivora* females have abdo-

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men with dorsal cross bars. The length of alatae ranges between 1.4 and 2.1 mm (Blackman and Eastop, 2000).

Aphis craccivora is associated with about 50 crops and weed species belonging to 19 plant families (i.e., Amaranthaceae, Araceae, Asteraceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Malpighiaceae, Malvaceae, Nyctaginaceae, Pedaliaceae, Portulacaceae, Ranunculaceae, Rosaceae, Rutaceae, Solanaceae, Sterculiaceae, Zingiberaceae) from which the aphid mainly attacks Fabaceae (Blackman and Eastop, 2007; Kavallieratos *et al*., 2007; Mehrparvar *et al*., 2012; Yovkova *et al.,* 2013; CABI data base, 2016). The species is probably palearctic warm temperate in origin but it has now a cosmopolitan distribution; it is abundant in subtropical and tropical regions, and in the Mediterranean. It is one of the commonest aphid species with a high pest status in the tropics (Blackman and Eastop, 2000).

Aphis craccivora is generally anholocyclic (wingless and winged females), ovoviviparous. In the tropics the aphid reproduces parthenogenetically throughout the year while in areas with colder winters, overwintering may be as egg or hibernation. In Europe, males (alate) and sexual forms have been recorded in Germany (Falk, 1960). Temperatures that range between 24 and 28.5°C and 65% relative humidity (= RH) are optimal conditions for the development of *A. craccivora* (Réal, 1955; Mayeux, 1984), which is capable of rapid population development. Formation of winged individuals is triggered by the reduction in the intensity of hydrocarbon translocation (Mayeux, 1984). Young colonies concentrate on growing points of plants and are regularly attended by ants (mutualism with ants) (Soans and Soans, 1971; Hamid *et al*., 1977; Takeda *et al.,* 1982; Patro and Behera, 1991).

The spectrum of natural enemies that are associated with *A. craccivora* is wide. For instance, Kavallieratos *et al.* (2004, 2016) reported 13 parasitoid species (Hymenoptera: Braconidae: Aphidiinae) that parasitize this aphid in agricultural and non-agricultural ecosystems in southeastern Europe, i.e., *Aphidius colemani* Viereck, *Aphidius matricariae* Haliday, *Binodoxys acalephae* (Marshall), *Binodoxys angelicae* (Haliday), *Diaeretiella rapae* (M'Intosh), *Ephedrus pericae* Froggat, *Lipolexis gracilis* Förster, *Lysiphlebus confusus* Tremblay and Eady, *Lysiphlebus fabarum* (Marshall), *Lysiphlebus orientalis* Starý and Rakhshani, *Lysiphlebus testaceipes* (Cresson), *Praon abjectum* (Haliday), *Praon volucre* (Haliday). Important predators include coccinellid beetles [*Cheilomenes sexmaculata* (F.), *Coccinella septempunctata* (L.) (Coleoptera: Coccinelidae)], syrphid larvae [*Ischiodon scutellaris* (F.) (Diptera: Syrphidae)] Neuroptera larvae [*Micromus timidus* Hagen (Neuroptera: Hemerobiidae)] and Diptera larvae [*Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyidae)]. Spiders may also be important in some areas (CABI data base, 2016). Recorded fungal pathogens include *Fusarium pallidoroseum* (Cooke) Sacc. (Hypocreales: Nectriaceae) (Hareendranath *et al.,* 1987) and *Neozygites fresenii* (Nowak.) Remaud. and S. Keller (Entomophthorales: Neozygitaceae) (Zhang, 1987; Sewify, 2000).

Most of the major chemical groups of insecticides have been used against this aphid species, including organophosphates, carbamates and pyrethroids (CABI data base, 2016). However, decisions concerning the chemical treatment against *A. craccivora* should take into account the identity and abundance of local populations of its natural enemies in the context of an integrated pest management, so as to avoid outbreaks of this important pest.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή της αφίδας *Aphis craccivora* Koch (Hemiptera: Aphididae) σε καλλιέργεια αρωνίας στο Μαυροβούνιο

Ν. Latinović, Φ. Καραμαούνα και Ν.Γ. Καβαλλιεράτος

Περίληψη Η αφίδα *Aphis craccivora* καταγράφηκε να προσβάλλει την καλλιέργεια της αρωνίας, *Aronia melanocarpa*, στο Μαυροβούνιο, τον Ιούνιο των ετών 2015 και 2016. Πρόκειται για την πρώτη καταγραφή του *A. craccivora* επί του *A. melanocarpa* στο Μαυροβούνιο.

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Reaction of the native Greek tomato varieties 'Chondrokatsari Messinias' and 'Katsari Santorinis' to *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* infection

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Summary Plants have to cope with a number of biotic stresses among which, infectious diseases. The present study was conducted to investigate the reaction of two native Greek tomato vars, 'Chondrokatsari Messinias' and 'Katsari Santorinis', to infection by *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani*. Disease symptoms, disease incidence and severity were recorded and the effects of infection on the number of flowers, the biomass production (fresh and dry weight), CO_2 assimilation, stomatal conductance and transpiration were also evaluated. Both tomato varieties were susceptible to *F. oxysporum* f. sp. *lycopersici* and *R. solani* infection. However, 'Chondrokatsari Messinias' was found to be less susceptible to *F. oxysporum* f. sp. *lycopersici* compared to 'Katsari Santorinis'. Both pathogens negatively affected biomass production of var. 'Chondrokatsari Messinias' but not that of 'Katsari Santorinis'. The number of flowers produced by 'Chondrokatsari Messinias' was negatively affected by *R. solani* but not by *F. oxysporum* f. sp. *lycopersici*. Infection of both varieties by *R. solani* also caused reduction in the CO_2 assimilation, stomatal conductance and transpiration.

Additional Keywords: dry weight, Fusarium wilt, native tomato varieties, photosynthesis, stem canker, transpiration

Introduction

Native plant varieties have been extensively examined throughout the modern human history (Teshome *et al.,* 1997; Zeven, 1998). Such plant material is usually selected and maintained by traditional farmers as part of their social, economic, cultural and ecological history. Louette *et al.* (1997) described a native variety as a farmer's variety which has not been improved by any formal breeding programme. Native varieties contain much more genetic diversity than modern cultivars or hybrids (Zeven, 1998; Terzopoulos and Bebeli, 2008; Terzopoulos and Bebeli, 2010). Therefore, they are among the most important sources of genetic variation for breeders. So far, a large number of native varieties grown in the Mediterranean region have been morphologically and genetically studied (Terzopoulos and Bebeli, 2008; Mazzucato *et al.,* 2010; Cebolla-Cornejo *et al.,* 2013; Corrado *et al.,* 2014). For example, seven out of 33 native Greek tomato varieties comprise 27 different morphotypes (Terzopoulos and Bebeli, 2008). However, most of them have not yet been genetically classified or morphologically described.

Plants have to cope with a number of biotic and abiotic stresses during their growth and development (Kai *et al.,* 2007). Fusarium wilt diseases, caused by the pathogenic soil-inhabiting fungus *Fusarium oxysporum* Schlectend.:Fr., can cause severe losses in a wide range of cultivated and non-cultivated plants (Larkin *et al.,* 1998). On tomato, two forms of the pathogen, *F. oxysporum* f. sp. *lycopersici* W.C. Snyder & H.N. Hans. and *F. oxysporum* f. sp. *radicis-lycopersici* W.R. Jarvis & Shoemaker, cause two symptomatologically distinct diseases, i.e. vascular wilt and crown and root rot, respectively. *F. oxysporum* f. sp. *lycopersici* invades the vascular system of the plant through natural openings or damaged tissue of the roots (Bishop and Cooper, 1983; Agrios, 1997; Di Pietro *et al.,*

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2003). Initial symptoms of Fusarium wilt are described as vein clearing of the younger leaves and leaf epinasty, followed by stunting, yellowing of the lower leaves, progressive wilting of leaves and stem, defoliation and finally plant death. In cross-sections of the stem, a brown ring is evident in the area of the vascular bundles (Bishop and Cooper, 1983; Di Pietro *et al.,* 2003).

The soil-borne pathogen *Rhizoctonia solani* Kühn [teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk] causes serious damage to many economically important horticultural crops (Baker, 1970; Anderson, 1982; Sneh *et al.,* 1996). In the past few years, the importance of the disease caused by this pathogen has increased dramatically in Europe (Grosch *et al.,* 2005). *R. solani* strains occur ubiquitously and are either saprophytic or pathogenic to more than 500 plant species. Damping-off diseases caused by *R. solani* in greenhouse-grown vegetables are commonly encountered (Lumsden and Locke, 1989). Symptoms develop as dark brown to black cankers on the base of the plant, which increase in size over time resulting in plant collapse (Baker, 1970; Agrios, 1997).

No information is available in the literature with respect to the reaction of the native Greek tomato varieties 'Chondrokatsari Messinias' and 'Katsari Santorinis' to the infection by soil-borne fungal pathogens or on the effects of infection on plant growth and development. The objectives of the present study were to investigate *F. oxysporum* f. sp. *lycopersici* and *R. solani* infection process on the native tomato vars 'Chondrokatsari Messinias' and 'Katsari Santorinis', record the symptomatology of the diseases and correlate disease intensity (incidence and severity) with plant growth decline after infection.

Materials and Methods

Plant material, cultivation practices and experimental design

Untreated tomato (*Lycopersicon esculentum* L.) seeds of vars 'Chondrokatsari Messinias' and 'Katsari Santorinis' obtained from local growers were sown in 60 × 20 cm plastic trays (INA plastics, Athens, Greece) filled with sterile white peat moss (TS2 Klasmann-Deilmann, Geeste, Germany; pH 6.0). Tomato seedlings were grown inside a non-heated greenhouse located at the premises of the Technological Educational Institute of Peloponnese (lat. 37° 20′ 20′′N, long. 22° 60′ 51′′E) for 35 d and until they reached the 4-true-leaf stage (approx. 30 cm in height). The young plants were then transplanted individually into 5 lt plastic pots filled with a mixture of white peat moss (TS2 Klasmann-Deilmann, Geeste, Germany; pH 6.0) and perlite (Perloflor, Isocon SA, Athens, Greece) at 1:1 (v/v). The pots were then placed on aluminium benches (0.2 m width x 15 m length x 0.5 m height) in a non-heated greenhouse in a completely randomised design. Standard cultivation practices, such as plant tie-up, irrigation and fertilization, were applied to all plants. The nutrient solution used for the fertilization of the plants consisted of (in mmol/lt) 5.10 Ca^{2+}, 2.40 Mg^{2+}, 7.00 K^{+}, 1.50 NH_4^+, 3.60 SO_4^{2}, 14.30 NO_3^-, 1.50 $H_2PO_4^-$ and (in µmol/lt) 20 Fe 10 Mn, 5 Zn, 0.80 Cu, 35 B and 0.5 Mo. Electrical conductivity (EC) and pH of the nutrient solution ranged between 2.4-2.5 mS/cm and 5.8-6.0, respectively. Three hundred ml of the nutrient solution was provided to the plants every two days during the experimental period.

Two individual experiments, Experiment 1 and Experiment 2, were conducted starting out at the end of February 2015 and finishing 95 d later. In Experiment 1, tomato plants of vars 'Chondrokatsari Messinias' and 'Katsari Santorinis' were challenged with *F. oxysporum* f. sp. *lycopersici,* whereas in Experiment 2, the same varieties were challenged with *R. solani*. In each experiment, six plants per variety and time of assessment [i.e. 40 or 60 days post inoculation (dpi)] were used as replicates.

Pathogen isolates, inoculum preparation and plant inoculation

For the inoculation of experimental plants, strain BPIC2550 of *F. oxysporum* f. sp. *lycopersici* isolated from tomato plants (*Lyc-*

opersicon esculentum L.) and strain BPIC2531 of *R. solani* isolated from potato plants (*Solanum tuberosum* L.) were used. Both strains were provided by the Benaki Phytopathological Institute (Kifissia, Athens, Greece).

Tomato plants at the stage of 4 true leaves (approx. 30 cm in height) were inoculated with *F. oxysporum* f. sp. *lycopersici* by applying a conidial suspension at the basal stem-end of each plant (Dhingra and Sinclair 1995; Akköprü and Demir, 2005). The fungal inoculum was prepared as follows: initially the fungus was cultured on potato dextrose agar (PDA, Oxoid Ltd., Basingstoke, Hampshire, UK) medium in Petri plates at 26°C in the dark for 12 d. The conidial suspension, which consisted of both micro- and macroconidia, was prepared by pouring 20 ml of sterile distilled water containing 0.01% Tween 80 (Sigma, St. Louis, USA) in each plate. The conidia were dislodged by gently rubbing the fungal colony surface with a sterile razor blade. The suspension was filtered through two layers of fine, nylon, sterile cheesecloth to remove mycelia. The final conidial concentration was adjusted to 4.5 x 10^6 conidia/ml using a haemocytometer. Twenty ml of the conidial suspension were applied to each plant approx. 3 cm below the surface of the growing substrate and at a contact with the stem base using a 5 ml plastic syringe (i.e. 4 applications around the plant stem) without wounding the roots (Akköprü and Demir, 2005). Control plants were treated with 20 ml of sterile distilled water.

Tomato plants at the stage of 4 true leaves (approx. 30 cm in height) were inoculated with *R. solani* using mycelium plugs (Dhingra and Sinclair, 1995). Initially, *R. solani* cultures were prepared by placing mycelium plugs cut from the edges of 12-d-old cultures at the centre of PDA (Oxoid Ltd., Basingstoke, Hampshire, UK) plates. The inoculated plates were incubated at 25°C for 12 d in the dark. Mycelium plugs, 5 mm in diameter, were then cut from the edges of the growing colonies using a cork borer. Inoculation of tomato plants was carried out by placing three, 5 mm in diameter, mycelium plugs 3 cm below the surface of the growing substrate and at a distance of approximately 1 cm from the stem base. Control plants were treated with non-inoculated PDA plugs.

Disease assessments

In both experiments, disease symptoms were recorded 40 and 60 dpi. In Experiment 1, disease severity index (DSI) and disease incidence (DI) on tomato plants inoculated with *F. oxysporum* f. sp. *lycopersici* were assessed on the root system and stem base. DSI was determined using the arbitrary scale of: 0: no symptoms, 1: 1% of roots with symptoms, 2: >1-5% of roots with symptoms, 3: 6-10% of roots with symptoms, and 4: >10% of roots with symptoms. DI was calculated according to the following formula:
Disease incidence (DI) = (number of symptomatic plants/total number of inoculated plants) x 100 (1)

In Experiment 2, DI, number of cankers (CN) and average canker diameter (ACD) were recorded. DI was calculated according to formula (1) above. Canker diameter was measured in cm using a digital micrometer (Stock No. 600-880, Mitutoyo, Japan).

Biomass production, number of flowers and physiological parameters of tomato plants

Plant biomass production was recorded 40 and 60 dpi. Prior to assessment, the growing substrate was completely removed by gentle washing the root system of the plants under running tap water. Biomass was determined by measuring the fresh weight (FW; gr) of the aerial plant parts (i.e. stems, leaves and inflorescences) and the root system using a digital balance (Kern & Sohn GmbH, Balingen, Germany). Then, the same plant parts were dried separately in an oven (Daihan Labtech Co. Ltd, Gagok-ri, Korea) at 75°C for 72 h and the dry weights (DW; gr) were also measured. The number of flowers was recorded once every week (total of eight counts over the 60 dpi period).

The physiological parameters of CO_2 assimilation (A_s; µmol $CO_2/m^2/s$), stomatal conductance (g_s; mmol/m^2/s) and transpiration (E; mmol/m^2/s) were recorded 16 and 27 dpi

at anthesis and fruit set, respectively. A_s, g_s and *E* were measured using a LC*pro*+ portable photosynthesis system (ADC BioScientific Ltd. Great Amwell, Herts, UK). Recordings were made between 10:00 and 12:00 a.m on fully expanded young leaves of similar size. Photosynthetic photon flux density (PPFD) in the leaf chamber was set at 1100 μmol/m^2/sec with a halogen lamp at 25°C, while CO_2 reference ranged between 380 and 437 ppm.

Statistical analysis

Both experiments were factorial with variety and time of assessment (i.e. dpi) as the main factors. Experimental data were subjected to one-way ANOVA and means were separated using the Duncan's multiple range test at $P = 0.05$. Prior to analysis, DI percentage data were transformed to logarithmic values (i.e. Log_{10}) to highlight significant differences between means, although, the untransformed data are presented in the tables. Scale data of DSI were analysed using the Kruskal-Wallis non-parametric test. Statistical analysis was performed with SPSS for Windows, Version 12.0 (Chicago, SPSS Inc., USA).

Results and Discussion

Disease symptoms

F. oxysporum f. sp. *lycopersici* infected the root and the vascular system of tomato vars 'Chondrokatsari Messinias' and 'Katsari Santorinis' (Figure 1). No visual symptoms were observed on the experimental plants 40 dpi. However, tomato plants of 'Chondrokatsari Messinias' and, to a lesser extent, 'Katsari Santorinis' showed a limited degree of leaf epinasty and leaf yellowing 60 dpi. Di Pietro *et al.* (2003) described the symptoms caused on tomato plants infected by *F. oxysporum* as leaf epinasty, followed by stunting, yellowing of the lower leaves, progressive wilting, defoliation and finally plant death. In the present study, symptoms were also observed on the surface of the roots as dark brown to black, necrotic, circular or irregular lesions (Figure 1A). According to Olivain and Alabouvette (1999), *F. oxysporum* f. sp. *lycopersici* was able to perform a vascular infection of tomato root tissue producing lesions on the roots. However, these lesions had limited expansion probably due to intense defense reactions occurring in the superficial cell layers (Olivain and Alabouvette, 1999). Brown discoloration of the vascular system of the plants was also observed in the present study indicating colonization of xylem vessels by the pathogen (Figure 1B). This is considered a typical symptom of infection of tomato plants by *F. oxysporum* f. sp. *lycopersici* following root tissue penetration and colonization of the vascular system by the pathogen (Bishop and Cooper, 1983; Agrios, 1997; Olivain and Alabouvette, 1999).

R. solani infected the plants of both varieties at the stem base (Figure 2). Symptoms were recorded as volcano-like cankers of various sizes, with a brown centre and dark brown to black margin (Figure 2A). Cankers increased in size with time resulting in plant collapse 40 dpi (Figure 2B). Usually, *R. solani* infection progresses quickly, especially when conditions are favourable (i.e. low temperatures and increased soil humidity) (Baker, 1970; Agrios, 1997).

Disease assessments

DSI on tomato plants of vars 'Chondrokatsari Messinias' and 'Katsari Santorinis' inoculated with *F. oxysporum* f. sp. *lycopersici* was low and ranged between 0.5 and 2.5 (on a 0-4 scale). In general, DSI and DI did not significantly ($P < 0.05$) increase with time (from 40 to 60 dpi) with the exception of DSI on var. Katsari Santorinis (Table 1). More specifically, DSI on 'Katsari Santorinis' increased by 5-fold from 40 to 60 dpi (Table 1).

In general, CN, ACD and DI on 'Chondrokatsari Messinias' and 'Katsari Santorinis' tomato plants inoculated with *R. solani* significantly ($P < 0.05$) increased with time (from 40 to 60 dpi) with the exception of CN on 'Katsari Santorinis' (Table 2). More specifically, CN, ACD and DI on 'Chondrokatsari Messinias' increased with time by 7-, 23- and 67%, respectively (Table 2). ACD on var. 'Katsari Santorinis' increased by 4-fold from

40 to 60 dpi. Nevertheless, 40 dpi, all experimental plants of var. 'Katsari Santorinis' showed disease symptoms (DI=100%) (Table 2).

Based on the above-mentioned results, var. 'Katsari Santorinis' was found to be more susceptible to *F. oxysporum* f. sp. *lycopersici* infection compared to 'Chondrokatsari Messinias', as the former showed significantly ($P < 0.05$) higher disease levels 60 dpi compared to the latter (Table 1). The results of the present study also showed that, 60 dpi, vars 'Chondrokatsari Messinias' and 'Katsari Santorinis' showed similar susceptibility to infection by *R. solani* (Table 2).

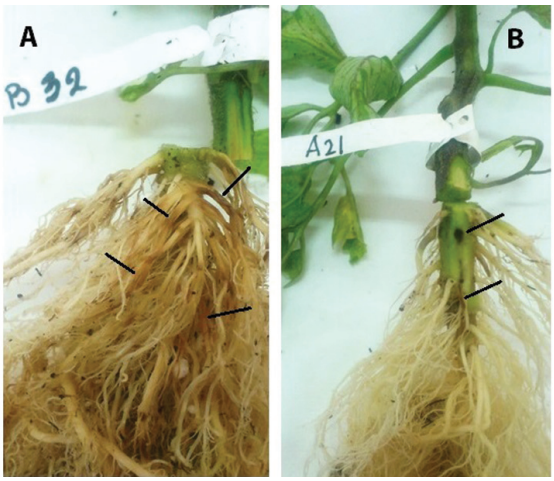

Figure 1. Light to dark brown lesions (arrows) on roots of 'Katsari Santorinis' tomato plants (A) and discoloration (arrows) of the vascular system of 'Chondrokatsari Messinias' tomato plants (B) inoculated with *Fusarium oxysporum* f. sp. *lycopersici* 60 dpi.

Effects of *F. oxysporum* f. sp. *lycopersici* infection of tomato plants on biomass production, number of flowers A_s, g_s and *E*

The results of the present study showed that FW and DW of the aerial parts of var. 'Chondrokatsari Messinias' inoculated with *F. oxysporum* f. sp. *lycopersici* were significantly ($P < 0.05$) lower compared to those of the control plants 60 dpi (Figure 3). However, FW

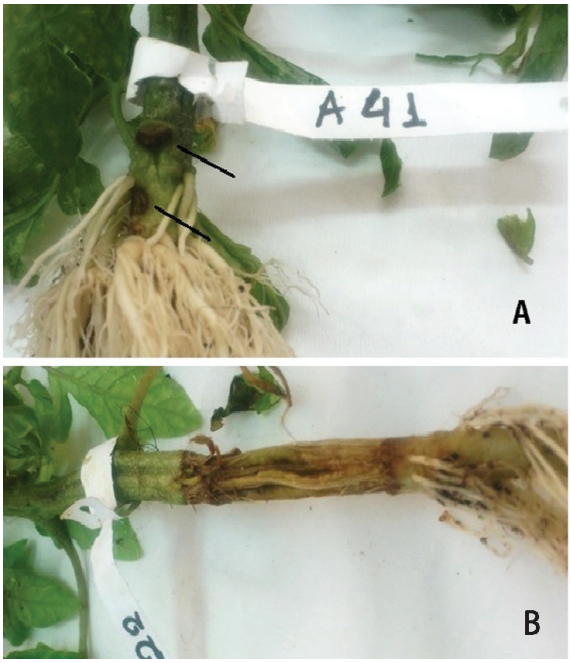

Figure 2. Dark brown cankers (arrows) on the stem base of 'Chondrokatsari Messinias' tomato plants as a result of their infection by *Rhizoctonia solani* (A) 60 dpi. Collar rot symptoms on 'Katsari Santorinis' tomato plants inoculated with *R. solani* (B).

Table 1. Disease severity index (DSI; scale 0–4) and disease incidence (DI; % plants with symptoms) on tomato plants of vars 'Chondrokatsari Messinias' and 'Katsari Santorinis' inoculated with *Fusarium oxysporum* f. sp. *lycopersici.* DSI and DI are means of six replicates and were recorded 40 and 60 days post- inoculation (dpi). Means followed by different letters are statistically different according to the Duncan's Multiple Range test ($P = 0.05$)).

Variety	Treatment	DSI (scale 0–4)		DI (%)	
		dpi			
		40	60	40	60
'Chondrokatsari Messinias'	Control	0	0	0	0
	F. oxysporum f. sp. *lycopersici*	0.67 a	1.00 a	33 a	50 a
'Katsari Santorinis'	Control	0	0	0	0
	F. oxysporum f. sp. *lycopersici*	0.50 a	2.50 b	67 ab	100 b

Table 2. Number of cankers (CN), average canker diameter (ACD; cm) and disease incidence (DI; % plants with symptoms), on tomato plants of vars 'Chondrokatsari Messinias' and 'Katsari Santorinis' inoculated with *Rhizoctonia solani*. CN, ACD and DI are means of six replicates and were recorded 40 and 60 days post inoculation (dpi). Means followed by different letters are statistically different according to the Duncan's Multiple Range test ($P = 0.05$).

Variety	Treatment	CN		ACD (cm)		DI (%)	
		dpi					
		40	60	40	60	40	60
'Chondrokatsari Messinias'	Control	0	0	0	0	0	0
	R. solani	0.67 a	4.67 b	0.13 a	3.00 bc	33 a	100 b
'Katsari Santorinis'	Control	0	0	0	0	0	0
	R. solani	3.00 b	4.50 b	1.00 ab	4.00 c	100 b	100 b

and DW of the root system of 'Chondrokatsari Messinias' were not significantly affected by the infection of the pathogen. Infection of var. 'Katsari Santorinis' by *F. oxysporum* f. sp. *lycopersici* did not significantly ($P < 0.05$) affect FW and DW of the aerial parts and the root system of the experimental plants (Figure 3).

The number of flowers of 'Katsari Santorinis' plants inoculated with *F. oxysporum* f. sp. *lycopersici* was significantly ($P < 0.05$) lower compared to that of the control plants (Table 3). However, *F. oxysporum* f. sp. *lycopersici* did not significantly affect the number of flowers of var. 'Chondrokatsari Messinias' (Table 3).

A_s, g_s and E of both tomato varieties were not significantly ($P < 0.05$) affected by *F. oxysporum* f. sp. *lycopersici* infection (Table 3). Pshibytko *et al.* (2006) showed that Fusarium wilt led to suppression of the photosynthetic activity of 4- to 6-month-old tomato plants of var. Kunera. Although, only in the case of a slowly developed pathogen could damage the photosystem. Significant differences in A_s, g_s and E between tomato plants inoculated with *F. oxysporum* f. sp. *lycopersici* and the non-inoculated controls were reported by Lorenzini *et al.* (1997). A_s, g_s and E, were negatively affected by *F. oxysporum* f. sp. *lycopersici* infection and correlations were made between the time post-inoculation (i.e. dpi) and the values of A_s, g_s and E. As dpi increased, A_s, g_s and E were reduced (Lorenzini *et al.,* 1997). In the present study, A_s, g_s and E were measured earlier (i.e. 16 and 27 dpi) than DS and DI (i.e. 40 and 60 dpi). Even at 40 dpi, DSI and DI on both varieties were very low (Table 1) which may explain the insignificant effect of *F. oxysporum* f. sp. *lycopersici* infection on A_s, g_s and E.

Effects of *R. solani* infection of tomato plants on biomass production, number of flowers A_s, g_s and E

FW and DW of the aerial parts of var. 'Chondrokatsari Messinias' inoculated with *R. solani* were significantly ($P < 0.05$) lower compared to those of the control plants 60 dpi (Figure 4). However, *R. solani* did not significantly affect FW and DW of the aerial parts of var. 'Katsari Santorinis' even 60 dpi (Figure 4). No significant ($P < 0.05$) differences in FW and DW of roots were observed 60 dpi between the inoculated plants of both varieties and the controls (Figure 4).

The number of flowers of var. 'Chondrokatsari Messinias' inoculated with *R. solani* was significantly ($P < 0.05$) lower compared to the controls (Table 4). However, tomato plants of var. 'Katsari Santorinis' inoculated with *R. solani* produced significantly ($P < 0.05$) more flowers than the control plants (Table 4).

A_s, g_s and E of both tomato varieties inoculated with *R. solani* were significantly ($P < 0.05$) lower than those of the control plants (Table 4).

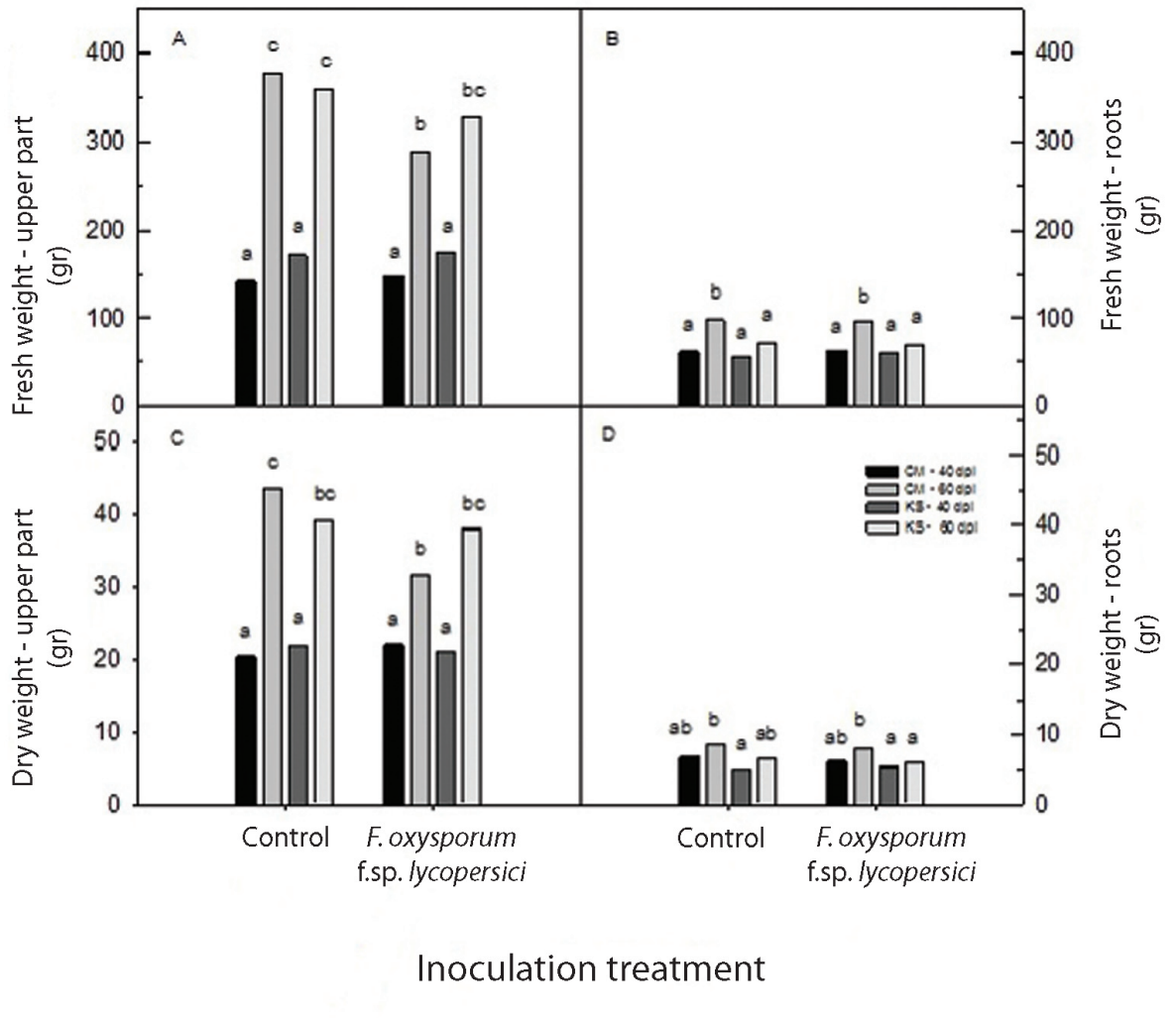

Figure 3. Fresh weight (FW) and dry weight (DW) of the aerial parts (A, C) and the root system (B, D) of 'Chondrokatsari Messinias' (A, B) and 'Katsari Santorinis' (C, D) tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici*. Data are means of six replicates. FW and DW were measured 40 and 60 dpi. Columns followed by different letters are statistically different according to the Duncan's Multiple Range test ($P = 0.05$).

Table 3. Effect of *Fusarium oxysporum* f. sp. *lycopersici* on the number of flowers, CO_2 assimilation (A_s), stomatal conductance (g_s) and transpiration (E) of tomato plants of vars 'Chondrokatsari Messinias' and 'Katsari Santorinis'. Data are means of six replicates and were recorded 16 and 27 dpi. Means followed by different letters are statistically different according to the Duncan's Multiple Range test ($P = 0.05$).

Variety	Treatment	Variables			
		Number of flowers	A_s (μmol CO_2/m²/s)	g_s (mmol /m²/s)	E (mmol /m²/s)
'Chondrokatsari Messinias'	Control	0.65 a	9.60 a	0.23 ab	2.58 ab
	F. oxysporum f. sp. *lycopersici*	1.68 a	8.84 a	0.21 a	2.36 a
'Katsari Santorinis'	Control	8.85 c	10.43 a	0.32 b	2.98 b
	F. oxysporum f. sp. *lycopersici*	5.00 b	9.89 a	0.30 b	3.32 b

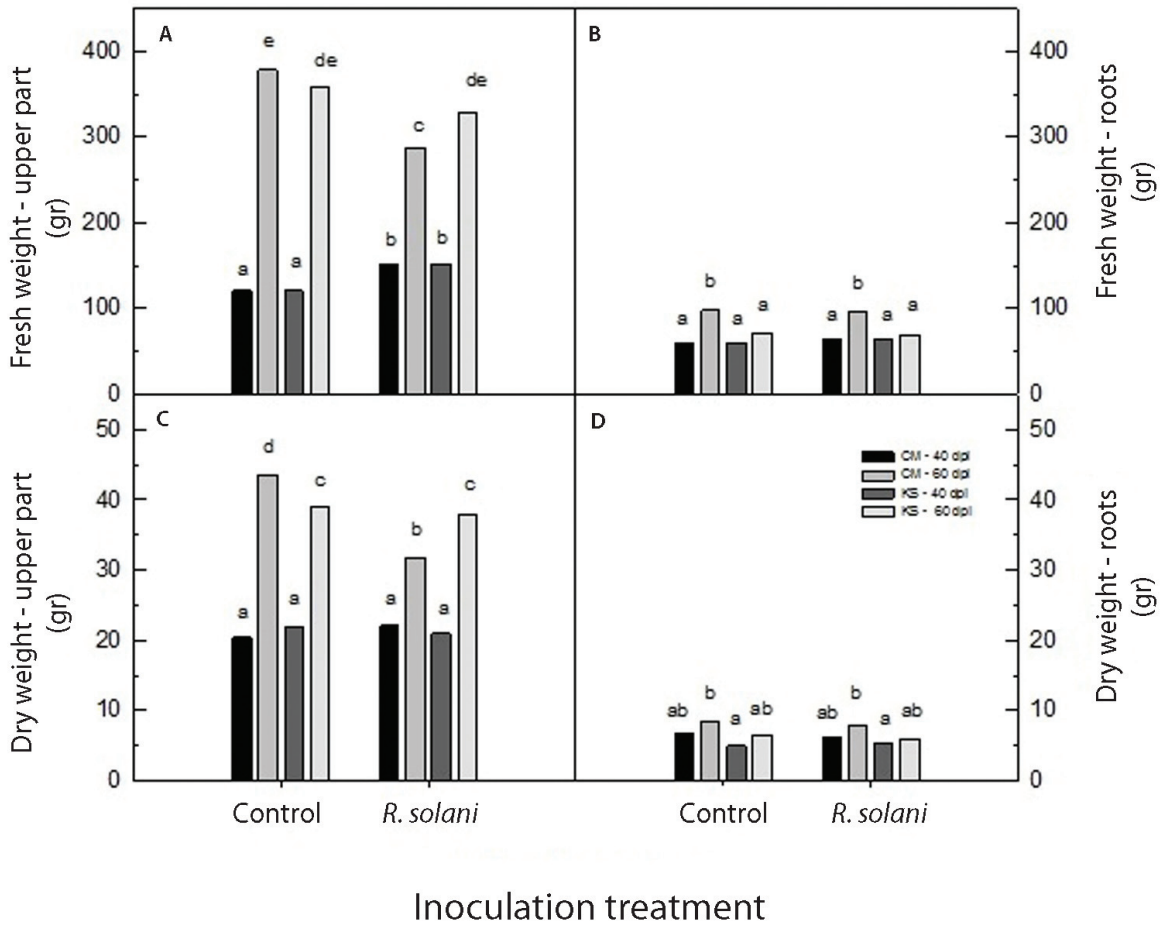

Figure 4. Fresh weight (FW) and dry weight (DW) of the aerial parts (A, C) and the root system (B, D) of 'Chondrokatsari Messinias' (A, B) and 'Katsari Santorinis' (C, D) tomato plants inoculated with *Rhizoctonia solani*. Data are means of six replicates. FW and DW were measured 40 and 60 dpi. Columns followed by different letters are statistically different according to the Duncan's Multiple Range test ($P = 0.05$).

Table 4. Effect of *Rhizoctonia solani* on the number of flowers, CO_2 assimilation (A_s), stomatal conductance (g_s) and transpiration (E) of tomato plants of vars 'Chondrokatsari Messinias' and 'Katsari Santorinis'. Data are means of six replicates and were recorded 16 and 27 dpi. Means followed by different letters are statistically different according to the Duncan's Multiple Range test ($P = 0.05$).

Variety	Treatment	Variables			
		Number of flowers	A_s (μmol CO_2/m²/s)	g_s (mmol/m²/s)	E (mmol/m²/s)
'Chondrokatsari Messinias'	Control	2.40 b	9.08 b	0.19 bc	2.77 b
	R. solani	1.55 a	7.80 a	0.15 a	2.30 a
'Katsari Santorinis'	Control	7.95 c	11.01 c	0.23 c	3.01 c
	R. solani	8.80 d	8.35 ab	0.18 ab	2.50 ab

Conclusions

The present study was the first attempt to investigate the reaction of two native Greek tomato varieties, 'Chondrokatsari Messinias' and 'Katsari Santorinis', to infection by *F. oxysporum* f. sp. *lycopersici* and *R. solani*. Results showed that both tomato varieties were susceptible to *F. oxysporum* f. sp. *lycopersici* and *R. solani* infection. However, 'Chondrokatsari Messinias' was found to be less susceptible to *F. oxysporum* f. sp. *lycopersici* compared to 'Katsari Santorinis'. Both of the pathogens negatively affected biomass production of var. 'Chondrokatsari Messinias' but not that of 'Katsari Santorinis'. The number of flowers produced by 'Chondrokatsari Messinias' was negatively affected by *R. solani* but not by *F. oxysporum* f. sp. *lycopersici*. Infection of both tomato varieties by *R. solani* also caused reduction in the CO_2 assimilation, stomatal conductance and transpiration.

Additional work is required on the interaction between the two native Greek tomato vars 'Chondrokatsari Messinias' and 'Katsari Santorinis' and the soil-borne fungi *F. oxysporum* f. sp. *lycopersici* and *R. solani* as well as on the management of the diseases caused by these pathogens.

The Benaki Phytopathological Institute is gratefully acknowledged for providing the strains of Fusarium oxysporum f. sp. lycopersici (BPIC2550) and Rhizoctonia solani (BPIC2531) used in the present study.

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Προσβολή φυτών τομάτας των Ελληνικών παραδοσιακών ποικιλιών 'Χοντροκατσαρή Μεσσηνίας' και 'Κατσαρή Σαντορίνης' από τους μύκητες *Fusarium oxysporum* f. sp. *lycopersici* και *Rhizoctonia solani*

Α.Ι. Δάρρας, Α. Κώτσιρας, Κ. Δελής, Κ. Νηφάκος, Ε. Παυλάκος και Β. Δημόπουλος

Περίληψη Τα φυτά συχνά πρέπει να αντιμετωπίσουν καταπονήσεις που οφείλονται σε βιοτικούς παράγοντες μεταξύ των οποίων είναι και οι ασθένειες. Η παρούσα μελέτη πραγματοποιήθηκε με σκοπό να εξετάσει την αντίδραση δύο Ελληνικών παραδοσιακών ποικιλιών τομάτας, της 'Χοντροκατσαρής Μεσσηνίας' και της 'Κατσαρής Σαντορίνης', στην προσβολή από τους μύκητες *Fusarium oxysporum* f. sp. *lycopersici* και *Rhizoctonia solani*. Καταγράφηκαν τα συμπτώματα και η ένταση κάθε ασθένειας και εκτιμήθηκε η επίδραση των προσβολών στην παραγωγή βιομάζας, στον αριθμό των ανθέων, στη φωτοσυνθετική δραστηριότητα, στη στοματική αγωγιμότητα και στη διαπνοή των φυτών. Και οι δύο ποικιλίες ήταν ευπαθείς στη μόλυνση από τους μύκητες *F. oxysporum* f. sp. *lycopersici* και *R. solani.* Εντούτοις η ποικ. 'Χοντροκατσαρή Μεσσηνίας' ήταν λιγότερο ευπαθής στην προσβολή από το μύκητα *F. oxysporum* f. sp. *lycopersici* σε σχέση με την 'Κατσαρή Σαντορίνης'. Και τα δύο παθογόνα επηρέασαν αρνητικά την παραγωγή βιομάζας των φυτών της ποικ. 'Χοντροκατσαρή Μεσσηνίας' αλλά όχι της ποικ. 'Κατσαρή Σαντορίνης'. Ο αριθμός των ανθέων της ποικ. 'Χοντροκατσαρή Μεσσηνίας' επηρεάστηκε αρνητικά από την προσβολή από το μύκητα *R. solani* αλλά όχι από τον *F. oxysporum* f. sp. *lycopersici*. Η προσβολή από το μύκητα *R. solani* είχε ως αποτέλεσμα τη μείωση της φωτοσυνθετικής δραστηριότητας, της στοματικής αγωγιμότητας και της διαπνοής των φυτών και των δύο ποικιλιών.

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SHORT COMMUNICATION

The pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) in Greece

P.G. Milonas* and G.K. Partsinevelos

Summary The invasive pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), is reported for first time in Greece. Individuals of the mealybug were found infesting *Hibiscus rosa-sinensis* (Linnaeus) (Malvaceae) in private and public gardens in the urban environment in Rhodes, Dodecanese islands, East Greece. This is the first report of genus *Maconellicoccus* in Greece.

Additional Keywords: hibiscus, invasive species, mealybug

Introduction

The genus *Maconellicoccus* Ezzat (Hemiptera: Pseudococcidae) includes eight species that are distributed in Australian, Oriental and Ethiopian regions but only *Maconellicoccus hirsutus* has been reported in Palaearctic region (García Morales *et al*., 2016). *Maconellicoccus hirsutus* Green, known as pink hibiscus mealybug, is a highly polyphagous species native to southern Asia, that feeds on 212 genera in 75 host plant families, including important crops such as bean (*Phaseolus vulgaris*), chrysanthemum (*Chrysanthemum* spp.), hibiscus (*Hibiscus* spp.), rose (*Rosa* spp.), pumpkin (*Cucurbita pepo*), avocado (*Persea americana*), citrus (*Citrus* spp.), coconut (*Cocos nucifera*), coffee (*Coffea* spp.), cotton (*Gossypium* spp.), corn (*Zea mays*), vegetables, grape (*Vitis vinifera*) and peanuts (*Arachis hypogaea*) (Chong *et al*., 2015; García Morales *et al*., 2016). For a complete list of *M. hirsutus* host plants see Chong *et al*. (2015).

Pink hibiscus mealybug is considered a highly invasive species. Although it is believed to originate from India, it has been accidentally introduced into other parts of the world, i.e. North America, the Caribbean and Africa. Overall *M. hirsutus* distribution includes 75 countries in all over the world (EPPO, 2005). In Europe, it was reported for first time in Cyprus in 2011 (EPPO, 2011). Upon its introduction into several countries, it has caused substantial economic damages through the cost of control operations and impact on trade. In the US, it has been estimated that without control, it may cause a damage of 163 million dollars only in Florida (Chong *et al*., 2015).

Adult females are 2.5-4 mm long, wingless, soft-bodied, elongate oval and flattened. Females can lay more than 500 eggs. Eggs are orange initially but turn into pink before hatching. Crawlers are 0.3 mm long, pink, oval in shape with well-defined legs and antennae, and lack the waxy body coating; young adult females turn greyish-pink, dusted with mealy white wax that covers their bodies; adult males are gnat-like 1 mm long, pink to orange, with a single pair of wings and two pairs of filaments. They are weak flyers, lack mouthparts and live only one day or two. Entire colony is covered by white, waxy ovisac material (Chong *et al*., 2015; García Morales *et al*., 2016). One generation is completed in approximately five weeks in warm conditions. In Jordan, nymphs have three peaks and adult females two peaks, in early February and mid-July, respectively (Al-Fwaeer *et al*., 2014). Here

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we report the first presence of *M. hirsutus* in Greece.

Feeding by larvae and adults causes characteristic symptoms on the damaged plants. During feeding both larvae and adults inject toxic saliva that causes curling and contortion of leaves. Infested plants become stunted, swollen with leaf curl, shortened internodes or malformed stems. Damage varies according to the susceptibility of each host species; in highly susceptible plants feeding can ultimately cause the death of the plant (EPPO, 2005; Vitullo *et al*., 2009; Hoy *et al*., 2014; García Morales *et al*., 2016).

Materials and Methods

Infested hibiscus plants were found on the island of Rhodes (36°26'1.49"N and 28°13'28.54"E) in September 2014 and samples were sent to BPI. New samples were sent to BPI collected from the Municipality of Rhodes coming from *Hibiscus* sp. (Malvaceae), *Ceratonia silique* (Fabaceae), *Erythrina* sp. (Fabaceae) and *Bauhinia* sp. (Fabaceae) in autumn of 2016. All of them are known host plants of *M. hirsutus*. Microscopic slides were prepared following the procedure described by Kosztarab and Kozár (1988) and identified according to description and illustration by Williams and Watson (1988). Specimens are deposited at the Biological Control Laboratory of BPI.

Material examined: Rhodes (Dodecanese islands); 02.ix.2014, 2 ♀♀, *Hibiscus* sp. (Malvaceae); Rhodes, 02.ix.2014 1 ♀♀ *Hibiscus* sp. (Malvaceae); Rhodes; 13.xii.2016, 2 ♀♀, *Hibiscus* sp (Malvaceae); Rhodes; 13.xii.2016, 1 ♀♀, *Hibiscus* sp. (Malvaceae).

Results and Discussion

This is the first record of the pink hibiscus mealybug, *M. hirsutus* in Greece. Reports from local authorities from the island of Rhodes had pointed out that infestation has expanded within the island during the years 2014-2016. No other outbreaks or records of *M. hirsutus* have been reported outside Rhodes. The specific pathway of introduction of the species into Rhodes is unknown. However, long distance dispersal of the pest is likely to occur through movement of infested plant material and to a lesser extend with infested fruits and cut flowers (EPPO 2005).

The introduction of the pink hibiscus mealybug in Rhodes without its natural enemies could pose a serious threat for several crops of high economic importance, such as vegetables, vineyards and ornamental plants in urban areas and in nurseries. Control efforts of the mealybug should principally focus on the identification and mapping of the actual infested area on the island of Rhodes in order to design an efficient management plan that would restrict further spread of the pest in the area. A sustainable solution should definitely include the careful introduction of its natural enemies following all the appropriate procedures for such an approach.

Because of the minimum tolerance level for *M. hirsutus* damage, intense management approaches are often required. Management tactics include monitoring, cultural, biological and chemical treatments. Observations for typical damage symptoms like bunchy top, honeydew and sooty-mold presence can help in the identification of infestation spots. The pheromone of *M. hirsutus* has been identified and can be used in pheromone traps for monitoring and detection especially in areas with low infestation density. Monitoring with pheromone traps is also useful for timing insecticide applications.

After its introduction into a new area *M. hirsutus* is usually difficult to eradicate due to its high reproductive ability and polyphagy. In areas where it has been established, long standing sustainable management has been provided by biological control. Especially, classical biological control attempts have been quite successful (Kairo *et al.*, 2000). Several natural enemies have been associated with *M. hirsutus*, including specialist and generalist parasitoids and predators. In classical biological control the

predatory species *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) and the parasitoids *Anagyrus kamali* Moursi and *Gyranusoidea indica* Shafee, Alam and Agarwal (Hymenoptea: Encyrtidae) are regarded as most commonly used biological control agents (Chong *et al*., 2015). The releases of the above parasitoids and predators have resulted in very successful control of *M. hirsutus* in Central America (Chong *et al*., 2015).

The use of contact insecticides may result in scarce control of the scale population due to the cryptic behaviour and the waxy covering of the mealybug bodies. Therefore, any applications should follow after careful monitoring for the presence of crawlers which are the most susceptible stage. Systemic insecticides might have a higher efficacy on reducing *M. hirsutus* populations. Nevertheless, application of insecticides should be avoided when biological control efforts are taking place. At present, no insecticide against *M. hirsutus* is registered in Greece.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) στην Ελλάδα

Π.Γ. Μυλωνάς και Γ.Κ. Παρτσινέβελος

Περίληψη Το είδος *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), καταγράφεται για πρώτη φορά στην Ελλάδα. Ενήλικα άτομα του ψευδόκοκκου συλλέχθηκαν σε φυτά ιβίσκου *Hibiscus rosa-sinensis* (Linnaeus) (Malvaceae) σε ιδιωτικούς και δημόσιους κήπους στο αστικό περιβάλλον, στη Ρόδο. Αυτή είναι η πρώτη καταγραφή του γένους *Maconellicoccus* στην Ελλάδα.

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SHORT COMMUNICATION

A catalogue of the Coleoptera of the G.P. Moazzo Collection in the Goulandris Natural History Museum. Part III (Scarabaeidae)

J. Tylianakis[1], M. Dimaki[2*] and V. Perdiou[2]

Summary This is a detailed list of 223 species (727 specimens) of the family Scarabaeidae, the subfamilies Dynastinae (21 species), Melolonthinae (11 species), Rutelinae (29 species), Aphodiinae (104 species), Cetoniinae (59 species), represented in G.P. Moazzo's collection at the Goulandris Natural History Museum. All label data for each specimen are given. The aim of this paper is to present this collection as reference data for comparison with recent entomological material.

Additional keywords: Scarabaeidae, Dynastinae, Melolonthinae, Rutelinae, Aphodiinae, Cetoniinae

Introduction

The Entomological Collection of the Goulandris Natural History Museum (GNHM) was established in 1973. One particularly significant collection is that of Georgi Polychronis Moazzo, which contains insects (Coleoptera in their majority) collected since 1910 (Goulandris, 1977). Moazzo's entomological collection of GNHM includes 5500 specimens. Among them there are 1312 beetle species belonging to 58 families (Dimaki and Tylianakis 2006; Tylianakis and Dimaki 2006, Dimaki *et al.,* 2016). This is part III of the aforementioned collection, part I containing the families Carabaeidae, Cicindelidae, and Dytiscidae (Dimaki and Tylianakis, 2006) and part II containing part of the Scarabaeidae family (Tylianakis and Dimaki, 2006).

The aim of this paper is to present the species of the Scarabaeidae subfamilies Dynastinae, Melolonthinae, Rutelinae, Aphodiinae and Cetoniinae kept in the entomological collection of GNHM (Moazzo's collection). The catalogue would be of particular interest for use by entomologists seeking for reference information regarding insect specimens of Scarabaeidae.

The material is of historical importance, with some specimens over 100 years old with a wide geographical distribution across 5 continents (Europe, Africa, Asia, America, Australia). It is published with the information given exactly as it appears on the individual labels. In some cases data may be missing such as collection date and exact locality. An effort has been made to provide the current nomenclature, where possible. The specimens are in a very good condition and their identification is still possible.

Materials and methods

The specimens were collected during fieldwork and have all been mounted on pins and arranged in unit trays within cabinet drawers at the GNHM. A determination label accompanies each specimen as well as a database number. This number corresponds to the information of each specimen as well as the current name. The material has been examined and described mainly by G.P. Moazzo.

This collection includes material from collectors such as A. Carneri, G. Louvet, B. Alpes, Talbiele, Petroff, Schuster, Winkler, McMaygiore, Efflatoun and G.P. Moazzo

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himself.

We followed the systematics of Ratcliffe and Jameson (2004) and Triplehorn and Johnson (2005).

Catalogue of Coleoptera, Scarabaeidae

In the list below, we record the species and the relevant metadata: the number of specimens (spm), the place of the collection, and the year and collector's name, when available.

Dynastinae (21 species, 105 spm)

Pentodon dispar (syn. *P. algerinum* sbsp. *dispar)* 23 spm, EGYPT: Alexandria, Victoria?, Mariout, Ramleh, Rond-Point, Rounleh, Abou el Chekark, 1917, 1921, 1924, 1929, 1935, 1938 leg. Efflatoun
Pentodon punctatus 1 spm, ITALY: Gerace Calabria, leg. Schuster
Pentodon idiota 1 spm, AUSTRIA: Neusiedi lake, leg. Schuster
Pentodon bispinosus 1spm, MOROCCO: Casablanca, 1919, leg. Antoine
Pentodon sp.1 spm, TURKESTAN: Samarkand
Pentodon sp.1 spm, TURKESTAN: Samarkand
Dyscinetus rugifron 2 spm, ARGENTINA: Buenos Aires
Pachononyx fuscodeneus 2 spm, ARGENTINA: Rio Salodo
Phyllognathus silenus 12 spm, FRANCE: Toulouse; TURKEY: Adana, Cilicie; SPAIN: Tenerife; EGYPT: Mariout, Ramleh, Palais; ALGERIA: Bou-Berak, 1921, 1924, 1935, 1953, leg.V.M. Duchon, Efflatoun, A. Chobaut, Thery, A. Carneri
Heteronychus parumpunctatus 16 spm, EGYPT: Alexandria, 1921, 1922, 1924, 1929
Heteronychus dilatatus 2 spm, ARGENTINA: Tucuman
Heteronychus spp. 3 spm, EGYPT: Mariout, 1933
Oryctomorphus maculicollis 1 spm, CHILE
Oryctomorphus sp. 2 spm, SOUDAN: FRANCE
Temnorrhynchus baal 21 spm, EGYPT: Alexandria, Victoria?, Mazarita, Mandara Cairo, Rond Point, Nousha, 1919, 1921, 1923, 1924, 1932, 1942, leg. A. Carneri
Phileurus valgus 4 spm, VENEZUELA
Scaptophilus dasipleurus 2 spm, ARGENTINA: Tucuman
Oryctes rhinoceros 2 spm, INDONESIA: Amboine, Iles Moluques, leg. A. Carneri
Oryctes spp. 5 spm, ARGENTINA: Tucuman
Oryctes sp. 2 spm, ITALY, 1950, leg. Gentile
Oryctes nasicornis 1 spm

Melolonthinae (11 species, 30 spm)

Europtron gracile 4 spm, ALGERIA: Ghardaia, leg. A. Chobaut
Haplidia nitidula 7 spm
Hoplia coerulea 1 spm, FRANCE: Prats de Mollo, leg. Waegner
Hoplia dubia 5 spm, ITALY: Macarese, Rome, 1919, leg. P. Crozet
Hoplia africanum 4 spm, MOROCCO: Casablanca, leg. Antoine
Hoplia farinosa 2 spm, AUSTRIA: Graz; FRANCE: Besançon
Hoplia philanthus 1 spm
Hoplia graminicola 1 spm
Hoplia praticola 1 spm, ALGERIA: Ain Sefra, 1923, leg. A. Chobaut
Hoplia pubicollis 1 spm, CORSICA: Ajaccio
Hoplia spp. 3 spm, ALGERIA: Bou-berak; MOROCCO: Casablanca, leg. A. Chobaut, Antoine

Rutelinae (29 species, 71 spm)

Anisoplia flavipennis 1 spm
Anisoplia segetum var. straminea (syn. *Chaetopteroplia segetum straminae*) 1 spm, TURKEY: Konya, 1899, leg. Korh
Anisoplia segetum (syn. *Chaetopteroplia segetum)* 1 spm, IRAN: Kopet-Dagh, Descht, 1902, leg. F. Hauser
Anisoplia pallidipennis 2 spm, ALGERIA: Ain Sefra, 1923, leg. A. Chobaut
Anisoplia cyathigera 1 spm, BOSNIA: Sanskimost
Anisoplia biguttata 1 spm, INDONESIA: Java Breanger Tjigembong, leg. Corporaai
Anisoplia lata 2 spm, AUSTRIA: Graz, CROATIA: Pola Istria
Anisoplia leucaspis 1 spm, IRAN: Kermanschah, 1909, leg. F. Hauser
Anisoplia agricola 1 spm, GERMANY: Offenbach
Anisoplia sp.1 spm, CROATIA: Velezgb, leg. Krauss

Anisoplia monticola 2 spm, CROATIA: Pola Istria
Anisoplia syriaca 1 spm, TURKEY: Ephesos
Anisoplia flavocincta 1 spm, ITALY: Emilia, 1897, leg. Fiori
Anisoplia graminivora (syn. *Anisoplia tempestiva*) 2 spm, FRANCE: Ste Baume, 1920, leg. A. Chobaut
Anisoplia sabulicola (syn. *Brancoplia pumila)* 2 spm, EGYPT: Saba Pacha, 1918
Anisoplia deserticola 4 spm, ALGERIA: Bou Saada, Ain Sefra, 1923, leg. A. Chobaut, Martin
Anisoplia floricola 4 spm, ALGERIA: Bou-Berak; MOROCCO: Casablanca; SPAIN: Valencia, leg. Antoine
Anisoplia austriaca 1 spm, Nd. Tesders?
Anisoplia sp. 2 spm, TURKEY: Marmara, leg. Thery
Anisoplia bromicola var. *nigra* 1 spm, ITALY: Cerchio
Anisoplia bromicola 1 spm, CROATIA: Velezgb, leg. Krauss
Anisoplia austriaca var. *major* 1 spm
Anisoplia tempestiva 1 spm, FRANCE: Ste Baume, 1920, leg. A. Chobaut
Anisoplia sabulicola 24 spm, EGYPT: Abou kir, Rond Point, Cherbine, Mariout, Smouha, Nouzha, Alexandria, Sidi Bishu 1919, 1920, 1938, 1940, 1943, 1944
Pelidnota paraguayensis 1 spm, ARGENTINA: Tucuman
Pelidnota sp. 1spm, SUDAN
Blitopertha horticola (syn. *Phyllopertha horticola)* 6 spm, FRANCE: Rouen; BOSNIA, 1921
Blitopertha horticola (var. *ustulatipennis*) 1 spm, AUSTRIA: Wien
Blitopertha arenaria (syn. *Blitopertha lineolata)* 1 spm, GREECE: Korinth
Blitopertha hirtella 1 spm, GREECE: Attica
Blitopertha lineolata 1 spm, IRAN: Dagh, 1902, leg. F. Hauser

Aphodiinae (103 species, 294 spm)
Aphodius contaetus 3 spm, EGYPT: Barages de Caire
Aphodius punctifer 1 spm
Aphodius sordidus (syn. *Bodilopsis rufus)* 1 spm, SWITZERLAND: Geneva
Aphodius punctipennis 4 spm, EGYPT: Mariout, Dekhela, Palais, 1919, 1921, 1923, leg. A. Carneri
Aphodius spp. 5 spm EGYPT: Palais, Alexandria, 1923, 1925, leg. A. Carneri
Aphodius (Bodolius) hydrochoeris 17 spm, EGYPT: Palais, Victoria?, Bulgley, 1919, 1923, leg. A. Carneri, Fleming, M. Gantes
Aphodius (Volinus) hieroglyphicus 10 spm, EGYPT: Mariout, Dekhela, Rond Point, 1921, 1923, 1925
Aphodius granarius (syn. *Calamosternus granarius)* 29 spm, EGYPT: Mariout, Dekhela, Rond Point, Cairo, Victoria?, Smouha, Sidi Bishr, Alexandria, ALGERIA: Bou-Berak; RUSSIA: Transcaspia Kisil Arwat, 1898,1920, 1921, 1923, 1939, 1944, leg. A. Chobaut, F. Hauser
Aphodius varians 9 spm, EGYPT: Mariout, Rond Point, Smouha 1920,1923, 1938,1939
Aphodius (Erytus) sp. 29 spm, EGYPT: Mariout, Dekhela, Alexandria, Saba Pacha Montaza; ALGERIA: Batra; RUSSIA: Transcaspia, 1900, 1919, 1920, 1921, 1922, 1925, leg. F. Hauser
Aphodius (Erytus) brunneus 3 spm, 1919, leg. A. Chobaut
Aphodius (Erytus) lividus (syn. *Labarrus lividus)* 5 spm, leg. Montaza, 1921, 1922
Aphodius (Erytus) leucopterus (syn. *Mecynodes leucopterus*) 4 spm, EGYPT: Mariout, 1925, 1944, leg. Garbaniat
Aphodius (Eremazus) sp. 3 spm
Aphodius (Eremazus) punctatus 2 spm
Aphodius (Eremazus) sp.1 spm, leg. A. Chobaut
Aphodius (Eremazus) unistriatus 2 spm, EGYPT: Mariout, leg. A. Chobaut
Aphodius thermicola 8 spm, ITALY; AUSTRIA; MOROCCO: Rabat, 1917, 1922, leg. A. Chobaut, P. Crozet
Aphodius luridus var. *nigripes* 6 spm, ITALY, 1917, leg. P. Crozet
Aphodius erraticus 6 spm, FRANCE: Cote D'Or; ALGERIA: Bou-Berak
Aphodius erraticus var. *fumigatus* 1 spm, ITALY: Cerchio
Aphodius subterraneus 6 spm, FRANCE: Rennes, Hungbor? 1920, leg. P. Crozet, V. Foufal
Aphodius bonnaizei (Type) 13 spm, FRANCE: Carrieres des Angles; ITALY: Valgares; TUNIS, 1922, leg. A. Chobaut

Aphodius merdarius 1 spm, PORTUGAL: Leiria Lusitania
Aphodius scrofa (syn. *Trichonotulus scrofa*) 1 spm, GERMANY?: Marburg, leg. Krauss
Aphodius pusillus 1 spm, SLOVENIA: Carniole, leg. Krauss
Aphodius maculatus 1 spm, GREECE: Nemea
Aphodius obscurus 1 spm, SWITZERLAND: Faulhorn
Aphodius obscurus var. *olichrous* 1 spm, AUSTRIA: Obir Carinthia
Aphodius guttatus (syn. *Agolinus guttatus)* 2 spm, ALGERIA: Bou-Berak, leg. A. Chobaut
Aphodius lineolatus (syn. *Chilothorax lineolatus*) 2 spm, SPAIN: Cadiz, ALGERIA: Bou-berak, leg. A. Chobaut
Aphodius spp. 9 spm, MOROCCO: Rabat, Taja; ALGERIA: Ougda Thery
Aphodius stricticus (syn. *Volinus stricticus)* 1 spm
Aphodius depressus 1 spm, AUSTRIA: Stiria bor., leg. Krauss
Aphodius distinctus (syn. *Chilothorax distinctus)* 3 spm, FRANCE: Les Angles; AUSTRIA: Umgeb. Graz, 1922, leg. A. Chobaut
Aphodius tessulatus 1 spm, GERMANY, 1922, leg. A. Chobaut
Aphodius obliteratus 1 spm, GERMANY: Reichenau island, leg. Ponecke
Aphodius prodromus 3 spm, ITALY: Valcares; PORTUGAL: Leiria Lusitania, 1922, leg. A. Chobaut
Aphodius punctatosulcatus 1 spm, SPAIN: Ronda
Aphodius punctatosulcatus var. *marginalis* 1 spm, SPAIN: Valencia
Aphodius limbatus 1 spm, AUSTRIA: Neusiedlersee
Aphodius maenlatus 1 spm, Valegb Hz., leg. Krauss
Aphodius zenkeri 2 spm, LIBERIA; GERMANY, leg. Grunwld
Aphodius miaetus 1 spm, ROMANIA: Paring, leg. Krauss
Aphodius miaetus var. *unicolor* 1 spm, ROMANIA: Paring, leg. Krauss
Aphodius praecose 1 spm, Penecke st. leg. H. Reichart
Aphodius satellitius 1 spm, CROATIA: Pola Istria
Aphodius rufipes 1 spm, AUSTRIA: Trifail Styria
Aphodius lividus 5 spm, ALGERIA: Beni Ounif de Figule, 1923, leg. A. Chobaut
Aphodius spp. 2 spm, GERMANY: Marburg; TURKEY: Adana
Aphodius depressus var. *atramentarius* (syn. *Aphodius atramentarius)* 1 spm, AUSTRIA: Koralpe, leg. Penecke
Aphodius licardi 1 spm, ALGERIA
Aphodius sp. 1 spm, TUNISIA
Aphodius vitellinus (syn. *Subrinus vitellinus)* 2 spm, MOROCCO: Casablanca, Rabat, leg. Antoine
Aphodius unicolor (=eastaneus) 4 spm, ALGERIA: Bou-Berak, leg. A. Chobaut
Aphodius (Bodilus) longispina 1 spm, MOROCCO: Casablanca, 1920, leg. Antoine
Aphodius serutator (syn. *Teuchestes serutator)* 3 spm, ITALY: Maggiore, leg. P. Crozet
Aphodius fossor 1 spm, AUSTRIA: Graz, leg. Krauss
Aphodius brevis 1 spm, AUSTRIA: Neusiedler
Aphodius conjugatus 1 spm, ROMANIA: Klausenberg
Aphodius (Otophorus) haemorrhoidolis 1 spm
Aphodius (Otophorus) sanguinolentus (syn. Aphodius (Otophorus) haemorrhoidolis) 4 spm, Marrier de la Mer; TURKEY: Kizil-Dagh, Taurus, leg. A. Chobaut
Aphodius fimetarius 1 spm, ALGERIA: Bou-Berak
Aphodius foetens 3 spm, BOSNIA; CYPRUS
Aphodius scybalarius var. *conflagratus* 1 spm, TUNIS: Bel, 1900
Aphodius (Bodilus) ictericus (=nitidulus) 6 spm, FRANCE: Le Cailar, CROATIA: Sebenico, 1923
Aphodius hydrochoeris 2 spm, ALGERIA: Ghardaia; MOROCCO: Tanger
Aphodius sordidus 1 spm, FRANCE
Aphodius rufus 1 spm, HUNGARY
Aphodius rufus var. *arcuatus* 1 spm, ITALY: Mte Baldo
Aphodius lugeus 1 spm, GREECE: Nemea
Aphodius immundus 1 spm, GERMANY: Marburg, leg. Krauss
Aphodius ater 1 spm
Aphodius piceus 1spm, FINLAND
Aphodius gibbus 1 spm

Aphodius nemoralis 1 spm, AUSTRIA: Rekawinkel
Aphodius putridus 1spm
Aphodius alpinus 1 spm, SWITZERLAND Faulhorn
Aphodius plagiatus var. *immaculatus* 1 spm, ROMANIA: Mezo-Zah
Aphodius sturmi 1 spm, AUSTRIA: Umgeb. Zara, leg. Muller
*Aphodius niger*1 spm, AUSTRIA: Neusiedler, leg. Krauss
Aphodius lapponum 1 spm
Aphodius corvinus 1 spm, AUSTRIA: Umgeb. Graz
Aphodius varians (=*ambiguus)* 2 spm BOSNIA, GREECE: Korfu
Aphodius tristis 1 spm GERMANY
Aphodius biguttatus 1 spm
Aphodius cribricollis 1 spm, TUNIS: Le kef
Aphodius (Amidorus) cribarius 1 spm, CROATIA: Kistange, leg. D. Muller
Aphodius contaminatus 1 spm,, TURKEY
Aphodius tingens 3 spm, ALGERIA: Bou-Berak, Philippevile
Aphodius consputus 1 spm, TURKEY
Aphodius affinis 1 spm, TURKEY: Adana
Aphodius kraatzi (syn. *Liothorax kraatzi)* 1 spm, KAZAKHSTAN: Thian – Shan, Musart
Aphodius rhododactylus 1 spm, CROATIA: Velezgb, leg. Krauss
Aphodius sp. 2 spm
Aphodius lividus 1 spm
Enpleureus subterraneus 2 spm, Marrier de la Mer, leg. A. Chobaut
Euheptaulacus sus 1 spm, GERMANY
Heptaulacus alpinus (syn. *Oromus alpinus)* 1 spm, CROATIA: Brokovo
Heptaulacus porcellus 1 spm AUSTRIA: Wien
Psammobius porcicollis 1 spm, HUNGARY
*Pleurophorus baebus*1 spm, ALGERIA: Bou-Berak
Pleurophorus caesus 1 spm, Zoppa Tr.
Aegialia sabuleti 1 spm
Aegialia arenaria 1 spm, GERMANY: Borkum, leg. Schneider
Rhyssemus germanus 1 spm, EGYPT: Kabushia leg. Alfieri
Rhyssemodes reilleri 1 spm, ALGERIA: Ghardaia, leg. A. Chobaut

Cetoniinae (59 species, 227 spm)
Stalagmosoma abbela 1 spm, Wadioff, 1927, leg. Petroff
Tropinota squalida 37 spm, EGYPT: Saba Pacha, Alexandria, Mex, Mariout, Siouf, Avignon, St. Genes, Sidi Bishr, Mandara, 1918, 1923, 1924, 1934, 1935, 1936, 1943, 1944, 1949, leg. A. Chobaut
Tropinota hirta (syn. *Epicometis hirta*) 2 spm, St Gemes de lo Molus; GREECE: Rhodus, 1924, leg. Plason
Lasiotrichius succinctus 4 spm, EGYPT: Montaza Dekhela, Mandara, Agami, 1919, 1927, 1938, 1940
Oxythyrea abigail 22 spm, EGYPT: Avignon, TURKEY: Istanbul, SLOVENIA: Carniolia, GERMANY: Wippach, FRANCE: St. Genie's de Co., Rouen, 1924
Oxythyrea funesta 9 spm, MOROCCO: Casablanca, 1921, leg. Antoine
Oxythyrea funesta var. Wagner 3 spm, UNITED ARAB EMIRATES: Wadi, SWITZERLAND: Stadt?, TURKEY: Istanbul, 1927
Oxythyrea cinctella 3 spm, SOUDAN, MOROCCO: Casablanca, 1918, leg. Antoine
Oxythyrea spp. 3 spm, FRANCE: Tonkin, AUSTRIA: Wien , leg. H Fruhsturfer
Trichius bifasciatus 5 spm
Trichius bifasciatus var. *dubius* 1 spm, TURKESTAN: Djarkent Semirjetschensk, leg. Winkler
Trichius bifasciatus var. *vulgaris* 1 spm, AUSTRIA: Dobratsch
Trichius bifasciatus var. *succinctus* 1 spm, IRAN: Astrabad=Gorgan, 1899, leg. Hauser
Trichius abdominalis 1 spm, SLOVENIA: Wochein Carniolia
Trichius sexualis 1 spm, FRANCE: Rouen
Trichius gallicus 1 spm, USA: Pennsylvania
Trichius affinis 2 spm, ALGERIA: Bou-Berak leg. D. Chobaut
Trichius zonatus var. *fortunatarum* 2 s p m , FRANCE: St. Saurent Htes Pyrenees
Gnorimus nobilis 2 spm, AUSTRIA, leg. Schuster
Gnorimus variabilis 1 spm, CHILE
Euphoria lurida 2 spm, MEXICO
Euphoria bascalis 1 spm, MADAGASCAR: Tananarive, ERITREA, 1918, leg. A. Mochi
Gnorimus variabilis 16 spm, AUSTRIA: Prze-

mysl Galizieu, FRANCE: Rouen, ITALY, leg. Vogel
Valgus hemipterus 4 spm
Osmoderma eremita 1 spm, CANADA: Joliette, 1906
Osmoderma scabra 2 spm, ARGENTINA: Tucuman
Diplognatha gagates 1 spm, SRI LANKA: south, 1889, leg. H Fruhsturfer
Glycyphana versicolor var. *luduosa* 2 spm, East Africa, leg. A. Heyne
Smaragdesthes viridis (syn. *Smaragdesthes africana viridis)* 1 spm, VIETNAM: Saigon
Protaetia sp. 2 spm, east Africa
Gnathocera cruda 2 spm, MADAGASCAR
Euryomia argentea 2 spm, GREECE: Vrilissia, ITALY: Ragusa, 1958, leg. Schuster
Potosia speciosissima (=*aeruginosa*) 2 spm, SLOVENIA: Carniolia GERMANY: Wippach
Potosia affinis 1 spm, TURKESTAN: Djarkent Semirjetschensk, leg. Winkler
Potosia aurata var. *viridiventris* 1 spm, SYRIA
Potosia chrysosoma 1 spm, IRAN: Kopet-Dag
Potosia aeratula 1 spm, FRANCE: Bonifacio
Potosia carthami (syn. *Cetonia carthami*) 2 spm, MOROCCO: Casablanca, leg. Antoine
Aethiessa floralis var. *barbara* 2 spm, TURKEY: Istanbul, leg. F. Charles, Yervanh
Potosia hungariga 4 spm, Caucasus, TURKEY: Istanbul, leg. Yervanh
Potosia hungariga var. *armeniaca* 3 spm, ARMENIA, MOROCCO, leg. Chobaut, Thery
Aethiessa floralis 4 spm, EGYPT: Alexandria, Rond-Point, 1919
Aethiessa inhumata 1 spm, EGYPT: Nouzha, Alexandria, TURKEY: Taurus, 1920, 1922, 1927
Potosia cuprea 1 spm, KYRGHYZSTAN: Ketmen Tjube, TURKEY: Sussamyr
Potosia cuprea ssp. *ignicollis* 9 spm, TURKEY: Taurus
Potosia floricola var. *phoebe* (syn. *Potosia phoebe*) 1 spm, TURKEY: Istanbul, leg. F. Charles
Potosia floricola (syn. *Cetonia floricola*) 5 spm, EGYPT: Avignon
Potosia floricola var. *marginicollis* 1 spm, ITALY
Potosia floricola var. *incerta* 3 spm, CHINA: Kalgan
Potosia floricola var. *daurica* 1 spm
Potosia floricola var. *metallica* 1 spm, CROATIA: Pola Istria, 1920
Potosia floricola var. *obscura* 2 spm, ITALY: Rome, GREECE: Itea, 1918
Potosia floricola var. *florentine* 3 spm, IRAN: Kopet-Dagh
Potosia floricola var. *hieroglyphica* 1 spm
Potosia angustata 1 spm, SYRIA: Damascus, leg. Schuster
Potosia afflicta 2 spm, FRANCE: St Lucie, leg. Schuster
Potosia oblonga 1 spm, TURKEY: Istanbul, leg. Chobaut, F. Charles
Potosia vidua 4 spm, SYRIA: Aleppo, FRANCE: Bouchat (Drome), leg. Winkler, Chobaut
Potosia opaca 2 spm, Bumfacio, ITALY: Rome, leg. Chobaut
Potosia morio 4 spm, MOROCCO, 1921, leg. Antoine, Thery
Potosia morio var. *punctate* 2 spm, NIGIRIA: east, leg. E.V. Bodemeyer
Potosia funeraria 3 spm, FRANCE: Bouchat (Drome), leg. Antoine
Potosia cardui 2 spm, (syn. *Potosia opaca*), EGYPT: Avignon, leg. Chobaut
Potosia marmorata (liocala) 1 spm, ITALY: Sicily, leg. Schuster
Aethiessa inhumata 2 spm, ARGENTINA: Tucuman
Stephanorrhina guttata 2 spm, GREECE: Parnitha, 1921, leg. Sophrone, Schuster
Diplognatha gagates 2 spm, AUSTRALIA: Freshwater Queensland
Celidota stephensis 2 spm, KENYA: Nairobi
Pachnoda fasciata 7 spm, ZAMBIA: Katona, 1909
Pachnoda petersi 2 spm, ERITREA

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Καταγραφή ειδών Κολεοπτέρων εντόμων της Συλλογής G.P. Moazzo του Μουσείου Γουλανδρή Φυσικής Ιστορίας. Μέρος III (Scarabaeidae)

J. Tylianakis, Μ. Δημάκη και Β. Περδίου

Περίληψη Σε αυτήν την εργασία προυσιάζεται ο κατάλογος 223 ειδών (727 δείγματα) της οικογένειας Scarabaeidae των υποοικογενειών Dynastinae (21 είδη), Melolonthinae (11 είδη), Rutelinae (29 είδη), Aphodiinae (104 είδη) και Cetoniinae (59 είδη), της Συλλογής του G.P. Moazzo του Μουσείου Γουλανδρή Φυσικής Ιστορίας. Παρέχονται όλες οι πληροφορίες των δειγμάτων που υπάρχουν. Σκοπός αυτής της εργασίας είναι η παρουσίαση της εντομολογικής συλλογής ως βάση αναφοράς για συγκριτικές μελέτες με σύγχρονο εντομολογικό υλικό.

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