Volume 9, Issue 1, January 2016
ISSN 1791-3691

Hellenic Plant Protection Journal

A semiannual scientific publication of the
BENAKI PHYTOPATHOLOGICAL INSTITUTE

Editorial Policy

The *Hellenic Plant Protection Journal (*HPPJ) (ISSN 1791-3691) is the scientific publication of the Benaki Phytopathological Institute (BPI) replacing the *Annals of the Benaki Phytopathological Institute* (ISSN 1790-1480) which had been published since 1935. Starting from January 2008, the *Hellenic Plant Protection Journal* is published semiannually, in January and July each year.

HPPJ publishes scientific work on all aspects of plant health and plant protection referring to plant pathogens, pests, weeds, pesticides and relevant environmental and safety issues. In addition, the topics of the journal extend to aspects related to pests of public health in agricultural and urban areas.

Papers submitted for publication can be either in the form of a complete research article or in the form of a sufficiently documented short communication (including new records). Only original articles which have not been published or submitted for publication elsewhere are considered for publication in the journal. Review articles in related topics, either submitted or invited by the Editorial Board, are also published, normally one article per issue. Upon publication all articles are copyrighted by the BPI.

Manuscripts should be prepared according to instructions available to authors and submitted in electronic form on line at http://www.hppj.gr. All submitted manuscripts are considered and published after successful completion of a review procedure by two competent referees.

The content of the articles published in HPPJ reflects the view and the official position of the authors. The information and opinions contained herein have not been adopted or approved by the HPPJ Editorial Board. The HPPJ Editorial Board neither guarantees the accuracy of the information included in the published articles nor may be held responsible for the use to which information contained herein may be put.

For all parties involved in the act of publishing (the author(s), the journal editor(s), the peer reviewers, and the publisher) it is necessary to agree upon standards of expected ethical behavior. HPPJ follows the ethics statements of De Gruyter journals, which are based on the Committee on Publication Ethics (COPE) Code of Conduct guidelines available at www.publicationethics.org.

For back issues, exchange agreements and other publications of the Institute contact the Library, Benaki Phytopathological Institute, 8 St. Delta Str., GR-145 61 Kifissia, Attica, Greece, e-mail: m.kitsiou@bpi.gr.

This Journal is indexed by: *AGRICOLA, CAB Abstracts*-Plant Protection Database, *INIST* (Institute for Scientific and Technical Information) and *SCOPUS*.

The olive tree of Plato in Athens is the emblem of the Benaki Phytopathological Institute

DE GRUYTER
OPEN

Hellenic Plant Protection Journal **9:** 1-15, 2016
DOI 10.1515/hppj-2016-0001

REVIEW ARTICLE

The role of silicon (Si) in increasing plant resistance against fungal diseases

N. Sakr

Summary The use of silicon (Si) in agriculture has attracted a great deal of interest from researchers because of the numerous benefits of this element to plants. The use of silicon has decreased the intensity of several diseases in crops of great economic importance. In this study, the relationship between silicon nutrition and fungal disease development in plants was reviewed. The current review underlines the agricultural importance of silicon in crops, the potential for controlling fungal plant pathogens by silicon treatment, the different mechanisms of silicon-enhanced resistance, and the inhibitory effects of silicon on plant pathogenic fungi *in vitro*. By combining the data presented in this paper, a better comprehension of the relationship between silicon treatments, increasing plant resistance, and decreasing severity of fungal diseases could be achieved.

Additional keywords: pathogenic fungi, severity of fungal disease, silicon treatment

1. Introduction

Diseases caused by different fungal pathogens are among the major constraints of plant production (Semal, 1989). The use of resistant varieties/rootstocks and fungicides are therefore the simplest and most effective methods to reduce the severity of fungal diseases (Dubin and Rajaram, 1996; Shephard, 1997). However, resistance is overcome by the genetic diversity of fungal pathogens as well as by genotype × environmental interactions (Bayles *et al.,* 2000). Repeated fungicide treatments generate important economic losses, emergence of resistant pathogen populations, and potential environmental impacts (Ma and Michailides, 2005). Therefore, alternative environment-friendly methods for the management of fungal plant pathogens remain to be urgently investigated. Soil fertilizers with nutritional elements were shown to have disease suppressing effects on various pathosystems (Datnoff *et al.,* 2007). In fact, the application of silicon (Si) has been proposed as a viable alternative to conventional control techniques. Silicon can improve environmental stress tolerance and increase crop productivity (Ma and Yamaji, 2006; Datnofft *et al.,* 2007). Moreover, silicon application is a preventive measure against a number of fungal diseases (Fauteux *et al.,* 2005; Datnoff *et al.,* 2007; Van Bockhaven *et al.,* 2013).

In the literature, two hypotheses for silicon-enhanced resistance to fungal diseases have been proposed (Fauteux *et al.,* 2005; Datnoff *et al.,* 2007; Van Bockhaven *et al.,* 2013). The first one is associated with the higher deposit of silicon in the leaf so as to form physical barrier to impede pathogen penetration. The second one is related to its biologically active role in the expression of natural defense mechanisms. However, the first mechanism (physical defense) may partly explain the prophylactic effects of silicon, the second one (biochemical defense) is more accepted for explaining the protective role of silicon against many plant pathogens (Fauteux *et al.,* 2005; Datnoff *et al.,* 2007).

To date, several studies have documented the ability of silicon to control and reduce the incidence and severity of fungal diseases in both monocotyledons and dicotyle-

Department of Agriculture, A.E.C., P.O. Box 6091, Damascus, Syria
e-mail: ascientific@aec.org.sy

dons (Table 1). The exact nature of protective effects of silicon in plants is uncertain and presently a subject of debate (Van Bockhaven *et al.,* 2013). However, functions including physical and/or biochemical protection systems have been proposed (Datnoff *et al.,* 2007). The aims of this review are to underline the agronomic importance of silicon in plant crops, to present the potential for controlling fungal plant pathogens by silicon treatment, to refer to different mechanisms of silicon-enhanced resistance, and to explain the inhibitory effects of silicon on phytopathogenic fungi *in vitro*.

2. Silicon and plants

Although silicon has not been considered as an essential element for plant nutrition, according to the classical definition of essentiality (Arnon and Stout, 1939), it is regard-

Table 1. Pathosystems on which the role of silicon in reducing the fungal disease incidence has been studied.

Host plant	Fungal pathogen	Reference
Barley	*Alternaria* spp.	Kunoh and Ishiazaki (1975)
Wheat	*Septoria nodorum* *Erysiphe graminis* *Blumeria graminis* f. sp. *tritici*	Leusch and Buchenauer (1989) Leusch and Buchenauer (1989) Guevel *et al*. (2007)
Rice	*Pyricularia oryzae* *Bipolaris oryza* *Magnaporthe grisea* *Rhizoctonia solani*	Domiciano *et al*. (2015) Dallagnol *et al*. (2011) Rodrigues *et al*. (2005) Zhang *et al.* (2013)
Corn	*Pythium aphanidermatum* *Fusarium graminearum* *Fusarium moniliforme*	Sun *et al*. (1994) Sun *et al.* (1994) Sun *et al*. (1994)
Banana	*Mycospaerella fijiensis* *Fusarium oxysporum* f. sp. *cubense*	Kablan *et al*. (2012) Fortunato *et al*. (2012)
Pearl millet	*Sclerospora graminicola*	Deepak *et al*. (2008)
Arabidopsis	*Erysiphe cichoracearum*	Ghanmi *et al*. (2004)
Rose	*Diplocarpon rosae* *Podosphaera pannosa*	Gillman *et al.* (2003) Shetty *et al.* (2012)
Common bean	*Colletotrichum lindemuthianum*	Polanco *et al.* (2014)
Soya bean	*Phakospora pachyrhizi*	Arsenault-Labrecque *et al*. (2012)
Bean	*Pseudocercospora griseola*	Rodrigues *et al*. (2010)
Pea	*Mycospaerella pinodes*	Dann and Muir (2002)
Strawberry	*Sphaerotheca aplanis*	Kanto *et al. (2006)*
Cherry	*Penicillium expansum* *Monilinia fructicola*	Qin and Tian (2005) Qin and Tian (2005)
Potato	*Fusarium sulphureum*	Li *et al*. (2009)
Belle pepper	*Phytophthora capsici*	French-Monar et al. (2010)
Tomato	*Pythium aphanidermatum* *Fusarium oxysporum* f. sp. *radicis-lycopersici*	Heine *et al.* (2007) Huang *et al.* (2011)
Cucumber	*Pythium ultimum* *Pythium aphanidermatum* *Sphaerotheca fuliginea* *Podosphaera xanthii* *Colletotrichum lagenarium*	Cherif *et al.* (1992) Cherif *et al*.,(1992) Menzies *et al*. (1991) Fawe *et al*. (1998) Liang *et al*. (2005)
Bitter melon	*Pythium aphanidermatum*	Heine *et al*. (2007)

ed as one of the most beneficial elements that increases plant resistance against abiotic and biotic stresses. However, the mechanisms responsible for alleviating biotic and abiotic stresses remain unclear because they may act in the soil, at the root surface and *in planta* (roots and shoots) (Liang *et al.,* 2007; Van Bockhaven *et al.,* 2013).

2.1. Absorption of silicon by plants

Silicon is as important as phosphorus and magnesium (0.03%) in the biota (Exley, 1998). It is the second most abundant element on the earth's crust after oxygen. It comprises up to 70% of the soil mass in the form of minerals and water-soluble monosilicic acid (H_4SiO_4) (Lowenstam, 1981). In soil solution, silicon occurs mainly as monosilicic acid in concentrations between 0.1 and 0.6 mM (Savant *et al.,* 1997).

Silicon is taken up by plant roots as noncharged monosilicic acid (Ma and Yamaji, 2006), when pH of the soil solution is below 9 (Ma and Takahashi, 2002). Monosilicic acid uptake is passive and largely determined by transpiration rate (Datnoff *et al.,* 2007). Once it reaches a concentration of around 2 mM, monosilicic acid is polymerized into insoluble silica, known as species-specific solid bodies (phytoliths) (Mitani *et al.,* 2005). It is deposited in cell walls, intercellular spaces and as a subcuticular layer outside the cells of leaves (Datnoff *et al.,* 2007). Moreover, silicon accumulates in higher amounts in mature leaves than in young ones (Ma and Takahashi, 2002). Plants absorb a significant fraction of dissolved silicon that originates from litterfall decomposition i.e phytolith dissolution (Datnoff *et al.,* 2007). The concentration level of absorbed silicon in plants ranges from 0.1 to 10% dry weight, depending on the plant genotype, the concentration of silicon in soil and the environmental conditions (Ma and Yamaji, 2006).

2.2. Agronomic importance of silicon in plant crops

Silicon is reported to increase and enhance yield, growth and production of plants. It improves some morphological and mechanical characteristics (height, stature, root penetration into the soil, exposure of leaves to light, resistance to lodging) in several plant species. Silicon reduces transpiration and enhances plant resistance to drought stress, salinity and metal toxicity, and increases enzyme activity (Datnofft *et al.,* 2007). On the other hand, regarding biotic stresses, the accumulation of silicon in plant plays an important role in plant defense against insect herbivores. Several herbivorus insects suffer adverse effects when feeding on silica-rich plants (Reynolds *et al.,* 2009). Moreover, silicon has been shown to improve resistance in many plants to various fungal, viral and bacterial pathogens (Rodriges and Datnoff, 2005; Silva *et al.,* 2010; Zellner *et al.,* 2011; Van Bockhaven *et al.,* 2013). Most interesting, silicon protects plants against a multitude of stresses without the occurrence of resistance trade-offs and/or growth and yield penalties (Fauteux *et al*., 2005; Ma and Yamaji, 2006; Epstein, 2009; Van Bockhaven *et al.,* 2013).

2.3. Accumulation of silicon in plant species

In the absence of abiotic and/or biotic stresses, silicon was believed to have a negligible effect on metabolism of healthy plants, which suggests its nonessential role (Epstein, 2009). However, silicon nutrition promoted agronomic yields of unstressed crops such as rice, as demonstrated by Rodriges and Datnoff (2005). According to Ma and Yamaji's (2006) agricultural point of view, silicon uptake in graminaceous plants, such as wheat, oat, rye, barley, sorghum, maize, and sugarcane, was much higher than its uptake in other plant species. One typical example was rice, which absorbed 150-300 kg Si/ha. High accumulation of silicon in rice has been demonstrated to be necessary for healthy plant growth, and high and stable production (Snyder *et al.,* 2006). Moreover, graminaceous plants absorb silicon at concentration levels equal to or greater than some of the essential nutrients like N and K (Savant *et al*., 1997). In rice, for example, silicon accumulation was about 108% greater than

nitrogen (Rodriges and Datnoff, 2005). The majority of dicotyledonous plants, such as cucumbers, melons, strawberries, and soybeans, absorb silicon inertly (Ma and Yamaji, 2006). Nonetheless, some plants, especially dicotyledons, such as tomatoes, beans, and other plant species, are not able to absorb silicon from soil (Ma and Yamaji, 2006). The Si/Ca ratio is another criterion used to determine whether a plant species is classified as a silicon absorber (Datnoff *et al.,* 2007).

3. Silicon controls fungal plant pathogens

In plant species, the association between silicon and reduced severity of fungal diseases has been documented in several studies. Another interesting association is the seemingly stronger efficacy of silicon against biotrophic and hemibiotrophic pathogens (e.g. rice blast, powdery mildews) compared to necrotrophs (Belanger *et al.,* 2014).

Adding silicon to plants as a fertilizer makes them more resistant to various pathogenic fungi (Datnoff *et al.,* 2007). There are several silicon fertilizers (solid and liquid sources) that could be used for agronomic purposes (Heckman, 2013; Datnoff and Heckman, 2014). To be beneficial for plants, silicon fertilizers should provide a high percentage of silicon in a soluble form, be cost effective, have physical properties that will facilitate storage ability, ease their application, be uncontaminated with heavy metals, and perhaps have the ability to raise soil pH (Heckman, 2013; Datnoff and Heckman, 2014). Calcium silicate ($CaSiO_3$) incorporated into soil has been used successfully as a solid source. Liquid sources, which are primarily used as a foliar spray, include potassium silicate (K_2SiO_3) or sodium silicate (Na_2SiO_3). Silicon has proved effective in controlling both soil- and air-borne fungal diseases in several plant crops.

3.1. Air- and soil- borne fungi

Numerous studies have shown increased plant resistance to foliar fungal pathogens as a response to silicon application. For example, soil treatments with silicon-rich materials reduced the incidence of diseases caused by *Erysiphe graminis* (powdery mildew) and *Septoria nodorum (*leaf and glume blotch*)* on wheat, as reported by Leusch and Buchenauer (1989). Foliar sprays of potassium silicate at concentrations ≥17 mM effectively reduced the number of powdery mildew (*Sphaerotheca fuliginea on* cucumber and muskmelon*; Erysiphe cichoracearum on* zucchini squash*)* colonies on leaves (Menzies *et al.,* 1992). Bowen *et al.* (1992) also reported that foliar sprays of potassium silicate at 1.7 mM reduced the number of powdery mildew (*Uncinula necator*) colonies on grape leaves by more than 60%. Soil silicon fertilization at 100 mg/l appeared to increase wheat resistance to *Blumeria graminis* (powdery mildew), *Mycosphaerella graminicola* (septoria leaf blotch), *Phaeosphaeria nodorum* (leaf spot), and *Puccinia recondita* (brown rust) only under high disease pressure (Rodgers-Gray and Shaw, 2004). A 40% reduction in the incidence of neck blast (*Pyricularia oryzae*) on rice plants supplied with silicon was reported by Seebold *et al.* (2004). Foliar application of 1% sodium metasilicate solution to sweet cherry reduced blue mold decay (*Penicillium expansum*) by 63% and brown rot decay (*Monilinia fructicola*) by 87% (Qin and Tian, 2005). The root application of silicon reduced powdery mildew severity on cucumber (Liang *et al.,* 2005). Potassium silicate application to soil reduced strawberry powdery mildew (*Sphaerotheca aphanis*) by 86% in the first year and by 60% in the second year (Kanto *et al.,* 2006). In the *Colletotrichum lindemuthianum*-bean pathosystem, silicon reduced both the area under the anthracnose incidence progress curve and the area under the anthracnose severity progress curve (Moraes *et al.,* 2006). Root applications of 1.7 mM Si reduced the severity of powdery mildew disease (*Blumeria graminis* f. sp. *tritici*) on wheat by as much as 80% (Guevel *et al.,* 2007). Guo *et al.* (2007) reported that sodium silicate reduced significantly the severity of post-harvest pink rot of Chinese cantaloupe caused by *Trichoth-*

ecium roseum. Potassium silicate solutions (pH 5.5 and 10.5) applied to bean plants reduced the intensity of angular leaf spot (*Pseudocercospora griseola*) by 42 and 30%, respectively (Rodrigues *et al.,* 2010). Supply of silicon to wheat plants reduced the severity of spot blotch caused by *Bipolaris sorokiniana* (Domiciano *et al.,* 2010). Regarding the wheat–*Pyricularia oryzae* interaction, Xavier *et al.* (2011) demonstrated that supply of silicon to plants decreased the area under the blast progress curve and the number of lesions per cm^2 of leaf area. Application of 2mM silicon solution decreased the area under brown spot progress curve and the number of brown epidermal cells caused by *Bipolaris oryzae* on rice plants (Dallagnol *et al.,* 2011). Kablan *et al.* (2012) showed that sodium metasilicate added to banana plants at a concentration of 1.7 mM reduced the severity of the disease caused by *Mycosphaerella fijiensis* (black sigatoka). Soybean plants supplied with soluble silicon exhibited a near absence of symptoms of Asian rust caused by *Phakopsora pachyrhizi* (Arsenault-Labrecque *et al.,* 2012). The efficacy of silicon applied at 0.5 and 1.0 l/ha in controlling apple scab (*Venturia inaequalis*) on leaves and fruit ranged from 67 to 81% and from 78 to 80%, respectively (Meszka and Wilk, 2014). Foliar sprays with 2 mM potassium silicate applied to common bean plants reduced the severity of the disease caused by *Colletotrichum lindemuthianum* (anthracnose) by 34% (Polanco *et al.,* 2014). Regarding the perennial ryegrass–*Magnaporthe oryzae* pathosystem, Rahman *et al.* (2015) found that calcium silicate applied at the rate of 5 metric ton/ha suppressed significantly gray leaf spot in plants achieving a reduction in disease incidence and severity by 39.5 and 47.3%, respectively. Application of 2 mM silicon reduced the severity of blast disease (*Pyricularia oryzae*) in rice plants (Domiciano *et al.,* 2015). Silicon treatment of soybean was highly associated with increased plant resistance to target spot caused by *Corynespora cassiicola (*Fortunato *et al.,* 2015). Root application of silicon was more effective compared to foliar application in reducing the severity of powdery mildew (*Podosphaera xanthii*) on melon (Dallagnol *et al.,* 2015).

A number of studies have indicated that silicon application can also reduce the severity of soil-borne fungal diseases. For example, potassium silicate at a concentration of 1.7mM amended to nutrient solutions of cucumber plants significantly reduced the incidence of *Pythium ultimum* and *P. aphanidermatum*, the causal agents of root rot (Cherif *et al.,* 1992). The application of potassic and siliceous fertilizers increased resistance of corn to stalk rot caused by *Pythium aphanidermatum*, *Fusarium graminearum* (syn. *Gibberella zeae*) and *F. moniliforme* (syn. *G. fujikuroi*) (Sun *et al.,* 1994). Soil silicon fertilization applied to wheat plants reduced severity of brown foot rot (*Fusarium culmorum*) and eyespot (*Oculimacula yallundae*), under high disease pressure (Rodgers-Gray and Shaw, 2004). Application of 100 and 200 mM sodium silicate solutions decreased the diameter of dry rot (*Fusarium sulphureum*) lesions in potato tubers by 44 and 45%, respectively (Li *et al.,* 2009). Regarding the belle pepper–*Phytophthora capsici* pathosystem, French-Monar *et al.,* (2010) reported that supply of silicon to plant roots can potentially reduce the severity of Phytophthora blight while enhancing plant growth. Huang *et al.* (2011) showed that foliar application of silicon at the dose of 100 mg Si/l to tomato plants significantly reduced the severity of Fusarium crown and root rot (*Fusarium oxysporum* f.sp. *radicis-lycopersici*); data suggested that the decrease in disease severity was probably due to a delay in the onset of the initial infection of roots and the movement of the pathogen from roots to stems (Huang *et al.,* 2011). Silicon amended to soil at a rate of 0.39 g/kg soil reduced the symptoms of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* on banana plants (Fortunato *et al.,* 2012). Zhang *et al.* (2013) demonstrated that application of 1.5 mM silicon improved resistance of rice to sheath blight (*Rhizoctonia solani*). Moreover, silicon solution at a concentration of 2 mM decreased the area under relative lesion expansion progress curve of sheath blight (*R.*

solani) by 34.2% in rice plants (Schurt *et al.,* 2014).

For air- and soil-borne fungi, the mode of silicon action in a number of components of host plant resistance could be summarized as follows (Datnoff *et al.,* 2007; Datnoff and Heckman, 2014): silicon delays the incubation and latent periods, decreases conidial production, and reduces some features of the lesions produced by the fungal pathogens (expansion rate, size and number). Subsequently, disease development and/or definitive disease incidence is dramatically decreased, and the resistance of susceptible cultivars is, in some cases, raised to nearly the same level as that of cultivars with complete or partial resistance. Moreover, for susceptible and partially resistant rice cultivars, the observed disease resistance is greatest when silicon is applied to the soil and is root-absorbed as oppose to when it is applied to the foliage (Rezende *et al.,* 2009). This is mainly due to the silicon transporters which are not expressed in the leaves. Regarding foliar sprays, the disease suppressive effects observed are probably due to silicon being deposited on the leaf surface and thus, having an osmotic or pH effect. However, the underlying mechanisms that govern disease protection when silicon is root-absorbed remain largely unclear (Datnoff *et al.,* 2007; Datnoff and Heckman, 2014).

4. Mechanisms of silicon-enhanced resistance

In spite of the many scientific reports about silicon effects on fungal pathogens, the properties, spectrum of efficacy and mode of action of silicon remain largely speculative (Ghanmi *et al.,* 2004; Fauteux *et al.,* 2005; Datnoff *et al.,* 2007; Van Bockhaven *et al.,* 2013). Under controlled hydroponic conditions, silicon does not affect plant growth or development (Ma and Yamaji, 2006). However, where plants are exposed to multiple stresses, silicon plays an important role in plant health (Epstein, 2009). Generally, the effect of silicon on resistance of plants to diseases is considered to be due to either an accumulation of absorbed silicon in the epidermal tissue, or an expression of metabolic or pathogenesis-mediated host defense responses (Fauteux *et al.,* 2005; Datnoff *et al.,* 2007; Van Bockhaven *et al.,* 2013).

4.1. Physical defense

For the first hypothesis of silicon physical enhanced resistance, silicon deposited on the tissue surface acts as a physical barrier that protects plants from fungal infection. In this model, the increase of resistance has been associated with several factors, such as (1) the density of the long and short silicified cells present in the epidermis of the leaves, (2) the thick silica layer below the cuticle, (3) the double cuticular layer, (4) the thickened silicon-cellulose membrane, and (5) the papilla formation (Fauteux *et al.,* 2005; Datnoff *et al.,* 2007; Van Bockhaven *et al.,* 2013).

Silicon prevents physical penetration by pathogenic fungi, strengthens plants mechanically, and / or makes the plant cell less susceptible to enzymatic degradation by fungal pathogens. Yoshida *et al.* (1962) reported that a thick layer of silica is formed beneath the cuticle of rice leaves and sheaths after polymerization of monosilicic acid. This silicon layer beneath the cuticle might be partially responsible for impeding pathogen penetration. Furthermore, silicon might also form complexes with organic compounds in the wall of the epidermal cells, thus increasing their resistance to degradation by enzymes released by plant pathogenic fungi (Volk *et al.,* 1958). It was also suggested that silicon may be associated with lignin-carbohydrate complexes present in the cell wall of epidermal cells (Inanaga *et al.,* 1995).

Regarding cytological and pathogenic features associated with physical resistance, silicon deposited on the tissue surface decreases the number of lesions on leaf blades, or increases the incubation period, as reported for the *Pyricularia grisea*– and *Rhizoctonia solani*–rice pathosystems (Rodrigues *et al.,* 2001; Seebold *et al.,* 2004). Moreover, Kim *et al.* (2002) reported that silicified epidermal cell walls were closely as-

sociated with the reduced severity of the blast disease (*Magnaporthe grisea*) in susceptible and partially resistant rice cultivars, although the thickness of the epidermal cell wall was not significantly affected by the presence of silicon. For the cucumber–*Podosphaera xanthii* pathosystem, the foliar applied silicon produced only physical barrier and osmotic effect. However, the root applied silicon led to systemic acquired resistance when plants were infected by the powdery mildew pathogen (Liang *et al.,* 2005). Moreover, Hayasaka *et al.* (2008) confirmed that silicon in the rice leaf epidermis may confer resistance against *M. grisea* (blast) appressorial penetration. However, the prophylactic effect against powdery mildew was lost when silicon feeding to cucumber plants was interrupted (Samuels *et al.,* 1991). Heine *et al.* (2007) reported that the accumulation of silicon in root cell walls did not represent a physical barrier to the spread of *Pythium aphanidermatum* in the roots of bitter gourd and tomato. Although these authors concluded that silicified epidermal cell walls in leaves could be the main factor for the reduction in severity of plant diseases caused by fungal pathogens, they did not report that this was sufficient evidence to explain the impediment of fungal penetration in the leaves. Based on these results, it was suggested that resistance to fungal pathogens in plants treated with silicon was much more complex than a physical resistance, which was strongly contested and doubted in recent years (Van Bockhaven *et al.,* 2013).

4.2. Biochemical defense

Regarding the second hypothesis of silicon biochemical enhanced resistance, the soluble silicon in plant tissue may be associated with an increase in resistance to fungal diseases. In this model, the enhancement of resistance is due to (1) increased activity of defense-related enzymes in leaves, such as polyphenoloxidase, peroxidase, phenylalanine ammonia-lyase, and glucanase, (2) increased production of antifungal compounds, such as phenolic metabolism products (lignin), flavonoids, phytoalexins and pathogenesis-related proteins in plants, and (3) activation of some plant defense-related genes (Fauteux *et al.,* 2005; Datnoff *et al.,* 2007; Van Bockhaven *et al.,* 2013). When infected with necrotizing pathogens, many plants developed an enhanced resistance against further pathogen attack, which is referred to as systemic acquired resistance (SAR) (Conrath, 2006). The two mechanisms involved in increasing the activity of enzymes and antifungal compounds due to silicon application on plants could induce defense response similar to SAR (Cai *et al.,* 2009). Moreover, there might be other biochemical and physiological mechanisms involved in the silicon-mediated resistance of plants to diseases. For example, higher levels of salicylic acid, jasmonic acid, and ethylene have been reported to be induced by silicon supplements in some host-pathogen interactions: powdery mildew *of Arabidopsis* caused by *Golovinomyces cichoracearum (*Vivancos *et al.,* 2015) and rice-brown spot caused by *Cochliobolus miyabeanus* (Van Bockhaven *et al.,* 2015).

4.2.1. Defense-related enzymes

Defense-related enzymes are important in relation to disease resistance. Several studies indicated that lower disease intensity in the silicon-treated plants was related to higher activity of protective enzymes. Silicon has been demonstrated to stimulate accumulation of defense-related enzymes in plant leaves after fungal infection (Fauteux *et al.,* 2005; Datnoff *et al.,* 2007; Van Bockhaven *et al.,* 2013).

Activities of chitinase, peroxidases and polyphenoloxidases in cucumber plants infected by *Pythium* spp. were enhanced as a result of silicon root application (Cherif *et al.,* 1994; Liang *et al.,* 2005). Increased activity of chitinase and β-1,3-glucanase in pea seeds supplied with potassium silicate reduced incidence of *Mycosphaerella pinodes* (Dann and Muir, 2002). Enhanced peroxidase activity in wheat leaves, due to silicon treatment, decreased the severity of powdery mildew caused by *Blumeria graminis*

f.sp.*tritici* (Yang *et al.,* 2003). Regarding the rice–*M. oryzae* interaction, increased resistance against the blast pathogen is characterized by higher accumulation of glucanase, peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase (Rodrigues *et al*., 2003, 2004, 2005; Cai *et al*., 2008). Liang *et al.* (2005) found that enhanced peroxidases, polyphenoloxidases and chitinases activities due to silicon root application were effective in reducing powdery mildew severity in cucumber. Regarding *Cryptococcus laurentii*-sweet cherry interaction, increased polyphenol oxidase activity reduced disease severity in fruit treated with 1% silicon (Qin and Tian, 2005). Enhanced peroxidase activity in melon plants treated with sodium silicate decreased incidence of pink rot caused by *Trichothecium roseum* (Bi *et al.,* 2006). Increased rice resistance due to silicon treatment against the brown spot pathogen (*Bipolaris oryzae*) seems to be the result of higher levels of chitinase and peroxidase (Dallagnol *et al*., 2011). Enhanced peroxidase and phenylalanine ammonia lyase activities in sodium silicate-treated Chinese cantaloupe decreased the severity of pink rot (*Trichothecium roseum*) (Guo *et al.,* 2007). Xavier *et al.* (2011) reported that higher activities of chitinases and peroxidases contributed to the increase in wheat resistance to blast (*Pyricularia oryzae*). Increased activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in common bean plants reduced severity of *Colletotrichum lindemuthianum* (Polanco *et al.,* 2014). Schurt *et al.* (2014) found that the increased activities of phenylalanine ammonia-lyases, peroxidases, polyphenoloxidases and chitinases in the leaf sheaths of rice plants supplied with silicon led to the reduction in the progress of sheath blight lesions (*R. solani*). Increased activation of chitinase, superoxide dismutase, peroxidase and β-1,3-glucanase reduced the severity of powdery mildew (*Podosphaera xanthii*) in melon plants (Dallagnol *et al*., 2015). Perennial ryegrass grown in silicon-amended soil exhibited greater activities of peroxidase and polyphenol oxidase following infection by *Magnaporthe oryzae* (Rahman *et al.,* 2015). High activities of superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and lypoxigenase contributed to the increase in rice resistance to *Pyricularia oryzae* (Domiciano *et al.,* 2015). In leaves of soybean plants supplied with silicon, higher activities of chitinases, β-1-3-glucanases, phenylalanine ammonia-lyases, peroxidases, and polyphenol oxidases reduced the incidence of target spot (*Corynespora cassiicola*) *(*Fortunato *et al.,* 2015).

4.2.2. Antifungal compounds

Antifungal compounds play important role in plant fungal resistance (Fauteux *et al.* 2005; Datnoff *et al.* 2007; Van Bockhaven *et al.* 2013). Defense-related enzymes have an important role in regulating the production and accumulation of lignin, flavonoids, and phytoalexins (Cai *et al.,* 2009). Rodrigues *et al.* (2005) reported the strong induction of pathogenesis-related protein transcripts following infection by *Magnaporthe grisea*, which corresponded to an increase in the concentration of lignin in rice plants. Moreover, Xavier *et al.* (2011) showed that the high peroxidase activity following leaf blast infection of wheat leaves supplied with silicon was associated with an increase in the concentration of lignin. However, the biochemical pathways by which the phenolic metabolism might mediate silicon-enhanced plant resistance to fungi remains unclear (Fauteux *et al.,* 2005; Datnoff *et al.,* 2007; Van Bockhaven *et al.,* 2013). Silicon application induces the production of antifungal compounds after pathogen penetration of the epidermal cells (Cai *et al.,* 2009).

Enhanced accumulation of phenolic substances impeded the penetration of *Pythium ultimum* hyphae into the vascular system of cucumber plants (Cherif *et al.,* 1992). Phenolics extracted from silicon-treated cucumber plants displayed a strong fungistatic activity against *Pythium* spp. (Cherif *et al.,* 1994). Increased flavonoid phytoalexin aglycone rhamnetin in cucumber plants due to silicon treatment decreased severity of powdery mildew caused by *Podosphaera xanthii*

(Fawe *et al.,* 1998). Enhanced glycosylated phenolics and lignin activities in epidermal cells of silicon-treated wheat reduced severity of *Blumeria graminis* f.sp. *tritici* (Belanger *et al.,* 2003; Yang *et al.,* 2003). Increased silicon-induced resistance of rice to blast (*M. grisea*) was related to higher production of phytoalexin (Rodrigues *et al.,* 2004; 2005). Higher accumulation of fungitoxic phenolic compounds due to silicon treatment protected *Arabidopsis* from powdery mildew caused by *Erysiphe cichoracearum* (Ghanmi *et al*., 2004; Fauteux *et al*., 2005). Increased activity of antimicrobial glycosylated phenolics, diterpenoid phytoalexins, and lignin decreased severity of blast disease in silicon-treated rice plants (Cai *et al.,* 2008). Dallagnol *et al.* (2011) found that decreased level of rice brown spot (*Bipolaris oryzae*) was due to enhanced accumulation of lignin and soluble phenolics. High concentrations of lignin-thioglycolic acid derivatives increased wheat resistance to blast caused by *Pyricularia oryzae* (Xavier *et al.,* 2011). Regarding the rice–*Rhizoctonia solani* pathosystem, silicon-induced enhancement of phenolic metabolism contributed to the improved resistance to sheath blight of a susceptible rice cultivar (Zhang *et al.,* 2013). Enhanced production of flavonoids in wheat leaves reduced incidence of blast caused by *Pyricularia oryzae* (Rodrigues *et al.,* 2014). Fortunato *et al.* (2015) found that higher activity of total soluble phenolics and lignin-thioglycolic acid derivatives in leaves of soybean plants supplied with silicon led to reduced incidence of target spot (*Corynespora cassiicola).* Regarding the perennial ryegrass–*Magnaporthe oryzae* interaction, Rahman *et al.* (2015) found that several phenolic acids, including chlorogenic acid and flavonoids, and relative levels of genes encoding phenylalanine ammonia lyase and lipoxygenase were significantly increased in silicon-amended plants compared with non-amended control plants. Increased lignin concentration reduced the incidence of *Podosphaera xanthii* (powdery mildew) in melon plants (Dallagnol *et al.,* 2015)

4.2.3. Molecular mechanism

Silicon acts as a modulator of host resistance to pathogens (Fauteux *et al*., 2005; Van Bockhaven *et al.,* 2013). However, the biochemical and physiological mechanisms that are potentiated by silicon are complex phenomena (Rodrigues *et al.,* 2005). Under optimum conditions, gene expression had no significant difference between silicon-treated and non-treated plants (Watanabe *et al.,* 2004). A study by Kauss *et al.* (2003) conducted on cucumber leaves and investigating the process of plant infection showed that resistance to infection can be acquired by the expression of a protein rich in proline together with the presence of silica at the site of pathogen penetration. Fauteux *et al.* (2006) stated that only two genes were up-regulated when silicon alone was applied to *Arabidopsis* plants. Brunings *et al.* (2009) studied the gene expression of silicon-treated rice using a microarray and found differential regulation of 221 genes compared to untreated control, including some transcription factors. Chain *et al.* (2009) demonstrated a comparable differential response with 47 genes of varying function in silicon-treated wheat. It has been suggested that silicon could act as a potentiator of defense responses or as an activator of protein-mediated cell signaling (Fauteux *et al*., 2005; Van Bockhaven *et al.,* 2013).

It has been proposed that in a cell, silicon controls the signaling events that guide the synthesis of antimicrobial compounds, and could also control the generation of systemic signals. In this way, silicic acid, without being a second messenger, might play a role in resistance, both local and systemic (Fauteux *et al*., 2005; Bockhaven *et al.,* 2013). By using Agilent 44K oligo DNA arrays, it has been shown that silicon increased significantly the level of photorespiration in rice leaves infected by *Cochliobolus miyabeanus* (Van Bockhaven *et al.,* 2014). Genome-wide studies on tomato, rice, *Arabidopsis* and wheat grown in soil amended with silicon and compared to non-amended control plants have shown a differential and unique expression of a large number of genes involved in host

plant defense mechanisms or metabolism (Watanabe *et al.,* 2004; Fauteux *et al.,* 2006; Chain *et al.,* 2009; Brunings *et al.,* 2009; Ghareeb *et al.,* 2011).

5. *In vitro* inhibition of fungal pathogens by soluble silicon

Some studies have been carried out to determine whether silicon has fungicidal activity *in vitro*. Hyphal growth of *Magnoporthe grisea* on silicic acid-amended water agar was 62% less compared to growth on non-amended water agar (Maekawa *et al.,* 2003). Bekker *et al.* (2006) reported that mycelial growth of 11 phytopathogenic fungi (*Phytophthora cinnamomi, Sclerotinia sclerotiorum, Pythium* F-group, *Mucor pusillus, Drechslera* spp., *Fusarium oxysporum, F. solani, Alternaria solani, Colletotrichum coccodes, Verticillium theobromae, Curvularia lunata* and *Stemphylium herbarum*) was inhibited on potassium silicate (20.7% $Si0_2$)-amended PDA, at concentrations ≥ 20 ml Si/l agar. The level of mycelial inhibition was dependant on dose indicating different tolerance of the tested fungi to potassium silicate. Bi *et al.* (2006) reported that 100 mM sodium silicate completely inhibited mycelial growth of *Alternaria alternata*, *F. semitectum*, and *Trichothecium roseum*. Sodium silicate inhibited spore germination and mycelial growth of *Penicillium digitatum (Liu et al., 2010)*. Nevertheless, Shen *et al.* (2010) indicated that the inhibition of *Rhizoctonia solani*, *F. oxysporum*, *F. oxysporum* f. sp. *fragariae* and *Pestalotiopsis clavispora* colony growth on PDA plates amended with low concentrations of potassium silicate (1.67, 3.33, 5 or 6.67 mM) was due to a pH effect. The range of potassium silicate concentrations tested is suitable for field application (Shen *et al.,* 2010). Also, the potassium silicate concentrations used by Bekker *et al.* (2006) were 50 to 60 times higher than those in Shen's *et al.* (2010) study. Moreoever, these concentrations (Bekker *et al.,* 2006; Bi *et al.,* 2006) are unrealistic for field use because the high pH of the resulting potassium silicate solutions could cause phytotoxicity. Shen *et al.* (2010) concluded that the reduction in fungal diseases following treatment of field plants with silicon is probably not due to the fungistatic effects of silicon, but to other biochemical and physical mechanisms mentioned previously.

6. Conclusions

Silicon application could be one of the most promising approaches for sustainable, environmentally sound and broad-spectrum control of fungal diseases in plants in various agricultural contexts. That is why in the last few decades, extensive studies have been carried out to investigate its protective role in numerous pathosystems. However, its effect on enhancing plant resistance against fungal pathogens is not limited to high silicon-accumulators as it has also been described in low silicon-accumulators. The role of silicon as a modulator of plant defense-related gene expression in combination with biotic stress is dominant over its function as a mechanical barrier. Silicon does not seem to directly affect phytopathogenic fungi, as fungicides, and therefore exerts no selective pressure. The in-depth understanding of silicon in plants will be helpful to effectively use silicon to increase crop yield and enhance resistance to fungal pathogens.

I would like to thank Professor I. Othman, Director General of AECS, and the Head of the Agriculture Department for their support.

Literature cited

Arnon, D. and Stout, P. 1939. The essentiality of certain elements in minute quantity for plants with special reference to copper. *Plant Physiology*, 14: 371-375.

Arsenault-Labrecque, G., Menzies, J.G. and Belanger, R.R. 2012. Effect of silicon absorption on soybean resistance to *Phakopsora pachyrhizi* in different cultivars. *Plant Disease*, 96: 37-42.

Bayles, R.A., Flath, K., Hovmoller, M.S. and de Valla-

vieille-Pope, C. 2000. Breakdown of the Yr17 resistance to yellow rust of wheat in northern Europe. *Agronomie*, 20: 805-811.

Bekker, T.F., Kaiser, C. and Labuschagne, N. 2006. The antifungal activity of potassium silicate and the role of pH against selected plant pathogenic fungi *in vitro*. *South African Journal of Plant Soil*, 26: 55-57.

Belanger, R.R., Benhamou, N. and Menzies, J.G. 2003. Cytological evidence of an active role of silicon in wheat resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*). *Phytopathololgy*, 93: 402–412.

Belanger, R.R, Vivancos, J., Wilkinson, J.A., Belzile, F. and Menzies, J.G. 2014. Silicon influence on biotic stress in plants. In *Proceedings of the 6th International Conference on Silicon in Agriculture*, Stockholm, Sweden, 26-30 August, p. 42.

Bi, Y., Tian, S.P., Guo, Y.R., Ge, Y.H. and Qin, G.Z. 2006. Sodium silicate reduces postharvest decay on Hami melons: Induced resistance and fungistatic effects. *Plant Disease*, 90: 279-283.

Bowen, P., Menzies, J. and Ehret, D. 1992. Soluble silicon sprays inhibit powdery mildew development on grape leaves. *Journal of the American Society for Horticultural Science*, 117: 906–912.

Brunings, A.M., Datnoff, L.E., Ma, J.F., Mitani, N., Nagamura, Y., Rathinasabapathi, B. and Kirst, K. 2009. Differential gene expression of rice in responses to silicon and the rice blast fungus *Magnaporthe oryzae*. *Annals of Applied Biology*, 155: 161-170.

Cai, K., Gao, D., Chen, J. and Luo, S. 2009. Probing the mechanisms of silicon-mediated pathogen resistance. *Plant Signaling and Behavior*, 4: 1-3.

Cai, K.Z., Gao, D., Luo, S.M., Zeng, R.S., Yang, J.Y. and Zhu, X.Y. 2008. Physiological and cytological mechanisms of silicon-induced resistance in rice against blast disease. *Physiological Plantarum*, 134: 324–333.

Chain, F., Cote-Beaulieu, C., Belzile, F., Menzies, J.G. and Belanger, R. 2009. A comprehensive transcriptomic analysis of the effect of silicon on wheat plants under control and pathogen stress conditions. *Molecular Plant-Microbe Interactions*, 22: 1323-1330.

Cherif, M., Asselin, A. and Belanger, R.R. 1994. Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathology*, 84: 236–242.

Cherif, M., Menzies, J.G., Benhamou, N. and Belanger, R.R. 1992. Studies of silicon distribution in wounded and *Pythium ultimum* infected cucumber plants. *Physiological and Molecular Plant Pathology*, 41:371–385.

Conrath, U. 2006. Systemic acquired resistance. *Plant Signal and Behavior*, 4: 179-84.

Dallagnol, L.J., Rodrigues, F.A., DaMatta, F.M., Mielli, M.V.B. and Pereira, S.C. 2011. Deficiency in silicon uptake affects cytological, physiological, and biochemical events in the rice-*Bipolaris oryzae* interaction. *Phytopathology*, 101: 92–104.

Dallagnol, L.J., Rodrigues, F.A., Pascholati, S.F., Fortunato, A.A. and Camargo, L.E.A. 2015. Comparison of root and foliar applications of potassium silicate in potentiating post-infection defences of melon against powdery mildew. *Plant Pathology,* 64: 1085–1093.

Dann, E. and Muir, S. 2002. Peas grown in media with elevated plant-available silicon levels have higher activities of chitinases and b-1,3-glucanase, are less susceptible to a fungal leaf spot pathogen and accumulate more foliar silicon. *Australian Plant Pathology*, 31: 9-13.

Datnoff, L., Elmer, W. and Huber, D. 2007. Mineral nutrition and plant disease. The American Phytopathological Society, St. Paul, USA. 278 p.

Datnoff, L.E. and Heckman, J.R. 2014. Silicon fertilizers for plant disease protection. In *Proceedings of the 16th World Fertilizer Congress of CIECRio*. De Janeiro-RJ, Brazil, 20-24 October, p. 37-38.

Deepak, S., Manjunath, G., Manjula, S., Niranjan-Raj, S., Geetha, N.P. and Shetty, H.S. 2008. Involvement of silicon in pearl millet resistance to downy mildew disease and its interplay with cell wall proline/hydroxyproline-rich glycoproteins. *Australasian Plant Pathology*, 37: 498-504.

Domiciano, G., Cacique, I., Freitas, C., Filippi, M., DaMatta, F.M., Vale, F. and Rodrigues, F. 2015. Alterations in gas exchange and oxidative metabolism in rice leaves infected by *Pyricularia oryzae* are attenuated by silicon. *Phytopathology*, 105: 738-747.

Domiciano, G.P., Rodrigues, F.A., Vale, F.X.R., Xavier, F.M.S., Moreira, W.R., Andrade, C.C.L. and Pereira S.C. 2010. Wheat resistance to spot blotch potentiated by silicon. *Journal of Phytopathology*, 158: 334-343.

Dubin, H.J. and Rajaram, S. 1996. Breeding disease-resistant wheats for tropical highlands and lowlands. *Annual Review of Phytopathology*, 34: 503-526.

Epstein, E. 2009. Silicon: Its manifold roles in plants. *Annals of Applied Biology*, 155: 155-160.

Exley, C. 1998. Silicon in life: a bioinorganic solution to bioorganic essentiality. *Journal of Biological Inorganic Chemistry*, 69: 139–144.

Fauteux, F., Chain, F., Belzile, F., Menzies, J.G. and Belanger, R.R. 2006. The protective role of silicon in the Arabidopsis-powdery mildew pathosystem. In *Proceedings of the National Academy of Sciences of the United States of America*, 103: 17554–17559.

Fauteux, F., Rémus-Borel, W., Menzies, J. and Belanger, R. 2005. Silicon and plant disease resistance against pathogenic fungi. *FEMS Microbiology Letters*, 249: 1-6.

Fawe, A., Abou-Zaid, M., Menzies, J.G. and Belanger, R.R. 1998. Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. *Phytopathol-*

ogy, 88: 396–401.

French-Monar, R.D., Rodrigues, F.A., Korndorfer, G.H. and Datnoff, L.E. 2010. Silicon suppresses *Phytophthora* blight development on bell pepper. *Journal of Phytopathology*, 158: 554-560.

Fortunato, A.A., Debona, D., Bernardeli, A.M.A. and Rodrigues, F.A. 2015. Defense-related enzymes in soybean resistance to target spot. *Journal of Phytopathology*, 163: 731–742.

Fortunato, A.A., Rodrigues, F.A., Baroni P.J.C., Soares B.G.C., Rodriguez, D.M.A. and Pereira, O.L. 2012. Silicon suppresses *Fusarium* wilt development in banana plants. *Journal of Phytopathology*, 160: 674-679.

Ghareeb, H., Bozso, Z., Ott, P.G., Repenning, C., Stahl, F. and Wydra, K. 2011. Transcriptome of silicon-induced resistance against *Ralstonia solanacearum* in the silicon non-accumulator tomato implicates priming effect. *Physiological and Molecular Plant Pathology*, 75: 83–89.

Ghanmi, D., McNally, D.J., Benhamou, N., Menzies, J.G. and Belanger, R.R. 2004. Powdery mildew of *Arabidopsis thaliana:* a pathosystem for exploring the role of silicon in plant–microbe interactions. *Physiological and Molecular Plant Pathology*, 64: 189–199.

Gillman, J., Zlesak, D. and Smith, J. 2003. Applications of potassium silicate decrease black spot infection of *Rosa hybrida* 'Meilpelta'. *HortScience*, 38: 144-1147.

Guo, Y., Liu, L., Zhao, J. and Bi, Y. 2007. Use of silicon oxide and sodium silicate for controlling *Trichothecium roseum* postharvest rot in Chinese cantaloupe (*Cucumis melo* L.). *International Journal of Food Science and Technology*, 42: 1012-1018.

Guevel, M.H., Menzies, J.G. and Blanger, R.R. 2007. Effect of root and foliar applications of soluble silicon on powdery mildew control and growth of wheat plants. *European Journal of Plant Pathology*, 119: 429-436.

Hayasaka, T., Fujii, H. and Ishiguro, K. 2008. The role of silicon in preventing appressorial penetration by the rice blast fungus. *Phytopathology*, 98: 1038-44.

Heckman, J. 2013. Silicon: A Beneficial Substance. *Better Crops*, 97: 14-16.

Heine, G., Tikum, G. and Horst, W. 2007. The effect of silicon on the infection by and spread of *Pythium aphanidermatum* in single roots of tomato and bitter gourd. *Journal of Experimental Botany*, 58: 569-577.

Huang, C.H., Roberts, P.D. and Datnoff, L.E. 2011. Silicon suppresses *Fusarium* crown and root rot of tomato. *Journal of Phytopathology*, 159: 546–554.

Inanaga, S., Okasaka, A. and Tanaka, S. 1995. Does silicon exist in association with organic compounds in rice plant? *Japanese Society of Soil Science and Plant Nutrition*, 11: 111-117.

Kablan, L., Lagauche, A., Delvaux, B. and Legreve, A. 2012. Silicon reduces black sigatoka development in banana. *Plant Disease*, 96: 273-278.

Kanto, T., Miyoshi, A., Ogawa, T., Maekawa, K. and Aino, M. 2006. Suppressive effect of liquid potassium silicate on powdery mildew of strawberry in soil. *Journal of General Plant Pathology*, 72: 137-142.

Kauss, K., Franke, R., Gilbert, S., Dietrich, A. and Kroger, N. 2003. Silica deposition by a strongly cationic proline-rich protein from systemically resistant cucumber plants. *Plant Journal*, 33: 87-95.

Kim, S., Kim, W., Park, E. and Choi, D. 2002. Silicon-induced cell wall fortification of rice leaves: a possible cellular mechanism of enhanced host resistance to blast. *Phytopathology*, 92: 1095-1103.

Kunoh, H. and Ishizaki, H. 1975. Silicon levels near penetration sites of fungi on wheat, barley, cucumber and morning glory leaves. P*hysiological and Plant Pathology*, 5: 283-287.

Leusch, H. and Buchenauer, H. 1989. Effect of soil treatments with silica-rich lime fertilizers and sodium trisilicate on the incidence of wheat by *Erysiphe graminis* and *Septoria nodorum* depending on the form of N-fertilizer. *Journal of Plant Diseases and Protection*, 96: 154-172.

Li, Y.C., Bi, Y., Ge, Y.H., Sun, X.J. and Wang, Y. 2009. Antifungal activity of sodium silicate on *Fusarium sulphureum* and its effect on dry rot of potato tubers. *Journal of Food Science*, 74: M213-M218.

Liang, Y.C., Si, J. and Romheld, V. 2005. Silicon uptake and transport is an active process in *Cucumis sativus*. *New Phytologist*, 167: 797–804.

Liang, Y., Sun, W., Zhu, Y.G. and Christie, P. 2007. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: A review. *Environmental Pollution*, 147: 422-428.

Liu, J., Zong, Y., Qin, G., Li, B. and Tian, S. 2010. Plasma membrane damage contributes to antifungal activity of silicon against Penicillium digitatum. *Current Microbiology*, 61: 274-279.

Lowenstam, H.A. 1981. Minerals formed by organisms. *Science*, 211: 1126–1131.

Ma, Z. and Michailides, T.J., 2005. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Protection*, 24: 853-863.

Ma, J.F. and Takahashi, E. 2002. Soil, fertilizer, and plant silicon research in Japan. Elsevier Science, Amsterdam, The Netherlands, 294 p.

Ma, J.F. and Yamaji, N. 2006. Silicon uptake and accumulation in higher plants. *Trends in Plant Science*, 11: 392-397.

Maekawa, K., Watanabe, K,. Kanto, T., Aino, M. and Saigusa, M. 2003. Effect of soluble silicic acid on suppression of rice leaf blast. *Japanese Journal*

of soil sciences and Plant Nutrition, 74: 293-299.

Mitani, N., Ma, J.F. and Iwashita, T. 2005. Identification of silicon form in xylem sap of rice (*Oryza sativa* L.). *Plant and Cell Physiology*, 46: 279-283.

Moraes, S.R., Pozza, E.A., Alves, E., Pozza, A.A., Carvalho, J.C., Lima, P.H. and Botelho, A.O. 2006. Effects of silicon sources on the incidence and severity of the common beans anthracnose. *Fitopatologia Brasileira*, 31: 283-291.

Menzies, J., Bowen, P., Ehret, D.L. and Glass, A.D.M. 1992. Foliar applications of potassium silicate reduce severity of powdery mildew on cucumber, muskmelon, and zucchini squash. *Journal of the American Society for Horticultural Science,* 117: 902–905.

Menzies, J., Ehret, D., Glass, A. and Samuels, A. 1991. The influence of silicon on cytological interactions between *Sphaerotheca fuliginea* and *Cucumis sativus*. *Physiological and Molecular Plant Pathology*, 39: 403-414.

Meszka, B. and Wilk, R. 2014. Indirect effect of silicon product against apple scab and strawberry diseases. In *Proceedings of the 6th International Conference on Silicon in Agriculture*, Stockholm, Sweden, 26-30 August, p. 128.

Polanco, L.R., Rodrigues, F.A., Nascimento, K.J.T., Cruz, M.F.A., Curvelo, C.R.S., DaMatta FM, Vale, FXR. 2014. Photosynthetic gas exchange and antioxidative system in common bean plants infected by *Colletotrichum lindemuthianum* and supplied with silicon. *Tropical Plant Pathology*, 39:035-042.

Qin, G.Z. and Tian, S.P. 2005. Enhancement of biocontrol activity of *Cryptococcus laurentii* by silicon and the possible mechanisms involved. *Phytopathology*, 95: 69-75.

Rahman, A., Wallis, C. and Uddin, W. 2015. Silicon induced systemic defense responses in perennial ryegrass against infection by *Magnaporthe oryzae*. *Phytopathology*, 105: 748-757.

Reynolds, O.L., Keeping, M.G. and Meyer, J.H. 2009. Silicon-augmented resistance of plants to herbivorous insects: a review. *Annals of Applied Biology*, 155: 171–186.

Rezende, D.C., Rodrigues, F.A., Carre-Missio, V., Schurt, D.A., Kawamura, I.K. and Korndorfer, G.H. 2009. Effect of root and foliar applications of silicon on brown spot development in rice. *Australasian Plant Pathology*, 38: 67-73.

Rodgers-Gray, B. and Shaw, M. 2004. Effects of straw and silicon soil amendments on some foliar and stem-base diseases in pot-grown winter wheat. *Plant Pathology*, 53: 733-740.

Rodrigues, F., Benhamou, N., Datnoff, L., Jones, J. and Belanger, R. 2003. Ultrastructural and cytochemical aspects of siliconmediated rice blast resistance. *Phytopathology*, 93: 535-546.

Rodrigues, F.A. and Datnoff, L.E. 2005. Silicon and rice disease management. *Fitopatologia Brasileira*, 30: 457-469.

Rodrigues, F.A., Datnoff, L.E., Korndorfer, G.H., Seebold, K.W. and Rush, M.C. 2001. Effect of silicon and host resistance on sheath blight development in rice. *Plant Disease*, 85, 827–32.

Rodrigues, F.A., Duarte, H.S.S., Rezende, D.C., Filho W.J.A, Korndo, G.H. and Zambolim, L. 2010. Foliar spray of potassium silicate on the control of angular leaf spot on beans. *Journal of Plant Nutrition*, 33: 2082–2093.

Rodrigues, F.A., Jurick, W.M., Datnoff, L.E., Jones, J.B. and Rollins, J.A. 2005. Silicon influences cytological and molecular events in compatible and incompatible rice-*Magnaporthe grisea* interactions. *Physiolgical and Molecular Plant Pathology*, 66: 144–159.

Rodrigues, F.A., McNally, D., Datnoff, L., Jones, J., Labbe, C., Benhamou, N., Menzies, J. and Bélanger, R. 2004. Silicon enhances the accumulation of diterpenoid phytoalexins in rice: a potential mechanism for blast resistance. *Phytopathology*, 94: 177-183.

Rodrigues, F.A., Silva, D.W.L., Cruz, M.F.A. and Fortunato, A.A. 2014. Histochemical aspects of wheat resistance to leaf blast mediated by silicon. In *Proceedings of the 6th International Conference on Silicon in Agriculture*, Stockholm, Sweden, 26-30 August, p. 156.

Samuels, A.L., Glass, A.D.M., Ehret, D.L. and Menzies, J.G. 1991. Mobility and deposition of silicon in cucumber plants. *Plant, Cell & Environment*, 14: 485-492.

Savant, N., Snyder, G. and Datnoff, L. 1997. Silicon management and sustainable rice production. *Advances in Agronomy*, 58: 151-199.

Schurt, D.A., Cruz, M.F.A., Nascimento, K.J.T., Filippi, M.C.C. and Rodrigues, F.A. 2014. Silicon potentiates the activities of defense enzymes in the leaf sheaths of rice plants infected by *Rhizoctonia solani*. *Tropical Plant Pathology*, 39: 457-463.

Seebold, K.W., Datnoff, Jr.L.E., Correa-Victoria, F.J., Kucharek, T.A. and Snyder, G. H. 2004. Effects of silicon and fungicides on the control of leaf and neck blast in upland rice. *Plant Disease*, 88: 253-258.

Semal, J. 1989. Traité de Pathologie Végétale. Les Presses Agronomiques de Gembloux, Gembloux, Belgium, 621 p.

Shen, G.H., Xue, Q.H., Tang, M., Chen, Q., Wang, L.N., Duan, C.M., Xue, L. and Zhao, J. 2010. Inhibitory effects of potassium silicate on five soil-borne phytopathogenic fungi *in vitro*. *Journal of Plant Diseases and Protection*, 117: 180–184.

Shetty, R., Jensen, B., Shetty, N.P., Hansen, M., Hansen, C.W., Starkey, K.R. and Jorgensen, H.J.L. 2012. Silicon induced resistance against powdery mildew of roses caused by *Podosphaera pannosa*. *Plant Pathology*, 61: 120–131.

Shephard, M.C. 1997. Screening for Fungicides. *Annual Review of Phytopathology*, 25: 189-206.

Silva, I.T., Rodrigues, F.A., Oliveira, J.R., Pereira, S.C., Andrade, C.C.L., Silveira, R.P. and Conceic, M.M. 2010. Wheat resistance to bacterial leaf streak mediated by silicon. *Journal of Phytopathology*, 158: 253–262.

Snyder, G.H., Matichenkov, V.V. and Datnoff, L. E. 2006. *Plant Nutrition*. Belle Glade, Fla, USA: Taylor & Francis; Silicon; pp. 551-562.

Sun, X., Sun, Y., Zhang, C., Song, Z., Chen, J., Bai, J., Cui, Y. and Zhang, C. 1994. The mechanism of corn stalk rot control by application of potassic and siliceous fertilizers. *Acta Phytophysiologica Sinica*, 21: 102-108.

Van Bockhaven, J., Vleesschauwer, D.D. and Hofte, M. 2013. Towards establishing broad-spectrum disease resistance in plants: silicon leads the way. *Journal of Experimental Botany*, 64: 1281–1293.

Van Bockhaven, J., Kikuchi, S., Asano, T., Hofte, M. and Vleesschauwer, D. 2014. Transcriptome analysis of silicon-induced brown spot resistance in rice reveals central role of photorespiration. In *Proceedings of the 6th International Conference on Silicon in Agriculture*, Stockholm, Sweden, 26-30 August, 174 p.

Van Bockhaven, J., Spichal, L., Novak, O., Strnad, M., Asano, T., Kikuchi, S., Hofte, M. and Vleesschauwer, D.D. 2015. Silicon induces resistance to the brown spot fungus *Cochliobolusmiyabeanus* by preventing the pathogen from hijacking the rice ethylene pathway. *New Phytologist*, 206: 761–773.

Volk, R., Kahn, R. and Weintraub, R. 1958. Silicon content of the rice plant as a factor influencing its resistance to infection by the rice blast fungus, *Pyricularia oryzae*. *Phytopathology*, 48: 179-184.

Vivancos, J., Labbe, C., Menzies, J.G. and Belanger, R.R. 2015. Silicon-mediated resistance of Arabidopsis against powdery mildew involves mechanisms other than the salicylic acid (SA)-dependent defence pathway. *Molecular Plant Pathology*, 16: 572–582.

Watanabe, S., Shimoi, E., Ohkama, N., Hayashi, H., Yoneyama, T., Yazaki, J., Fujii, F., Shinbo, K., Yamamoto, K., Sakata, K., Sasaki, T., Kishimoto, N., Kikuchi, S. and Fujiwara, T. 2004. Identification of several rice genes regulated by Si nutrition. *Soil Science and Plant Nutrition*, 50: 1273-1276.

Xavier M.S.Fa., Rodrigues, F.A., Domiciano, G.B., Oliveira, H.V., Silveira, P.R. and Moreira, W.R. 2011. Wheat resistance to leaf blast mediated by silicon. *Australian Plant Pathology*, 40:28-38.

Yang, Y.F., Liang, Y.C., Lou, Y.S. and Sun, W.C. 2003. Influences of silicon on peroxidase, superoxide dismutase activity and lignin content in leaves of wheat *Tritium aestivum* L. and its relation to resistance to powdery mildew. *Scientia Agricultura Sinica*, 36: 813-817.

Yoshida, S., Ohnishi, Y. and Kitagishi, K. 1962. Chemical forms, mobility, and deposition in the rice plant. *Soil Science and Plant Nutrition*, 8: 107-113.

Zellner, W., Frantzb, J. and Leisnera, S. 2011. Silicon delays Tobacco ringspot virus systemic symptoms in *Nicotiana tabacum*. *Journal of Plant Physiology*, 168: 1866– 1869.

Zhang, G., Cui, Y., Ding, X. and Dai, Q. 2013. Stimulation of phenolic metabolism by silicon contributes to rice resistance to sheath blight. *Journal of Plant Nutrition and Soil Sciences*, 176: 118–124.

Received: 17 August 2015; Accepted: 11 December 2015

ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Ο ρόλος του πυριτίου (Si) στην αύξηση της αντοχής των φυτών σε μυκητολογικές ασθένειες

A. Sakr

Περίληψη Η χρήση του πυριτίου (Si) στη γεωργία έχει προσελκύσει το ενδιαφέρον πολλών ερευνητών εξ' αιτίας των πολλών οφελειών που έχει το στοιχείο αυτό στα φυτά. Η εφαρμογή του πυριτίου έχει μειώσει την ένταση πολλών ασθενειών σε καλλιέργειες μεγάλης οικονομικής σημασίας. Στην παρούσα μελέτη γίνεται ανασκόπηση της βιβλιογραφίας όσον αφορά στη σχέση μεταξύ της θρέψης των φυτών με πυρίτιο και της εμφάνισης και εξέλιξης των μυκητολογικών ασθενειών στα φυτά. Η παρούσα ανασκόπηση υπογραμμίζει τη γεωργική σημασία του πυριτίου στις καλλιέργειες, τη δυνατότητα αντιμετώπισης φυτοπαθογόνων μυκήτων με την εφαρμογή πυριτίου, τους διάφορους μηχανισμούς μέσω των οποίων το πυρίτιο επάγει την αντοχή των φυτών στα παθογόνα, και την *in vitro* ανασταλτική επίδραση

του πυριτίου στην ανάπτυξη φυτοπαθογόνων μυκήτων. Ο συνδυασμός των δεδομένων που παρουσιάζονται στο παρόν άρθρο θα μπορούσε να συμβάλει στην καλύτερη κατανόηση της σχέσης μεταξύ της εφαρμογής πυριτίου, της αύξησης της αντοχής των φυτών στις μυκητολογικές ασθένειες και της μείωσης της έντασης των ασθενειών αυτών.

Hellenic Plant Protection Journal **9:** 1-15, 2016

DE GRUYTER
OPEN

Hellenic Plant Protection Journal **9:** 16-27, 2016
DOI 10.1515/hppj-2016-0002

REVIEW ARTICLE

Swarming motility in plant-associated bacteria

A. Venieraki*, P.Ch. Tsalgatidou, D.G. Georgakopoulos, M. Dimou and P. Katinakis*

Summary Plant-associated environments harbor a huge number of diverse bacteria that compete and/or cooperate for the occupation of the most nutrient-rich ecological niches. Motility, a common trait among bacteria, has long been assumed to provide a survival advantage to skilful bacteria in invading these environments. Bacterial surface motility, such as swarming, a flagella-driven type of surface movement, although mostly observed and studied on agar substrates, is emerging as a major trait involved in many functions of plant-associated bacteria in regard to their ability to colonize and spread on their host. In this review, we address some novel swarming motility strategies, which enable bacteria to colonize, disperse and compete in plant surfaces.

Additional keywords: Competition, cooperation, fungi

Introduction

Plants harbor epiphytic or endophytic communities of bacteria that colonize almost all tissues (roots, leaves, stems, vascular tissues, seeds and fruit). In general, plant-associated prokaryotes can be grouped based on the nature of their interaction with host into commensal, mutualistic, and pathogenic; mutualistic, when it is beneficial for both organisms, commensal, when one organism benefits and the other is not affected and pathogenic, when only the microbe benefits at the expense of the host or host damage. Mutualistic and commensal bacteria in association with plants are either so-called ectophytes or endophytes, if their location is outside or within plant tissues, respectively (Mentes *et al*., 2013; Berg *et al*., 2015).

Bacteria move from one location to another in natural niches and this movement is referred as motility. Plant colonization is a complex process and motility of bacteria in soil and/or on plant surfaces is a basic component of this process. Bacterial motility has been classified into discrete types, based on structural surface appendages or internal structures involved, and bacterial species may employ more than one type for translocation and colonization (Jarrell and McBride, 2008). Most bacteria are able to swim in aquatic environments powered by rotating flagella. This type of motility is referred as swimming motility. However, a range of different mechanisms have evolved that facilitate movement and spreading on a variety of surfaces (Jarrell and McBride, 2008). Swimming motility is considered to be an individual bacterial behavior (Jarrell and McBride, 2008; Harshey, 2003; Kearns, 2010). Surface movement can depend on the presence of flagella (i.e., swarming), the extension and retraction of type IV pili (i.e., twitching motility), the involvement of rearrangements in the shape of the cell that generate standing waves, the secretion of material from the poles, and localized focal adhesion complexes between cells and the substrate (i.e., gliding), or "passive" surface translocation where the expansive force of cell proliferation moves cells at the periphery of a cell mass (i.e., sliding).

Swarming is a multicellular movement of flagellated bacteria over solid surfaces and this trait is displayed by dozens of bacterial species under laboratory conditions.

Laboratory of General and Agricultural Microbiology, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, Votanikos, GR-118 55 Athens, Greece
* Corresponding authors: venieraki@aua.gr
katp@aua.gr

Such a mode of motility allows bacteria to escape local stresses, translocate to a better nutritional environment and efficiently invade host tissue (Harshey, 2003). Thus, we can infer that swarming motility must be an important mean for overriding surface impediments and claiming more space in the bacteria's natural habitat. However, despite the benefits, this trait is energy expensive and is dependent on surface wetness. The loss of motility may be considered as another adaptive strategy of bacteria to cope with harsh environmental conditions

Swarming regulatory mechanisms and strategies are diverse among the different bacteria species and have recently been reviewed (Harshey, 2003; Kearns, 2010; Partridge and Harshey, 2013; Harshey and Partridge, 2015). In plant-associated bacteria the ability to swarm can play an important role in colonization of interior and exterior surfaces of plants, in biofilm formation and in virulence or protective functions (Xu *et al*., 2012). In this review, we focus on highlighting the recently emerging novel tactics of plant-associated swarming bacteria to occupy, disperse and duel and/or cooperate on plant surfaces.

Swarming bacteria dispersed over fungi

While numerous studies have been focused on identifying bacterial genes involved in root colonization, limited attention was given to the involvement of fungi in facilitating migration of bacteria (Hannula *et al*., 2011). Soils are heterogeneous particulate systems exhibiting chemical heterogeneity. In the majority of soils, the patchiness and thickness of the liquid films restrict the dispersal of individual cells or populations. Flagellum-driven swimming requires bacterial cells to be fully immersed in liquid while swarming is restricted to a narrow range of wet conditions (Partridge and Harshey, 2013; Partridge and Harshey, 2015). Thus, flagellated bacteria would be expected to swim or to swarm under certain soil saturation levels. Recent studies demonstrated that displacement of *Bradyrhizobium japonicum* is achieved in 80% saturated soil (Covelli *et al*., 2013) while *Pseudomonas fluorescens* strain X (Kremmydas *et al*., 2013) displayed a fast movement in 50% saturated soil (Fig. 1). Movement of bacteria in bulk unsaturated soils or rhizosphere, conditions that limit the dispersal of microbes due to environments of low water potential or discontinuous water films, may not be achievable without additional aid. Several lines of evidence suggested that mycelia may also provide the appropriate conditions for motile bacteria migration in unsaturated soils. First, the abundance of fungi which is ranging from 100 to 700 mgr per g of soil, the extensive network of growing mycelia which according to estimates sum up to 20.000 km per m^3 of soil (Simon *et al*., 2015). Second, their ability to colonize both water-saturated and air-filled voids between soil particles (Wösten, 2001). Third, flagellated bacterial strains could move along the hyphal surface (Kohlmeier *et al*., 2005).The role of fungi in facilitating the dispersal of bacteria was further substantiated in a recent work, where it was shown that fungal mycelia facilitate the spread of motile bacte-

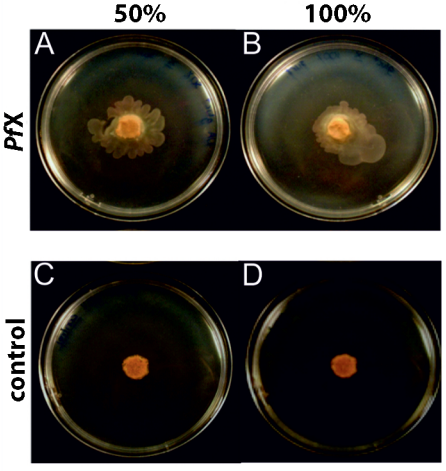

Figure 1. Motility of *Pseudomonas fluorescens* strain X in 100% and 50% saturated soil.
The soil tablets included in solid (0.5% agar) Nutrient Agar (NA) medium were inoculated with 3 µl of bacteria in the center of each tablet and growth was recorded at the NA–soil interfaces after incubation for 20 h.

ria in the soil, acting as highways for motile bacteria (Nazir *et al.*, 2010). In a recent study, this concept was further extended; the authors using the bacterium *Paenibacillus vortex* have shown that *P. vortex* swarms can transport conidia of the *Aspergillus fumigatus* over long distances. Inoculation of *A. fumigatus* conidia near to an artificial air revealed that the fungi grown across the gap, permitting a successful cross of *P. vortex*, suggesting a role for swarming and/or flagella in this mutually facilitating migration process (Ingham *et al.*, 2011).

Dispersal along fungal hyphae appears to be a widespread trait of swarming bacteria (Bravo *et al.*, 2013; Furuno *et al.*, 2102; Pion *et al.*, 2013; Simon *et al.*, 2015). *In vitro* studies have shown that migration along mycelium surface facilitated the bacterial degradation of organic soil pollutants (Banitz *et al.*, 2013) and the migration of *Burkholderia terrae* BS001 along mycelium surface (Warmink *et al.*, 2011). Shiga toxin-producing *Escherichia coli* was found to spread over several food-related fungi (Lee *et al.*, 2013). Enhanced colonization of rhizosphere by saprotrophic fungi stimulated root surface colonization by indigenous rhizosphere inhabiting biocontrol bacteria (de Boer *et al.*, 2014).

Bacterial swarms recruit cargo bacteria or facilitate the migration of fellow swarmers

Swarming offers a competitive advantage to some bacteria in invading some plant habitats (Barak *et al.*, 2009). However, co-swarming or transporting other bacterial species may expand the abilities of the partners in occupying and exploiting new territories. This combination of properties is well illustrated by recent studies at laboratory conditions. A non-swarming gentamycin resistant *Burkholderia cepacia* (cargo bacterium) allowed the gentamycin-sensitive proficient-swarming *Pseudomonas aeruginosa* to swarm and colonize a gentamycin-containing area of the plate, dispersing both bacteria (Venturi *et al.*, 2010). Moreover, it has been shown that an ampicillin-sensitive *Paenibacillus vortex* was capable to swarm and colonize an ampicillin plate using non-motile ampicillin resistant *Escherichia coli* as a cargo organism; one species provides an enzyme that detoxifies the antibiotic (a sessile cargo bacterium carrying a resistance gene), while the other (*P. vortex*) moves itself and transports the cargo bacterium *(*Finkelshtein *et al.*, 2015*).* Fast-swarming *Myxococcus xanthus* strains cooperated with slower isolates, allowing the latter to keep pace with faster strains in mixed groups (Kraemer and Velicer, 2014).

Whether these cooperative phenomena between swarming bacteria may occur in natural habitats is not clear. However, in situ experiments have provided evidence that non-motile bacteria may behave as hitchhikers and thus are able to move along fungal hyphae only with the aid of other motile bacteria acting as 'community migrators' (Simon *et al.*, 2015). Cooperation among non-competitive swarming bacterial strains provide a mechanism for mixing, thus it would be predicted that they may form mixed strains biofilm (Reichenbach *et al.*, 2007). The ability of three species (an antibiotic-producing *Pseudomonas aeruginosa* strain P1, a resistant *Raoultella ornithinolytica* strain R1 and a sensitive *Brevibacillus borstelensis* strain S1) to establish biofilms (Narisawa *et al.*, 2008), and the recent demonstration that kin but not identical swarming *B. subtilis* strains were able to co-exist in biofilms formed in *Arabidopsis thaliana* roots (Stefanic *et al.*, 2015), suggested that co-swarming but non-competitive bacterial strains may lead to the establishment of an non-transitive competitive network in natural habitat.

Interspecies communication affecting swarming motility

In most environmental niches, multiple bacterial species coexist as dynamic communities. Bacteria have developed intercellular signaling to adapt and survive in natural environments and to detect each other as

they colonize different surfaces (Park, 2003). Many bacteria secrete small diffusible as quorum sensing (QS) signaling molecules such as acyl-homoserine lactones (AHLs) (Rajput *et al*., 2015) or volatile signal molecules such as terpenoids, alkenes, aldehydes (Piechulla and Degenhardt, 2014), thus mediating different types of cell-to-cell communication among physically separated microorganisms (Schmidt *et al*., 2015).

AHLs are commonly synthesized by members of the LuxI family of proteins and are sensed by members of the LuxR family of transcriptional regulators (Daniels *et al*., 2004). Above threshold concentrations which is dependent by the cell population density (quorum sense signals), AHLs are involved in the activation of expression of certain genes which confer the ability to the bacterium to migrate in a wide range of ecological niches.

The complexity of AHL signal molecules (QS signals) produced by bacteria are limited, thus there is considerable opportunity for cross talk among bacteria, as in most environmental niches, multiple bacterial species coexist as dynamic communities. Gantner and colleagues, using a reporter engineered *Pseudomonas putida* strain on plant surfaces, showed that some AHL signals were able to travel relatively long distances (up to 78 mm) but was most commonly detected only a few micrometers away from the producing strain (Gantner *et al*., 2006). This was referred to as the cell-to-cell calling distance. Culturable rhizosphere bacteria of wheat also produced signal that could inhibit QS in *P. chlororaphis* (*aureofaciens*) via mechanisms that remain uncharacterized (Morello *et al*., 2004).

The ability of QS signals to influence QS regulated networks is an important mechanism in modulating the QS-controlled surface motility of physically separated bacteria. This conjecture was elegantly demonstrated in recent studies where it was shown that bacterial epiphytes produced signals capable of interfering with the QS system of *Pseudomonas syringae* pv. *syringae* (Pss) affected its swarming motility (Dulla *et al*., 2009). The plant epiphytic pathogenic bacterium Pss grows and survives on leaf surfaces, invades into the leaf tissue and contributes to brown spot disease, thus the assessment of lesion formation was considered as sensitive marker of its motility behavior; non-motile bacteria were less virulent (Haefele and Lindow, 1987). In this respect, proficiency in swarming motility on plant surface has been categorized as a virulence factors.

QS suppresses swarming in Pss and QS-deficient hyperswarmer strains invade leaves more readily than wild-type strains, thereby causing a higher incidence of brown spot lesions on bean, suggesting that swarming motility of Pss strongly contributes to its ability to invade leaves and incite disease on the leaves (Quinones *et al*., 2005). Nonmotile mutants of Pss are less able to survive desiccation stresses on leaves, apparently because they cannot access protected sites in or on the leaf surface (Quinones *et al*., 2004). Microarray analysis of Pss gene expression during growth in epiphytic versus apoplastic sites, revealed that genes involved in motility were relatively expressed at higher levels when bacteria are located in former site, suggesting that bacteria are requiring active motility for relocation on leaves surface compared to a the apoplast where these traits were less expressed (Yu *et al*., 2013). Co-inoculation of wild type Pss along with selected AHLs-producing epiphytic bacteria which produced large amounts of AHLs identical to those produced by Pss, decreased mobility Pss B728a on the leaf surface caused by inappropriate induction of Pss B728a QS system, resulting in less invasion into the tissue (Dulla *et al*., 2009). Similarly, premature induction of *Xylella fastidiosa* by its QS diffusible signal factor which however was produced by the transgenic host plant enforced the pathogen to prematurely adopt a suite of phenotypes that would restrict its ability to move in the plant. Diffusible signal factor is also produced by other beneficial endophytic bacteria such as *Stenotrophomonas maltiphila* (Fouhy *et al*., 2007; Zhu *et al*., 2012), thus it may represent an alternative to inhibit the migration of *X. fastidiosa*.

Volatile organic compounds (VOCs) produced by plant-associated bacteria are involved in their interaction with plant associated microorganisms as well as with host plants, providing a new source of compounds with antibiotic and plant growth-promoting activities. Rhizobacterial VOCs have been shown to inhibit microbial plant pathogens, induce systemic resistance in plants and trigger plant growth promotion (Bitas *et al.*, 2013). Volatiles emitted by *Serratia plymuthica* decreased the cell–to-cell communication quorum-sensing (QS) network mediated by AHLs produced in several plant pathogenic and plant-beneficial bacteria (Chernin *et al.*, 2011).

The ability of bacterial volatiles signals to influence motility of physically separated bacteria demonstrated in a recent study, where the authors presented data showing that volatiles produced by bouquet of phylogenetically different bacterial isolates (*Collimonas pratensis, S. plymuthica, Paenibacillus* sp. and *Pedobacter* sp.) affected the expression of genes involved in *P. fluorescens* motility (Garbeva *et al.*, 2014). Recent studies further substantiated swarming motility among the traits influenced by VOCs. Co-inoculation experiments of physically separated *P. vortex* and the non-motile plant pathogen *Xanthomonas perforans* revealed a massively spread of both bacteria on the plates (Hagai *et al.*, 2014). The influence of diffusible and/or volatile signals on mobilization of *X. perforans* appears also to occur in planta; fluorescence-stained *X. perforans* spotted on a leaf surface seems to swarm towards the distantly located *P. vortex* (Hagai *et al.*, 2014). Modulation of swarming motility by interspecies or cross- cross-kingdom signaling appears a quite common phenomenon among bacteria. Volatiles emitted by *B. subtilis* 168 to modulate the swarming motility of an array of bacterial species including *E. coli* (Kim *et al.*, 2013). Several *Xanthomonas* species affected *Paenibacillus vortex, Paenibacillus dendritiformis* and *Proteus mirabilis* surface motility through volatiles (Hagai *et al.*, 2014). Volatile metabolites such as farnesol produced by *Candida albicans* reduce the swarming motility of *Pseudomonas aeruginosa* (McAlester *et al.*, 2008).

Swarmers dueling

Plant surfaces are the habitat to a complex and competitive microbiota. The root surface and surrounding rhizosphere are significant carbon sinks which is produced by the plant (Compant *et al.*, 2005). Thus, along root surfaces, there are various suitable nutrient-rich niches attracting a great diversity of microorganisms, including phytopathogens (Nelson *et al.*, 2004). Competition for these nutrients and niches is a fundamental mechanism by which biological control agents such as biocontrol bacteria protect plants from phytopathogens. Thus proficiency in surface motility can provide a competitive advantage to the invading bacterial populations over other swarming or non-swarming microorganisms that are colonizing similar plant niches and either intercept and/or kill the opponents or merge with them.

In vitro studies using converging swarming colonies between less or more phylogenetically related bacteria revealed the presence of a complex social behavior pattern ranging from discriminatory aggression, by forming a boundary between the two advancing swarms, to cooperating merging of the two swarms. This phenomenon provided evidence that there is a general tendency for discrimination of self and non-self between phylogenetically unrelated interacting swarms, and have been studied in the soil bacterium *Myxococcus xantus* (Vos and Velicer, 2009; Rendueles *et al.*, 2015), *Burkholderia pseudomallei* (Ngamdee *et al.*, 2015), the pathogen *Proteus mirabilis* (Gibbs *et al.*, 2008; Alteri *et al.*, 2013; Wenren *et al.*, 2013) and in *B. subtillis* (Stefanic *et al.*, 2015).

Different strains of the soil inhabiting bacterium *B. subtilis*, isolated from 1-cm^3 soil samples, examined on swarm plates in pairwise combinations were found to form either distinct boundaries (phylogenetically unrelated, nonkin strains) or the swarms merge (phylogenetically related, kin strains).

Interestingly, the nonkin bacteria competed with each other and only one was able to colonize plant roots. The possible lack of alive cells in many swarm boundaries and the competition for root surface colonization between nonkin strains may suggest that antagonistic mechanisms preventing coexistence of nonkin *B. subtilis* on roots (Stefanic *et al*., 2015). On the other hand, the kin strains demonstrated the ability to merge on agar substrate and in situ - colonization of the same root surface suggests that co-swarming on root surface is taking place which permitted the formation of mixed biofilms. In contrast, genetically identical strains (siblings) of *Paenibacillus dendritiformis* swarming colonies mutually inhibit growth through secretion of a toxic protein termed sibling lethal factor (Slf) (Be'er *et al*., 2009; Be'er *et al*., 2010). Slf is produced in an isolated, nutrient-starved colony. This protein is not toxic for other phylogenetically related bacteria such as *B. subtilis*. However Slf is produced along with subtilisin, a biosurfractant, in swarming colonies and not in immobile isolated colonies; thus, we suggest that the Slf represents a new class of toxins that are most effective for regulating swarming interspecies competition.

Proteus mirabilis is capable of movement on solid surfaces by swarming motility. Swarms of independent *P. mirabilis* isolates can recognize each other as nonkin and establish a visible boundary where they meet. In contrast, genetically identical swarms merge (Gibbs *et al*., 2003; Alteri *et al*., 2013). In an elegant study, Alteri and coworkers have shown that in *P. mirabilis* upon initiation of swarming differentiation, the type VI secretion system (T6SS) apparatus is assembled and appears to fire when opposing swarms meet by injecting the toxin into the cytosol of the rival strain. The Dienes line represents a zone of dead bacteria of the less dominant strain. The dominant strain infiltrates deeply beyond the boundary of the two swarms and continues to assemble and discharge the T6SS (Alteri *et al*., 2013; Sarris *et al*., 2013).

The T6SSs are prevalent and conserved among plant pathogenic and plant beneficial Gram-negative bacteria (Loper *et al*., 2012; Sarris *et al*., 2013). By now multiple cases have been described where T6SS-harboring plant associated bacteria are able to duel and outcompete competitor bacterial cells. For example, the plant beneficial *Pseudomonas protegens* strains were competed under cell contact-promoting conditions against *P. putida*, a bacterium that inhabits similar environments, whereas those lacking *tge2*, a type VI effector resembling glycoside hydrolase protein, were 6-fold less fit compared with the wild-type (Whitney *et al*., 2003). Hemolysin-coregulated protein (Hcp) function is also required for *P. syringae* pv. *tomato* DC3000 antibacterial activity against other Gram-negative bacteria, yeast and amoeba during contact on a solid surface (Haapalainen *et al*., 2012).

In a recent study, the competitive advantage of plant associated bacteria harboring T6SS in dueling with other bacteria in planta has been illustrated (Ma *et al*., 2014). The authors presented data showing that *Agrobacterium tumefaciens* (a Gram-negative soil inhabiting bacterium that causes crown gall in infected plants) T6SS is important in interspecies completion with the soil bacterium *P. aeruginosa*. When *A. tumefaciens* and *P. aeruginosa* duels *in vitro* under cell contact-promoting conditions, the former was efficiently outcompeted. The competitive advantage of *P. aeruginosa* included a T6SS-mediated counterattack. However, co-infection experiments conducted in tobacco plants revealed that the outcome of the duel was reverted within the plant habitat.

Pseudomonas fluorescens strain MFE01, under cell contact-promoting conditions, outcompete *E. coli, P. aeruginosa* PA14, *P. fluorescens* Pf0-1 and *P. fluorescens* MFE1032, whereas a MFE01Δhcp2 and MFE01Δhcp1 mutants demonstrated a significant loss in their ability to reduce prey cell population (Decoin *et al*., 2014; Decoin *et al*., 2015). The bacterial killing capacity of MFE01 against *E. coli* cells was neutralized by the constitutive expression of a T6SS- mediated-injected immunity cognate protein from *Serratia marcescens*. MFE01 was also able to outcompete the

competitor under swarming conditions possibly through the combined action of secreted Hcps; Hcp1 could reduce the motility of prey cells and the killing conducted by Hcp2 (Decoin *et al.*, 2015). The capacity of MFE01 to duel and kill other bacteria was also tested in physiological relevant environments, coinfection of potato tuber with a mixture of MFE01 and *Pectobacterium atrosepticum* protected efficiently potatoes against soft-rot symptoms caused by *P. atrosepticum* whereas MFE01Δhcp2 was unable to confer protection (Decoin *et al.*, 2014). However, *P. atrosepticum* harbor the T6SS and excretes Hcps (Mattinen *et al.*, 2008), thus it will be of interest to examine the dueling of this bacterium with MFE01 *in vitro* on plates.

Because T6SS is dependent on cell-to-cell contact, it would seem beneficial for bacteria exhibiting multicellular behavior to employ the T6SS to discriminate and kill competitors rather than indiscriminately secrete bactericidal agents when competing for resources in their natural habitats. Plant beneficial swarming bacteria harboring T6SS may encounter each other or competitor pathogenic bacteria when are migrating on plant surface or rhizosphere, thus among the traits of a biocontrol bacterium may be its efficient T6SS against a wide range of competitor bacteria thereby indirectly supporting the protection for the plant.

A recent study in our laboratory has shown that *Pseudomonas fluorescens* strain X (*Pf.* X) displayed a killing aggression against prokaryotic and eukaryotic competitors on agar substrate. The *Pf.* X, a nonpathogenic rhizobacterium, was isolated from the rhizosphere of sugar been (Georgakopoulos *et al.*, 2002). This strain harbors a gene cluster coding for a cyclic lipopeptide surfactant similar to massetolide A, which is important for swarming motility and T6SS core component genes (Venieraki *et al.*, unpublished observations). *Pf.* X forms discrete boundaries between neighboring swarms on an agar substrate with different competitor pathogenic and beneficial *Pseudomonas* strains, the boundary demarcation is occupied by killed bacteria and a one-sided invasion by the dominant *Pf.* X swarm within the swarm of the opponents (Fig. 2). To quantify the killing, competition assays were conducted between *Pf.* X and the other *Pseudomonas* sp. For example, wild-type *Pf.* X killed *Pseudomonas* strain P21 by at least 5-logs when plated together on agar to permit swarming or non-swarming conditions and co-cultured for 20 h (unpublished data).

Although strain *Pf.* X clearly exhibited antagonism against *Fusarium* sp., *Botrytis* sp. and *Rhizoctonia solani* on 1.5% agar nutrient plates (Fig. 3), all fungi ended up occupying more than 90% of the plate surface area. In contrast, mycelium spreading of fungi was safely hampered when co-cultivated with the surface-motile *Pf.* X (Fig. 3). Once the *Pf.* X colony started to expand, contact between the spreading bacterial front and the fungus was achieved within a few hours. In some cases, a boundary line was observed and the advancing *Pf.* X swarm colonies moved along the hyphae. The trapped fungus was no longer viable as judged by its failure to resume mycelium growth when transfer to a new nutrient plate or in liquid culture.

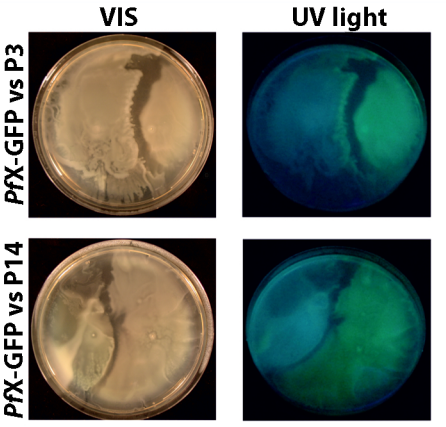

Figure 2. Warfare between swarming *Pseudomonas fluorescens* strain X (green) GFP-tagged and *Pseudomonas* sp. strain P3 and P14 (bluish).
Pseudomonas fluorescens strain X-GFP was cultured in pairs with *Pseudomonas* sp. strain P3 or P14 on swarming agar plates. The boundary formed between the different strains is referred as Dienes line. Note the presence of *Pseudomonas fluorescens* strain X-GFP (green) within the swarms of the rival strains.

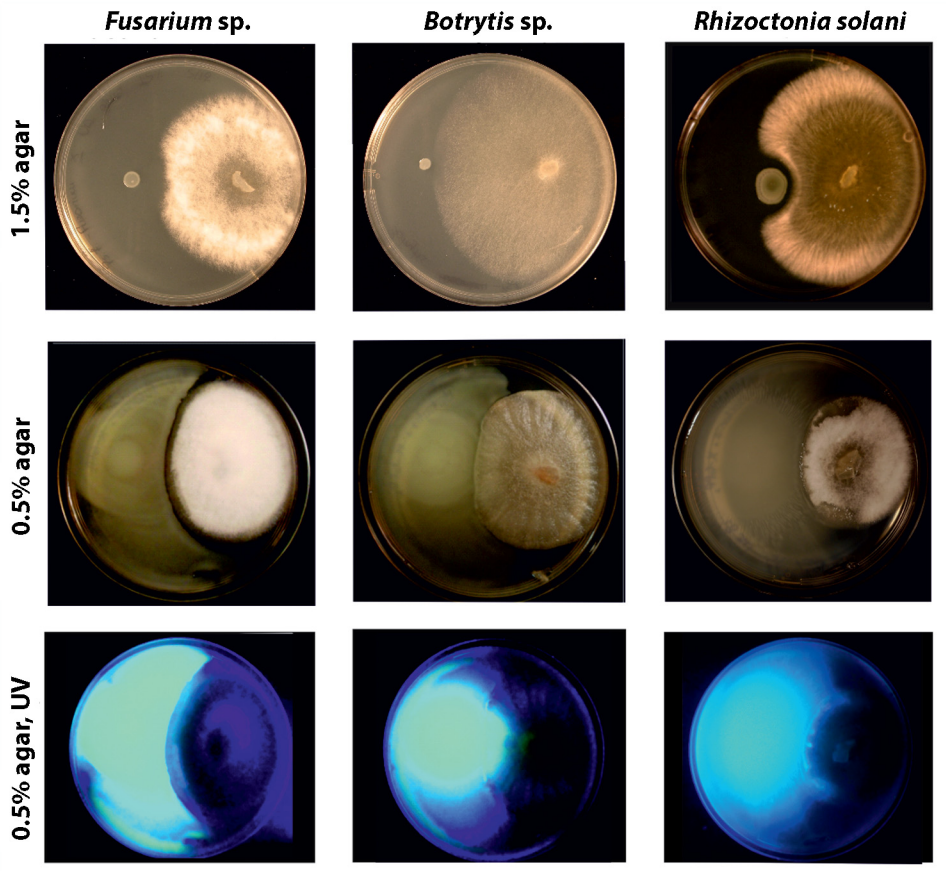

Figure 3. Antifungal properties of swarming (0.5% agar) and non-swarming (1.5% agar) of *Pseudomonas fluorescens* strain X against *Fusarium* sp., *Botrytis* sp. and *Rhizoctonia solani*.

Conclusions

This review has focused on resent research concerning bacterial surface motility as a trait that provides a survival advantage to competitive environments. Furthermore, it is stressing the point that flagella- or type IV pili-driven surface motility appears to provide a competitive advantage to plant pathogenic or biocontrol bacteria for colonization of plant tissues. These motility modalities may be harnessed for beneficial tasks through novel and ecologically safe strategies. Aiming at plant growth and health future challenges should therefore concentrate in exploring the genetic basis of these phenomena placing emphasis on biocontrol bacteria, to discover appropriate partners for the fungus-driven bacterial dispersal and resolve the importance of microbially produced volatiles in plant protection.

Literature cited

Alteri, C.J., Himpsl, S.D., Pickens, S.R., Lindner J.R, Zora, J.S., Miller, J.E, Arno P.D., Straight, S.W. and Mobley, H.L. 2013. Multicellular bacteria deploy the type VI secretion system to preemptively strike neighboring cells. *PLoS Pathogens*, 9(9): e1003608

Banitz, T., Johst, K., Wick, L.Y., Schamfuß, S., Harms, H. and Frank, K. 2013. Highways versus pipelines: contributions of two fungal transport mechanisms to efficient bioremediation. *Envi-*

ronmental Microbiology Reports, 5: 211–8.

Barak, J.D., Gorski, L., Liang, A.S. and Narm, K-E. 2009. Previously uncharacterized *Salmonella enteric* genes required for swarming play a role in seedling colonization. *Microbiology*, 15: 3701-3709.

Be'er, A., Ariel, G., Kalisman, O., Helman, Y., Sirota-Madi, A., Zhang, H.P., Florin, E-L., Payne, S.M., Ben-Jacob, E. and Swinney H. L. 2010. Lethal protein produced in response to competition between sibling bacterial colonies. *Proceedings of the National Academy of Sciences of the United States of America*, 107(14): 6258–6263.

Be'er, A., Zhang, H.P., Florin, E.L., Payne, S.M., Ben-Jacob, E. and Swinney, H.L. 2009. Deadly competition between sibling bacterial colonies. *Proceedings of the National Academy of Sciences of the United States of America*, 106: 428–433.

Berg, G., Rybakovam, D., Grube, M. and Köberl M. 2015. The plant microbiome explored: implications for experimental botany. *Journal of Experimental Botany,* doi: 10.1093/jxb/erv466

Bitas, V., Kim,H-S., Joan W. Bennett, J.W. and Kang S. 2013. Sniffing on microbes: Diverse roles of microbial volatile organic compounds in plant health. *Molecular Plant-Microbe Interactions*, 26: 835–843

Bravo, D., Cailleau, G., Bindschedler, S., Simon, A., Job, D., Verrecchia, E. and Junier, P. 2013. Isolation of oxalotrophic bacteria able to disperse on fungal mycelium. *FEMS Microbiology Letters*, 348: 157–66.

Chernin, L., Toklikishvili, N., Ovadis, M., Kim, S., Ben-Ari, J., Khmel, I. and Vainstein, A. 2011. Quorum-sensing quenching by rhizobacterial volatiles. *Environmental Microbiology Reports*, 3: 698–704.

Compant, S., Duffy, B., Nowak, J., Clément, C. and Barka, E.A. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology*, 71: 4951–4959.

Covelli, J.M., Althabegoiti M.J, López, M.F. and Lodeiro, A.R. 2013. Swarming motility in *Bradyrhizobium japonicum*. *Research in Microbiology*, 164: 136-44.

Daniels, R., Vanderleyden, J. and Michiels, J. 2004. Quorum sensing and swarming migration in bacteria. *FEMS Microbiol Reviews*, 28(3): 261–89.

de Boer, W., Hundscheid, M.P.J., Gunnewiek K., P.J.A., de Ridder-Duine, A.S., Thion, C., van Veen, J.A., and van der Wal A. 2015. Antifungal Rhizosphere Bacteria can increase as Response to the Presence of Saprotrophic Fungi. *PLoS ONE*, 10(9): e0137988.

Decoin, V., Barbey, C., Bergeau, D., Latour, X., Feuilloley, M.G., Orange, N. and Merieau, A. 2014. A type VI secretion system is involved in *Pseudomonas fluorescens* bacterial competition. *PLoS ONE*, 9(2): e89411.

Decoin V, Gallique M, Barbey C, LeMauff F, Duclairoir Poc C, Feuilloley M.G.J., Orange, N. and Merieau, A. 2015. A *Pseudomonas fluorescens* type 6 secretion system is related to mucoidy, motility and bacterial competition. *BMC Microbiology*, 15: 72.

Dulla, G. and Lindow, S. 2009. Acyl-homoserine lactone mediated cross talk among epiphytic bacteria modulates behavior of *Pseudomonas syringae* on leaves. *The ISME Journal*, 3: 825–834.

Finkelshtein, A., Roth, D., Ben Jacob, E. and Ingham, C.J. 2015. Bacterial swarms recruit cargo bacteria to pave the way in toxic environments. *mBio* 6(3): e00074-15.

Fouhy, Y., Scanlon, K., Schouest, K., Spillane, C., Crossman, L., Avison, M.B., Ryan, R.P. and Dow, J.M. 2007. Diffusible signal factor-dependent cell-cell signaling and virulence in the nosocomial pathogen *Stenotrophomonas maltophilia*. *Journal of Bacteriology*, 189: 4964–4968.

Furuno, S., Remer, R., Chatzinotas, A., Harms, H. and Wick, L.Y. 2012. Use of mycelia as paths for the isolation of contaminant-degrading bacteria from soil. *Microbial Biotechnology*, 5(1): 142-8.

Gantner, S., Schmid, M., Durr, C., Schuhegger, R., Steidle, A., Hutzler, P., Langebartels C, Eberl, L., Hartmann, A., Dazzo, F.B.. 2006. In situ quantitation of the spatial scale of calling distances and population density-independent N-acylhomoserine lactone mediated communication by rhizobacteria colonized on plant roots. *FEMS Microbiology Ecology*, 56: 188–194.

Garbeva, P., Hordijk, C., Gerards, S. and De Boer, W. 2014. Volatile-mediated interactions between phylogenetically different soil bacteria. *Frontiers in Microbiology*, 5: 285–290.

Georgakopoulos, D.G., Fiddaman, P., Leifert, C. and Malathrakis, N.E. 2002. Biological control of cucumber and sugar beet damping-off caused by *Pythium ultimum* with bacterial and fungal antagonists. *Journal of Applied Microbiology*, 92: 1078–86.

Gibbs, K.A., Urbanowski, M.L. and Greenberg, E.P. 2008. Genetic determinants of self-identity and social recognition in bacteria. *Science*, 321(5886): 256–259.

Haapalainen, M., Mosorin, H., Dorati, F., Wu, R.F., Roine, E., Taira, S., Nissinen, R., Mattinen, L., Jackson, R., Pirhonen, M. and Lin N.C. 2012. Hcp2, a secreted protein of the phytopathogen *Pseudomonas syringae* pv. tomato DC3000, is required for fitness for competition against bacteria and yeasts. *Journal of Bacteriology*, 194: 4810–4822.

Haefele, D.M. and Lindow, S.E. 1987. Flagellar motility confers epiphytic fitness advantages upon *Pseudomonas syringae*. *Journal of Applied Microbiology*, 53: 2528–2533.

Hagai, E., Dvora, R., Havkin-Blank, T., Zelinger, E., Porat, Z., Schulz, S. and Helman, Y. 2014. Surface-

motility induction, attraction and hitchhiking between bacterial species promote dispersal on solid surfaces. *The ISME Journal*, 8: 1147–1151.

Hannula, S.E, de Boer, W. and van Veen, J.A. 2010. In situ dynamics of soil fungal communities under different genotypes of potato, including a genetically modified cultivar. *Soil Biology & Biochemistry*, 42: 2211–2223.

Harshey, R.M. 2003. Bacterial motility on a surface: Many ways to a common goal. *Annual Review of Microbiology,* 57: 249–273.

Harshey, R.M. and Partridge, J.D. 2015. Shelter in a Swarm. *Journal of Molecular Biology*, 427(23): 3683-94.

Ingham, C.J., Kalisman, O., Finkelshtein, A. and Ben-Jacob, E. 2011. Mutually facilitated dispersal between the nonmotile fungus *Aspergillus fumigates* and the swarming bacterium *P. vortex*. *Proceedings of the National Academy of Sciences of the United States of America*, 108: 19731–19736.

Jarrell, K.F. and McBride, M.J. 2008. The surprisingly diverse ways that prokaryotes move. *Nature Reviews Microbiology*, 6: 466–476.

Kearns, D.B. 2010. A field guide to bacterial swarming motility. *Nature Reviews Microbiology,* 8: 634–644.

Kim, K-S., Lee, S. and Ryu, C-M. 2013. Interspecific bacterial sensing through airborne signals modulates locomotion and drug resistance. *Nature Communications*, 4: 1809-1815.

Kohlmeier, S., Smits, T.H.M., Ford, R.M., Keel, C., Harms, H. and Wick, L.Y. 2005. Taking the fungal highway: mobilization of pollutantdegrading bacteria by fungi. *Environmental Science and Technology*, 39: 4640–46.

Kraemer, S.A. and Velicer, G.J. 2014. Social complementation and growth advantages promote socially defective bacterial isolates. *Proceeding of the Royal Society B*, 281: 20140036.

Kremmydas, G. F., Tampakaki, A. P. and Georgakopoulos, D. G. 2013. Characterization of the biocontrol activity of *Pseudomonas fluorescens* strain X reveals novel genes regulated by glucose. *PLoS ONE*, 8(4): e61808.

Lee, K., Kobayashi, N., Watanabe, M., Sugita-Konishi, Y., Tsubone, H., Kumagai, S. and Hara-Kudo, Y. 2014. Spread and change in stress resistance of Shiga toxin-producing *Escherichia coli* O157 on fungal colonies. *Microbial Biotechnology*, 7: 621–629.

Loper, J.E., Hassan, K.A., Mavrodi, D.V. *et al.*, 2012. Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. *PLoS Genetics*, 8(7): e1002784.

Ma, L.S., Hachani, A., Lin, J.S., Filloux, A., and Lai, E.M. 2014. *Agrobacterium tumefaciens* deploys a superfamily of type VI secretion DNase effectors as weapons for interbacterial competition in planta. *Cell Host and Microbe*, 16: 94–104.

Mattinen, L., Somervuo, P., Nykyri, P., Nissinen, R., Kouvonen, P., Corthals, G., Auvinen, P., Aittamaa, M., Valkonen, J.P.T. and Pirhonen, M. 2008. Microarray profiling of host-extract induced genes and characterization of the type VI secretion cluster in the potato pathogen *Pectobacterium atrosepticum*. *Microbiology,* 154: 2387–2396.

McAlester, G., O'Gara, F. and Morrissey J.P. 2008. Signal-mediated interactions between *Pseudomonas aeruginosa* and *Candida albicans*. *Journal of Medical Microbiology*, 57: 563–569.

Mendes, R., Garbeva, P. and Raaijmakers, J.M. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, 37: 634-63.

Morello, J.E., Pierson, E.A., and Pierson, L.S. 2004. Negative cross-communication among wheat rhizosphere bacteria: effect on antibiotic production by the biological control bacterium *Pseudomonas aureofaciens* 30–84. *Applied and Environmental Microbiology*, 70: 3103–3109.

Narisawa, N., Haruta, S., Arai, H., Ishii, M. and Igarashi, Y. 2008. Coexistence of antibiotic-producing and antibiotic sensitive bacteria in biofilms is mediated by resistant bacteria. *Applied and Environmental Microbiology*, 74: 3887–3894.

Ngamdee W, Tandhavanant S, Wikraiphat C, Reamtong O, Wuthiekanun V, Salje J, Low D.A., Peacock, S.J. and Chantratita N. 2015. Competition between *Burkholderia pseudomallei* and *B. thailandensis*. *BMC Microbiology*, 15: 56-52.

Nazir, R, Warmink, J.A., Boersma, H. and van Elsas, J.D. 2010. Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. *FEMS Microbiology Ecology*, 71(2): 169-85.

Nelson, E.B. 2004. Microbial dynamics and interactions in the spermosphere. *Annual Review of Phytopathology*, 42: 271–309.

Park, S. 2003. Influence of topology on bacterial social interaction. *Proceedings of the National Academy of Sciences of the United States of America*, 24: 13910–13915.

Partridge, J.D. and Harshey, R.M. 2013. Swarming: Flexible roaming plans. *Journal of Bacteriology*, 195: 909–918.

Piechulla, B. and Degenhardt, J. 2014. The emerging importance of microbial volatile organic compounds. *Plant, Cell and Environment*, 37: 811–812.

Pion, M., Bshary, R., Bindschedler, S., Filippidou, S., Wick, L.Y., Job, D. and Junier P. 2013. Gains of bacterial flagellar motility in a fungal world. *Applied and Environmental Microbiology*, 79: 6862–6867.

Quinones, B., Dulla, G. and Lindow, S.E. 2005. Quorum sensing regulates exopolysaccharide pro-

duction, motility, and virulence in *Pseudomonas syringae. Molecular Plant-Microbe Interactions*, 18: 682–693.

Quinones, B., Pujol, C.J. and Lindow, S.E. 2004. Regulation of AHL production and its contribution to epiphytic fitness in *Pseudomonas syringae*. Mol *Molecular Plant-Microbe Interactions*, 17: 521–531.

Rajput, A., Kaur K. and Kumar, M. 2015. SigMol: repertoire of quorum sensing signaling molecules in prokaryotes. *Nucleic Acids Research*, 1: doi: 10.1093/nar/gkv1076.

Rendueles, O., Zee, P.C., Dinkelacker, I., Amherd, M., Wielgoss, S. and Velicer, G.J. 2015. Rapid and widespread de novo evolution of kin discrimination. *Proceedings of the National Academy of Sciences of the United States of America*, 112(29): 9076–9081.

Reichenbach, T., Mobilia, M. and Frey, E. 2007. Mobility promotes and jeopardizes biodiversity in rock-paper scissors games. *Nature*, 448:1046–1049.

Sarris, P.F., Trantas, E.A., Baltrus, D.A., Bull, C.T., Wechter, W.P., Yan, S., Ververidis, .F, Almeida, N.F., Jones, C.D., Dangl J.L, Panopoulos N.J, Vinatzer, B.A. and Goumas, D.E. 2013. Comparative Genomics of Multiple Strains of *Pseudomonas cannabina* pv. *alisalensis*, a Potential Model Pathogen of Both Monocots and Dicots. *PLoS ONE*, 8(3): e59366.

Sarris, P.F., Trantas, E.A., Skandalis, N., Tampakaki, A.P., Kapanidou, M., Kokkinidis, M. and Panopoulos, N.J. 2011. Phytobacterial Type VI Secretion System: Gene Distribution, Phylogeny, Structure and Biological Functions. 53-84 pp. In: Plant Pathology, C.J. R. Cumagan (ed). InTech - Open Access Publisher. ISBN: 978-953-307-933-2. Croatia

Simon, A., Bindschedler, S., Job, D., Wick, L.Y., Filippidou, S., Kooli, W.M., Verrecchia, E.P. and Junier, P. 2015. Exploiting the fungal highway: development of a novel tool for the in situ isolation of bacteria migrating along fungal mycelium. *FEMS Microbiology Ecology*, 91: 1-13.

Stefanic, P., Kraighera, B., Lyonsb, N.A., Kolter, R. and Mandic-Mulec, I. 2014. Kin discrimination between sympatric Bacillus subtilis isolates. *Proceedings of the National Academy of Sciences of the United States of America*, 112(45): 14042-7

Schmint, R., Cordovez, V., deBoer, W. and Raaijmamakers, L. 2015. Volatille affairs in microbial interactions. *The ISME Journal*, 9(11): 2329-35.

Yu X., Lund, S.P., Greenwald, J.W., Angela H., Records, A.H., Scott, R.A., Nettleton,D., Lindow, S.E., Gross D.C. and Beattie, G.A. 2013. Transcriptional Analysis of the Global Regulatory Networks Active in *Pseudomonas syringae* during Leaf Colonization. *mBio*, 5(5): e01683-14.

Venturi, V., Bertani, I., Kerenyi, A., Netotea, S. and Pongor, S. 2010. Co-Swarming and Local Collapse: Quorum Sensing Conveys Resilience to Bacterial Communities by Localizing Cheater Mutants in *Pseudomonas aeruginosa*. *PLoS ONE*, 5(4): e9998.

Vos, M. and Velicer, G.J .2009. Social conflict in centimeter- and global-scale populations of the bacterium *Myxococcus xanthus*. *Current Biology*, 19(20): 1763–1767.

Warmink, J.A. and van Elsas, J.D. 2009. Migratory response of soil bacteria to *Lyophyllum* sp. strain Karsten in soil microcosms. *Applied and Environmental Microbiology,* 75(9): 2820-2830.

Warmink, J.A., Nazir, R., Corten, B. and van Elsas J.D. 2011. Hitchhikers on the fungal highway: the helper effect for bacterial migration via fungal hyphae. *Soil Biology & Biochemistry*, 43 :760–765.

Wenren, L.M., Sullivan, N.L., Cardarelli, L., Septer, A.N. and Gibbs, K.A. 2013. Two independent pathways for self-recognition in *Proteus mirabilis* are linked by type VI-dependent export. *mBio*, 4(4): e00374-13.

Whitney, J.C., Chou, S., Russell, A.B., Biboy, J., Gardiner, T.E., Ferrin, M.A., Brittnacher, M., Vollmer, W., and Mougous, J.D. 2013. Identification, structure, and function of a novel type VIsecretion peptidoglycan glycoside hydrolase effector-immunity pair. *Journal of Biological Chemistry*, 288: 26616–26624.

Wösten HAB 2001. Hydrophobins: multipurpose proteins. *Annual Review of Microbiology*, 55: 625–46.

Xu, J., Plat, T.G., Fuqua, C. 2012. Regulatory Linkages between Flagella and Surfactant during Swarming Behavior: Lubricating the Flagellar Propeller? *Journal of Bacteriology*, 194: 1283-86.

Zhu, B., He Liu, H., Tian, W.X., Fan, X.Y., Bin Li, B., Zhou X.P., Jin G.L., and Xie G.L. 2012. Genome sequence of *Stenotrophomonas maltophilia* RR-10, isolated as an endophyte from rice root. *Journal of Bacteriology*, 194: 1280-1.

Received: 13 December 2015; Accepted: 7 January 2016

ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Η ομαδική κινητικότητα των βακτηρίων στις επιφάνειες των φυτών

Α. Βενιεράκη, Π.Χ. Τσαλγατίδου, Δ.Γ. Γεωργακόπουλος, Μ. Δήμου και Π. Κατινάκης

Περίληψη Το φυτά φιλοξενούν πλήθος βακτηριακών ειδών τα οποία ανταγωνίζονται ή/και συνεργάζονται για την εγκατάστασή τους στο πλέον κατάλληλο, κατά περίπτωση, οικολογικό ενδιαίτημα. Η κινητικότητα, ένα κοινό χαρακτηριστικό των βακτηρίων, εικάζεται ότι παρέχει στους βακτηριακούς πληθυσμούς πλεονεκτήματα επιβίωσης, έναντι άλλων μη-κινητικών, όσον αφορά την εγκατάστασή τους σε ανταγωνιστικά περιβάλλοντα. Η ομαδική κινητικότητα των βακτηρίων σε επιφάνειες (swarming), τύπος κινητικότητας που οφείλεται στην ύπαρξη μαστιγίων, αν και έχει παρατηρηθεί και μελετηθεί σε τεχνητά θρεπτικά υποστρώματα, φαίνεται να αποτελεί βασικό λειτουργικό χαρακτηριστικό των βακτηρίων των φυτών αναφορικά με την ικανότητά τους να αποικίζουν και να εξαπλώνονται στον ξενιστή τους. Στο άρθρο αυτό, παραθέτουμε ορισμένες νέες στρατηγικές ομαδικής κινητικότητας των βακτηρίων οι οποίες τους παρέχουν τη δυνατότητα αποικισμού, εξάπλωσης και διαχείρισης της ανταγωνιστικής ικανότητας τους στις επιφάνειες των φυτών.

Hellenic Plant Protection Journal **9:** 16-27, 2016

DE GRUYTER
OPEN

Hellenic Plant Protection Journal **9:** 28-34, 2016
DOI 10.1515/hppj-2016-0003

Trichoderma viride and *Pseudomonas fluorescens* CHA0 against *Meloidogyne javanica* in the rhizosphere of tomato plants

A. Saeedizadeh

Summary Root-knot nematodes are among the most important pests that reduce tomato yield in greenhouses and fields in Iran. The scope of this research was to evaluate the antagonistic effect of *Trichoderma viride* and *Pseudomonas fluorescens* CHA0 on the reproduction and galling rate of *Meloidogyne javanica* in tomato roots. A pot experiment was conducted on seedlings of tomato cultivars Bony best, Falat, Mobile and Walter grown in sterilized sandy loam soil. Inocula used for artificial inoculation were 3 J_2/g of soil for the nematode, 1×10^6 spores/ml for the fungus and 1×10^9 cfu/ml for the bacterium. The nematicide RUGBY® 10 G (cadusafos) was used as a reference product at 2g per each pot. Two months after inoculation, the number of knots and egg masses per root in the treatments were (with descending order): control (nematode), nematode+bacterium, nematode+fungus, nematode+fungus+bacterium and nematode+nematicide. The combination fungus+bacterium enhanced the biocontrol effect against *M. javanica* activity as compared to the fungus and bacterium stand-alone treatments except for the cases of the cultivars Mobile and Bonny best in which the effect was similar to the one by the fungus alone. The *fungus* + *bacterium* combined treatment was equally effective to the nematicide treatment for all cultivars. The highest and lowest rate of nematode activity was observed in Walter and Mobile cultivars, respectively.

Additional keywords: biocontrol, fungus, knot, nematode

Introduction

Root-knot nematodes are the most important plant parasitic nematodes in the world, due to the wide host range, worldwide distribution and interactions with phytopathogenic fungi and bacteria (Sasser, 1979). Tomato (*Solanum lycopersicum* L.) is a suitable host for most *Meloidogyne* species. The most important Root-knot nematode species in Iran are *M. incognita* and *M. javanica* with a broad host range and great dispersal, making plant roots more vulnerable to soil pathogens (Hosseini-Nejad and Khan, 2001).

Today, numerous microorganisms have been introduced as antagonists of plant parasitic nematodes (Akhtar and Malik, 2000). Some species of *Trichoderma* have been widely applied as biological control agents against various soil-borne plant pathogens (Whipps, 2001), and several isolates have been successful as biocontrol agents of root-knot nematodes (Sharon *et al.*, 2001). On the other hand, bacteria are the most abundant microorganisms in the soil, and some genera such as *Pasteuria*, *Pseudomonas* and *Bacillus* have also considerable potential as biological control agents against nematodes (Meyer, 2003).

The scope of the study was to evaluate *T. viride* and *P. fluorescens* CHA0 as biocontrol agents against *M. javanica* in four tomato cultivars.

Materials and Methods

Preparation of *M. javanica* inoculum

Plants infected with *M. javanica* were obtained from a population maintained in the glasshouse on tomato plants (cv. Walter) in Varamin, southern Tehran, Iran. Extraction and preparation of the nematode inoculum were applied according to the Hussey and Barker (1973) method using the single egg mass method. According to the morpholog-

Assistant Professor, Department of Plant Protection, Faculty of Agriculture, Shahed University, P.O. Box: 33191-18651, Tehran, Iran
e-mail: ayatsaeed314@gmail.com

ical and morphometrical characteristics of body and perineal pattern, the nematodes were initially identified (Hunt, 1993; Jepson, 1987; Siddiqi, 2000). Then, nematodes were multiplied on the rhizosphere of local tomato cultivars and second stage juveniles (J_2) were finally obtained in the glasshouse. Infected tomato roots bearing large egg masses were incubated in water for three days at 28±2°C and hatched J_2 were collected and counted. Nematode inoculum level for each of the treatments was determined as 3 J_2/g of soil (McClure *et al*., 1973).

Biocontrol of nematode using *T. viride*

Trichoderma viride was obtained from the culture collection of the Department of Plant Pathology, Shahed University, Tehran, Iran. It was cultured on potato dextrose agar (PDA) for 14 days at 25°C. To prepare the inoculum suspension of *T. viride*, about 15ml distilled water were added to the growing colony on PDA medium in a Petri dish. The spores were suspended in distilled water using a sterile glass rod and applied gently on the surface of the colonies. After that, the suspension passed through two layers of sterile net fiber. The number of spores per ml was estimated by a hemocytometer. A spore concentration basis of 1×10^6 spores/ml was adjusted by adding distilled water and suspending (Sahebani and Hadavi, 2008).

Biocontrol of nematode using *P. fluorescens*

Pseudomonas fluorescens CHA0 was obtained from the culture collection of the Department of Plant Pathology, Shahed University, Tehran, Iran. The bacterial inoculum suspension was prepared according to Weller and Cook (1983). A full loop of 48-hour culture of the bacterium on King's medium B (King B) was transferred to a flask containing 100ml King B liquid medium and incubated for 48 hours on shaker (120 RPM) at 27°C. Bacterial suspension was centrifuged for 10 min at 6000 x g, and washed for 2-3 times with a natural salt solution (NaCl 0.14M) to remove residual nutrient medium. Bacterial cells were extracted by recentrifuging and suspending to a solution of 1×10^9 cfu/ml, which was prepared using the standard curve spectrophotometrically in a carboxymethyl cellulose solution (Weller and Cook, 1983).

Plant material and inoculation

Tomato seedlings of four cultivars including Bonny best, Falat, Mobile and Walter were cultivated in sterilized sandy loam soil in the greenhouse. Seedlings were inoculated at the six-leaf stage (aerial parts were intact, and about 20 cm long). Each pot containing one plant, represented one replication and was kept under natural light and 25-27°C. In this experiment, the nematode inoculum was set at 3 J_2/g of soil, the fungus at 1×10^6 spores/ml, the bacterium at 1×10^9 cfu/ml, while 2g of nematicide were added at the appropriate pots. Each pot was filled with 2000g plant substrate. The nematicide and each inoculum were individually applied to each pot in a volume of 5ml suspension in three separate holes around the plants to a depth of 3cm.

Evaluation of *M. javanica* activity on tomato plants

According to the proposed method of Hussey and Johnson (2002) activity of *M. javanica* was evaluated as the number of egg masses and knots per root. The roots of each plant were washed with tap water and drained on blotting paper. To determine the number of egg masses, the roots were divided into 3-4cm parts, then egg masses stained with Floxin solution B (0.15g/l of water), bleached with lactophenole and counted under a dissecting microscope (Hussey and Janssen, 2002; Taylor and Sasser, 1978).

Experimental design and statistical analysis

This experiment was based on a completely randomized design with four tomato cultivars, six treatments for the control of *M. javanica* and five replicates, including: nematode+fungus (NF), nematode+bacterium (NB), nematode+nematicide (NN), nematode+fun-

gus+bacterium (NFB) and control (Cntr). The nematicide RUGBY® 10 G (cadusafos) was used as reference product. Two months after inoculation, the plants were taken from the soil and the number of knots and egg masses per root were counted. The data were then subjected to one way analysis of variance (ANOVA). The means of the treatments were compared using Duncan multiple range test (Steel and Torrie, 1980). All analyses were done by using SAS software version 9.1.

Results

The mean number of knots and egg masses produced by the nematode was significantly different among the studied cultivars (P≤0.0001). The highest number of knots and egg masses belonged to the cultivar Walter while Mobile showed the lowest numbers. In this regard, the cultivars were ranked as Walter> Falat> Bony best> Mobile (Figure 1). Also, the treatments for the control of the nematode had significant effect on the knots and egg masses (P≤0.0001). The highest nematode control based on number of knots was observed when the nematicide was added to the soil (NN) (Figure 2). No statistically significant difference was observed between the NFB and the NN treatments regarding the number of nematode egg masses (Figure 2). In the treatment NFB, the number of nematode knots and egg masses on the roots of different cultivars was lower than in the treatments NF and NB.

The interactive effect of cultivar and treatment was significant (P≤0.0001) suggesting that the cultivars responded differently to the various treatments of nematode control. The lowest number of knots and egg masses (namely the highest nematode control effect) was found at all cultivars under the effect of the nematicide and the NFB treatment (Figure 3). The number of knots and egg masses did not differ between the NFB treatment and the NF treatment for the cultivars Mobile and Bonny best (Figure 3).

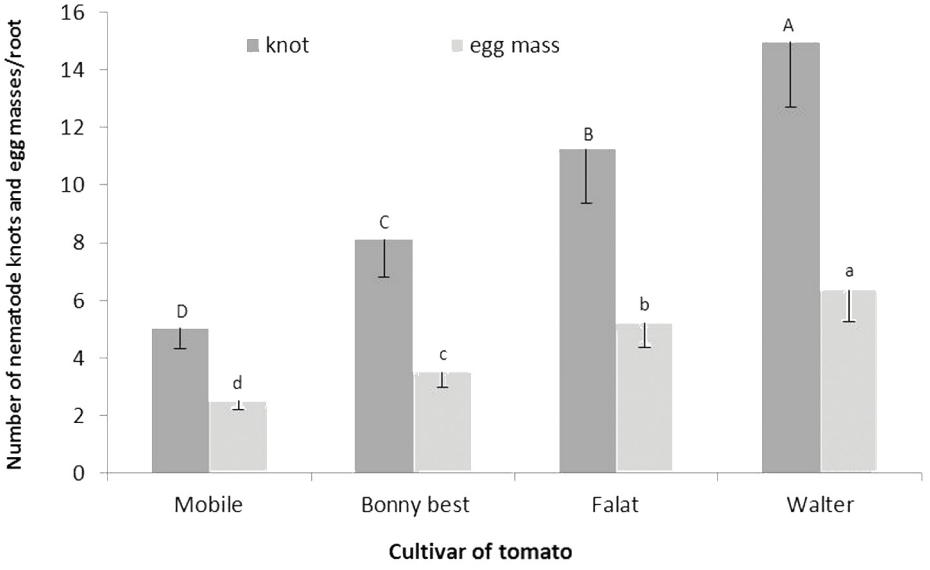

Figure 1. Mean number of nematode knots and egg masses of *Meloidogyne javanica* on the roots of four tomato cultivars (Mobile, Bonny best, Falat, and Walter) treated with *Trichoderma viride* and *Pseudomonas fluorescens* (pooled data of Control, nematode+fungus, nematode+bacterium, nematode+nematicide, nematode+fungus+bacterium). Means with different capital and small letters on the columns (knot and egg mass, respectively) are significantly different (Duncan Test, $P \leq 0.05$; $n = 5$).

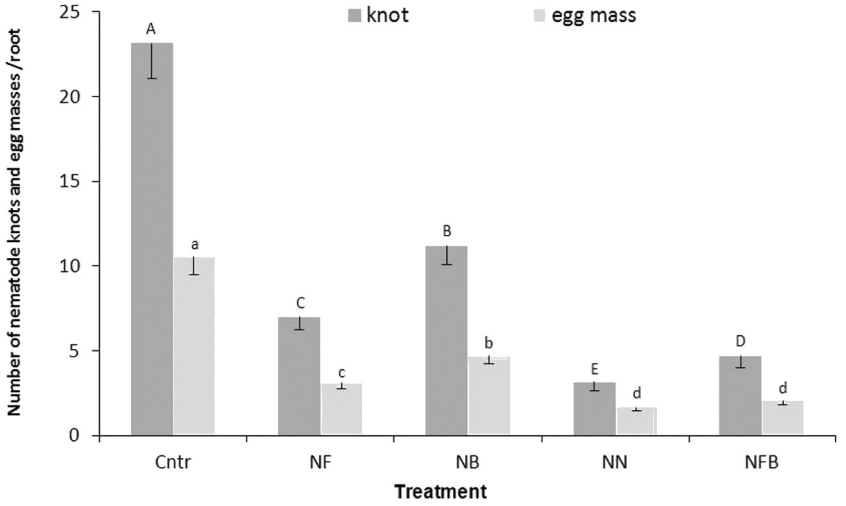

Figure 2. Mean number of nematode knots and egg masses of *Meloidogyne javanica* on roots of four tomato cultivars (pooled data) treated with *Trichoderma viride* and *Pseudomonas fluorescens* (Cntr: Control, NF: nematode+fungus, NB: nematode+bacterium, NN: nematode+nematicide, NFB: nematode+fungus+bacterium). Means with different capital and small letters on the columns (knot and egg mass, respectively) are significantly different (Duncan Test, $P \leq 0.05$; $n = 5$).

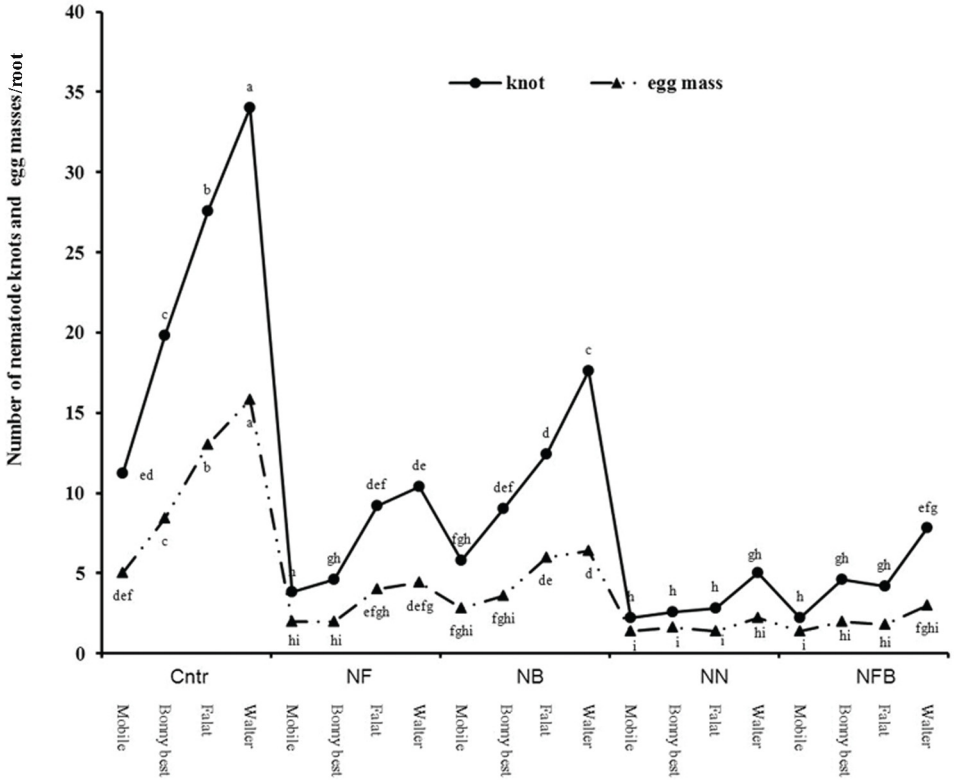

Figure 3. Mean number of nematode knots and egg masses of *Meloidogyne javanica* on roots of four tomato cultivars treated with *Trichoderma viride* and *Pseudomonas fluorescens* (Cntr: Control, NF: nematode+fungus, NB: nematode+bacterium, NN: nematode+nematicide, NFB: nematode+fungus+bacterium). Means with different letters at each of the knot and egg mass lines are significantly different (Duncan Test, $P \leq 0.05$; $n = 5$).

Discussion

Many studies (Ashoub and Amara, 2010; Dababat and Sikora, 2007; Golzari *et al*., 2011; Sahebani and Hadavi, 2008; Sharon *et al*., 2001; Siddiqui and Shaukat, 2003; Siddiqui *et al*., 2005; Siddiqui *et al*., 2004; Zaki *et al*., 2009) have been reported in the field of biological control of plant parasitic nematodes, especially against *Meloidogyne* spp., through the application of several microorganisms, including bacteria and fungi. Natural enemies of nematodes are successful in reducing plant parasitic nematode activity through parasitism, toxins, antibiotics production, enzyme production and competition, inducing systemic resistance in plants and stimulation of plant growth (Tian *et al*., 2007).

In the present study, combination of *T. viride* and *P. fluorescens* CHA0 enhanced the biocontrol effect against *M. javanica* activity as compared to the fungus and bacterium stand alone treatments except for the cases of the cultivars Mobile and Bonny best in which the effect was similar to the one by the fungus alone. Our results also indicated that the *T. viride* and *P. fluorescens* CHA0 combined treatment was equally effective to the nematicide treatment for all cultivars. The combined effect of *P. fluorescens* CHA0 and other fungi species has been evaluated on *Meloidogyne* species control. For example, Siddiqui *et al.* (2004) studied the interaction of six species of *Aspergillus* and bacterial strains *P. fluorescens* CHA0 and *P. fluorescens* CHA0/pME3424 on the control of *M. javanica*, showing that compounds such as methanol and ethyl acetate secreted by *A. niger* enhanced the nematicidal effect of bacterial strains. Zaki *et al.* (2009) investigated the effect of antagonistic fungi and plant growth promoting rhizobacteria (PGPRs) on *M. incognita* in the rhizosphere of tomato. *Aspergillus niger, Paecilomyces lilacinus* and *Penicillium chrysogenum* fungi and *Azetobacter chroococcum, Bacillus subtilis* and *Pseudomonas putida* bacteria were shown to be efficient in diminuting the number of nematode knots and female counts. It was also shown that soil incorporation of *P. lilacinus* had the highest impact on nematode control.

Several studies have indicated that the bacteria have antagonistic effects on the plant parasitic nematodes e.g. *Pseudomonas* on *Meloidogyne* spp. (Kerry, 2000; Jayakumar *et al*., 2002; Siddiqui and Shaukat, 2002, 2003; Andreogloua *et al*., 2003; Siddiqui *et al*., 2005). In recent years, Ashoub and Amara (2010) found that *B. thuringiensis*, *P. fluorescens* RR and *Rhizobium leguminosarum* have been fatal for *M. incognita* juveniles. The latter two species of bacteria also have been efficient in increasing plant growth. Meyer *et al*. (2001) used inoculums of *Burkholderia cepacia* and *T. virens* to manage the activity of *M. incognita* in the rhizosphere of pepper.

Golzari *et al*. (2011) studied the effect of bacterial metabolites of *Pseudomonas aeruginosa* in the control of *M. javanica* on tomato, showing that the 7NSk2, UTPF92 and UTPF86 strains of *P. aeruginosa* produced hydrogen cyanide, protease and salicylic acid, which caused mortality and prevention of nematode egg hatching. In these greenhouse trials, roots of Early Bana tomato cultivar inoculated with the UTPF86 and 7NSK2 strains exhibited the highest plant growth activity and lowest nematode penetration rate, respectively. Sahebani and Hadavi (2008) were able to control *M. javanica* using *T. harzianum* in the greenhouse. This antagonist of the nematode has also been used for the control of *M. incognita* in the rhizosphere of tomato (Al-Fattah *et al*., 2007).

In conclusion, according to our results, the use of a combination of *Trichoderma* spp. and *P. fluorescens* can be more effective against *M. javanica* and will reduce the level of its pathogenic activity. Biocontrol with these two nematode antagonists in a joint application could provide similar results to the use of commercial pesticides at controlled greenhouse conditions.

I wish to acknowledge and appreciate Shahed University, Tehran, Iran for the financial support of this research.

Literature cited

Akhtar, M. and Malik, A. 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. *Bioresource Technology*, 74: 35-47.

Andreogloua, F.I., Vagelasa, I.K., Woodb, M., Samalievc, H.Y. and Gowena, S.R. 2003. Influence of temperature on the motility of *Pseudomonas oryzi* habitans and control of *Globodera rostochiensis*. *Soil Biology and Biochemistry*, 35: 1095–1101.

Ashoub, A.H. and Amara, M.T. 2010. Biocontrol Activity of Some Bacterial Genera Against Root-Knot nematode, *Meloidogyne incognita. Journal of American Science*, 6(10): 321-328.

Dababat, A. and Sikora, R.A. 2007. Use of *Trichoderma harzianum* and *Trichoderma viride* for the Biological Control of *Meloidogyne incognita* on Tomato. *Jordan Journal of Agricultural Sciences*, 3: 297-309.

Golzari, H., Panjekeh, N., Ahmadzade, M., Salari, M. and Sedaghati, A. 2011. Study the role of fluorescent Pseudomonas bacteria metabolites root-knot nematode *Meloidogyne javanica* on tomatoes in control. *Iranian Journal of Plant Protection Science*, 42(1): 113-124.

Hosseini-Nejad, S.A. and Khan, M.W. 2001. Interaction of root-knot nematode, *Meloidogyne incognita* (race 1), on chickpea cultivars. *Applied Entomology and Phytopathology*, 68: 1-11.

Hunt, D.J. 1993. *Aphelenchida, Longidoridae and Trichodoridae: Their Systematics and Bionomes*. C. A. B. International, Hertfordshine, UK, 352 p.

Hussey, R.S. and Barker, K.R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. Including a new technique. *Plant Disease Reports*, 57: 1025-1028.

Hussey, R.S. and Janssen, G.J.W. 2002. Root-Knot. Nematodes: *Meloidogyne* species. Pp: 43-70. In: J.L. Starr, J. Bridge and R. Cook, (eds). Plant resistance to parasitic nematodes. CAB1 Publishing, Walling ford, UK.

Jayakumar, J., Ramakrishnan, S. and Rajendran, G. 2002. Bio-control of reniform nematode, *Rotylenchulus reniformis* through fluorescent Pseudomonas. *Pesttology*, 26: 45–46.

Jepson SB. 1987. Identification of root–knot nematodes (*Meloidogyne* spp.), C.A.B. International, UK, 265 p.

Kerry, B.R. 2000. Rhizosphere interactions and exploitation of microbial agents for the biological control of plant-parasitic nematodes. *Annual Review of Phytopathology*, 38: 423–441.

McClure, M.A., Kruk, T.H. and Misaghi, I. 1973. A method for obtaining quantities of clean *Meloidogyne* eggs. *Journal of Nematology*, 5: 230.

Meyer, S.L. 2003. United States Department of Agriculture – Agricultural Research Service research programs on microbes for management of plant-parasitic nematodes. *Pest Management Science*, 59: 665–670.

Meyer, S.L.F., Roberts, D.P., Chitwood, D.J., Carta, L.K., Lumsden, R.D. and Mao, W. 2001. Application of *Burkholderia cepacia* and *Trichoderma virens*, alone and in combinations, against *Meloidogyne incognita* on bell pepper. *Nematropica*, 31: 75-86.

Sahebani, N. and Hadavi, N. 2008. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Soil Biology and Biochemistry*, 40: 2016–2020.

Sasser, J.N. 1979. Pathologencity, host ranges and variability in *Meloidogyne* spp. E. Lamberti and C.E. Taylor (eds.). Root–knot nematodes (*Meloidogyne* spp.) systematics, biology and control Academic press, New York, 477 p.

Sharon, E., Bar-Eyal, M., Chet, I., Herrera-Estrella, A., Kleifeld, O. and Spiegel, Y. 2001. Biological Control of the Root-Knot Nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopathology*, 91(7): 687- 693.

Siddiqi, M.R. 2000. Tylenchida, Parasites of Plants and Insects, 2nd edition. CABI Publishing, Wallingford, Oxon, UK, 833 p.

Siddiqui, I.A. and Shaukat, S.S. 2002. Rhizobacteria-mediated induction of systemic resistance (ISR) in tomato against *Meloidogyne javanica*. *Journal of Phytopathology-phytopathologische Zeitschrift*, 150: 469–473.

Siddiqui, I.A. and Shaukat, S.S. 2003. Suppression of root-knot disease by *Pseudomonas fluorescens* CHA0 in tomato: importance of bacterial secondary metabolite 2,4- diacetylphloroglucinol. *Soil Biology and Biochemistry*, 35: 1615–1623.

Siddiqui, I.A., Haas, D. and Heeb, S. 2005. Extracellular Protease of *Pseudomonas fluorescens* CHA0, a Biocontrol Factor with Activity against the Root-Knot Nematode *Meloidogyne incognita. Applied and Environmental Microbiology*, 5646–5649.

Siddiqui, I.A., Shaukat, S.S. and Khan, A. 2004. Differential impact of some *Aspergillus* species on *Meloidogyne javanica* biocontrol by *Pseudomonas fluorescens* strain CHA0. *Letters of Applied Microbiology*, 39:74–83.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc. New York, USA, 481 p.

Taylor, A.L. and Sasser, J.N. 1978. Biology, identification and control of root–knot nematodes (*Meloidogyne* spp.). Coot Publ. Dep. Plant Pathol., North Carolina State university and U.S. Agency Int. Dev. Raleigh, N. C., 111 p.

Tian, B., Yang, J. and Zhang, K. 2007. Bacteria used in the biological control of plant parasitic nematodes: populations, mechanisms of action, and future prospects. *FEMS Microbiology and Ecology*, 61 : 197–213.

Weller, D.M. and Cook, R.J. 1983. Suppression of Take-all of Wheat by Seed Treatments with Fluorescent Pseudomonads. *Phytopathology*, 78: 463-469.

Whipps, J.M. 2001. Microbial Interactions and Biocontrol in the rhizosphere. *Journal of Experimental Botany*, 52:487-511.

Zaki, A., Siddiquia, B. and Futaib, K. 2009. Biocontrol of *Meloidogyne incognita* on tomato using antagonistic fungi, plant-growth-promoting rhizobacteria and cattle manure. *Pest Management Science*, 65: 943–948.

Received: 6 June 2015; Accepted: 13 December 2015

Trichoderma viride και *Pseudomonas fluorescens* CHA0 κατά του κομβονηματώδη *Meloidogyne javanica* στη ριζόσφαιρα φυτών τομάτας

A. Saeedizadeh

Περίληψη Οι κομβονηματώδεις συγκαταλέγονται μεταξύ των πιο σημαντικών εχθρών που προκαλούν μείωση της παραγωγής στην καλλιέργεια θερμοκηπιακής και υπαίθριας τομάτας στο Ιράν. Σκοπός αυτής της μελέτης ήταν η αξιολόγηση της ανταγωνιστικής δράσης του *Trichoderma viride* και του *Pseudomonas fluorescens* CHA0 στην αναπαραγωγή του *Meloidogyne javanica* και το σχηματισμό κόμβων από τον κομβονηματώδη σε ρίζες τομάτας. Πραγματοποιήθηκε πείραμα με σπορόφυτα των ποικιλιών τομάτας Bonny best, Falat, Mobile και Walter grown σε αποστειρωμένο αμμοπηλώδες έδαφος σε γλάστρες. Έγινε τεχνητή μόλυνση με 3 J_2/g εδάφους για τον νηματώδη, 1×10^6 σπόρια/ml για το μύκητα και 1×10^9 cfu/ml για το βακτήριο. Το σκεύασμα RUGBY® 10 G (cadusafos) χρησιμοποιήθηκε σε ποσότητα 2g ανά γλάστρα. Δύο μήνες μετά την μόλυνση, ο αριθμός των κόμβων και των ωόσακκων ανά ρίζα στις διάφορες επεμβάσεις ήταν (κατά φθίνουσα σειρά): μάρτυρας (νηματώδης), νηματώδης+βακτήριο, νηματώδης+μύκητας, νηματώδης+μύκητας+βακτήριο και νηματώδης+νηματωδοκτόνο. Ο συνδυασμός μύκητας+βακτήριο αύξησε την αποτελεσματικότητα της βιολογικής αντιμετώπισης του *M. javanica* σε σύγκριση με τις απλές επεμβάσεις με το μύκητα και το βακτήριο, με εξαίρεση τις ποικιλίες Mobile and Bonny best, στις οποίες η επίδραση ήταν ανάλογη με αυτή του μύκητα. Η συνδυασμένη επέμβαση μύκητας + βακτήριο ήταν εφάμιλλης αποτελεσματικότητας με την επέμβαση του νηματωδοκτόνου για όλες τις ποικιλίες. Η μεγαλύτερη και η μικρότερη δραστηριότητα του νηματώδους παρατηρήθηκε στις ποικιλίες Walter και Mobile, αντίστοιχα.

Hellenic Plant Protection Journal **9:** 28-34, 2016

DE GRUYTER
OPEN

Hellenic Plant Protection Journal **9:** 35-43, 2016
DOI 10.1515/hppj-2016-0004

Effectiveness of salicylic acid, *Pseudomonas fluorescens* CHA0 and *Trichoderma viride* to control *Meloidogyne incognita* race 2 on different tomato cultivars

L. Esfahani[1], S. Jamali[1*], A. Saeedizadeh[2] and H. Pedramfar[1]

Summary The effects of salicylic acid (SA), *Trichoderma viride* and *Pseudomonas fluorescens* CHA0 were studied on the root-knot nematode *Meloidogyne incognita* race 2 in resistant and susceptible tomato cultivars (Gina VF, Falat CH, Falat 111, Karoon) during 2012-2013. Four-leaf tomato seedlings were used, grown in pots containing 1000 g of sterilized soil; each seedling receiving 20 ml of *T. viride* suspension containing 1×10^6 spores, 30 ml of *P. fluorescens* CHA0 with 10^9 cfu/ml, 5mM of salicylic acid and 2000 second stage nematode juveniles. Parameters relevant to nematode population and plant growth were evaluated. The biocontrol agents and salicylic acid were effective in nematode control in combined and single treatments. High reductions in root galling and egg mass indices were observed with combination of SA and biocontrol agents. The greatest increase in plant growth was obtained when cv. Falat CH was treated with SA followed by *P. fluorescens* CHA0 and *T. viride*. The highest number of galls was recorded in cv. Karoon, followed by cvs. Falat 111, Gina VF and Falat CH. *Pseudomonas fluorescens* CHA0 provoked the highest increase in fresh and dry root weight, fresh and dry shoot weight and plant length in all free nematode treatments. The results indicated that chemical inducer (salicylic acid), in combination with biocontrol agents (*T. viride* and *P. fluorescens* CHA0), stimulated and eventually increased plant growth.

Additional keywords: biocontrol, *Lycopersicon esculentum*, reproduction factor, root-knot nematode

Introduction

Root knot nematodes are one of the most important plant parasites. They have a wide host range including more than 2000 plant species (Gugino *et al*., 2008). The primary symptom of root-knot nematode infection is the formation of typical root galls on the roots of susceptible host plants. Nutrient and water uptake are substantially reduced because of the damaged root system, resulting in weak and low-yielding plants (Abad *et al*., 2003). *Meloidogyne* spp. can severely damage important crops including tomato, cucumber and melon in many areas. The nematodes are major pests on tomatoes causing considerable yield losses (Sahebani and Hadavi, 2008).

Biological control is a safe alternative to pesticides for phytonematode management, likely to be free from toxic residual effects. There are numerous microbial antagonists of root-knot nematodes and their application results in significant decrease in the nematode populations (Khan *et al*., 2007). In fact, a wide range of bacteria (Hallmann *et al*., 2001) and fungal agents (Meyer *et al*., 2001) have been used to reduce damages of plant parasitic nematodes. *Pseudomonas fluorescens* CHA0 is a root-colonizing biocontrol bacterium that protects several plant species from root diseases caused by soil-borne fungi and root-knot nematodes (Siddiqui and Shaukat, 2002). Some species of *Trichoderma* spp. have widely been used as biocontrol agents against soil-borne plant diseases (Whipps, 2001) and root-knot nematodes (Sharon *et al*., 2001). Nandy *et al*. (2003) found that salicylic acid decreased gall and egg numbers of *Meloidogyne incognita* on okra and cowpea. Numerous studies have separately inves-

[1] Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, P.O. Box: 41635-1314, Rasht, Iran
[2] Faculty of Agriculture, Shahed University, P.O. Box: 33191-18651, Tehran, Iran
* Corresponding author: Jamali_s2002@yahoo.com

tigated the impact of biocontrol agents to control root-knot nematodes on susceptible cultivars. However, comprehensive research about the effects of salicylic acid (SA), *Trichoderma* and *Pseudomonas* simultaneously on *M. incognita* race 2 in both susceptible and resistance tomato cultivars has not yet been implemented. The present study examined the effect of a chemical inducer (SA) and two biocontrol agents (*T. viride* and *P. fluorescens* CHA0), when used independently and in combination, in controlling *M. incognita* race 2 on different tomato cultivars.

Materials and Methods

Nematode Inocula

Infected root samples were collected from naturally infested tomato plants in a greenhouse. Roots were rinsed with water and nematodes were extracted from egg masses present on the galled tissue. The identification of the root-knot nematode species was based on the shape of the perineal pattern (Jepson 1987) according to the methods of Taylor and Netscher (1974). The races of root-knot nematode populations were identified on the basis of differential hosts tests (Hartman and Sasser, 1985). A single egg mass was used to establish a population on tomato plants. Eggs were extracted from galled tomato roots using 1.5% NaOCl (Hussey and Barker, 1973), collected on a 25 µm mesh sieve and transferred to a beaker containing distilled water. Hatched juveniles of *M. incognita* were obtained by placing the eggs in sterile distilled water for 5 days at 28 ± 2°C. The activities of juveniles were evaluated under a stereomicroscope.

Biocontrol Agents

Trichoderma viride and *P. fluorescens* CHA0 were a kind offer of the Plant Pathology Department, Faculty of Agriculture, Shahed University. *T. viride* was cultured on potato dextrose agar (PDA) containing 150 mg l^{-1} streptomycin and 150 mg l^{-1} of chloramphenicol, and was kept at 25°C. The mycelia and formed conidia were carefully scraped from the media and suspended in 100 ml of distilled water. Spores were separated from mycelia by sieving through a 50 µm sieve. The spore suspensions were then adjusted to the desired concentration after counting spore density using a haemocytometer (Sahebani and Hadavi, 2008).

The bacterium isolate was stored in sterile distilled water and was cultured in nutrient agar medium. Density of 10^9 colony forming unit (cfu) per milliliter of water of 24 h bacterium culture was prepared by serial dilution (10^1- 10^9) in 590 nm (Zhang *et al*., 2002).

Chemical Agent

Salicylic acid (SA) (Merck) was prepared and dissolved in ethanol and water in concentrations of 5 mM. Firstly, the necessary amounts of SA (0.69 g) were dissolved in 100 ml 96% methanol solution. The volume of the solution was brought to one liter with distilled water. Then, the concentration of 5 mM was used and sprayed on the leaves (Zhang *et al*., 2002).

Plant Cultures

The tolerant tomato cultivars Gina VF, Falat 111 and the susceptible Falat CH and Karoon were used (Gharabadiyan *et al*., 2012). Seeds were sown in the pots containing 1000 g of sterilized mixture of field soil, leaf compost and sand at the rate of 1:1:2 in 24-27°C under greenhouse conditions. Three-week-old (four-leaf stage) seedlings were used in the experiments. The seedlings were inoculated with *T. viride, P. fluorescens* and salicylic acid, separately and jointly. Each seedling received 20 ml of a liquid suspension of *T. viride* containing 1×10^6 spores, 30 ml of bacterial suspension 10^9 cfu/ml and lastly 5mM salicylic acid. Four replicates were maintained for each treatment. The biocontrol agents were used by soil drenching, but SA was applied with foliar spray. The next day, 2000 J_2 (two stage juveniles) of root-knot nematode per plant were used for the artificial inoculation. The inoculum was injected into 3 holes approximately 2 cm deep around the stem base.

Forty five days after inoculation, nematode infestations as well as plant growth parameters were assessed. Two control sets were included, namely uninoculated and artificially inoculated plants with nematodes. The uninoculated control did not receive nematodes and biocontrol agents and SA and the inoculated control received only nematodes. The experiment consisted of 12 treatments: 1) non-inoculated (negative control); 2) only nematode (positive control); 3) T (*Trichoderma*); 4) SA; 5) P (*Pseudomonas*); 6) N+T; 7) N+SA; 8) N+P; 9) N+T+SA; 10) N+P+SA; 11) N+P+T; 12) N+P+T+SA. At harvest, roots were removed, washed free of soil and stained by 0.015% Phloxine B for 20 minutes to facilitate egg mass counting. The number of galls, egg masses per plant and length, fresh and dry weight of plants and nematode population in the soil were measured and recorded (Hussey and Janssen, 2002).

During the experiment, tomato plants were maintained in a greenhouse randomizing the position of the blocks and, at the same time, repositioning each plant within a block every week to avoid a block position effect and the factor 'position of the plant within the block'.

Statistical Analysis

The experimental design was factorial randomized complete block with 4 tomato cultivars, 12 treatments and 4 replications per treatment. The experiment was repeated for two years (2012 and 2013) under the same conditions. Statistical analysis proved that there was no statistically significant difference between the two years in recorded data and hence the results of two years were pooled. Comparison of the means was performed by ANOVA and Tukey test ($P \leq 0.01$). According to Oostenbrinks equation [Oostenbrink, 1966], reproduction factor was calculated for each of the treatments based on R = PF/PI formula where PI is the initial population and PF is the final population. The Final Population density (PF) was obtained by counting second stage juvenile (J_{2s}) population in the soil. All data was analyzed by SAS Software Version 9.00.

Results

Detailed observations of (n=10-20) female nematodes indicated that the perineal pattern characteristics and differential host tests correspond to *Meloidogyne incognita* race 2.

The data presented in Table 1 revealed that the combined inoculation of *T. viride* and *P. fluorescens* and SA improved the tomato plants' growth characters compared to the inoculated control.

According to Figure 1, the soil application of biocontrol agents and chemical inducer significantly ($P \leq 0.01$) reduced galling and egg mass production compared to the inoculated control. Treatment 12 (soil drenching of biocontrol agents + foliar spray of SA) exhibited the lowest number of galls and egg masses compared to positive control and other treatments (Figure 1, Figure 2). Application of SA was also found to be effective against the nematode but less effective than *T. viride* and *P. fluorescens* (Figure 1). The greatest numbers of galls were recorded in the inoculated control pots of tomato cv. Karoon, followed by cvs. Falat 111, Gina VF and Falat CH. (Figure 1). Egg masses at all treatments were significantly ($P \leq 0.01$) different compared to the control. There were differences between the four tomato cultivars (Figure 2). Reproduction factor of *M. incognita* race 2 on the cultivars is shown in Figure 3.

The results showed that inoculation with the root-knot nematode caused significant reductions ($P \leq 0.01$) in fresh weight of roots (23%, 30%, 27% and 49%), fresh weight of shoots (45%, 51%, 48% and 54%), plant length (25%, 31%, 34% and 27%), dry weight of shoots (40%, 51%, 47% and 53%) and dry weight of roots (24%, 48%, 26% and 41%), in the tomato cultivars Gina VF, Falat CH, Falat 111 and Karoon, respectively, in comparison to the uninoculated control. The data presented in Table 1 revealed that the treatment of the soil with the biocontrol agents or salicylic acid without nematodes significantly increased plant growth parameters.

Table 1. Effects of salicylic acid, *Trichoderma viride* and *Pseudomonas fluorescens* on growth parameters of four tomato cultivars (pooled data) infested with *Meloidogyne incognita*. C: uninoculated (negative control), N: Nematode (positive control), T: *Trichoderma viride*, SA: Salicylic acid, P: *Pseudomonas fluorescens*. Each value is the mean of four replicates in 2012 and 2013.

Tomato cultivar	Treatments	Plant length (cm)	Fresh weight (g)		dry weight (g)	
			Shoot	Root	Shoot	Root
Gina VF	C	50.5±0.57	51.3±0.31	10.44±0.10	10.27±0.32	2.23±0.31
	N	37.75*±0.50	28.1*±0.12	8*± 0.08	6.07*±0.10	1.58*±0.03
	T	52.75± 1.70	53±1.41	10.82±0.21	10.7±0.47	2.83*±0.11
	SA	54±2.16	53.25± 1.5	10.75±0.29	10.89±0.74	2.65*±0.04
	P	55.75±2.5	55±2.44	11*±0.0816	11.16±1.06	2.92*±0.04
	N+ T	40*±2.94	34.5*±0.57	7.90*±0.08	7.04*±0.65	1.42*±0.04
	N+ SA	41*±2.16	36*±0.81	7.01*± 0.11	7.32*±0.25	1.25*±0.04
	N+ P	44.25*±2.62	40.75*±1.70	8.15*±0.12	8.28±0.94	1.69*±0.03
	N+ T+ SA	50.25± 0.50	49.5±1.73	10.05*±0.03	10.02±0.76	2.01±0.01
	N+ P+ SA	50.25±0.5	50.5±1.29	10.28±0.06	10.19±1.1	2.09±0.02
	N+ P+ T	52.5±0.57	50.75±0.95	10.1±0.081	10.29±0.73	2.07±0.01
	N+ P+ T+ SA	53.5±0.57	51.5±0.57	10.35±0.07	10.45±0.52	2.2±0.03
Falat CH	C	53.5± 0.57	24.80±0.32	8.61±0.13	5.78±0.33	2.01±0.01
	N	36.75*±0.50	12*±0.81	6*± 0.08	2.8*±0.78	1.03*±0.02
	T	57.5± 2.08	28.80*±0.37	8.88±0.07	6.73±1.08	2.29±0.03
	SA	58.5± 2.88	29.41*±0.69	8.75±0.23	6.9±0.85	2.11±0.01
	P	60±2.82	33.06*±0.67	9.04*±0.03	7.76*±1.60	2.43±0.05
	N+ T	40*±1.41	16.16*±0.39	5.79*±0.17	3.86±0.17	1.01*±0.08
	N+ SA	43*± 2.94	17.34*±1.14	5.81*± 0.16	4.01±0.74	0.98*±0.01
	N+ P	46*±1.63	19.43*±2.20	6.3*±0.216	4.58±0.98	1.06*±0.02
	N+ T+ SA	53±0.81	24.55± 0.54	8*± 0.01	5.76±0.59	1.4*±0.05
	N+ P+ SA	54±1.63	23.34±1.08	8.2±0.08	5.47±0.36	1.55*±0.42
	N+ P+ T	54.5±1.73	24.45±0.39	8.01*±0.02	5.76±0.37	1.42*±0.24
	N+ P+ T+ SA	55.5±2	25.00±1.96	8.5±0.31	5.91±0.81	1.96±0.06
Falat 111	C	48.25±0.50	29.17±1.66	9.82±0.04	9.85±0.40	2.15±0.02
	N	31.75*±0.50	15*±0.81	7.15*±0.12	5.13*±0.22	1.59*±0.15
	T	51± 2.16	31.73±2.66	10.04±0.03	10.85±0.44	2.19±0.03
	SA	52±1.63	32.78±0.76	9.94± 0.12	11.23±0.71	2.17±0.02
	P	55±0.81	35.92*±2.84	10.15*±0.13	12.25*±0.94	2.23*±0.02
	N+ T	38.25*±0.95	19.53*±1.92	6.94*± 0.05	6.75*±1.08	1.56*±0.04
	N+ SA	39.25*±1.70	20.33*±1.55	6.45*±0.10	6.98*±0.14	1.46*±0.05
	N+ P	43*±2.16	23.05*±1.51	7.33*±0.17	7.94±1.17	1.65*±0.04
	N+ T+ SA	46±0.81	28.97± 0.57	9.19*±0.11	9.91±0.34	2.02*±0.03
	N+ P+ SA	48.5±1.29	28.78±1.63	9.32*±0.04	9.88±0.39	2.05±0.06
	N+ P+ T	49.25±1.70	29.6±0.29	9.18*±0.01	10.26±0.87	2.02*±0.04
	N+ P+ T+ SA	52±1.63	30.02±0.29	9.69±0.01	10.31±0.55	2.13±0.02
Karoon	C	44.5±0.57	23.97±2.53	4.27±0.17	11.2±0.46	1.05±0.11
	N	32.25*±1.70	11*±0.81	2.15*±0.12	5.21*±0.16	0.61*±0.08
	T	47.75±1.25	24.95±1.34	4.48± 0.12	11.82±0.85	1.09±0.04
	SA	48.5±0.57	25.01± 2.13	4.34±0.12	11.95±1.36	1.05±0.03
	P	51.25±0.5	27.22*±1.63	4.65*±0.129	12.87*±1.16	1.11±0.06
	N+ T	38*±0.81	15*±0.81	2.26*±0.12	7.28*±0.97	0.64*±0.26
	N+ SA	39*± 0.81	16.88*±0.73	2.15*±0.10	7.60*±0.36	0.61*±0.12
	N+ P	40*±2.16	19.29*±0.54	3.14*±0.12	9.13±0.69	0.82±0.10
	N+ T+ SA	41±2.16	21.85±1.30	3.99±0.01	10.37±0.88	0.99±0.03
	N+ P+ SA	44±0.81	22.43±0.40	4.15±0.07	10.63±0.20	1.03±0.02
	N+ P+ T	44.75±0.95	23.47±1.44	4.03±0.02	11.15±0.84	1±0.07
	N+ P+ T+ SA	45.5±0.57	24.10±1.89	4.19±0.12	11.43±0.47	1.04±0.10

* Values are significantly different from the control (Tukey test, P≤0.01).

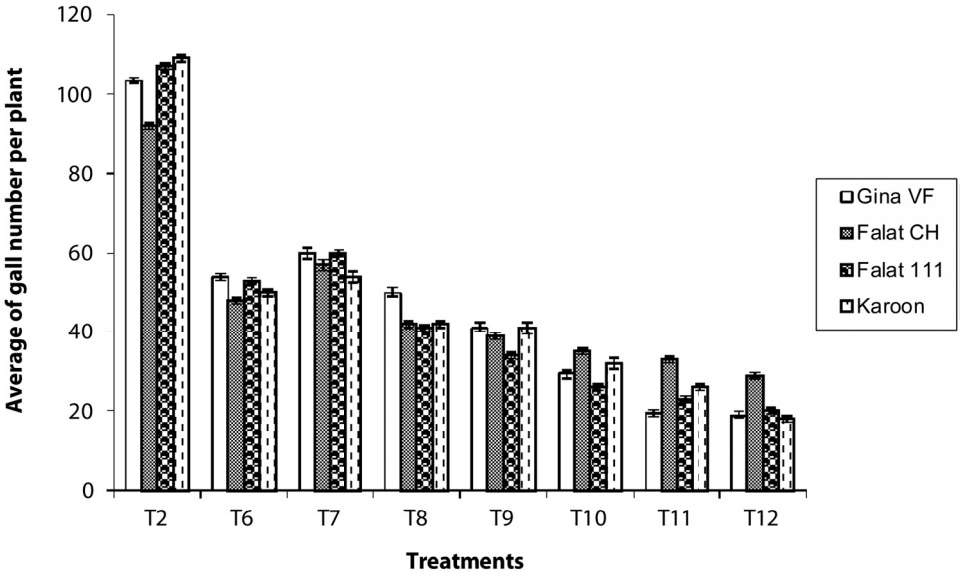

Figure 1. Mean gall numbers of *Meloidogyne incognita*/plant on the roots of four tomato cultivars (pooled data) treated with salicylic acid, *Trichoderma viride* and *Pseudomonas fluorescens* (Treatments: T2: N, T6: N+T, T7: N+SA, T8: N+P, T9: N+T+SA, T10: N+P+SA, T11: N+P+T, T12: N+P+T+SA; N: Nematode, T: *Trichoderma viride*, SA: Salicylic acid, P: *Pseudomonas fluorescens*). Each number is a mean of four replications in two years.

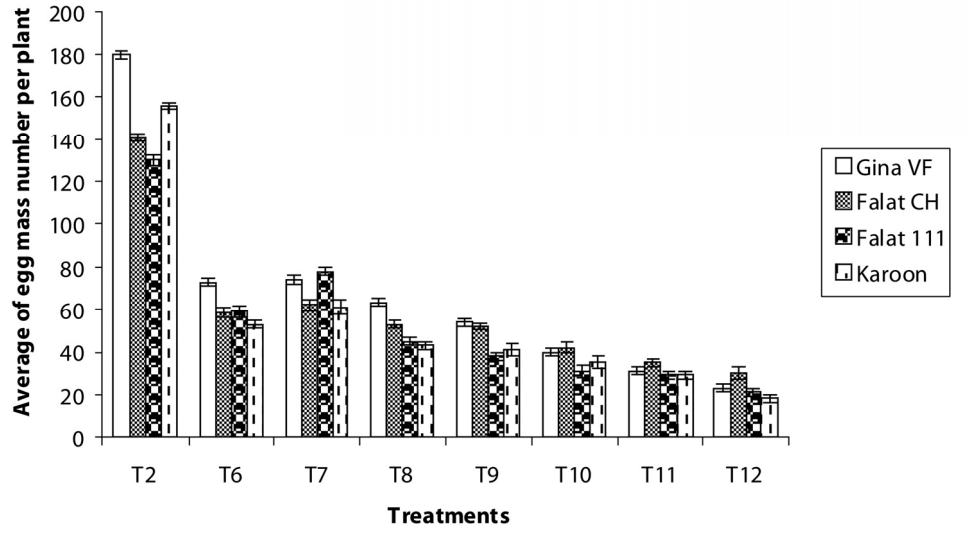

Figure 2. Mean egg mass number of *Meloidogyne incognita*/plant on the roots of four tomato cultivars (pooled data) treated with salicylic acid, *Trichoderma viride* and *Pseudomonas fluorescens* (Treatments: T2: N, T6: N+T, T7: N+SA, T8: N+P, T9: N+T+SA, T10: N+P+SA, T11: N+P+T, T12: N+P+T+SA; N: Nematode, T: *Trichoderma viride*, SA: Salicylic acid, P: *Pseudomonas fluorescens*). Each number is a mean of four replications in two years.

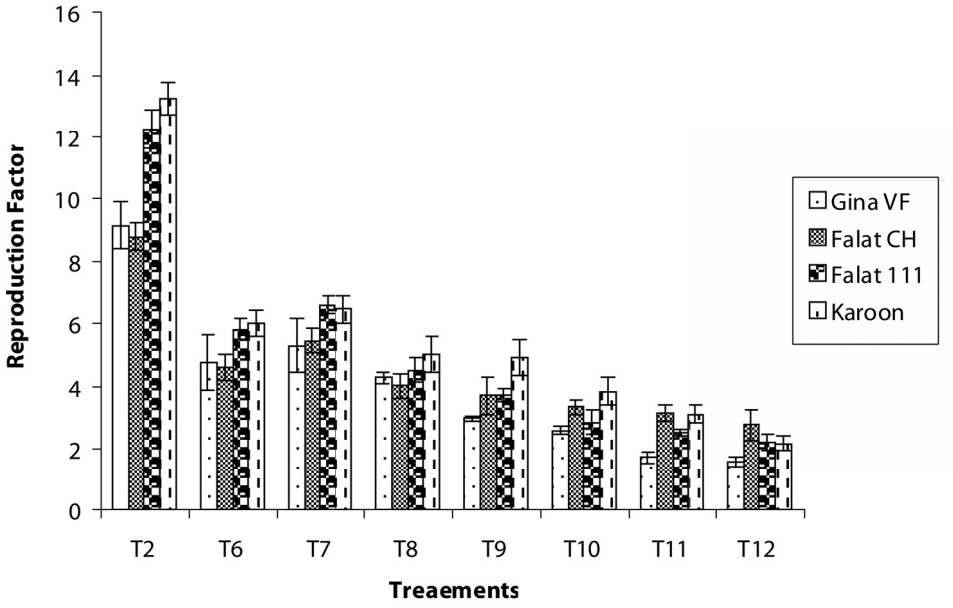

Figure 3. Reproduction factor of *Meloidogyne incognita* on the roots of four tomato cultivars (pooled data) treated with salicylic acid, *Trichoderma viride* and *Pseudomonas fluorescens* (Treatments: T2: N, T6: N+T, T7: N+SA, T8: N+P, T9: N+T+SA, T10: N+P+SA, T11: N+P+T, T12: N+P+T+SA; N: Nematode, T: *Trichoderma viride*, SA: Salicylic acid, P: *Pseudomonas fluorescens*). Each number is a mean of four replications in two years.

The greatest increase in plant growth was obtained in cv. Falat CH treated with *P. fluorescens* CHA0 followed by SA. Fresh weights of shoots were higher in nematode-free plants in all cultivars compared to both *M. incognita*-infested plants and the above-mentioned treatments. The results showed that combined inoculation of fungi, bacteria and SA caused significant reductions (P≤0.01) in fresh weight of root (0.6%, 1%, 1% and 1.6%), dry weight of root (1.3%, 2%, 0.9% and 0.9%) in all cultivars and fresh weight of shoots (0.5 and 0.8) in two cultivars (Gina VF and Falat CH); plant length (5.9%, 3.7%, 7.7% and 2.2%), dry weight of shoots 1.75%, 2.24%, 4.6% and2%) in all cultivars and fresh weight of shoots (0.8%, 2% and 0.1%) in three cultivars of tomato (Falat CH, Falat 111 and Karoon) increased; galling (81%, 68%, 80% and 83%), egg mass (87%, 78%, 83% and 88%) and reproduction factor (83%, 69%, 82% and 84%) were reduced compared to the inoculated control in all cultivars (Gina VF, Falat CH, Falat 111 and Karoon, respectively) (Figure 1, Figure 2, Figure 3 and Table 1).

The data indicated that chemical inducer (salicylic acid), in combination with *T. viride* and *P. fluorescens* CHA0, stimulated plant response and increased plant growth especially in susceptible cultivars. Inoculation with the *M. incognita* race 2 caused significant reduction ($P \leq 0.01$) in plant length (25%), fresh weight of shoots (45%) and roots (23%) of tomato cv. Gina VF ($P \leq 0.01$) in comparison to the uninoculated control (Table 1). The nematode infection caused 31% reduction in plant length, 52% in fresh weight of shoot and 30% in root fresh weight of cv. Falat CH. The cv. Falat 111 exhibited significant decreases in any of the plant parameters (length (34%), fresh weights of shoots (49%) or roots (27%) at $P \leq 0.01$). In the cv. Karoon decreases in plant length (27%), fresh weight of shoots (54%) and roots (49%) were recorded. Soil treatments with *T. viri-*

de, P. fluorescens and foliar spray of SA gave the greatest recovery of the plant length and dry weight of shoots in all cultivars and in shoot fresh weigh in three cultivars (Falat CH, Falat 111 and Karoon) compared to uninoculated control. The maximum reduction in the numbers of host root galls and egg masses was observed in Karoon cultivar representing the most susceptible cultivar.

According to growing characteristics assessments, the cultivar Gina VF, artificially inoculated with 2000 J_2 of root-knot nematode, had the highest root fresh weight, shoot fresh weight, shoot dry weight and length. Thus, this cultivar can be characterized as relatively tolerant. The lowest root fresh weight, shoot fresh and dry weight, shoot length and the maximum number of galls were counted in the cultivar Karoon that was the most susceptible cultivar. In the case of two cultivars, Falat CH and Falat 111, the Falat 111 had a greater number of galls and higher reproductive factor but its growth characters were less than Falat CH.

Discussion

Biological control of soil-borne plant pathogens and nematodes using biocontrol microorganisms is a potential non-chemical means in crop protection (Stirling, 1991).

Treatment of the soil with salicylic acid, *P. fluorescens* and *T. viride* resulted in a reduction in the galls and egg mass number of *M. incognita*. Similar results were obtained by other researchers. *Trichoderma* spp. has been reported to produce chitinases into the culture (Chet and Baker, 1981), which might help the inhibition of egg hatching. Dos Santos *et al*. (1992) reported *Trichoderma harzianum* as an effective egg parasite of *M. incognita*; the fungus was able to grow on the egg surface and penetrate the egg shell. In a study by Naserinasab *et al*. (2011), treatment of the soil with the *T. harzianum* B1 or salicylic acid resulted in a reduction in the galls, eggs and egg masses of *M. javanica*. Application of *T. harzianum, T. hamatum* or *T. virens* has also demonstrated potential to suppress root-knot nematodes (Siddiqui and Shaukat, 2004).

Pseudomonas fluorescens was one of the effective agents in reducing J_2 population and prevented the formation of galls on tomato roots. Similar results have been reported (Siddiqui and Shaukat, 2003; Ali *et al*., 2002). Siddiqui *et al*. (2006) found that there is a direct relation between the production of HCN by *P. fluorescens* CHA0 and mortality of the nematode. Akhtar *et al*. (2013) suggested that the inoculation with *P. fluorescens* increases the root and shoot length of black gram (*Vigna mungo* L.) infected with *M. incognita*. Khan and Haque (2011) reported the greatest plant growth and biomass of tobacco after treatment with *P. fluorescens* compared with *T. harzianum* and two nematicides.

Our results confirm the findings of Gharabadiyan *et al*. (2012) sustaining Gina as tolerant cultivar and Karoon as susceptible cultivar, while Falat 111 is similarly tolerant to Falat CH. The combined treatment of the soil with *T. viride, P. fluorescens* CHA0 and the chemical inducer salicylic acid clearly improved nematode control of the root-knot nematode *M. incognita* race 2. In addition, they stimulate plant response and increase plant growth. The study suggests that there is considerable potential for exploiting SA, *P. fluorescens*, and *T. viride* for the management of root-knot nematodes particularly in susceptible cultivars of tomato.

Literature Cited

Abad, P., Favery, B., Rosso M. and Castagnone-Sereno, P. 2003. Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. *Molecular Plant Pathology,* 4: 217–224.

Akhtar, A., Hisamuddin, R. and Abbasi Sharf. 2013. Study on Black Gram (*Vigna mungo* L.) Infected with *Meloidogyne incognita* under the Influence of *Pseudomonas fluorescens*, *Bacillus subtilis* and Urea. *Journal of Plant Pathology Microbiology,* 4: 202.

Ali, N.I., Siddiqui, I.A., Shaukat, S.S. and Zaki, M.J. 2002. Nematicidal activity of some strains of *Pseudomonas* spp. *Soil Biology and Biochemistry*, 34: 1051-1058.

Chet, I. and Baker, R. 1981. Induction of suppressiveness to *Rhizoctonia solani* in soil. *Phytopathology*, 70: 994-998.

Dos Santos, M.A., Ferraz, S. and Muchovez J.J. 1992. Evaluation of 20 species of fungi from Brazil for biocontrol of *Meloidogyne incognita* race-3. *Nematropica*, 22: 183-192.

Gharabadiyan, F., Jamali, S., Ahmadiyan yazdi, A. and Eskandari A. 2012. Source of resistance to root-knot nematode (*Meloidogyne javanica*) in tomato cultivars. *Journal of Agricultural Technology*, 8(6): 2011-2012.

Gugino, B.K., Ludwig, J.W. and Abawi G.S. 2008. An on-farm bioassay for assessing *Meloidogyne hapla* infestations as a decision management tool. *Crop Protection*, 27: 785-791.

Hallmann, J., Quadt-Hallmann, A., Miller, W.G., Sikora, R.A. and Lindow, S.E. 2001. Endophyte colonization of plants by biocontrol agent *Rhizobium etli* G12 in relation to *Meloidogyne incognita* infection. *Phytopathology*, 91(4):415-422.

Hartman, K.M. and Sasser, J.N. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. Pp. 69-77. In: An Advanced Treatise on *Meloidogyne*. Vol. 2. Methodology (Barker, K.R., Carter, C.C., Sasser, J.N., eds.). North Carolina State University Graphics, Raleigh, USA, 223pp.

Hussey, R.S. and Barker, K.R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Disease Reporter,* 57: 1025-1028.

Hussey, R.S. and Janssen G.J.W. 2002. Root-Knot. Nematodes: *Meloidogyne* species. In: Plant Resistance to Parasitic Nematodes (Starr, J.L., Bridge, J., Cook, R., eds.). CABI Publishing, Walling ford, UK, 258p.

Khan, M.R. and Haque, Z. 2011. Soil application of *Pseudomonas fluorescens* and *Trichoderma harzianum* reduces root-knot nematode, *Meloidogyne incognita*, on tobacco. *Phytopathology Mediterranea*, 50: 257-266.

Khan, M.R., Khan, S.M., Mohiddin, F.A. and Askary, T.H. 2007. Effect of certain phosphate-solubliziing bacteria on root-knot nematode disease of mungbean. In: *First International Meeting on Microbial Phosphate Solubilizers* (Velazuaz, E., Rodriguez, B, ed.). Springer-Verlag , Vienna.

Meyer, S.L.F., Roberts, D.P., Chitwood, D.J., Carta, L.K., Lumsden, R.D. and Mao, W. 2001. Application of *Burkholderia cepacia* and *Trichoderma virens*, alone and in combinations, against *Meloidogyne incognita* on bell pepper. *Nematropica*, 31: 75-86.

Nandy, B., Kundu, K., Banerjee, N. and Babu, S.P.S. 2003. Salicylic acid induced suppression of *Meloidogyne incognita* infestation of okra and cowpea. *Nematology*, 5: 742-752.

Naserinasab, F., Sahebani, N. and Etebarian, H.R. 2011. Biological control of *Meloidogyne javanica* by *Trichoderma harzianum* B1 and salicylic acid on Tomato. *African Journal of Food Science,* 5(3): 2765- 280.

Sahebani, N. and Hadavi, N. 2008. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Soil Biology and Biochemistry,* 40: 2016–2020.

Sharon, E., Bar-Eyal, M., Chet, I., Herra-Estrella, A., Kleified, O. and Spigel, Y. 2001. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopathology*, 91: 687- 693.

Siddiqui, I.A. and Shaukat, S.S. 2003. Suppression of root–knot disease by *Pseudomonas fluorescens* CHAO in tomato: importance of bacterial secondary metabolite, 2, 4- diacetylpholoroglucinol. *Soil Biology and Biochemistry*, 35: 1615 – 1623.

Siddiqui, I.A. and Shaukat, S.S. 2002. Mixtures of plant disease suppressive bacteria enhance biological control of multiple tomato pathogens. *Biology and Fertility of soils*, 36: 260- 268.

Siddiqui, I.A. and Shaukat, S.S. 2004. *Trichoderma harzianum* enhances the production of biocontrol of *Meloidogyne javanica* by *Pseudomonas fluorescens* in tomato. *Letters in Applied Microbiology,* 38: 169-175.

Siddiqui, I.A., Shaukat, S.S., Sheikh, I.H. and Khan, A. 2006. Role of cyanide production by *Pseudomonas fluorescens* CHA0 in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. *Microbiology and Biotechnology*, 22: 641-650.

Stirling, G.R. 1991. *Biological control of plant parasitic nematodes.* CAB International, Wallingford, UK. 282 PP.

Taylor, D.P. and Netscher, C. 1974. An improved technique for preparing perineal pattern of *Meloidogyne* spp. *Nematologica*, 20: 268.

Whipps, J.M. 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal Experess. Botanical*, 52: 487-511.

Zhang, S., Moyne, A.L., Reddy, M.S. and Kloepper, J.W. 2002. The role of salicylic acid in induced systemic resistance elicited by plant growth promoting rhizobacteria against blue mold of Tobacco. *Biological Control*, 25: 288-296.

Received: 16 June 2015; Accepted: 21 December 2015

Αποτελεσματικότητα σαλικυλικού οξέος, *Pseudomonas fluorescens* CHA0 και *Trichoderma viride* στην αντιμετώπιση του *Meloidogyne incognita* race 2 σε διάφορες ποικιλίες τομάτας

L. Esfahani, S. Jamali, A. Saeedizadeh και H. Pedramfar

Περίληψη Μελετήθηκαν οι επιδράσεις του σαλικυλικού οξέος (SA), του *Trichoderma viride* και του *Pseudomonas fluorescens* CHA0 στον κομβονηματώδη *Meloidogyne incognita* race 2 σε ανθεκτικές και ευαίσθητες ποικιλίες τομάτας (Gina VF, Falat CH, Falat 111, Karoon) κατά τα έτη 2012-2013. Χρησιμοποιήθηκαν σπορόφυτα τομάτας στο στάδιο των 4 φύλλων, τα οποία αναπτύχθηκαν σε γλάστρες με 1000 γραμμάρια αποστειρωμένου εδάφους, όπου έγινε εφαρμογή 20 ml εναιωρήματος του *T. viride* με 1×10^6 σπόρια, 30 ml του *P. fluorescens* CHA0 με 10^9 cfu/ml, 5 mM σαλικυλικού οξέος και 2000 προνυμφών δευτέρου σταδίου του νηματώδη. Έγινε εκτίμηση παραμέτρων που σχετίζονται με τον πληθυσμό των νηματωδών και την ανάπτυξη των φυτών. Οι παράγοντες βιολογικής αντιμετώπισης και το σαλικυλικό οξύ ήταν αποτελεσματικά στην καταπολέμηση των νηματωδών σε απλές ή συνδυασμένες επεμβάσεις. Μεγάλη μείωση του αριθμού των κόμβων στις ρίζες καθώς και των ωόσακκων παρατηρήθηκε μετά από τη συνδυασμένη επέμβαση του σαλικυλικού οξέος με τους βιολογικούς παράγοντες. Η μεγαλύτερη αύξηση στην ανάπτυξη των φυτών επιτεύχθηκε στην ποικιλία Falat CH μετά από επέμβαση με σαλικυλικό οξύ, ακολουθούμενη από τις επεμβάσεις με το *P. fluorescens* CHA0 και το *T. viride*. Ο μεγαλύτερος αριθμός κόμβων καταγράφηκε στην ποικιλία Karoon και ακολούθησαν οι ποικιλίες Falat 111, Gina VF και Falat CH. Η επέμβαση με *P. fluorescens* CHA0 προκάλεσε τη μεγαλύτερη αύξηση σε νωπό και ξηρό βάρος ρίζας, νωπό και ξηρό βάρος βλαστών και στο μήκος των φυτών σε όλες τις επεμβάσεις απουσία νηματωδών. Τα αποτελέσματα έδειξαν ότι το σαλικυλικό οξύ, σε συνδυασμό με τους βιολογικούς παράγοντες (*T. viride* και *P. fluorescens* CHA0), διήγειραν και τελικά αύξησαν την ανάπτυξη του φυτού.

Hellenic Plant Protection Journal **9:** 35-43, 2016

DE GRUYTER
OPEN

Hellenic Plant Protection Journal **9:** 44-50, 2016
DOI 10.1515/hppj-2016-0005

SHORT COMMUNICATION

First report of the nematodes *Filenchus orientalis* and *Hemicriconemoides californianus* on faba bean in Iran

S. Azimi[1], E. Mahdikhani-Moghadam[1*], H. Rouhani[1] and H. Rajabi Memari[2]

Summary During a survey in Iran, two known species of plant-parasitic nematodes of the families Tylenchidae and Criconematidae were reported for the first time. The morphological and morphometric characters of Iranian populations of the two recovered species are discussed and illustrated based on morphological and morphometrics data. Iranian population of *Filenchus orientalis* is characterized by having a 601-755µm body length, stylet length of 9.0-11.3 µm, lateral field with four incisures, tail length of 100-118 µm and males with 15-21 µm long spicules. *Hemicriconemoides californianus* population is characterized by having a body length of 430-550µm, lip region with two annuli, stylet length of 75-83µm and tail length of 20-28 µm. The morphological and morphometric characters of both species are in agreement with those in original descriptions.

Additional keywords: *Hemicriconemoides, Filenchus*, first record, , morphology, morphometric, plant-parasitic

Introduction

Plant parasitic nematodes cause severe damage to faba bean (*Vicia faba* L.) in many countries (Sikora and Greco, 1990). The root lesion nematodes (*Pratylenchus thornei*, *P. neglectus*, *P. pinguicaudatus*) and *Ditylenchus dipsaci* are major pests of this crop in the Mediterranean basin (Troccoli and Di Vito, 2002). Greco *et al*. (1984) recovered *Helicotylenchus* sp., *Tylenchus* sp. and *Tylenchorhynchus* sp. from soil samples of broad bean in Syria. *Tylenchorhynchus dubius*, *Merlinius brevidens*, *Pratylenchus* spp. and *Heterodera trifolii* have been reported on faba bean in Poland (Skwiercz *et al*., 1990). Di Vito *et al*. (1994) studied nematodes of cool-season food legumes in North Africa and found *Heterodera goettingiana* which occurs in high densities in faba bean fields. Faba bean can be attacked by *Ditylenchus dipsaci*, *H. goettingiana*, *Pratylenchus* spp. and *Meloidogyne* spp. but the most common and harmful parasitic nematode to this crop is the stem nematode *D. dipsaci* (Sillero *et al*., 2010). *Ditylenchus gigas* has been recorded from broad bean with economic importance in Italy, Spain and Lebanon (Vovlas *et al*., 2011).

During nematode surveys in Iran conducted during 2011-2014, we recovered seven species of the genus *Pratylenchus* associated with root and soil samples of faba bean (Azimi & Mahdikhani-Moghadam, 2013) and two species belonging to the rare genus, *Apratylenchoides* from the rhizosphere of faba bean (Azimi *et al*., 2014). Additional data about two species of the genera *Filenchus* Andrássy, 1954 and *Hemicriconemoides* Chitwood and Birchfield, 1957 associated with faba bean fields in Iran are presented herein.

Materials and methods

Soil samples were collected from the rhizosphere of faba bean fields in Khuzestan province, south-western Iran during 2011-2014. Nematodes were extracted from soil samples using the Jenkins (1964) method.

[1] Department of Plant Protection, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
[2] Department of Agronomy and Plant Breeding, College of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran
* Corresponding author: mahdikhani-e@ferdowsi.um.ac.ir

The collected specimens were killed in hot 4% formaldehyde solution, transferred to anhydrous glycerin according to De Grisse's (1969) method. Nematodes were mounted in a small drop of glycerin on permanent slides. Observations and measurements were done using a Leitz SM-LUX light microscope equipped with a drawing tube. Some of the best-preserved specimens were photographed using an Olympus DP72 digital camera attached to an Olympus BX51 light microscope. Nematode species were identified based on morphological and morphometric characters (Geraert, 2008, 2010).

Results and discussion

Filenchus orientalis Xie and Feng, 1996 (Figs 1 & 2)

MEASUREMENTS

See Table 1

DESCRIPTION

Female

Body slightly ventrally arcuate after fixation. Lateral field slightly more than one-fourth of maximum body diameter, with smooth four lines. Lip region rounded, smooth, continuous with body contour or separated with slight constriction. Amphidial apertures in form of longitudinal slits on lateral sides of lip region. Stylet delicate, 9-11 µm long, conus about one-third of entire stylet length, knobs directed posteriorly. Dorsal gland orifice opens at 1.5-2.0 µm posterior to stylet base. Procorpus narrow, cylindrical, median bulb oval with relatively prominent valve, posterior glandular region pyriform, not overlapping intestine. Excretory pore obvious, at 70-85 µm distance from anterior end. Reproductive system monodelphic-prodelphic, composed of an ovary which tip of the germinal zone sometimes reaching to terminal bulb and oocytes mostly in single row, short oviduct, spermatheca longer than wide, 35-41×11-14 µm size, filled with spheroid sperm cells, crustaformeria well visible, uterus with distinct lumen, post vulval uterine sac shorter than body width, vagina perpendicular to body axis and vulva a small transverse slit. Tail long-conical, narrowing evenly to filiform part and straight to slightly curved.

Male

General morphology similar to that of female except for character states associated with sexual differences. Body straight to slightly ventrally arcuate after fixation. Excretory pore 82-91 µm from anterior end. Testis a single continuous tube. Spicules slightly curved ventrad, 15-21 µm long. Gubernaculum simple. Tail narrowing evenly, straight to slightly curved, its end filiform as in female.

REMARKS

The species was originally described by

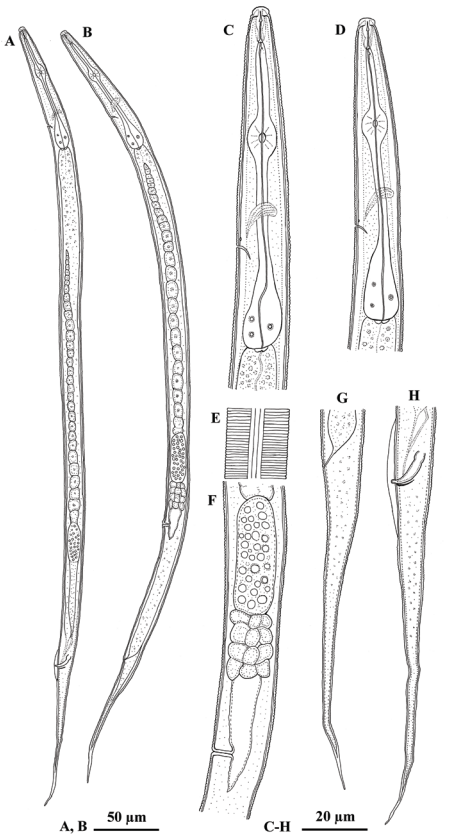

Figure 1. *Filenchus orientalis*. A: Male; B: Female; C: Anterior region of female; D: Anterior region of male; E: Female lateral field at mid-body; F: Part of reproductive system; G: Female tail; H: Male tail.

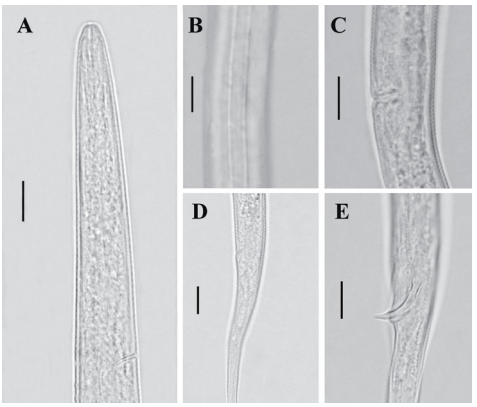

Figure 2. *Filenchus orientalis*. A: Anterior region of female; B: Female lateral field at mid-body; C: Vulval region; D: Female tail; E: Male tail (Scale bars: 10 µm).

Xie and Feng (1996) from soil around roots of water spinach (*Ipomoea aquatica*) in China. The morphological and morphometrics character of the recovered species are in agreement with those of original description. This population is characterized by having lateral field with four lines, head continuous to slightly constricted, 9-11 µm long stylet, and filiform tail. It can be distinguished from *F. vulgaris* (Brzeski, 1963) Lownsbery and Lownsbery (1985) by the shape of head region (rounded, from continuous to slightly constricted, smooth *vs* mostly trapezoid in outline, sometimes rounded, continuous with 4-5 annuli), the range of T/VA ratio (1-1.1 *vs* 1.2-1.7) and common of males. This species was recovered in 6.66% of soil samples (15 females and 8 males) from the

Table 1. Morphometrics of *Filenchus orientalis* Xie and Feng, 1996 from Iranian population and their comparison with the type population (data according to Geraert, 2008). All measurements are in µm and in the form: mean ± s.d. (range).

	Present study		Xie and Feng, 1996	
Character	Females	Males	Females	Males
n	15	8	-	-
L	643.3 ± 39.1 (601 -755)	616.6 ± 5.4 (610-626)	610-740	-
a	32.8 ± 1.2 (30.0-37.1)	34.2 ± 2.1 (31.7-38.5)	29-36	-
b	5.9 ± 0.3 (5.5-6.4)	5.7 ± 0.4 (5.1-6.2)	-	-
c	5.4 ± 1.1 (4.3-6.9)	4.4 ± 0.5 (3.8-5.5)	5.6-7.0	-
c´	10.3 ± 1.2 (8.1-13.0)	11.6 ± 1.4 (9.1-13.3)	7.5-11.0	-
V	63.6 ± 4.5 (58.6-69.7)	-	63-70	-
V´	78.4 ± 2.2 (76.2-81.3)	-	76-81	-
Stylet length	9.9 ± 0.9 (9.0-11.3)	9.9 ± 0.9 (9-11)	10-11	-
M	33.0 ± 2.4 (30-36)	33.0 ± 1.4 (31-36)	-	-
DGO	1.5 ± 0.2 (1.5-2.2)	1.5± 0.3 (1.4-2.1)	-	-
MB	37.3 ± 1.9 (34.0-40.7)	44.1 ±4.6 (37.9-51.8)	37-54	-
Body width	19.2 ± 1.5 (16.7-21.5)	17.9 ± 1.1 (16.2-19.2)	-	-
S. E. pore	80.3 ± 5.5 (70.0-85.5)	88.4 ± 4.9 (82.1-90.8)	67.6-91.0	-
Vulval body width	16 ± 1.9 (14.5-20.3)	-	-	-
Post vulval uterine sac	13 ± 2.9 (9.6-17.7)	-	-	-
Vulva-anus	113.9 ± 6.7 (101 -120)	-	-	-
Anal body width	11.4 ± 1.3 (9.6-13.1)	12.2 ± 0.8 (11.1-13.5)	-	-
Tail length	112.3± 6.0 (100.3-118.0)	113.0 ± 4.0 (106-118)	106-112	-
T/VA	1.1 ± 0.1 (0.9-1.3)	-	1.0-1.1	-
Spicule length	-	17.3 ± 2.3 (15-21)	-	15.5-21.0
Gubernaculum length	-	6.6 ± 0.7 (5.5-7.7)	-	7-8
Bursa length	-	35.5 ± 2.8 (30.0-38.5)	-	-

rhizosphere of faba bean fields in the vicinity of Shooshtar city in Khuzestan province, south-western Iran. This is the first report of the species from Iran, and the second report of it after the original description.

***Hemicriconemoides californianus* Pinochet and Raski, 1975**
(Figs 3 & 4)
MEASUREMENTS
See Table 2
DESCRIPTION
Female

Body slightly ventrally arcuate after fixation. Cuticular sheath attached to body at anterior end, and vulva generally closely fitting. Body annuli 3.5-5.0 µm wide at midbody. Lip region 11.5-12.5 µm wide and 5-6 µm high, anteriorly flatted, with two annuli, first annulus slightly smaller in diameter than second. Stylet very long, its conus comprising ca. 84% of total stylet length, knobs anchor-shaped, indented anteriad, rounded at base, 5.5-7.0 µm across. Dorsal pharyngeal gland orifice opens close to knobs. Excretory pore at 124-130 µm from anterior end. Pharynx typical of the genus. Nerve ring encircling isthmus. Reproductive system monodelphic-prodelphic, outstretched, composed of ovary with oocytes arranged in one or two rows, short oviduct, crustaformeric, oval spermatheca, 22-25 × 12-15 µm size, with spheroid sperm cells, vulval slit lacking flaps. Tail conoid with bluntly rounded terminus.

Male
Not found
REMARKS

The species was originally described by Pinochet and Raski (1975) from soil around roots of grape (*Vitis vinifera* L.) in California. Ye and Robbins (2000) examined 13 populations of this species from different hosts in California, no morphological or morphometric differences were found among examined populations. The general morphology of the recovered species closely resembles

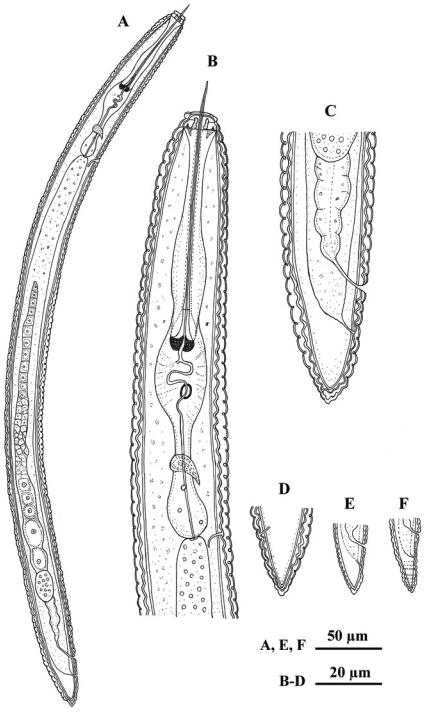

Figure 3. Female of *Hemicriconemoides californianus*. A: Entire body; B: Anterior region showing pharynx; C-F: Posterior end showing vulva and tail.

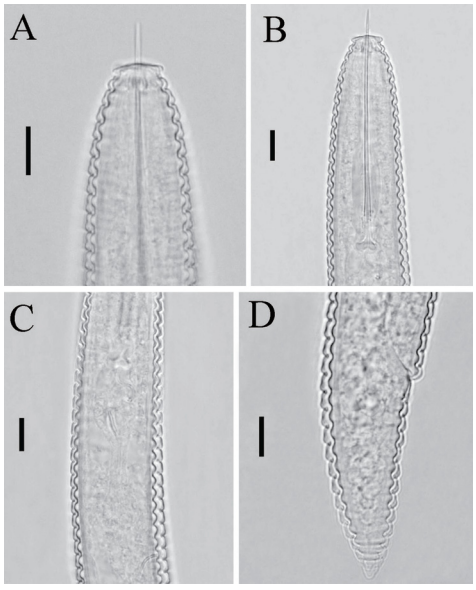

Figure 4. Female of *Hemicriconemoides californianus*. A, B: Anterior end; C: Pharynx, D: Vulval region and tail (Scale bars: 10 µm).

Table 2. Morphometrics of *Hemicriconemoides californianus* Pinochet and Raski, 1975 females from Iranian population and their comparison with the type population. All measurements are in µm and in the form: mean ± s.d. (range).

Character	Present study	Pinochet and Raski, 1975	Ye and Robbins, 2000	
n	20	10	5	10
L	469.8 ± 38.4 (430 -550)	440.0 (410-460)	436.0 ± 28.1 (400-480)	467.8 ± 23.8 (425-500)
a	17.4 ± 1.3 (15.6-20.0)	17.0 (16.0-19.0)	14.8 ± 1.5(12.5-17.1)	15.4 ± 1.4 (13.5-17.8)
b	4.2 ± 0.4 (3.4-4.7)	4.1 (3.4-4.6)	3.6 ± 0.3 (3.1-3.9)	3.6 ± 0.1 (3.4-3.8)
c	21.9 ± 1.9 (19 -26)	21.0 (17 -25)	24.4 ± 4.8 (19.2-30.3)	24.2 ± 2.8 (20.2-27.8)
c´	1.3 ± 0.1 (1.0-1.4)	-	1.1 ± 0.1 (0.9-1.3)	1.1 ± 0.1 (1.0-1.3)
V	92.0 ± 0.7 (91.0-93.1)	91.0 (90 -92)	91.8 ± 0.9 (90.3-92.8)	91.7 ± 0.6 (90.8-92.4)
Stylet length	80.0 ± 3.4 (75 -83)	80.0 (77-83)	77.6 ± 4.3 (74.0-86.0)	84.4 ± 1.6 (82-86)
m	86.3 ±2.2 (83.1-89.7)	-	86.2 ± 3.3 (81.4-89.3)	89.3 ± 1.5 (86.9-91.6)
DGO	4.1 ± 0.5 (3.5-4.8)	-	-	-
MB	66.2 ± 1.5 (63.7-68.0)	-	-	-
Body width	30.6 ± 1.7 (28.4-32.5)	-	29.5 ±1.9 (26.5-32.0)	30.4 ± 2.5 (27.5-35.0)
S. E. pore	126.7 ± 2.2 (124.1-130.2)	-	126.6 ±5.1 (120-135)	138.3 ± 8.2 (127-153)
Vulval body width	92.0 ± 1.3 (91.0-93.1)	-	-	-
Vulva-anus	15.0 ± 1.6 (13-18)	-	-	-
Anal body width	18.5 ± 2.4 (16-22)	-	-	-
Tail length	24.4 ± 3.1 (20-28)	-	-	-
R	126.8 ±6.4 (120-135)	119.0 (112-127)	114.6 ± 6.1 (103-121)	119.9 ± 2.0 (117-124)
RSt	20.7 ± 0.8 (20-22)	-	20.8 ± 1.1 (20-23)	23.0 ± 0.6 (22-24)
Roes	32.8 ± 3.1 (28-38)	-	30.0 ± 0.6 (29 -31)	33.3 ± 2.0 (31-37)
Rex	34.2 ± 2.9 (30-39)	35.0 (34 -37)	32.6 ± 1.7 (30-34)	36.2 ± 2.3 (33-40)
RV	12.2 ± 1.0 (11 -13)	11.0 (10-12)	12.4 ± 1.0 (11-14)	11.8 ± 0.6 (11-13)
RVan	4.2 ± 0.5 (4 -5)	6.0 (5-6)	4.8 ± 0.7 (4-6)	5.1 ± 0.5 (4-6)
Ran	6.6 ± 0.8 (6-8)	5.0 (4-6)	7.6 ± 0.8 (7-9)	6.7 ± 0.6 (6-8)
VL/VB	1.6 ± 0.1 (1.4-1.8)	1.7 (1.3-1.8)	1.5 ± 0.1 (1.4-1.8)	1.6 ± 0.1 (1.4-1.9)

to the characters given in the original description and the data given by Ye and Robbins, 2000 (see Table 2). However, the body length is slightly longer (430-550 *vs* 400-500 µm) and the number of body annuli is greater (120-135 *vs* 112-127). These differences can be attributed to the intraspecies variations due to geographical differences. The recovered population of the species is closely related to *H. chitwoodi*, Esser, 1960, *H. gaddi* (Loos, 1949) Chitwood & Birchfield, 1957 and *H. phoenicis*, Van den Berg, Tiedt, Inserra, Stanley, Vovlas, Palomares-Rius, Castillo and Subbotin (2015).

Our population differs from *H. chitwoodi* by lip region having the first annulus smaller than the second one, more broadly conoid tail with bluntly rounded terminus (*vs* conoid, gradually tapering to slender conoid outline, with finely rounded terminus. In our population, the first lip annulus is slightly smaller in diameter than the second, whereas it is always much smaller than the second in *H. gaddi*. Also, VL/VB ratio is 1.3-1.9 (*vs* 2.1-2.7). It can be distinguished from *H. phoenicis* by lacking the coarse cuticular ridges and grooves of the annuli.

Hemicriconemoides californianus has been reported from North Korea (Kornobis and Dobosz, 1997) and Taiwan (Chen *et al*.,

2007). This species was recovered in 4.0% of soil samples (20 females) from the rhizosphere of faba bean fields in the vicinity of Dizful city in Khuzestan province, southwestern Iran. This is the first report of *H. californianus* from Iran and the first report of the species in association with faba bean. The aim of the present study was originally to identify the plant parasitic fauna in Iran, therefore no further study was performed regarding the pathogenicity of the aforementioned plant parasitic nematode species found in association with faba bean roots. Some stunting and other symptoms such as yellowing, weakening etc, were observed on the plants, but, assigning these symptoms specifically to nematode damage needs further pathogenicity testing.

The authors thank Dr Majid Pedram (Tarbiat Modares University, Iran), who provided some of references for our study and Dr. Mehdi Esfandiari (Shahid Chamran University of Ahvaz, Iran) for his assistance in sampling.

Literature cited

Andrássy, I. 1954. Revision der Gattung *Tylenchus* Bastian, 1865 (Tylenchidae, Nematoda). *Acta Zoologica Hungaricae*, 1: 5-42.

Azimi, S. and Mahdikhani-Moghadam, E. 2013. Root lesion nematodes associated with faba bean fields in Iran with two new records of *Pratylenchus crassi* Das & Sultana, 1979 and *P. teres* Khan & Singh, 1974. *Advanced Crop Science*, 3: 398-404.

Azimi, S., Mahdikhani-Moghadam, E., Rouhani, H. and Rajabi Memari, H. 2014. The rare genus *Apratylenchoides* Sher, 1973 (Nematoda: Pratylenchidae) from faba bean in Iran. *Archives of Phytopathology and Plant Protection*, 47: 2288-2294.

Bastian, C.H. 1865. Monograph on the Anguillulidae, or free nematoids, marine, land and freshwater; with descriptions of 100 new species. *Transactions of the Linnean Society*, 25: 73-184.

Brzeski, M.W. 1963. On the taxonomic status of *Tylenchus filiformis* Butschli, 1873, and the description of *T. vulgaris* sp. n. (Nematoda: Tylenchidae). *Bulletin de l'Academie Polonaise des Sciences*, 11: 531-535.

Chen, D.Y., Ni, H.F., Tsay, T.T. and Yen, J.H. 2007. Identification of *Hemicriconemoides kanayaensis* and *H. californianus* (Nematoda: Criconematoidea, Criconematidae) among tea plantations in Taiwan. *Plant Pathology Bulletin*, 16: 181- 192.

Chitwood, B.G. and Birchfield, W. 1957. A new genus, *Hemicriconemoides* (Criconematidae: Tylenchina). *Proceedings of the Helminthological Society of Washington,* 24: 80-86.

De Grisse A.T. 1969. Redescription and modification of some techniques used in the study of nematodes phytoparasitaires. *Mededelingen Rijksfacultiet Landbouw Wetenschappe Gent*, 34: 351-369.

Di Vito, M., Greco, N., Halila, H.M., Mabsoute, L., Labdi, M., Beniwal, S.P.S., Saxena, M.C., Singh, K.B. and Solh, M. 1994. Nematodes of cool-season food legumes in North Africa. *Nematologia Mediterranea*, 22: 3-10.

Esser, R.P. 1960. Three additional species in the genus *Hemicriconemoides* Chitwood and Birchfield, 1957 (Nemata: Tylenchida). *Nematologica,* 5: 64-71.

Geraert, E. 2008. *The Tylenchidae of the world. Identification of the family Tylenchidae (Nematoda).* Ghent, Belgium, Academia Press, 530 p.

Geraert, E. 2010. *The Criconematidae of the world. Identification of the family Criconematidae (Nematoda).* Ghent, Belgium, Academia Press, 615 p.

Greco, N., Di Vito, M., Reddy, M.V. and Saxena, M.C. 1984. A preliminary report of survey of plant parasitic nematodes of leguminous crops in Syria. *Nematologia Mediterranea*, 12: 87-93.

Jenkins, W.R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. *Plant Disease Reporter*, 48: 692.

Kornobis, S. and Dobosz, R. 1997. Some species of Tylenchida (Nemata) from North Korea. *Journal of Plant Protection Research*, 37: 113-116.

Loos, C.A. 1949. Notes on free-living and plant-parasitic nematodes of Ceylon 4. *Journal of the Zoological Society of India*, 1: 17-22.

Lownsbery, J.W. and Lownsbery, B.F. 1985. Plant-parasitic nematodes associated with forest trees in California. *Hilgardia*, 53: 1-16.

Pinochet, J. and Raski, D.J. 1975. Four new species of the genus *Hemicriconemoides* (Nematoda: Criconematidae). *Journal of Nematology*, 7: 263-270.

Sikora, R.A. and Greco, N. 1990. *Nematode parasites of food legumes*, In: Luc, M., Sikora, R.A. and Bridge, J. (eds), *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, CAB International Wallingford, UK, p. 181-235.

Sillero, J.C., Villegas-Fernandez, A.M., Thomas, J., Rojas-Molina, M.M., Emeran, A.A., Fernandez-Aparicio, M. and Rubiales, D. 2010. Faba bean breeding for disease resistance. *Field Crops Research*, 115: 297-307.

Skwiercz, A.T., Zawiślak, K. and Adamiak, J. 1990. Study of plant parasitic nematodes in soil under faba beans in crop rotation and in long-term monoculture. *Zeszyts problemowe postepów Nauk Rolniczych*, 391: 97-103.

Troccoli, A. and Di Vito, M. 2002. Root lesion and stem nematodes associated with faba bean in North Africa. *Nematologia Mediterranea*, 30: 79-81.

Van den Berg, E., Tiedt, L.R., Inserra, R.N., Stanley, J.D., Vovlas, N., Palomares-Rius, J.E., Castillo, P. and Subbotin, S.A. 2015. Characterisation of a topotype and other populations of *Hemicriconemoides strictathecatus* Esser, 1960 (Nematoda: Criconematidae) from Florida with description of *H. phoenicis* sp. n. from the USA. *Nematology*, 17: 265-300.

Vovlas, N., Troccoli, A., Palomares-Rius, J.E., De Luca, F., Liébanas, G., Landa, B.B., Subbotin, S.A. and Castillo, P. 2011. *Ditylenchus gigas* n. sp. parasitizing broad bean: a new stem nematode singled out from the *Ditylenchus dipsaci* species complex using a polyphasic approach with molecular phylogeny. *Plant Pathology*, 60: 762-775.

Xie, H. and Feng, Z. 1996. Description of new species of the genus *Filenchus* (Andrassy, 1954) Meyl, 1961 (Nemata: Tylenchidae). III. *F. montanus* n. sp. and *F. orientalis* n. sp. *Acta Phytopathologica Sinica*, 26: 365-369.

Ye, W. and Robbins, R.T. 2000. Morphology of four species of *Hemicriconemoides* (Nematoda: Criconematidae) in the USA with the synonymy of *H. annulatus*. *International Journal of Nematology*, 10: 101-111.

Received: 14 December 2015; Accepted: 5 January 2016

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη αναφορά των νηματωδών *Filenchus orientalis* και *Hemicriconemoides californianus* σε κουκιά στο Ιράν

S. Azimi, E. Mahdikhani-Moghadam, H. Rouhani and H. Rajabi Memari

Περίληψη Σε μία επισκόπηση στο Ιράν αναφέρθηκαν για πρώτη φορά δύο ήδη γνωστά είδη φυτοπαρασιτικών νηματωδών, τα οποία ανήκουν στις Οικογένειες Tylenchidae και Criconematidae. Γίνεται συζήτηση και παρουσίαση των μορφολογικών και μορφομετρικών χαρακτήρων των Ιρανικών πληθυσμών των δύο ειδών με βάση μορφολογικά και μορφομετρικά δεδομένα. Ο Ιρανικός πληθυσμός του *Filenchus orientalis* χαρακτηρίζεται από μήκος σώματος 601-755μm, μήκος στιλέτου 9.0-11.3 μm, πλάγιο πεδίο με τέσσερις εγκολπώσεις, μήκος ουράς 100-118 μm και μήκος συζευκτικών ακάνθων αρσενικών 15-21 μm. Ο πληθυσμός του *Hemicriconemoides californianus* χαρακτηρίζεται από μήκος σώματος 430-550μm, περιοχή χειλέων με δύο ραβδώσεις, μήκος στιλέτου 75-83μm και μήκος ουράς 20-28 μm. Οι μορφολογικοί και μορφομετρικοί χαρακτήρες και των δύο ειδών είναι σε συμφωνία με αυτούς των αρχικών περιγραφών.

Hellenic Plant Protection Journal **9:** 44-50, 2016

Τόμος 9, Τεύχος 1, Ιανουάριος 2016
ISSN 1791-3691

Περιεχόμενα

Volume 9, Issue 1, January 2016
ISSN 1791-3691

Hellenic Plant Protection Journal

Contents

www.hppj.gr