

NEW PERSPECTIVES

Do interactions between plant roots and the rhizosphere affect parasitoid behaviour?

EMILIO GUERRIERI¹, GUIDO LINGUA², MARIA CRISTINA DIGILIO³, NADIA MASSA² and GRAZIELLA BERTA²

¹Istituto per la Protezione delle Piante, CNR, Sez. Portici (NA), ²Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale 'Amedeo Avogadro', Alessandria and ³Dipartimento di Entomologia e Zoologia agraria, Università di Napoli 'Federico II', Italy

Key words. Aphid parasitoids, arbuscular mycorrhizal, biological control, mycorrhizal symbioses.

Multitrophic interactions are powerful forces shaping the structure of living communities. Plants encounter a great diversity of organisms in their environment: some of these interactions are beneficial (e.g. symbiotic fungi and insect pollinators) while some are detrimental (e.g. herbivorous insects and pathogenic micro-organisms). Multitrophic interactions between below-ground and above-ground organisms are receiving increasing attention because they may influence plant defences against biotic and abiotic stresses (van Dam *et al.*, 2003). Plant defences can be constitutive or induced (Agrawal *et al.*, 1999; Walling, 2000), and may also be direct (e.g. toxic compounds like glucosinolates in Brassicaceae) or indirect. Indirect defences typically involve the production of volatile semiochemicals that are attractive towards natural enemies of herbivorous insects (Dicke, 1999). These semiochemicals have been referred to as synomones to stress the mutual benefit of the partners involved (Vet & Dicke, 1992). Some of these volatiles are released as a specific response to the attack of a specific herbivore (see Agrawal *et al.*, 1999 and references therein), a feature termed *induced indirect defence*. Several studies show that the release of induced volatiles is not confined exclusively to the organ attacked but involves all the plant through the circulation of systemic elicitors (Mattiacci *et al.*, 1995; Alborn *et al.*, 1997; Guerrieri *et al.*, 1999; Dicke & Dijkman, 2001). The growing evidence that any colonising organism alters the profile of plant volatiles suggests that this may have intriguing and often unpredictable consequences for the performance of higher trophic levels (Dicke *et al.*, 2003). In this paper we report on the interactions between below-ground interactions and indirect defences.

Correspondence: Emilio Guerrieri, Istituto per la Protezione delle Piante, CNR, Sez. Portici, Via Università 133, 80055 Portici (NA), Italy. E-mail: guerrieri@ipp.cnr.it

Arbuscular mycorrhizal symbioses are mutualistic interactions between plant roots and soil fungi, and have been reported for more than 80% of higher plants (Smith & Read, 1997). Colonisation by arbuscular mycorrhizal fungi induces resistance or tolerance to a variety of pathogens in tomato and in other plants (Cordier *et al.*, 1996; Trotta *et al.*, 1996; Lingua *et al.*, 2002). These changes are mediated by a variety of mechanisms, including the up-regulation and down-regulation of specific genes (Tahiri-Alaoui & Antoniw, 1996) that result in localised and systemic responses by the plant. These responses include the synthesis of new isoforms of chitinases and glucanases and the thickening of the cell walls (Azcón-Aguilar *et al.*, 2002; Pozo *et al.*, 2002) that may affect herbivore colonisation. The effects of arbuscular mycorrhizal symbiosis on above-ground herbivores has been investigated with contrasting results (van Dam *et al.*, 2003 and references therein). More recently, the effects of different species of arbuscular mycorrhizal fungi on parasitism rates have been reported (Gange *et al.*, 2003) but this study did not demonstrate a direct link between arbuscular mycorrhizae and attraction of insect parasitoids. In this study we tested the hypothesis that an arbuscular mycorrhizal symbiosis makes tomato plants significantly more attractive towards aphid parasitoids.

Fig. 1a shows the multitrophic system used for this study: at the base of the system is tomato (*Lycopersicon esculentum* Miller) whose roots were colonised by the arbuscular mycorrhizal fungus *Glomus mosseae* Nicol & Gerd (Gerde-mann & Trappe) BEG 12. Although tomato plants are characterised by high levels of constitutive defences (e.g. glandular trichomes, α -tomatine, Kennedy, 2003), induced defence mechanisms are nevertheless important (reviewed in Agrawal *et al.*, 1999 and references therein).

The herbivore (the potato aphid, *Macrosiphum euphorbiae* Thomas) is a key pest of tomato all over the world, causing direct and indirect damage to plants, including

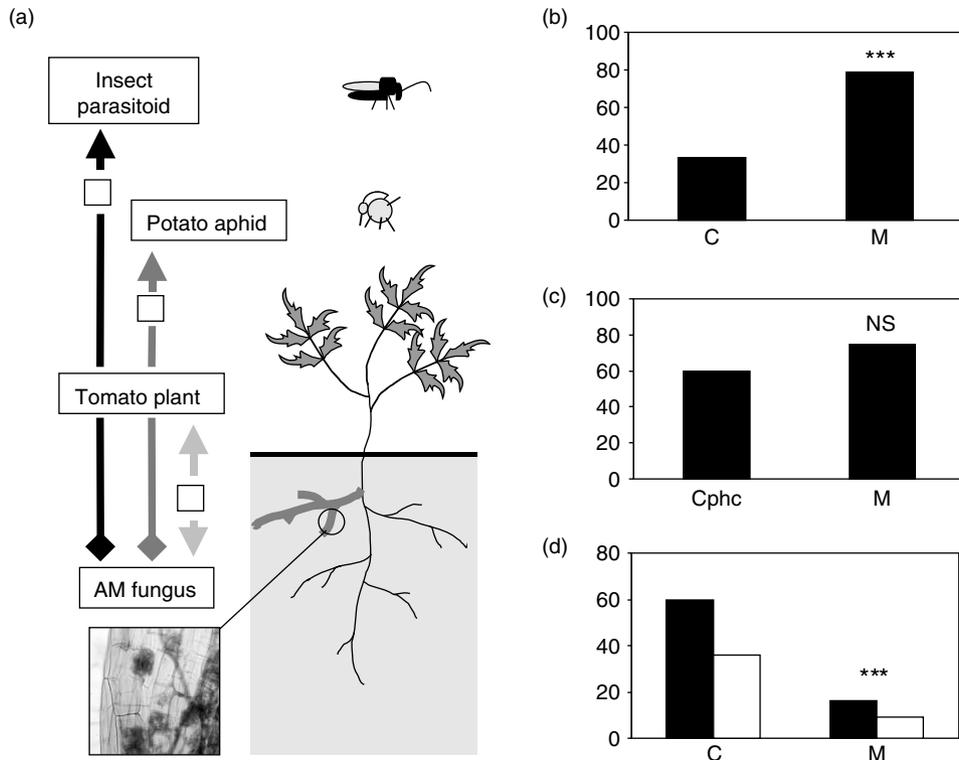


Fig. 1. (a) The multitrophic system used in this study consisted of tomato plant, cultivar M82, colonised by the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* (enlarged in the box, bottom left), the potato aphid *Macrosiphum euphorbiae* and its natural enemy, the braconid parasitoid *Aphidius ervi*; + indicates a positive interaction, - indicates a negative interaction. (b) Percentages of parasitic wasps ($n=200$) attracted by uninfested control (C) and uninfested mycorrhizal (M) plants of tomato in a wind tunnel bioassay. (***) $P < 0.001$, G -test for Independence, Sokal & Rohlf, 1995; Rohlf & Sokal, 1995). (c) Percentages of parasitic wasps ($n=200$) attracted by control tomato plants infested by *Macrosiphum euphorbiae* (Cphc) and by uninfested mycorrhizal (M) tomato plants in a wind tunnel bioassay (G -test for Independence, Sokal & Rohlf, 1995; Rohlf & Sokal, 1995). (d) Percentages of potato aphids ($n=100$) reaching adult stage (filled bars) and reproducing (open bars) on control (C) and mycorrhizal (M) plants of tomato (***) $P < 0.001$, G -test for Independence, Sokal & Rohlf, 1995; Rohlf & Sokal, 1995).

transmission of phytopathogenic viruses (Lange & Bronson, 1980). The parasitoid *Aphidius ervi* (Haliday) is well known for its efficiency against the potato aphid (van Lenteren *et al.*, 1997).

The parasitoid used in this study is considered to be a generalist as it can develop in several different aphid species attacking different plant families (mainly Solanaceae and Leguminosae). *Aphidius ervi* has become a model in the study of foraging behaviour (Guerrieri *et al.*, 1993, 1997, 1999, 2002), and is reported to discern between volatiles released by broad bean plant when attacked by host and non-host aphid species (Du *et al.*, 1997; Guerrieri *et al.*, 1999). Moreover, the increasing response of this parasitoid to plant volatiles following successful encounters with its host has shown the existence of a link between oviposition experience and foraging efficiency (associative learning) (Du *et al.*, 1997; Guerrieri *et al.*, 1997).

To test the hypothesis that mycorrhizal colonisation enhances the attractiveness of tomato plants towards the parasitic wasp *A. ervi*, arbuscular mycorrhizal and non-mycorrhizal plants were tested in a wind tunnel bioassay.

For each plant target, 200 female parasitoids were tested by releasing them individually in the glass chamber of the wind tunnel, 30 cm downwind from the target. The attractiveness of each target plant was calculated in terms of percentage of female parasitoids that flew directly upwind and landed on it. Bioassays were conducted on a minimum of 3 days and plant targets were presented in a random order to reduce any effect of temporal variability of behavioural responses. None of the plants was infested with aphids, allowing the effects of arbuscular mycorrhizae on parasitoid behaviour to be tested in the absence of their hosts. Mycorrhizal plants were twice as attractive towards the parasitoid as control plants, with percentages of attraction close to 80% (Fig. 1b), despite the fact that there were no host aphids feeding on these plants. Moreover, these percentages of parasitoids attracted to the mycorrhizal plants were not significantly different from those recorded in a second experiment against a non-mycorrhizal plant that had been infested by hundreds of *M. euphorbiae* for a week (Fig. 1c).

The hypothesis that the arbuscular mycorrhizal symbiosis would affect the development and reproduction

of the aphid hosts was also tested. Only 16% of aphids successfully completed their development on arbuscular mycorrhizal plants, and only 8% reproduced, percentages significantly lower than those recorded on control, non-mycorrhizal plants (Fig. 1d).

The results of these studies raise interesting questions about the reliability of plant signalling for insect parasitoids and the efficacy of biological control when plants have mycorrhizal symbioses. One of the most intriguing implications of this study is that it suggests that parasitoids may arrive on mycorrhizal plants that have few or no aphid hosts. In the system used here, *A. ervi* showed similar responses to plants with an arbuscular mycorrhizal symbiosis and to plants with large *M. euphorbiae* populations (65.5%, data not shown). Since *A. ervi* displays associative learning (Guerrieri *et al.*, 1997), the long-term implications of these results for biological control in this system are not clear. In other similar systems, a minimum threshold of aphid infestation (in terms of the number of aphids and the duration of their feeding activity) is required to make a host plant attractive towards *A. ervi* (Guerrieri *et al.*, 1999). In this study, arbuscular mycorrhizal plants and plants infested with aphids were significantly more attractive to parasitoids than plants without the symbiosis (Fig. 1b) or plants without aphids (32.3%, data not shown, $P < 0.01$, *G*-test for Independence, Rohlf & Sokal, 1995; Sokal & Rohlf, 1995), and hence the final outcome of the interaction between tomatoes and *A. ervi* was similar regardless of whether the plant was colonised by a beneficial soil fungus or populations of a sap-feeding herbivore. This combination of partial resistance to aphid infestation (Fig. 1d) and the production of semiochemicals attractive towards natural enemies of aphids, may result in an effective defence strategy for arbuscular mycorrhizal plants against herbivore populations.

Although this study suggests that tomato plants may benefit from the arbuscular mycorrhizal symbiosis the effects of this interaction on the performance of the parasitoid are less clear. A fascinating possibility is that the arbuscular mycorrhizal symbiosis may allow the parasitoids to locate hosts at low, and perhaps otherwise undetectable, aphid population densities.

It would also have been interesting to assess whether the quality of the few aphids that survived on the arbuscular mycorrhizal plants is high enough to support parasitoid development (Awmack & Leather, 2002), but this was beyond the scope of this study. Although it is possible that in the long term aphids will evolve resistance to the changes in plant quality induced by the arbuscular mycorrhizal symbiosis, the high levels of attractiveness of these plants to aphid parasitoids may provide an indirect plant defence that exerts no evolutionary pressure on the pest because it is not herbivore mediated. This apparent decrease in reliability of plant cues for the parasitoid may, however, be compensated for by the relatively high fecundity of braconid parasitoids of aphids compared with other parasitoids such as aphelinids (Digilio & Pennacchio, 1989; Couty *et al.*, 2001).

This study highlights the novel and complex net of interactions that exist even in this simplified model system.

Further experiments (especially field experiments) to investigate the long-term stability of the interactions would clearly be of value, as would an investigation to determine whether aphids and the mycorrhizal symbiosis have similar effects on the expression of genes associated with plant defences (Fidantsef *et al.*, 1999; Birkett *et al.*, 2000). Similarly, the results of a recent study suggesting that plant-mediated effects of mycorrhizal symbiosis on the higher trophic levels may be affected by the fungal species involved (Gange *et al.*, 2003) also merit further investigation.

The positive effect of mycorrhizal colonisation on the attraction of parasitic wasps to their host plants and a negative effect on insect pest populations observed in this study have important implications for our understanding of insect population dynamics. We will only be able to predict the course of the evolution of plant defence mechanisms that could be used to improve the efficacy of biological control if we understand these interactions.

Acknowledgements

This project was supported by the Italian CNR and by the Università degli Studi del Piemonte Orientale 'Amedeo Avogadro'. We would like to thank Caroline S. Awmack for helpful comments and discussions.

References

- Agrawal, A.A., Tuzun, S. & Bent, E. (1999) *Induced Plant Defenses Against Pathogens and Herbivores*. APS Press, The American Phytopathological Society, St Paul, Minnesota.
- Alborn, H.T., Turlings, T.C.J., Jones, T.H., Stenhagen, G., Loughrin, J.H. & Tumlinson, J.H. (1997) An elicitor of plant volatiles from beet armyworm oral secretion. *Science*, **276**, 945–949.
- Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**, 817–844.
- Azcón-Aguilar, C., Jaizme-Vega, M.C. & Calvet, C. (2002) Plant defense responses induced by arbuscular mycorrhizal fungi. *Mycorrhizal Technology in Agriculture* (ed. by S. Gianinazzi, H. Schüepp, and J. M. Barea), pp. 187–198. Birkhauser, Basel.
- Birkett, M.A., Campbell, C.A.M., Chamberlain, K., Guerrieri, E., Hick, A.J. & Martin, J.L. *et al.* (2000) New roles for *cis*-jasmone as an insect semiochemical and in plant defence. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 9329–9334.
- Cordier, C., Gianinazzi, S. & Gianinazzi-Pearson, V. (1996) Colonisation patterns of root tissues by *Phytophthora nicotianae* var. *parasitica* related to reduced disease in mycorrhizal tomato. *Plant and Soil*, **185**, 223–232.
- Couty, A., Clark, S.J. & Poppy, G.M. (2001) Are fecundity and longevity of female *Aphelinus abdominalis* affected by development in GNA-dosed *Macrosiphum euphorbiae*? *Physiological Entomology*, **26**, 287–293.
- van Dam, N.M., Harvey, J.A., Wäkers, F.L., Bezemer, T.M., van der Putten, W.H. & Vet, L.E.M. (2003) Interactions between

- aboveground and belowground induced responses against phytophages. *Basic and Applied Ecology*, **4**, 63–77.
- Dicke, M. (1999) The ecology and evolution of induced defenses. *Evolution of Induced Indirect Defenses of Plants* (ed. by R. Tollrian and C. J. Harvell), pp. 62–88. Princeton University Press, Princeton, New Jersey.
- Dicke, M. & Dijkman, H. (2001) Within-plant circulation of systemic elicitor of induced defence and release from roots of elicitor that affects neighbouring plants. *Biochemical Systematics and Ecology*, **29**, 1075–1087.
- Dicke, M., van Pecke, R.M.P. & de Boer, J.G. (2003) Inducible indirect defence of plants: from mechanisms to ecological functions. *Basic and Applied Ecology*, **4**, 27–42.
- Digilio, M.C. & Pennacchio, F. (1989) Analisi quantitativa di alcuni parametri biologici caratterizzanti una popolazione italiana di *Aphidius ervi* Haliday (Hymenoptera, Braconidae, Aphidiinae). *Bollettino del Laboratorio di Entomologia Agraria 'Filippo Silvestri'*, **46**, 59–74.
- Du, Y., Poppy, G.M., Powell, W. & Wadhams, L.J. (1997) Chemically mediated associative learning in the host foraging behavior of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *Journal of Insect Behavior*, **10**, 509–522.
- Fidantsef, A.L., Stout, M.J., Thaler, J.S., Duffey, S.S. & Bostock, R.M. (1999) Signal interactions in pathogen and insect attack: expression of lipoxigenase, proteinase inhibitor II, and pathogenesis-related protein P4 in the tomato *Lycopersicon esculentum*. *Physiological and Molecular Plant Pathology*, **54**, 97–114.
- Gange, A.C., Brown, V.K. & Aplin, D.M. (2003) Multitrophic links between arbuscular mycorrhizal fungi and insect parasitoids. *Ecology Letters*, **6**, 1051–1055.
- Guerrieri, E., Pennacchio, F. & Tremblay, E. (1993) Flight behaviour of the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) in response to plant and host volatiles. *European Journal of Entomology*, **90**, 415–421.
- Guerrieri, E., Pennacchio, F. & Tremblay, E. (1997) Effect of adult experience on in-flight orientation to plant and plant-host complex volatiles in *Aphidius ervi* Haliday (Hymenoptera, Braconidae). *Biological Control*, **10**, 159–165.
- Guerrieri, E., Poppy, G.M., Powell, W., Rao, R. & Pennacchio, F. (2002) Plant to plant communication mediating in-flight orientation of *Aphidius ervi*. *Journal of Chemical Ecology*, **28**, 1703–1715.
- Guerrieri, E., Poppy, G.M., Powell, W., Tremblay, E. & Pennacchio, F. (1999) Induction and systemic release of herbivore-induced plant volatiles mediating in-flight orientation of *Aphidius ervi* (Hymenoptera: Braconidae). *Journal of Chemical Ecology*, **25**, 1247–1261.
- Kennedy, G.G. (2003) Tomato, pests, parasitoids and predators: tritrophic interactions involving the genus *Lycopersicon*. *Annual Review of Entomology*, **48**, 51–72.
- Lange, W.H. & Bronson, L. (1980) Insect pests of tomato. *Annual Review of Entomology*, **26**, 345–371.
- van Lenteren, J.C., Roskam, M.M. & Timmer, R. (1997) Commercial mass production and pricing of organisms for biological control of pests in Europe. *Biological Control*, **10**, 143–149.
- Lingua, G., D'Agostino, G., Massa, N., Antosiano, M. & Berta, G. (2002) Mycorrhiza-induced differential response to a yellows disease in tomato. *Mycorrhiza*, **12**, 191–198.
- Mattiacci, L., Dicke, M. & Posthumus, M.A. (1995) β -Glucosidase: an elicitor of herbivore induced plant odor that attracts host-searching parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 2036–2040.
- Pozo, M.J., Slezacek-Deschaumes, S., Dumas-Gaudot, E., Gianinazzi, S. & Azcón-Aguilar, C. (2002) Plant defense responses induced by arbuscular mycorrhizal fungi. *Mycorrhizal Technology in Agriculture* (ed. by S. Gianinazzi, H. Schüepp and J. M. Barea), pp. 103–112. Birkhauser, Basel.
- Rohlf, F.J. & Sokal, R.R., eds (1995) *Statistical Tables*, 3rd edn. W.H. Freeman, New York.
- Smith, S.E. & Read, D.J. (1997) *Mycorrhizal Symbiosis*. Academic Press, London.
- Sokal, R.R. & Rohlf, F.J., eds (1995) *Biometry*, 3rd edn. W.H. Freeman, New York.
- Tahiri-Alaoui, A. & Antoniw, J.F. (1996) Cloning of genes associated with the colonisation of tomato roots by the arbuscular mycorrhizal fungus *Glomus mosseae*. *Agronomie*, **16**, 699–707.
- Trotta, A., Varese, G.C., Gnani, E., Fusconi, A., Sampò, S. & Berta, G. (1996) Interactions between the soil-borne root pathogen *Phytophthora nicotianae* var. *parasitica* and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. *Plant and Soil*, **185**, 199–209.
- Vet, L.E.M. & Dicke, M. (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, **37**, 141–172.
- Walling, L. (2000) The myriad plant responses to herbivory. *Journal of Plant Growth Regulation*, **19**, 195–216.

Accepted 6 April 2004