Chapter 5
Belowground Mycorrhizal Endosymbiosis and Aboveground Insects: Can Multilevel Interactions be Exploited for a Sustainable Control of Pests?

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5.1 Introduction

Terrestrial plants interact with an incredible variety of organisms. Some of these interactions are beneficial, some are detrimental; some develop in the aerial part of the plant, some at root level. The study of these interactions is a precious source of information that could be used to increase plant fitness, especially plant defence against insect pests and microbial pathogens.

Until the end of the last century, there had been a dramatic separation between research on belowground and aboveground interactions, although it has been possible to understand the basic rules of plant responses to beneficial and harmful organisms in both of the two “areas”.

In 1980 it was suggested for the first time to investigate plant-insect interactions following a multitrophic approach, that is by considering each species as being an element of a food chain, having at its base the plant, at the intermediate level the complex of herbivore species (consumers) and at the top level the complex of entomophagous species (carnivores) (Price et al. 1980). This milestone paper opened a completely new field of investigation, and led, in a relatively short period of time, to the identification of unexpected mechanisms that regulate plant defences against insects (Fig. 5.1). In fact, along with the well known physical (e.g. thorns) and chemical (e.g. anti-feedant and toxic compounds) defences that directly affect the development and the reproduction of an invading insect, it has been demonstrated that plants can *indirectly* reduce the populations of herbivore insects by recruiting or enhancing the efficiency of natural enemies, either predators (e.g. ladybirds) and/or parasitoids (e.g. Ichneumonidea and Chalcidoidea wasps). This is accomplished through the production/release of attracting volatile organic compounds (VOC), by supplying food (such as extra floral nectars) or by providing shelter (e.g. domatia) to entomophagous species (Agrawal and Karban 1997; Dicke et al. 2003, Wäckers et al. 2005).

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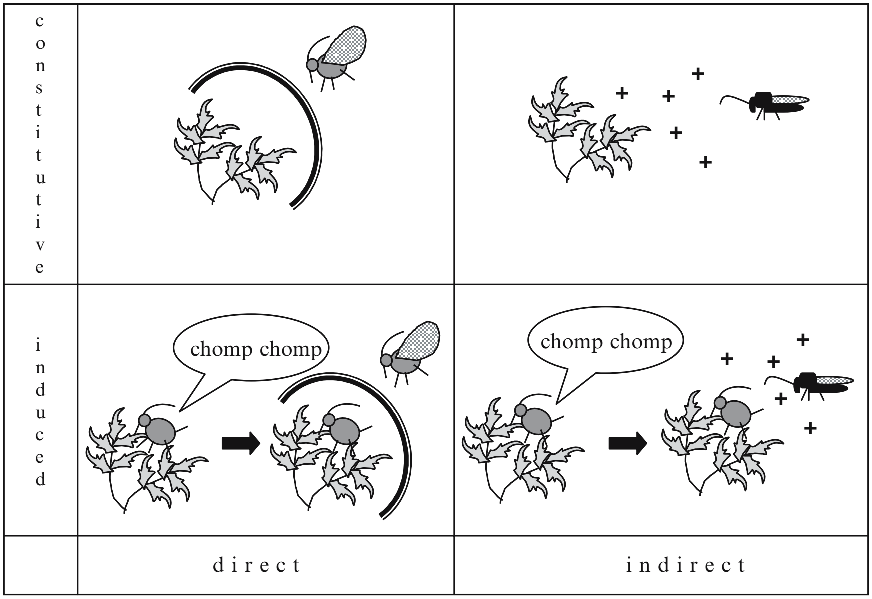

Fig. 5.1 Plant-defences against herbivore insects. Indirect defences are represented as the emission of VOC (+) attractive towards a parasitoid wasp

In many cases, plant defences, either direct or indirect, are activated only following herbivore damage, and for this reason they are referred to as *induced*, to distinguish them from *constitutive defences* that are always expressed, independently of herbivore attack (Agrawal et al. 1999 and references therein). It is documented that induced defences have a lower metabolic cost for the plant (Zangerl 2003) compared to constitutive ones, but they always need some plant damage to be activated; hence sometimes they can be economically inefficient. Nonetheless, the metabolic cost associated with induced defences is exacted only if pest attack occurs and can thus be less than that involved in constitutive defences (Simms and Fritz 1990). However, little is known about the ecological costs of induced defences that may include an increased susceptibility to untargeted herbivores (Cipollini et al. 2003).

The study of VOC that are attractive towards the natural enemies of insect pests has been one of the main research topics of agricultural entomology and biological control from the beginning of the 1990s (Vet and Dicke 1992). The theory predicted that for natural enemies of herbivore insects, the use of herbivore-induced VOC to locate their hosts/preys represents a winning strategy because they are both highly detectable and reliable (Vet and Dicke 1992). In any agricultural and forestry ecosystem the plant biomass is dominant; hence plant VOC are produced in large quantities that are easily detected by insect antennae. The release of such compounds in response to herbivore feeding activity makes them highly reliable for a natural enemy, given that there is a selective pressure on herbivore populations towards a strong reduction of the emission of individual (colony) odours (Vet and Dicke 1992).

So far, volatile compounds involved in these multitrophic interactions have been characterized in several herbaceous (e.g. Turlings et al. 1991; Birkett et al. 2003) and perennial systems (e.g. Scutareanu et al. 1997). This has led to the identification of insect elicitors (Mattiacci et al. 1995; Alborn et al. 1997), of metabolic pathways induced by plant damage (Walling 2000 and references therein; Schaller et al. 2005 and references therein), and most recently of the genes regulating the production/release of these semiochemicals (Schnee et al. 2006).

The time that the plant needs to activate the induced defences depends on the feeding habit of the invading herbivore. In this view, insects are usually divided into two main groups: chewers and suckers. Feeding larvae of Lepidoptera and Coleoptera are typical chewers, whose activity is always associated to a massive mechanical damage to plant tissues. Conversely, sap feeders like aphids and whiteflies, or cell-content feeders like thrips and spider mites, all belong to suckers, which cause low or null mechanical damage to infested tissues. As a consequence, evidence of plant response to chewers in terms of semiochemical production can be recorded in hours from the beginning of the attack (Turlings et al. 1998), whilst days are needed in the case of sap feeders (Guerrieri et al. 1999).

Interestingly, common patterns of plant responses to herbivore insects have been found regardless of the site of interaction. For example, maize root exudates released in response to the attack of a beetle (*Diabrotica virgifera*) selectively guide a parasitic nematode of this pest (*Heterorhabditis megidis*) to its host larvae (Rasmann et al. 2005). One of the compounds involved in this attractiveness was the terpene *E*-ß-caryophyllene that, in a different multitrophic system, is released by the aerial part of the plant in response to the attack/oviposition by a bug pest (*Nezara viridula*), and showed a similar attractive function towards the egg-parasitoid (*Trissolcus basalis*) of this pest (Colazza et al. 2004). More recently, this compound has been proved to regulate the flight behaviour of the aphid parasitoid *Aphidius ervi* towards tomato plants infested by aphids (Sasso et al. 2007).

Nonetheless, it has been demonstrated that plant responses to herbivores, regardless of the site of interaction, are usually systemic and thus their effects can also be recorded in the undamaged parts of the plant (Turlings and Tumlinson 1992; Rose et al. 1996; Guerrieri et al. 1999; Soler et al. 2007). These findings, along with the consideration that plants often suffer multiple attacks by different organisms, prompted a series of investigations where the plant was considered as a living connection between the two separated environments. Hence, the hypothesis was formulated that there could be a mutual influence between organisms living belowground and those living aboveground (Rillig 2004; Wardle et al. 2004; Bezemer et al. 2005).

Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) are among the most common microbial organisms in the soil, constituting endotrophic symbiotic associations with plant roots (Arbuscular Mycorrhizae, AM) reported for approximately 80% of vascular land plants. This symbiosis is considered a crucial factor for most terrestrial ecosystems and has a high potential of application to plant production and defence (Smith and Read 1997).

There are several advantages that the plant experiences from AM symbiosis, including phosphate and other nutrients supply (Marschner and Dell 1994; Harrison and van Buuren 1995, Joner et al. 2000; Hodge et al. 2001) especially in phosphorus-deficient soils, a better resistance to drought (Augè 2001) and a significant higher degree of bioprotection against various pathogens, including nematodes (Pinochet et al. 1996; Borowicz 2006 and references therein), fungi (Azcón-Aguilar and Barea 1997; Borowicz 2001; Fritz et al. 2006) and even insect pests (Guerrieri et al. 2004). A positive effect of AM fungi on soil structure has been indicated, making them a key component of sustainable agriculture (Johanson et al. 2004; van der Heijden et al. 2006). There is also evidence that defence responses induced by AM are systemic (see Liu et al. 2007 and references therein), thus indicating these symbioses as potential candidates for corroborating the hypothesis of mutual belowground-aboveground interactions.

Many parameters affect the final outcome of belowground AM symbiosis, one being the species-specificity of several plant-fungal associations (Kendrick 1992; Klironomos 2000). For example, by using a molecular approach it has been demonstrated that co-occurring grass species associate with a non-random set of AMF (Bever et al. 1996; Vandenkoornhuyse et al. 2003). Even on the same plant species, interesting differences in a number of physiological traits emerged by comparing different species/isolates of AMF (Hart and Reader 2002), leading to differences in the effects on plant physiology and growth (Klironomos 2003). Similarly, it has been recently demonstrated that the presence and the identity of AMF has a direct influence on the competitiveness between legume crops and weeds (Scheublin et al. 2007). On the other hand, the presence of plant growth promoting rhizobacteria (PGPR) seems to play a role in the outcome of plant-AM interaction (Filion et al. 1999; Gamalero et al. 2004 and references therein).

For all these reasons, it is not surprising that biological, genetic and chemical aspects of AM symbiosis have been thoroughly investigated (Franken and Requena 2001; Strack et al. 2003; Rillig 2004; Balestrini and Lanfranco 2006).

In this chapter we will focus on the mutual influence between aboveground insects and belowground AM fungi as mediated by the plant, indicating the outcome and the main parameters that regulate the top-down and bottom-up effects. We will also examine how modern techniques can be used to characterize these multilevel interactions as well as the signal-transduction pathways that are involved, indicating the possible cross-talk between them. Finally, we will discuss how the thorough characterization of these multilevel interactions among AM symbiosis, herbivore insects and their natural enemies, can be used in the postulation of novel and sustainable strategies to control insect pests.

5.2 The Effect of Aboveground Herbivory on AM Symbiosis

It is not always simple to separate clearly the top-down from the bottom-up effects during a contemporary presence of herbivore insects and AMF on the same plant, especially in long-term interactions. It is probably for this reason that so far, only two mycorrhizal systems have been deeply investigated to assess the influence of a herbivore insect on the development of the fungal symbiont (Gange et al. 2002; Wamberg et al. 2003), whilst several papers have been published in which aboveground damage was performed by vertebrate species or simulated by artificial clipping (reviewed by Ghering and Whitham 2002; Klironomos et al. 2004).

Following initial observations carried out in 1997, Gange and collaborators examined the influence of the leaf-chewing caterpillar *Arctia caja* (Lepidoptera) on root colonization of *Plantago lanceolata* by *Glomus intraradices* through both laboratory and field experiments (Gange and Bower 1997; Gange et al. 2002). In the laboratory, the feeding activity of the lepidopteran larvae nearly halved the levels of AM colonization, although this effect was reached after five events of defoliation. Similarly, in manipulative field experiments that included selective applications of insecticide and fungicide, a negative interaction between *A. caja* attack and *G. intraradices* colonization was recorded. The authors hypothesised that in this system the mycorrhizal symbiont suffered of the reduction of nutritive compounds following severe herbivory by insect chewers (Gange et al. 2002).

More recently, in a detailed study on pea plants, it was demonstrated that the effect of aboveground insect herbivory on root colonization by the same AMF species (*G. intraradices*) changes in relation to the physiological status of the plant (Wamberg et al. 2003). More precisely, the feeding activity by adult weevils of *Sitona lineatus* induced a marked increase of root colonization by *G. intraradices* during the nutrient acquisition phase, whilst the reverse was recorded during the reproductive phase of the plant (Wamberg et al. 2003). It was theorized that, during the vegetative phase (days 0–25), the plant compensates the loss of nutrients due to herbivory by investing on roots, i.e. by transferring more carbon belowground, that is exploited by AM symbiosis. During a later reproductive phase (from day 30 onwards), the plant transfers more resources to flowers and seeds; hence, there is a lack of carbon to be sent to roots and this leads to a progressive reduction of AM colonization (Wamberg et al. 2003).

A further piece of knowledge to the puzzle of top-down effects of aboveground herbivory on AM colonization has been recently added by Klironomos et al. (2004), although no living organism but artificial clipping was used to cause foliar damage to *Bromus* plants. In this study, it was demonstrated that the extent of the clipping effect was dependent on which fungal species was associated with the plant (Klironomos et al. 2004). The authors concluded that it is extremely important to know the composition of fungal inoculum because the response of individual AMF monocultures cannot be used to predict the response of multi-species AMF assemblages (van der Heijden et al. 1998a,b). In other words, the latter is neither a linear function of single AMF species responses nor a mirror of the most responsive AM fungal species. In this study, it was also demonstrated that there could be qualitative effects of aboveground stresses on AM development other than, or along with, quantitative ones. In this view, many other parameters (i.e. vesicular colonization, arbuscular colonization, extraradical hyphal length) must be measured in addition to the most commonly used “root length colonised” (percent AMF colonization × root length), because phenological changes in AMF do not necessarily occur in all mycorrhizal structures at the same time (Klironomos et al. 2004).

In a wider perspective, given the general negative influence of insect herbivory on the AM symbiosis, it cannot be excluded that in agricultural ecosystems at least a part of the "product losses" referred to insects are in fact caused by a reduction of AM symbiosis.

Finally, it must be noted that all the studies about the effect of aboveground herbivory on AM colonization have considered plant damage as caused exclusively by chewers, either insects or vertebrates (including artificial clipping). As a consequence, it remains completely unexplored whether feeding activity by aboveground insect suckers, e.g. aphids and whiteflies, has or has not consequences on the development/colonization by AMF.

5.3 The Effect of AM Symbiosis on Plant Direct Defences Against Herbivore Insects

Plant induced defences can turn into resistance against herbivores through either a compensating replacement of damaged tissues (tolerance) or a reduction of the herbivore's fitness (true resistance). In other words, the metabolic pathways involved in plant resistance belong to either primary or secondary metabolism of the plant.

The larger availability of soil nutrients, in particular of P, N and K, delivered to plant roots by AM fungi (Marschner and Dell 1994; Joner et al. 2000; Hodge et al. 2001), could be the ideal pre-requisite for an induced tolerance response by mycorrhizal plants towards herbivore insects, especially chewers (McNaughton and Chapin 1985).

So far, only two detailed studies, both involving plant chewers, have investigated this possibility with different results. Borowicz (1997) indicated that the presence of the AM fungus *Glomus etunicatum* did not change the tolerance level of soybean plants towards the Mexican bean beetle *Epilachna varivestis*. More recently, a different outcome of mycorrhizal influence on the tolerance of prairie plants towards grasshoppers was reported (Kula et al. 2005). In this study, root colonization of eight prairie plant species by a complex mix of AM fungi (including several species of *Glomus*) resulted in a compensatory regrowth after defoliation by the grasshopper *Melanoplus bivittatus* (Kula et al. 2005). In particular, the total aboveground plant biomass of mycorrhizal plants was nearly double the biomass production of non-mycorrhizal plants after they have been grazed by grasshoppers. This result was due principally to the response of two dominant C_4 grass species (Kula et al. 2005). It is not possible to compare the results of these two studies because they have been recorded in two completely different multitrophic systems. However, in the prairie experiments, the higher number of possible combinations between plant and mycorrhizal fungi species or a synergistic effect of "multiple partners" could have played a role in the final tolerance response towards the herbivore insect (van der Heijden 1998a,b).

In contrast, quite a few studies have investigated the effect of belowground AM symbiosis on plant true resistance towards aboveground herbivores, with results that pointed out the extreme complexity of these interactions. However, a general trend of negative impact of AM on chewer insect performances has been indicated.

In a pioneering paper, Rabin and Pacovsky (1985) reported a slower development and a lower weight at pupation in both *Spodoptera fungiperda* and *Helicoverpa zea* fed on excised leaves of soybean plants colonized by the AM fungus *Glomus fasciculatum*, in respect of larvae fed with leaves coming from P-fertilized plants. The authors then concluded that a true resistance response was induced by AM symbiosis in soybean plants (Rabin and Pacovsky 1985). Surprisingly, these results were not confirmed later on by Borowicz (1997), who investigated the effect of AM symbiosis on soybean resistance towards the Mexican beetle *Epilachna varivestis*. In fact, the presence of *G. etunicatum*, associated with low-P-fertilizer, resulted in higher larval mass and pupation rate of the herbivore (Borowicz 1997). In order to understand whether it is the species specificity of AM-plant interaction that plays a key role in the soybean response to chewers (see Sanders 2002 and references therein), it would be extremely interesting to cross test the development of *E. varivestis* on excised leaves of soybean plants colonised by *G. fasciculatum*, as well as the response of both *S. fungiperda* and *H. zea* when reared on soybean plants colonized by *G. etunicatum* and grown at low level of P-fertilizer. However, it must be noted that *Epilachna* feeds in a completely different way from *Spodoptera* (and *Helicoverpa*), and this can influence the plant response. The former usually scratches the leaf surface to feed on plant juices, more or less like thrips (Thripidae) whilst *Spodoptera* ingests plant tissues by using its powerful mandibles.

The same negative effect on the resistance of *Lotus corniculatus* towards a chewer insect, the common blue butterfly *Polymmatus icarus*, was reported by Goverde et al. (2000). However, a species-specific effect of AM fungus on herbivore fitness was demonstrated in this system (Goverde et al. 2000), as it was for the plant *Leucanthemum vulgare* and the leaf-miner *Chromatomyia syngensiae* (Diptera: Agromyzidae) in laboratory tests (Gange et al. 2003).

In detail, the percentage of plant leaves that were mined by the fly significantly varied with the AM fungal species considered, with the association *Glomus caledonium*+*G. fasciculatum* inducing the highest rate of attack and *G. caledonium*+*G. fasciculatum*+*G. mosseae* the lowest (Gange et al. 2003). Conversely, the theory of species specificity of plant-fungal associations did not apply to strawberry, where both root-feeding larvae and shoot-feeding adults of the black vine weevil (*Otiorrhyncus sulcatus*) were negatively affected by the presence of either *Glomus mosseae* or *G. fasciculatum* (Gange 2001). Similarly, the thistle gall fly was reported to reduce its performances, in terms of number of galls/plant and average weight of larvae, on mycorrhizal *Cirsium arvense* plants (Gange and Nice 1997).

An interesting link between the physiological state of the plant and the effect of AM plant on leaf-chewing insects has been reported for pea plants colonised by *Glomus intraradices* and attacked by adult weevils (*Sitona lineatus*) (Wamberg et al. 2003). During the vegetative phase (days 0–25) there was no difference in the range of leaf damage by chewing insects whilst a drastic reduction of *S. lineatus* attack was recorded during the reproductive phase (from day 30 onward). The authors hypothesised that the translocation of nutrients and carbon to the reproductive organs leads to a decrease of leaf quality, which in turn hampers herbivore attack (Wamberg et al. 2003).

Finally, field experiments with natural occurring populations of AM, shaped by means of application of fungicides, confirmed the trend of a negative effect of endomycorrhizal symbiosis on chewing insects, an outcome that resulted permanent on *Plantago lanceolata* attacked by *Arctia caja* (Lepidoptera) (Gange and West 1994) and transient on eucalyptus trees attacked by unidentified geometrid larvae (Lepidoptera) (Gange et al. 2005).

There are many possible explanations for these contrasting results, one being the repeatedly cited species specificity of different AM-plant associations effects. However, two more hypotheses have been formulated to justify either a positive or a negative influence of the mycorrhizal symbiosis on the performances of chewers, both based on the chemical alterations occurring in colonised plants. A detrimental effect on chewer development can be related to the production of toxic compounds induced by AM symbiosis, such as phenolics (Morandi 1996), terpenoids (Peipp et al. 1997) and isoflavonoids (Vierheilig et al. 1998). All these compounds are known to belong to the plant battery of defensive substances whose production is often associated to herbivore attack (Mullin et al. 1991; Dakora 1995). Conversely, the nutritional theory predicts that mycorrhizal plants are qualitatively (and often quantitatively) better that non-mycorrhizal ones, thus providing a better food for herbivores that results in better performances.

A contrasting scenario has been reported for the resistance response towards sap feeders induced in plant by AM symbiosis. This is probably due to the scarcity of studies, namely five, that have investigated this interaction (Pacovsky et al. 1985; Gange and West 1994; Gange et al. 1999; Guerrieri et al. 2004; Wurst et al. 2004).

Pacovsky et al. (1985) found no effect of colonization by *Glomus fasciculatum* on the reproduction of the aphid *Schizaphis graminum* developing on sorghum. In the field, with natural occurring populations of AM fungi, all the biological parameters considered (i.e. weight, embryo number and their development) of the generalist aphid species *Myzus persicae* on *Plantago lanceolata* were positively affected by the symbiosis (Gange and West 1994). These results were partially confirmed in a subsequent laboratory bioassay in which the same plant, two aphid species (*M. persicae* and *M. ascalonicus*) and a single symbiotic fungus (*Glomus intraradices*) were tested (Gange et al. 1999). In a more recent study on the same multitrophic system, the development time of *M. persicae* on *P. lanceolata* was delayed in the presence of the AM fungus *Glomus intraradices*, but was accelerated in the presence of both AM and earthworms (Wurst et al. 2004). Moreover, no effect of either AM or earthworms on aphid reproduction was recorded, and this was in clear contrast with the results of previous studies on the same system (Gange and West 1994; Gange et al. 1999). The sole difference between the two studies was the sterilization of the soil in the more recent one that could have mobilized more nutrients, thus attenuating the effects of either AM or earthworms on plant responses (Wurst et al. 2004).

In accordance with Wurst et al. 2004, Guerrieri et al. (2004) found that tomato plants colonised by the AM fungus *Glomus mosseae* became more resistant to the aphid *Macrosiphum euphorbiae* with respect to non-mycorrhizal plants, with only 16% of aphids reaching the adult stage and about 8% reproducing. One possible reason for this negative effect of mycorrhizae on aphid development is the synthesis of toxic compounds induced by AM in tomato plants that are known to be very rich in substances with defensive properties. However, another intriguing hypothesis can be formulated. It is known that in tomato, the aphid *M. euphorbiae* and the fungal pathogen *Phytophtora infestans* have similar effects on the expression of genes associated with plant defences, namely *P4* and *LOX* (Fidantsef et al. 1999) in accordance with modern theories that compare hemipteran herbivores (suborder Sternorrhyncha, including aphids, whiteflies, psyllids and scale insects) to plant pathogens (Kaloshian and Walling 2005). Also AM fungi do elicit a defensive response during the initial colonization, although this was noted to decline or to be subsequently downregulated as the symbiosis developed (Vierheilig 2004; Harrison 2005). However, this decline was not observed in the noncolonized regions of the root (Harrison and Dixon 1994).

5.4 The Effect of AM Symbiosis on Plant Indirect Defences Against Herbivore Insects

The possible interactions between AM symbiosis and the attraction of natural enemies of herbivore insects have been investigated by Guerrieri et al. (2004) (Fig. 5.2).

In the above-mentioned paper a multidisciplinary approach was followed by integrating the expertise of plant biologists, chemists and entomologists. In wind tunnel bioassays, the authors demonstrated that, in tomato plants, mycorrhizal symbiosis and aphid infestation produced similar results in terms of attractiveness towards an insect parasitoid, namely, the parasitic wasp *Aphidius ervi*, one of the most effective and studied natural enemies of the potato and tomato aphid (*Macrosiphum euphorbiae*)(Guerrieri et al. 1993, 1997, 1999, 2002). In detail, tomato plants colonised by the AM fungus *Glomus mosseae* became significantly more attractive towards *A. ervi* than control, non-mycorrhizal plants in complete absence of aphid infestation. Moreover, the percentage of female wasps landing on mycorrhizal plants was comparable to that recorded for tomato plants infested by *M. euphorbiae*. It was hypothesised that the possible basis of this similarity could be in the genes that are induced by both *M. euphorbiae* infestation and *Glomus mosseae* colonization (Guerrieri et al. 2004). A first step towards the characterization of tomato responses in terms of attractiveness towards parasitic wasps has recently led to the identification of the VOC released in response to aphid attack (Sasso et al. 2007). In accordance with other studies about tomato response to different herbivore species (Kant et al. 2004 and references therein), the differences

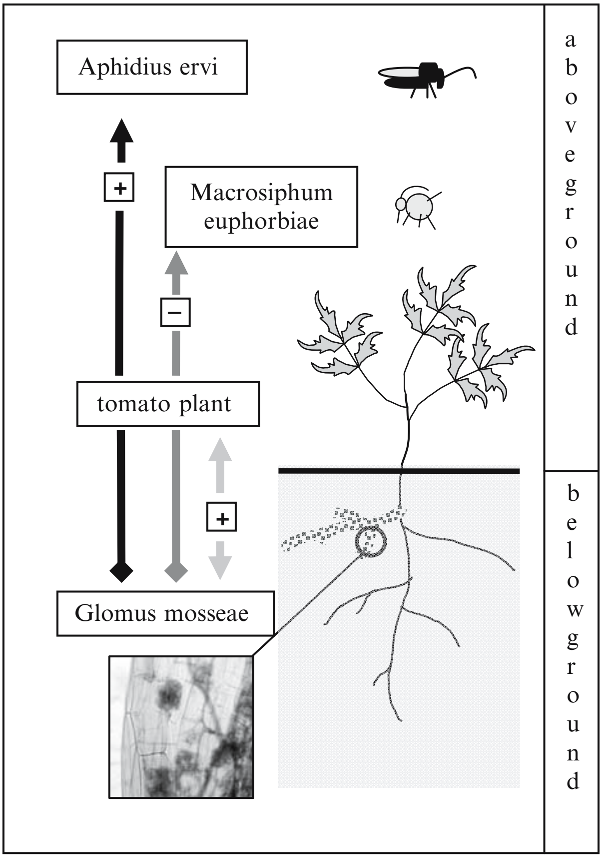

Fig. 5.2 Outcome of belowground-aboveground interactions in a multitrophic system. (+) indicates a positive interaction; (−) indicates a negative interaction (see text for explanation)

recorded between the emissions collected from uninfested and aphid-infested plants were only quantitative. Among the identified compounds whose release significantly increased following aphid attack, several terpenes, i.e. α-pinene, *E*-ß-ocimene and *E*-ß-caryophyllene, and methyl salicylate, were found to be involved in the long-range attractiveness of *A. ervi*. The identification of VOC released by tomato plants colonised by *G. mosseae* is currently in process and will help in understanding whether aphids and mycorrhizal fungi activate similar pathways of response (Guerrieri et al., in preparation).

The effect of mycorrhizal symbiosis on the final percentage of parasitism has been investigated in both field and laboratory tests by Gange et al. (2003). As reported for the attack rate by the leaf-miner *Chromatomyia syngensiae* (Diptera: Agromyzidae) on *Leucanthemum vulgare* (see above), in laboratory experiments the parasitism rate by *Digliphus isaea* (Hymenoptera: Eulophidae) was shown to be dependent on the AM fungal species involved (Gange et al. 2003). More precisely, mines on plants colonised with the single inoculum of *Glomus fasciculatum* suffered the highest rate of parasitism, whilst the inoculum of *G. mosseae* alone or together with *G. fasciculatum* caused a highly significant reduction in the parasitism rate (Gange et al. 2003). One possible explanation of these negative associations could have been the different plant response to diverse AMF species, in terms of growth and number of leaves that in turn could have affected the searching efficiency by an insect parasitoid (Cloyd and Sadof 2000). For example, the colonization by *G. mosseae* alone resulted in the highest number of leaves in *L. vulgare* plants, even though this was not associated to the lowest percentages of leaves mined, thus suggesting the existence of other mechanisms regulating these interactions (Gange et al. 2003).

5.5 Signal-Transduction Pathways Involved in Plant Response to AM and to Herbivore Insects

Induced responses to insects and pathogens rely on the circulation of signal molecules that alert the plant and eventually protect it from further attacks. Two sets of responses that regulate plant resistance have jasmonic acid (JA) and salicylic acid (SA) as key signal components (Fig. 5.3) (see Agrawal et al. 1999 and references

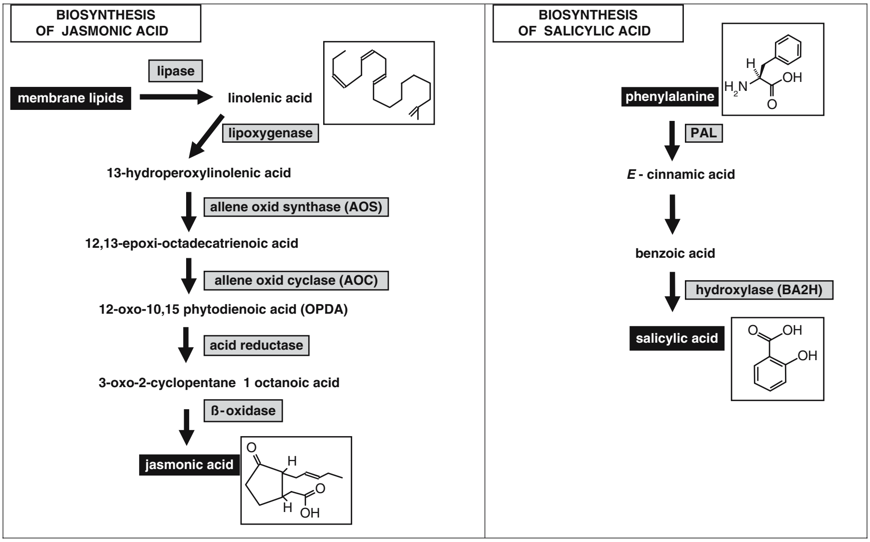

Fig. 5.3 Metabolic pathways of jasmonic acid and salicylic acid

therein). There is evidence that the JA pathway is mainly triggered by insects and heavy mechanical wounding of plant tissues whilst SA is switched on by pathogen infection.

Membrane disruption and liberation of lipids constitute the initial substrate of the octadecanoid pathway that starts with the production of linolenic and linoleic acids released from plastidial membranes by phospholypases and catabolized by enzymatic and nonenzymatic reactions to produce a set of oxygenated lipids (oxylipins), including JA and its methyl ester (MeJA) (Fig. 5.3) (see Schaller et al. 2005 and the entire special issue of J Plant Growth Regul 2005, volume 23, number 3). Circulation of JA induces the accumulation of proteinase inhibitors, polyphenol oxidase, and steroid glicoalkaloids, making the plant more resistant to further attacks by insects (Staswick and Lehman 1999). The activation of the octadecanoid pathway coordinates the contemporary release of VOC (terpenes, aldehydes and alcohols) that are known to be involved in the attractiveness of natural enemies of insect pests (Birkett et al. 2000; Thaler 2000).

The biosynthetic pathway of SA appears to begin with the conversion of the amino acid phenylalanine to *E*-cinnamic acid (*E*-CA) catalysed by phenylalanine ammonia lyase (PAL) (Fig. 5.3). The conversion of *E*-CA into SA proceeds via chain shortening to produce benzoic acid (BA), followed by hydroxylation to derive SA. The accumulation of SA is required for the induction of the systemic acquired resistance (SAR) which provides a protection against plant diseases (Ryals et al. 1996; Gozzo 2003 and references therein).

However, more recently it has been demonstrated that plant response towards invading organisms is far more complicated than this simplistic scenario. For example, insect suckers like aphids, whose intercellular stylets produce little or no mechanical damage, are perceived by the plant as if they were pathogens, which causes a concomitant activation of both SA and JA (Kaloshian and Walling 2005). In tomato it has been demonstrated that the attack of *Macrosiphum euphorbiae* or *Myzus persicae* induces the expression of both LOX that is correlated to JA pathway and pathogen related protein P4 that is linked to the activation of a SAR response following the accumulation of SA (Fidantsef et al. 1999).

The peculiarity of plant response to sap feeders can also be seen in the composition of volatiles released by plants attacked by aphids. In soybean and tomato, among the few compounds whose production is significantly higher in respect to that of uninfested plants, there are methyl salicylate, the volatile ester of SA, and several terpenes from the octadecanoid pathway (Zhu and Park 2005; Sasso et al. 2007). These findings confirm previous observations carried out on different systems, underlining the role of JA on direct and indirect defences against sap feeders (Birkett et al. 2000; Thaler 2000; Cooper and Goggin 2005) and have been recently reinforced by using tomato mutants (Corrado et al. 2007).

Recent evidence on the variegated role of jasmonates in plant responses have been reported (Peña-Cortés et al. 2005). In the model plant *Arabidopsis*, mutants defective in JA related processes showed susceptibility to normally non-pathogenic soil-borne oomycetes of the genus *Pythium*, increased susceptibility to *Fusarium oxysporum*, *Alternaria brassicola* and *Botrytis cynerea* and to the bacterial leaf pathogen *Erwinia carotovora*, as well as impairment of induced resistance against Cucumber mosaic virus (CMV) (Pozo et al. 2005). In accordance with these findings, several cases of increased resistance towards pathogens have been demonstrated in plants overexpressing JA related proteins (see Pozo et al. 2005 and references therein).

More interestingly, there is evidence that the recognition and the control of the intimate symbioses, such as AM and *Rhizobium*, involve the defence-related pathways. For example, it has been reported that treatments with JA significantly increased the percentage of AM infected root length and speeded up the process of colonization by *Glomus* inoculum in *Allium sativum* plants (Regvar et al. 1996). Moreover, the synergistic effect of AM+JA resulted in a greater shoot length and fresh weight in respect to either non-treated or non-inoculated plants (Regvar et al. 1996).

These findings were confirmed and reinforced later on by Hause et al. (2002). By following a molecular approach, these authors elegantly demonstrated that in barley roots the process of mycorrhization by *Glomus intraradices* is associated to a fivefold accumulation of endogenous jasmonates, as demonstrated by transcripts of AOS (see Fig. 5.3) and JIP23, a 23-kD protein that accumulates in barley leaves following JA treatment (Hause et al. 2002). The authors also demonstrated that transcripts and proteins of these two genes accumulate within arbuscule-containing cells, thus inferring a causal link to mycorrhization (Hause et al. 2002).

It must be noted that the bacterial phytotoxin *coratine* also elicits the expression of jasmonate-induced proteins in tomato (see Mithöfer et al. 2005 and references therein).

The increased attractiveness of mycorrhizal tomato towards the aphid parasitoid *Aphidius ervi* reported by Guerrieri et al. (2004) could represent a further demonstration of JA involvement in AM symbiosis. However, only the characterization of volatile compounds released by mycorrhizal tomato and their comparison with those released by aphid infested tomato could shed light on these intriguing interactions (Guerrieri et al., in preparation).

Far more complicated is the scenario in cases of multiple interactions such as those involving the contemporary presence of microbes (beneficial and/or pathogens) and insects. There have been a few studies investigating the possible cross-talk between JA and SA metabolic pathways in response to multiple "elicitation" on both model and agricultural plants (Kunkel and Brooks 2002; Thaler et al. 2002a,b; Glazebrook et al. 2003). Although it appears that the interaction between these two pathways is complex, there is evidence that in the majority of cases it results in a mutual antagonism (Gupta et al. 2000). For example, in tomato plants, SA and its related compound acetyl salicylic acid (ASA) have been shown not only to inhibit proteinase inhibitor synthesis induced by wounding and oligouronides (Doherty et al. 1988) and by linolenic acid (Peña-Cortés et al. 1993), but also to impair all the induced defensive mechanism based on JA and on the tomato hormone systemin (Doares et al. 1995). These SA effects have been reported to act at different sites of plant responses, either along the octadecanoid pathway (Fig. 5.3), thus stopping JA synthesis, or immediately after it, thus blocking the transcription of defensive genes (Doherty et al. 1988; Peña-Cortés et al. 1993; Doares et al. 1995). Similarly, it has been demonstrated that, in tobacco plants, JA inhibits the expression of SA-dependent genes encoding for pathogenesis-related (PR) proteins (Niki et al. 1998).

The same antagonism between SA and JA pathways has been further demonstrated in the model plant *Arabidopsis thaliana*. For example, *eds4* and *pad4* mutants, who are impaired in SA accumulation, exhibit enhanced responses to inducers of JA-dependent gene expression (Gupta et al. 2000). However, there are many parameters to be considered while assessing the final outcome of SA and JA interaction on plant defences against insect pests and pathogens. For example, concentration, timing of elicitation and life style of plant parasites all play a key role on tomato plant defensive performances (Thaler et al. 2002a,b). By using chemical elicitors, such as the SA functional analogue benzothiodiazole (BTH) and JA, these authors demonstrated that SA pathway had a stronger effect on JA pathway than did the reverse. Moreover, the negative interaction in the biochemical expression of the two pathways was most consistent in the case of simultaneous elicitation compared to when a two-day time lag passed between the applications of single elicitors. Interestingly, the application of BTH and JA at low concentration produced inconsistent antagonism (Thaler et al. 2002a).

Finally, it was demonstrated that the negative interaction between JA and SA pathways had biological consequences that varied among the herbivores and pathogens tested, thus making impossible to formulate a general theory that could be applicable in the control of plant parasites (Thaler et al. 2002b).

5.6 New Tools in the Study of Multitrophic Interactions

In this section we will indicate how the exploitation of techniques capable of identifying altered gene expression can point out genes possibly involved in plant defence. Actually, the exploitation of the emergent technologies made available for researchers of AM/plant interaction can easily accommodate one or more biotic levels, such as insect herbivores and their natural enemies.

An important instrument in the molecular approach to the study of plant/other organisms interactions is the crucifer *Arabidopsis thaliana*, whose genome has been elucidated. However, this classical model plant is not useful in the study of plant/AM fungi/insect interactions, because of its inability to host AM fungi, as the majority of Brassicaceae; therefore, the only model plant we can use with this purpose is *Medicago truncatula*, whose genome sequencing project is nearly completed and can be seen at the URL http://www.medicago.org. The roots of this legume can host, besides the nitrogen fixing rhizobial bacteria, additional symbiont microorganisms, including AM fungi. The presence of bacteria on the roots of legume species is certainly a further complication to be taken into account while studying belowground-aboveground interactions that could have beneficial effects on plant defence; indeed legume mutants resistant to AM fungus colonization (myc$^-$) have been obtained, for example, through chemical induction (Duc et al. 1989). A number of cases of mutant myc$^-$ plants species in legumes are reviewed by Peterson and Guinel (2000) and Rillig (2004).

Legumes, though, are not a typical mycorrhizae host, and non-legumes are a necessary instrument for the study of multilevel interactions. For this reason, plants resistant to AM fungus colonization (myc$^-$) have been obtained in other plant families. For example, several mutagenized myc$^-$ tomato plants are now available, preventing the establishment of AM symbiosis at different levels. In some cases, mutants are totally resistant to AMF infection and colonization and elicit a lower spore germination and appressoria formation in respect to wild type (myc$^+$) (David-Schwarz et al. 2001) and these effects can be displayed with different intensity (David-Schwarz et al. 2003).

Other myc$^-$ tomato plants stop the penetration of the root surface and symbiosis is associated with minimal accumulation of defence gene mRNAs, differently from myc$^+$ plants (Gao et al. 2004).

In the symbiosis between maize and *Glomus mosseae*, a series of mutants has been identified, lacking the ability to form appressoria, or forming appressoria of normal morphology and activity but in reduced number and unpaired in colonization (Paszkowski et al. 2006).

The use of myc$^-$ mutants could help in unravelling the intimate mechanisms regulating the plant resistance response towards herbivore insects that occurs at different stages of AM symbiosis. For example, it is still not clear whether it is the mechanical damage caused to plant tissues by the AM fungus penetration, or its growth into the roots or the formation of arbuscules and, when present, of vesicles, that triggers the cascade of events leading to plant resistance response, either direct and indirect.

In addition, experimental design can take advantage from the use of such mutants, allowing the cultivation in the same soil of mutant and control plant, as in the case of the same container (Neumann and George 2005) or also when upgrading from the pot to the field scale (Rillig 2004).

Tomato mutants have been collected over several decades and today more than a thousand monogenic stocks are described and deposited in the CM Rick Tomato Genetic Resource Center at University of California, Davis, and can be seen at the URL http://tgrc.ucdavis.edu/.These stocks are from several sources: spontaneous and induced mutants, natural variants from the edible tomato (*Lycopersicon esculentum*) and wild relatives (e.g. *L. hirsutum*). Tomato mutants have been particularly used to assess the metabolic pathways involved in plant response to biotic stresses (see above) and can equally be used to assess the genes involved in multilevel interactions (see Emmanuel and Levy 2002 and references therein).

All the advantages of the use of mutants apply equally to transgenic plants, in which either gene silencing or overexpression can help to dissect the outcomes of the plant response to the colonization of herbivore insects on the attacking organisms or on the attractiveness towards natural enemies.

Molecular biology techniques for the study of AM symbiosis are based on the establishment of cDNA libraries, deriving from mRNA extracted separately from fungal spores and from the roots of mychorrhizal plants and of non-mychorrhizal control plants. Several induced genes have been characterized by means of differential screening of cDNA libraries, differential RNA display or subtractive hybridization (Franken and Requena 2001). The use of DNA arrays is a powerful technique, allowing one to confront through hybridization the cDNAs from the three different organisms/symbionts and to analyse a large number of genes.

An interesting technique is based on the partial cloning of cDNAs, leading to the definition of expressed sequence tags (EST) that can be screened for similarity in DNA databases, in order to hypothesise a biological function. In the model plant *M. truncatula* EST analysis has been extensively applied, including root tissues colonized by AM fungi and other mutualistic or pathogenic microbes, and control roots. Similarly, Thompson and Goggin (2006) lately reviewed transcriptomics approaches to the study of the interactions between plant and phloem feeding insects (aphids, whiteflies, and planthoppers).

RNA probes constituted from 18 S rRNA deriving from plant and fungus can be used for Northern blot analysis; the use of 18 S rRNA probes in RNA protection assay (RPA) allows the discrimination and quantification of RNAs of fungal and plant origin (Maldonado-Mendoza et al. 2002).

The molecular approach in the study of multitrophic interactions even allows one to carry out *in silico* (i.e. computer-based) analyses and experiments (Strack et al. 2003). In the study of multilevel interactions, plant response to herbivore insects can be evaluated in presence and absence of AM fungi, in terms of overexpression or downregulation of genes associated with plant defence. Still another biotic level can be added to the system, as AM symbiosis positively affects the attraction of natural enemies of insect herbivores (often economic pests), through the altered profile of plant volatile emissions that constitute the necessary cues for parasitoid and predator insects to seek their hosts/prey (see the paragraph "The effect of AM symbiosis on plant indirect defences against herbivore insects"). Specific techniques are required for the study of the volatile compounds produced by the plant in presence or absence of AM fungi. In synthesis, plant odours are collected by air-tight systems (air entrainments) from the whole plant (head-space) or from a single leaf and directed towards a trap containing an adsorbent (e.g. Tenax). Volatile compounds are then released from the trap by either high temperature (thermal desorption) or solvents (chemical desorption) and analysed by a gas chromatograph usually coupled with a mass spectrometer (GC-MS). More recently, an innovative, solvent free technology has been made available, the solid phase microextraction (SPME). SPME utilises fiber coated with a liquid (polymer), a solid (sorbent), or a combination of both that removes the compounds from the sample by adsorption. The SPME fiber is then inserted directly into the gas chromatograph for desorption and analysis. Regardless of the type of collection and desorption, the peaks appearing in the chromatograms are identified by confrontation with available standards or databases (see, for example, Birkett et al. 2003). The ability to manipulate the searching behaviour of natural enemies is a most desirable prospect in modern plant protection (see below), and a better knowledge of the molecular basis for the changed plant odours would be very advantageous.

However, the power of the proteomic techniques depicted above might misleadingly suggest that, having once designed an experiment detecting AM and control plants, both infested and not, a search for the genes involved in plant defence is just a matter of technicalities. In fact, micro-array analysis, a very common gene profiling tool, requires the previous identification of a set of relevant transcripts (Thompson and Goggin 2006).

A possible start in the scrutiny of genes comes from the examination of the available literature. Balestrini and Lanfranco (2006) recently reviewed the genes transcriptionally induced or regulated in the different phases of the establishment of the symbiosis, from the fungal spores to the early stages of the interaction with root tissues, and to the symbiotic phase. AM symbiosis certainly has a huge impact on plant gene expression, and some of these might be appealing for plant protection applications; increased transcripts of chitinase have been reported in *M. truncatula* cells containing arbuscules, and a PR10 and a wound-induced protein are also reported (see Balestrini and Lanfranco 2006 and references therein). Those PR proteins, i.e. Pathogenesis Related proteins, are induced in the plant as a product of the metabolic Pathways activated in response to pest attack. The potential of chitinolytic enzymes in pest control is already established against pathogenic fungi, and more recently the possibility to damage the peritrophic matrix lining the insect midgut is being explored, as an adjuvant in conjunction with other toxins (Ding et al. 1998) or on its own (Gongora et al. 2001). Nonetheless, PR10 have been reported to be induced by the attack of herbivore mites and insects (Walling 2000).

5.7 Towards a Multilevel Approach of Pest Control in Agriculture

Sustainable control of insect pests in agriculture can be achieved by enhancing plant resistance and/or the activity of natural enemies, i.e. predators and parasitoids.

Plant resistance has been always considered the centre of the sustainable control of insect herbivores. For example, the use of a resistant rootstock (*Vitis labrusca* or other American native species), on which to graft a *Vitis vinifera* scion, as promoted by T.V. Munson at the beginning of the twentieth century, is still the most widespread technique to control the devastating infestations of phylloxera (*Daktulosphaira vitifoliae*, Aphidoidea). However, the concept of plant resistance has been recently widened to include those mechanisms which involve the activity of natural enemies, referred to as indirect defences (see above).

The study of direct resistance of plants towards insect is a very old discipline whilst the reverse is true for plant indirect resistance. These two mechanisms of plant resistance share some features. The first, and probably the most important, is that both are regulated by genes. As a consequence, we can select (or breed) to enhance both direct and indirect resistance in agricultural varieties although the characterization of genes related to plant attractiveness towards natural enemies is still in its infancy. To date, very few data are available about the "behaviour" of agricultural plant varieties towards either parasitoids and predators of insect pests (e.g. Lou et al. 2006 and references therein); hence it remains a crucial point to expand this kind of knowledge.

Moreover, as illustrated above, both these resistance mechanisms are frequently induced (see Fig. 5.1) and, in this optic, the study of multitrophic and multilevel interactions can be pivotal in unravelling the mechanisms of biological induction which can be used to increase the sustainable control of insect pests.

As for direct resistance, we have discussed above how there is often a species specificity of the effect of AM symbiont on a given herbivore species. For example, it has been reported that *Glomus mosseae* colonization dramatically reduced the fitness of the aphid *Macrosiphum euphorbiae* on tomato (Guerrieri et al. 2004) as did *Glomus intraradices* for insect chewers on soybean (Rabin and Pacovsky 1985). For all those associations whose final outcome is a reduction of herbivore fitness, a purified inoculum, of known composition, could be provided to plant roots in nurseries before transplanting them in the field, in order to induce a resistance response. In the case of perennial plants, the same "selection" of AM symbiont could be achieved by selective fungicide treatments and subsequent applications of purified inoculum.

Similarly, the higher attractiveness of mycorrhizal tomato plants towards the aphid parasitoid *A. ervi* opens new perspectives for the biological control of insect pests (Fig. 5.2). Following the same approach as reported for direct resistance, beneficial associations between AM fungal species and natural enemies could be artificially created to enhance the attractiveness of agricultural plants.

This point represents a bridge between the enhancement of plant resistance and natural enemies, given that among the classical biological control techniques there is the increase of natural enemies fitness (Van Driesche and Bellows 1996). However, a multidisciplinary approach to investigate plant-beneficial organisms interactions has only recently offered new tools to be used in the sustainable control of insect pests. For example, both jasmonic acid (JA) and salicylic acid (SA) are potent elicitors of plant responses, even though the former seems to be much involved not only in plant responses to insect herbivores (chewers and sap-feeders) but also in the identification and development of arbuscular endosymbiosis (Hause et al. 2002). There have already been field applications of JA (and derivates) that have determined a significant increase in the field presence of natural enemies of insect pests (James 2005). It could be possible to promote arbuscular endomycorrhizal symbiosis through radical or foliar application of this elicitor which in turn results in the production/release of semiochemicals attractive for natural enemies of insect herbivores.

On an a wider perspective, we can use these multilevel interactions as a further tool in the application of *push and pull* strategies that have been proved to be highly sustainable and exploitable in the Integrated Pest Management (IPM) practice (Cook et al. 2007). These strategies involve the behavioural manipulation of insects (pests and natural enemies) by using an integration of stimuli. By using chemical cues we can make the plant unattractive or unsuitable to the pests (push) while driving them towards an attractive source from which they are subsequently removed (pull). A similar concentration (pull) of natural enemies can be attained in those fields where their activity is most required by using a combination of chemical and visual stimuli (James 2005; Cook et al. 2007).

The positive bottom-up interaction between belowground AM symbiosis and aboveground natural enemies is not only played on the field of host/prey location cues but also on an enhanced availability of shelter and/or food. For example, it has been widely reported that AM plants are usually larger than non-mycorrhizal control ones and this leads to a larger availability of possible shelters for the natural enemies of insect pests. Similarly, it has been demonstrated that mycorrhizal symbiosis positively affect the flower number and size, along with the amount of pollen produced by plants (Gange and Smith 2005). This larger availability of food leads to an increase of the visitation rate by pollinating insects (Gange and Smith 2005), but also constitutes a precious source of carbohydrates and proteins available for parasitoids and predators of insect pests. For example, a 15-fold increase in the longevity of *Diadegma semiclausum*, a parasitoid of the diamond back moth *Plutella xylostella*, is determined by the presence of flowers (Wratten et al. 2003), while pollen has been indicated as a fundamental component in the diet of egg parasitoids (Zhang et al. 2004).

Finally, there is a wide literature about the ability of insect parasitoids to “learn” chemical compounds involved in host location and thus improving their searching ability at further encounters with their victims (see Meiners et al. 2003 and references therein). We can “enhance” the parasitoid response towards VOC induced by AM symbiosis in the laboratory before its release in the field, thus improving its fitness and in turn pest control.

5.8 Synthesis and Future Directions

It is well established that there is an incredible variety of interactions between soil and aerial organisms, with the plant mediating their final outcome. AM symbiosis is just one, though extremely complicated, of these interactions that could be exploited to enhance a sustainable control of insect pests and pathogens. However, in the perspective of practical application, many questions remain unanswered, although more than two decades have passed from the first pioneering paper by Rabin and Pacovsky (1985).

The first, and possibly the most urgent one is the assessment of the species-specificity effect induced by AM fungal symbiont on plant response, given that there are preferential associations in agricultural soils (Mathimaran et al. 2006). For example, it has been demonstrated that the parasitism rate by *Diglyphus isaea* is mycorrhizal-species dependent (Gange et al. 2003) but it is not possible to draw any conclusion about the influence of AM symbiosis on plant defences against either insect chewers or suckers, neither to have a clear view of whether insect specificity could be an important parameter to be considered. We know that, through AM symbiosis, more nutrients are transferred to the plant, especially in soils with a low content of P, but whether this goes towards an enhancement (e.g. tolerance) or a reduction (better performance of herbivore) of plant defences needs to be evaluated case by case.

Similarly, it has been recently reported that the production of essential oils in an aromatic plant is mycorrhizal species dependent (Copetta et al. 2006), and this could turn into a multiple defence response, given that many of these substances have both direct (Digilio et al. 2008) and indirect effect (Corrado et al. 2007; Sasso et al. 2007) on herbivore performances. Nonetheless, it needs to be tested whether plant cultivars respond in different ways to AM symbiosis, as it has been demonstrated for maize, in respect to the emission of induced VOC that are attractive towards the parasitoids of caterpillars (Gouinguené et al. 2001).

Overall, a standardization of methods is required because different results have been recorded on similar (if not identical) multitrophic systems. In fact, these discrepancies can be due to a transient effect of defensive responses induced by AM symbiosis, and this is probably another key point that needs to be demonstrated experimentally.

Regardless of the total number of papers published on the interactions between AM symbiosis and aboveground insects (both herbivores and their natural enemies), it must be noted that none of them includes a model plant. For example, many aspects of plant/insect and plant/AM interactions have been separately elucidated by using *Medicago truncatula,* a plant whose genome is close to being completely characterized (Harrison 2005; Leitner et al. 2005) but the contemporary presence of mycorrhizae and insects (herbivores and/or natural enemies) has never been investigated on this plant.

The role of bacteria associated with mycorrhizae (Gamalero et al. 2004) on plant defences against herbivores is still a completely unexplored field as is that of AM and nitrogen fixing bacteria that coexist in legume species (see Scheublin and van der Heijden 2006 and references therein). For example, a reduction of plant defence against a plant root fungal pathogen induced by a mycorrhiza helper bacterium has recently been reported (Lehr et al. 2007).

Still largely unknown and unexploited remain the mechanisms regulating plant-to-plant communication that has been demonstrated both belowground and aboveground (Bais et al. 2006). For example, root exudates of *Vicia faba* plants infested by the aphid *Acyrthosiphon pisum* elicit the release of host-induced VOC in neighbouring uninfested plants (Guerrieri et al. 2002). Although the specific compound involved in this interaction was not characterized, it remains a fact that a change in root exudates composition was determined by aboveground herbivory.

A similar induction of plant defence was demonstrated to happen at aerial level. In detail, host-induced VOC elicited the secretion of extrafloral nectar in neighbouring plants of Lima bean (*Phaseolus lunatus*), and (3Z)-hex-3-enylacetate was indicated as the compound seemingly most involved in this response (Kost and Heil 2006). Can we expect the same beneficial outcome of the plant-to-plant interactions in the event that the roots of at least one plant are colonized by AM symbiont?

From an ecological point of view, it would be essential to assess the long-term effect of these interactions, because there is a continuous and mutual influence of insect herbivory on AM colonization and vice versa (Ghering and Whitham 2002).

As outlined above, we can manipulate the temporal interaction between AM and herbivores by transplanting colonised plants in the field but how long will the plant defensive performance last? There is still an incredible lack of long-term field tests to assess whether the outcome of these interactions is stable or not.

All these questions lead to the big one: which are the genes involved in AM symbiosis that play a role in plant defence against aerial herbivores?

On the plant side, cDNA libraries have been established from mycorrhizal RNA using suppressive subtractive hybridization, and a large number of clones are being sequenced to obtain expressed sequence tags (EST). This constituted the base to characterize plant gene expression in response to AM symbiosis that now can be investigated with increasing precision by using modern approaches including proteomic, forward and reverse genetics and transgenic plants (see the paragraph "New tools in the study of multitrophic interactions"). Linking these observations to in vivo bioassay on plant defences against herbivore insects will be the fundamental step towards the understanding of the potentiality of these interactions. For example, it has been demonstrated that gene modulation induced by AM symbiosis happens not only in root tissues but also in the leaves and that the same plant response is recorded following a pathogenic infection (García-Rodriguez et al. 2005).

The thorough understanding of these fascinating interactions can only be achieved by following a multidisciplinary approach that involves plant physiologists, geneticists, microbiologists, chemists, entomologists and ecologists, each one participating with his own expertise and interacting with each other to formulate plausible theories. A typical flow of experiments and results between these different components is outlined in Fig. 5.4.

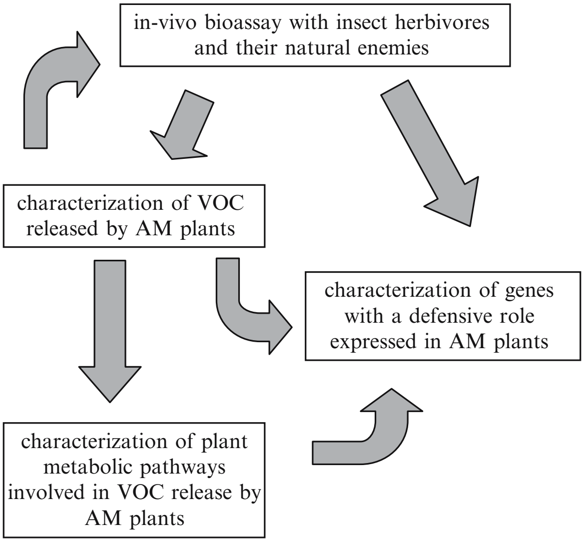

Fig. 5.4 Interdisciplinary approach to assess the role of AM symbiosis on plant defences against aboveground insect herbivores

The modern techniques available for plant breeding can be used to exploit the most updated findings in the field of beneficial interactions (see above). Apart from the controversial acceptance of GM plants by public and farmers of some countries (Herdt 2006), biotechnology can be used to mark the most promising genes for a sustainable control of insect pests to be transferred into commercial varieties even with conventional breeding programmes (Sharma et al. 2002). In this view, it is certainly "cheaper" to invest on single genes that are involved in multiple defence responses or on those whose expression, induced by beneficial microrganisms, results in a better resistance towards herbivores.

Only by unravelling the intimate mechanisms that regulate belowground symbiosis, plant resistance and biocontrol of insect pests, can we drive the plant responses and the behaviour of entomophagous insects towards a protection of agricultural crops that is environmentally friendly.

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