Tavole Rotonde sui maggiori problemi
riguardanti l'Entomologia Agraria in Italia
Sotto gli auspici del MIPAAF

XLII.
BIOLOGICAL PEST CONTROL: MANAGING MULTITROPHIC INTERACTIONS FOR SUSTAINABLE AGRICULTURE

Estratto da:
ATTI DELLA
ACCADEMIA NAZIONALE
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BIOLOGICAL PEST CONTROL: MANAGING MULTITROPHIC INTERACTIONS FOR SUSTAINABLE AGRICULTURE

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IMPACT OF CLIMATE CHANGE ON INVERTEBRATE BIOLOGICAL CONTROL

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Impact of climate change on invertebrate biological control

Climate change can have dramatic direct effects on the physiology, phenology and distribution of any living species but it may also indirectly affect them through the disruption of species interactions across trophic levels. The species with the highest specialization in terms of lifestyle or habitat are more vulnerable and due to the bottom-up amplification effect in trophic webs subjected to any kind of disturbance, natural enemies are expected to suffer the effects of CC to a greater extent than the herbivores they feed and develop on. The effects on biological control are discussed.

Key Words: natural enemies, parasitoid, predator, food web, Mediterranean basin

Climate change (CC) will most probably result in an increase in temperature extremes and aridity in the Mediterranean Basin (IPCC 2022), which is one of the most vulnerable regions to CC (Aguilar-Fenollosa and Jacas, 2014; Urbaneja-Bernat *et al.*, 2019) (Fig. 1).

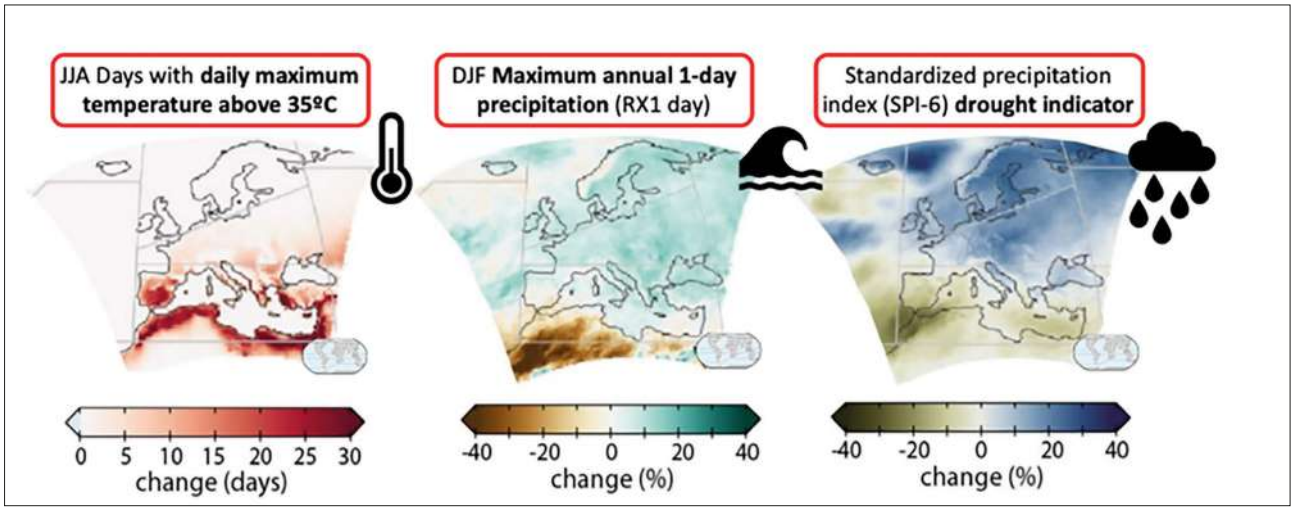

Fig. 1 - Projected changes for 2041-2060 relative to 1995-2014 according to IPPC (2022).

CC can have dramatic direct effects on the physiology, phenology and distribution of any living species but it may also indirectly affect them through the disruption of species interactions across trophic levels. This may eventually lead to a rearrangement of communities through asymmetric changes in competitive, 'bottom-up' and 'top-down' control effects. As a consequence, biological control may be impaired.

Foden *et al.* (2008) proposed a set of features that would make a species more susceptible to CC. These include species with (a) specialised habitat and/or microhabitat requirements, (b) narrow environmental tolerances or thresholds likely to be exceeded owing to CC, (c) dependence on specific environmental triggers or cues likely to be disrupted by CC, (d) dependence on interspecific interactions likely to be disrupted by CC and (e) poor ability or limited opportunity to disperse to, or colonise, a new or more suitable range. Therefore, those species with the highest specialisations in terms of lifestyle or habitat are typically more vulnerable. Because a bottom-up amplification effect often occurs in trophic webs subjected to any kind of disturbance, natural enemies are expected to suffer the effects of CC to a greater extent than their phytophagous hosts/preys, with indigenous generalist predators and

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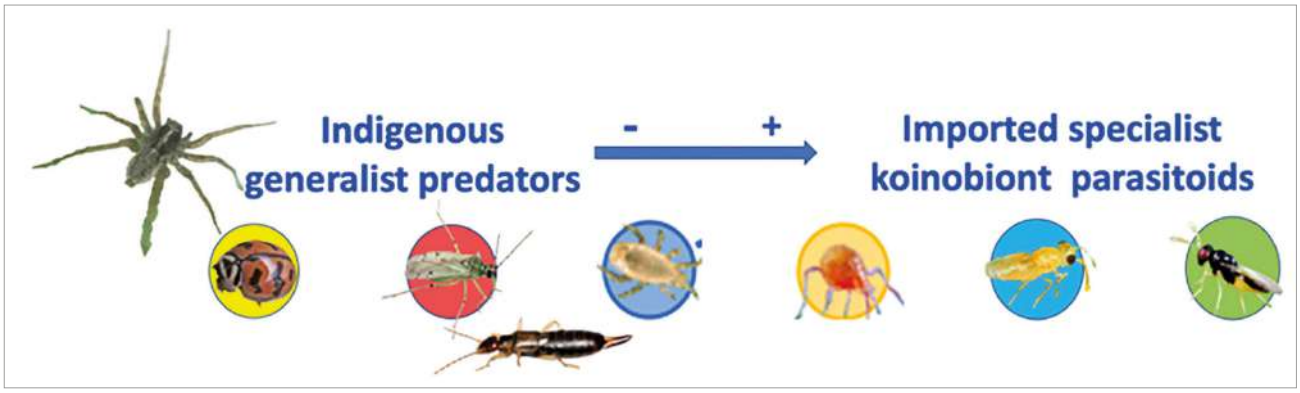

Fig. 2 - Increased vulnerability to CC of arthropod natural enemies according to their lifestyles.

imported specialist koinobiont parasitoids occupying extreme positions in a continuum of vulnerability to CC (Fig. 2).

Direct effects of CC on the physiology of plants include (a) increased sugar content, (b) reduced palatability/ nutritional quality as the C:N ratio increases, (c) higher content of secondary metabolites, and (d) changes in the activation of defense pathways, which overall may have opposite results on plant productivity. Those on the physiology of herbivores comprise (a) enhanced metabolism, (b) enhanced herbivory, (c) changes in chemical defenses accumulated from plants and (d) suicidal diapause, as photoperiod cues may no longer match thermal thresholds. Most of these physiological changes could also affect natural enemies and result in higher mortality from consuming toxic host or prey. The direct effects of CC on the phenology of any living being in temperate climates is an enlarged window for development, which in the case of insects would reduce generation time and increase voltinism, which could result in developmental traps due to asynchronous cycles with host plant/s for herbivores and with host/prey for natural enemies. All these direct changes may lead to the replacement of current crops/ cultivars no longer adapted to pre-CC environmental conditions. Such a replacement may lead to changes in the pest status of any herbivore, from non-economic to key pest and vice-versa and to losses of efficacy of biological control agents. Importantly, this may be coupled with an increased use of chemical control with all its side-effects.

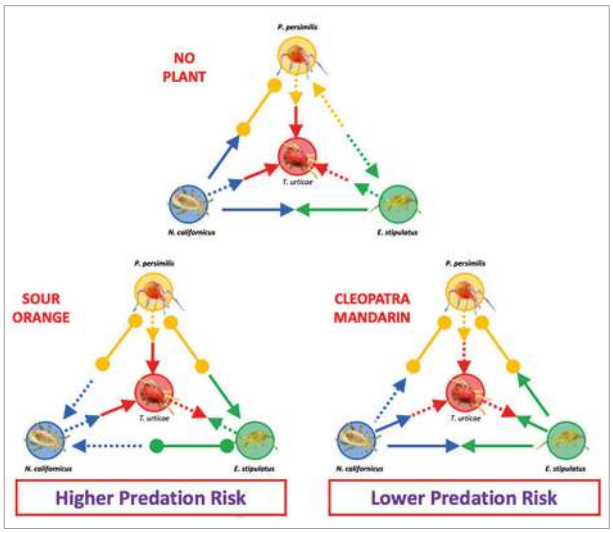

Fig. 3 - Interactions observed between the phytoseiid mites *Phytoseiulus persimilis*, *Neoseiulus californicus*, and *Euseius stipulatus*, and their shared prey, *Tetranychus urticae*. Arrowheads reflect behavioral responses of *P. persimilis* (orange), *N. californicus* (blue), *E. stipulatus* (green), and *T. urticae* (red) with no background odors and those corresponding to sour orange and Cleopatra mandarin. Arrowheads point at the preferred odor source. The results of these interactions include pursuit (gg), mutual attraction (gf), mutual avoidance (fg), avoidance (–g), and attraction (g). Continuous and dotted lines represent trends at $P \leq 0.05$ and $0.06 > P > 0.05$, respectively (figure from Cruz-Miralles et al., 2022)

In recent times, the replacement of the *Citrus Tristeza Virus* (CTV)-susceptible rootstock sour orange by CTV-tolerant ones prompted by the establishment of CTV in Spain, has been pointed out as the trigger for *Tetranychus urticae* Koch (Acari: Prostigmata) to change from a non-economic pest into a key-pest of mandarins (Brüssow *et al.*, 2010). As some CTV-tolerant rootstocks are also tolerant to osmotic stress, these rootstocks may become more common in a future arid Mediterranean. This could further increase the impact of *T. urticae* in the near future. Interestingly, this change of pest status should be attributed not only to a bottom-up effect through the host plant (Agut *et al.,* 2014, 2015, 2016) but also to a top-down effect through natural enemies (Cabedo-López *et al.*, 2019; Cruz-Miralles *et al.*, 2019, 2021a, 2021b, 2022). Indeed, these authors showed how citrus rootstocks affected attraction/repellence patterns of intraguild predators of *T. urticae* (phytoseiid mites), resulting in less frequent interference among phytoseiids in CTV-susceptible sour orange relative to CTV-tolerant Cleopatra mandarin. These effects combined with increased predation risk for *T. urticae* in sour orange relative to Cleopatra mandarin, could contribute to explaining the observed change in pest status when sour orange was massively replaced by CTV-tolerant rootstocks in Spain a few decades ago (Fig. 3). This example illustrates how indirect effects of CC through the disruption of species interactions may hamper biological control. In this case, disruption affected a system in which both the

Tab.1 - Types of biological control (BC) strategies based on the geographical origins of pest and natural enemy (NE) and the history of their association

Type of BC	Origin of		New environment?	Coevolution
	pest	NE		
Classical	Exotic	(same for both)	For both	Yes
New association, classical	Exotic	(different for both)	For both	No
Fortuitious	Exotic	Indigenous	For pest only	No
Neoclassical	Indigenous	Exotic	For NE only	No
Conservation, natural	Indigenous		No	Yes

pest and the natural enemies are indigenous and have coevolved. Coevolution should make this type of biological control more robust to the impact of CC. However, this is not always the case. According to the origin of the pest and the natural enemy, different types of BC can be defined (Table 1). The robustness of these systems depends on these origins and it may help predict how these associations may respond to CC. The potential of natural enemies to adapt to CC depend on their plasticity, which should be maximal for indigenous natural enemies (i.e., conservation biological control) but could be rather limited for imported natural enemies, either in the context of naturalized natural enemies established as a result of a classical biological control program, or when used in augmentative releases.

Adaptation to CC in the case of biological control methods exploiting exotic natural enemies should consider practices during collection, quarantine, rearing and releasing of these natural enemies aimed at maximizing the conservation of genetic diversity. Even in this case, though, these natural enemies may not be able to successfully control the pest and pest outbreaks could be increasingly frequent. This failure should make reliance on indigenous natural enemies and adoption of conservation biological control practices the cornerstone of future biological control, even of exotic pests. In this context, habitat manipulation, including the management of non-crop vegetation, could prove crucial to support diverse plant-herbivore-entomophagous food webs resulting in more resilient cropping systems. For this reason a thorough understanding of food web relationships is required to respond to the challenges posed by CC.

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RECENT ADVANCES IN CLASSICAL BIOLOGICAL CONTROL OF KEY HORTICULTURE PESTS: AFRICAN PERSPECTIVE

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Recent advances in classical biological control of key horticulture pests: African perspective

The spread and establishment of Alien invasive species (AIS) in new environments outside their native range are increasing worldwide at an unprecedented rate due to human activities and climate change. Once they arrive in new habitats, very often the population of AIS tend to multiply at exponential rate owing to several intertwined factors, key among them being the lack of efficient co-evolved natural enemies, thus posing a serious threat to fruit and vegetable production as well as biodiversity. This was the case with the oriental fruit fly, *Bactrocera dorsalis* and the tomato pinworm, *Phthorimaea* (=*Tuta*) *absoluta* in Africa which invaded the continent in 2003 and 2008, respectively. The invasion and spread of these two pests in Africa and beyond have led to massive economic losses due to direct damage to fruits and vegetables and as well as to quarantine restrictions of the African produce imposed by importing countries. To reunite these invaders with their respective natural enemies in Africa, classical biological program control against these pests were initiated. Two parasitiods *Fopius arisanus* and *Diachasmimorpha longicaudata* were imported from Hawaii for biological control of *B. dorsalis* in Africa, while *Dolichogenidea gelechiidivoris* was obtained from Peru targeting *T. absoluta*.

The pre-release evaluation of the introduced parasitoids against their respective target insect pest was undertaken under quarantine conditions and the interactions between these exotic parasitoids and the indigenous parasitoids and other biocontrol agents were assessed. Furthermore, suitable areas for the potential establishment of the three parasitoids were predicted under current and future climatic conditions using various modelling approaches to guide effective field releases of these natural enemies. Thereafter, parasitoids were released in the field to suppress the target pests. The performance of these parasitoids against their target host insect, the nature and multitude of the interaction with native parasitoid species as well as the suitable areas for their establishment under current and future scenario are discussed. The need for exploring the prospect of emerging invasive pest in the contestant is also highlighted.

Key Word: Invasive pest, classical biological control, climate change

Introduction

The African horticultural subsector continues to face myriad of biotic stressors hindering its transformation and the realization of its full potential in food security for the African populace. Key among these biotic stressors is insect pests that impede this subsector from realizing its full potential. In addition to the indigenous pests, alien invasive species have further compounded the production and economic constraints facing the horticultural sector (Mohamed *et al.,* 2008; Ekesi *et al.*, 2011; Gwokyalya *et al.*, 2022). The populations of the indigenous pests could be supressed by their co-evolved natural enemies to below economic threshold. However, this is not the case for the alien invasive pests, because these pests invade new territories and spread without their co-evolved natural enemies; hence, the uncontrolled populations result into serious damages to the horticultural crops (Mohamed *et al.*, 2016). Traditionally, the control of these pests involves indiscriminate and widespread application of synthetic chemicals. However, overreliance and excessive use of synthetic chemicals cause detrimental impact to One Health. Thus, Integrated Pest Management (IPM) strategies come in handy to offer eco-friendly and sustainable alternatives to manage the alien invasive pests. Of the numerous IPM strategies, classical biological control (CBC), which involves the introduction of co-evolved natural enemies of proven efficiency to the alien invasive pests into the invaded areas, have been employed in Africa (see Mohamed *et al.,* 2022; Mohamed *et al.*, 2016 and references therein). The International Centre for Insect Physiology and Ecology (*icipe*), as well as other research institutions have contributed immensely to the development and adoption of CBC of various horticultural pests such as *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) and *Phthorimaea absoluta* (Mey-

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rick) (Lepidoptera: Gelechiidae) among others. The outstanding achievements of using CBC in SSA's horticultural sector are presented.

Invasive fruit flies

Tephritids fruit fly pests are a major impediment to horticultural production in Africa. The problem is further compounded with invasion and wide spread of alien fruit flies. One of the most recent invaders which wreaked havoc in Africa, is the oriental fruit fly, *B. dorsalis* which was first recorded in Kenya in 2003(Lux *et al.*, 2003; Mohamed *et al*., 2008; Ekesi *et al*., 2011). Since then, this pest has spread to almost all the African countries causing enormous socioeconomic losses estimated at $2 billion annually (Ekesi *et al.*, 2011).

Another, species which is threating fruit production is the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). This pest so far has been recorded in Egypt, Sudan, Libya, Mauritius and La reunion (Eppo, 2010; Mahmoud *et al*., 2020; Zingore *et al*., 2020; Salah *et al*., 2012) where it is the dominant pest among horticultural crops. Considering the availability of host plants across the country borders, the fragile phytosanitary infrastructure and ill trained personnel the pest represents an imminent threat to the fruit industry across the continent (Ni *et al.*, 2012; Zingore *et al*., 2020)

Despite a recent report by Mahmoud *et al.,* (2020) that some native African parasitoids successfully parasitized *B. zonata*, the African continent has not been so resourceful in terms of parasitoid species potent enough for use as biological control agents of the invasive pests: earlier research reported zero adult parasitoid emergence of *P. cosyrae* and *Tetrastichus givardii* Silvestri (Hymenoptera: Eulophidae) reared on *B. dorsalis* despite the high host acceptability (Mohamed *et al.*, 2003, 2006; Ndlela *et al*., 2020; Gwokyalya *et al*., 2022). Consequently, efforts were channeled to importing co-evolved parasitoids of these pests from already established colonies outside Africa (Mohamed *et al*., 2008, 2010, 2016; Ndiaye *et al*., 2015).

Classical biological control of invasive fruit fly pests

Owing to the devastating effects of these pests across Africa, exacerbated by, among other factors, the lack of efficient resident natural enemies, co-evolved parasitoid of these pests were imported and released into the African ecosystem for CBC of these pests (reviewed in Mohamed *et al.*, 2016, 2022). These parasitoids include *Fopius arisanus* and *Diachasmimorpha longicaudata* targeting *B. dorsalis*. Prior to their release, these parasitoids were evaluated for their performance against the African population of *B. dorsalis* as well as other pestiferous flies native to Africa (Mohamed *et al*., 2008, 2010; Ndiaye *et al.*, 2015; Ndlela *et al*., 2020). *Fopius arisanus* and *D. longicaudata* readily parasitized *B. dorsalis* as well as other native *Ceratitis* species, however, adult emergence of both parasitoids was highly variable (Mohamed *et al.*, 2008, 2010; Ndlela *et al*., 2020; Gwokyalya *et al.*, 2022).

Investigation of the factors responsible for the distinct variations in parasitoid virulence has revealed that host intrinsic factors are a major driver of host-parasitoid interactions. Recently, Gwokyalya *et al.* (2022) demonstrated that differential host immune responses to parasitism is a key determinant of the outcome of host-parasitoid interactions. The authors further showed that parasitism by *D. longicaudata* led to lower total hemocyte and plasmatocyte counts and reduced cell viability and cell spreading indices compared to parasitism by *P. cosyrae*. These changes in cellular immunity led to low melanotic encapsulation and correspondingly higher adult parasitoid emergence in *D. longicaudata* parasitised hosts compared to those parasitised by *P. cosyrae* where no adult emerged was recorded. Further investigation of the molecular drivers of these immune phenotypes revealed that defense against the native parasitoid, *P. cosyrae* is mediated by the Toll and immune deficiency immune pathways whereas *D. longicaudata* interferes with host cytoskeleton reorganization and inhibits the Janus kinase-signal transducer and activator of transcription and c-Jun N-terminal kinase pathways thereby suppressing host immune defences (Gwokyalya *et al*. Unpublished)

On the other hand, host associated symbionts have been found to influence host-parasitoid interactions of *B. dorsalis*. *Lactococcus lactis*, a facultative symbiont of *B. dorsalis* increased parasitoid emergence whereas inoculation of *B. dorsalis* with *Providencia alcalifaciens* led to host protective phenotypes associated with improved fitness of the few successfully emerged adult parasitoids (Gwokyalya *et al*., 2023). These results shed light on the intricate mechanisms underpinning host-parasitoid interactions and present potential avenues for improving parasitoid efficacy, for example by using host-associated symbionts as probiotics to increase the number and fitness of parasitoids reared and released for CBC of invasive fruit fly pests. There is, however, dire need to investigate other aspects such as parasitoid associated-venoms, their evolutionary functions, and mechanism of action to better understand host-parasitoid evolutionary and functional ecology. This will guide pest-specific CBC programs for a more impactful outcome.

Using various modelling approaches, suitable areas for establishment of these parasitoids have been project for current and future climatic scenarios *(*Nanga Nanga *et al.,* 2021; Ndlela *et al.,* 2021*)*. Based on the outstanding performance of *F. arisanus and D. longicaudata* against the target pest, *B. dorsalis* and guidance by the outcome of these projections of suitable areas of establishment, these parasitoids have been released in several African countries with very promising outcomes (Mohamed *et al.*, 2016). For example, a recent study by Agboka *et al.* (2022a) which combined cellular automata and ecological niche models reported that *F. arisanus* has dispersed to cover an estimated area of 50.34 km^2 and 229.97 km^2 in Kenya and at the continental level, respec-

tively. In term of number of beneficiaries, the model estimated that 351,855 and 3,731,330 households in Kenya and across Africa, respectively, had benefited from *F. arisanus* releases.

Elsewhere in Africa, *Aganaspis daci* (Weld), *F. arisanus*, *Diachasmimorpha kraussii*, *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae) and *D. longicaudata* were imported to Egypt from Hawaii for the management of *B. zonata* (Mohamed *et al*., 2016). Of all these parasitoids, *A. daci* exhibited the highest adult emergence rate and was hence released in the field where post-release assessment revealed a 9.7% parasitism rate after one month. Additionally, *F. arisanus* was imported and released in La reunion and for CBC of *B. zonata* yielding high parasitism (>70%) (Rousse *et al.*, 2006).

Interaction of the introduced fruit fly parasitoids with other biocontrol agents

Once released in the field, the introduced parasitoid species are expected to interact with other natural enemies already in the system and those applied in the context of IPM. To understand the nature and the magnitude of these potential interactions, a couple of studies have been undertaken. For example, Karlsson *et al*. (2018) investigated the interaction between the introduced parasitoid *F. arisanus* and its congeneric, *Fopius caudatus* (Szépligeti) (Hymenoptera: Braconidae) and demonstrated that the fomer is the best performing parasitoid. Likewise, *Diachasmimorpha longicaudata* outcompeted the native parasitoid, *Pystallia cosyrae* in all exposure scenarios (Ndlela *et al*., 2020). Contrarily, Migani *et al*. (2017) showed that the presence of the predatory weaver ant reduces parasitism rates of *D. longicaudata* and *F. arisanus* on *B. dorsalis* by almost half, a phenomenon that could be attributed to the repellent effect of the semiochemicals produced by the weaver ant that reduce landing and patch residence time of the parasitoids (Appiah *et al.*, 2014). On the other hand, the combined use of the entomopathogenic fungus, *Metarhizium anisopliae* (ICIPE, 69, ICIPE 62, ICIPE 18) with either *F. arisanus* or *D. longicaudata* had no effect on the parasitoids (Mkiga *et al.* unpublished).

Classical Biological Control of the South American Tomato leafminer, *Phthorimaea absoluta* in Africa

Following the transatlantic invasion and widespread of *Phthorimaea absoluta* in Africa, the pest continues to cause huge economic losses in tomato farming. In an attempt to identify indigenous parasitoids that could be able to form new associations with this pest, field surveys were undertaken in several countries, during which several parasitoids (Idriss *et al*., 2018; Seydi *et al*., 2021; Mama Sambo *et al*., 2022a; Kinyanjui *et al.*, 2021) and predators (Sawadogo *et al*., 2022; Kinyanjui *et al.,* 2021) were reported to be associated with *P. absoluta*. Nevertheless, none of these natural enemies have been able reduce the population of the pest below the economic injury level. This necessitated the exploration for and introduction of the pest's natural enemy from its aboriginal home, Peru. In this regard, the Microgastrins parasitoid, *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae) was imported into Kenya for testing and subsequent field releases (Aigbedion-Atalor *et al.,* 2020). A host stage specificity test carried out under quarantine conditions at *icipe* showed that the parasitoid had a higher preference and better performance on the early larval instars of *P. absoluta* (Aigbedion-Atalor *et al*., 2022). A follow up study by Mama Sambo *et al*. (2022b) established that the performance of the parasitoid on its host follows a type II functional response. In another arena, for exploring the use of semiochmical for enhancement of parasitoid performance, Ayelo *et al.* (2021) demonstrated that *D. gelechiidivoris* was more attracted to volatiles of *P. absoluta*-infested compared to non-infested tomato plants, with the parasitoid preference increasing with increasing host density. The authors further illustrated that *D. gelechiidivoris* was attracted to larval frass volatiles and pheromones of *P. absoluta,* but not to larval odours.

Based on the outstanding laboratory performance of *D. gelechiidivoris*, *icipe* pioneered field releases of this parasitoid in Africa in 2020 (Shiraku, 2020); starting with East African countries i.e. Kenya, Uganda, and Ethiopia (Mohamed *et al.*, 2022). In a follow up survey in Kenya the parasitoid has been recovered at a 4.4 Km from the initial release (Mama Sambo *et al.*, unpublished data). Moreover, prediction of the ecological niche showed that the bigger part of Africa is suitable for field deployment of *D. gelechiidivoris* against *P. absoluta* under current and future scenarios (Aigbedion-Atalor *et al.*, 2022). A similar scenario was predicted using spatio-temporal dispersion modelling across Kenya tomato growing areas (Agboka *et al.*, 2022b).

Interaction of *D. gelechiidivoris* with other biocontrol agents

In an interaction study between *D. gelechiidivoris* and the indigenous ectoparasitoid *Stenomesius* sp near *japonicus, D. gelechiidivoris* laid more eggs and emerged more on first and second larval-stages than when the parasitoids was exposed to the host either simultaneously or sequentially (Mama Sambo *et al.*, 2022b). Also, Aigbedion-Atalor *et al.* (2021) reported that the performance of *D. gelechiidivoris* in terms of cocoon formation, adult emergence, and proportion of female progeny was not affected by the presence and density of *N. tenuis*. In the same study, the authors found that the combination of two *N. tenuis* and one female *D. gelechiidivoris* yielded up to 88% *P. absoluta* control.

In a separate study where the effect of *Metarhizium anisopliae* ICIPE 20 on *D. gelechiidivoris* adults was investigated, it was revealed that *M. anisopliae* negatively affects the longevity of the parasitoid. Although the infected adults parasitised up to 71% *P. absoluta* larvae in

comparison to 88% parasitism found when larvae were exposed to non-infected adults (Mama Sambo *et al.*, 2022a). In the same study, fungus-sprayed host plants and non-sprayed host plants were subjected to the parasitoid in both choice and non-choice assays and the highest parasitism rate was recorded on the non-sprayed host plants under choice scenario. However, the effect of the biocontrol agent seems to be synergistic whereby significantly higher *P. absoluta* mortality (80%) was observed on host plants sprayed with *M. anisopliae* ICIPE 20 and exposed to *D. gelechiidivoris* compared to when each biocontrol agent was applied alone.

In a field trial, the combination of these biocontrol methods (*M. anisopliae* ICIPE 20 and *D. gelechiidivoris*) yielded up to 70% marketable tomato fruits compared to *Tuta* pheromone TUA-Optima® impregnated with *M. anisopliae* ICIPE 20 dry conidia (52% marketable tomato fruits) and *D. gelechiidivoris* alone (41% marketable tomato fruits) (Mama Sambo *et al*., unpublished data).

Other invasive horticultural pests in Africa

In addition to *B. dorsalis* and *T. absoluta*, there are new and emerging alien invasive/and or invasive pests, which are expanding their geographical range in the continent that can be controlled through CBC. These pests include the spotted wing drosophila (*Drosophila suzukii* Matsumura (Diptera: Drosophilidae), Asian citrus psyllid (*Diaphorina citri* Kuwayama (Hemiptera: Liviidae), White mango scale *Aulacaspis tubercularis* (Hemiptera: Diaspididae); Japanese dodder, *Cuscuta japonica* (Solanales: Cuscutaceae), as well as the Potato Cyst Nematodes (PCN), *Globodera* spp. Also, the Papaya mealybug (*Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) and mango mealybug (*Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) are spreading to other part of the continent.

Concluding remarks

Although there is substantial progress made in terms of CBC in Africa, there are still constraints hindering the implementation of this approach. Few among them being lack of awareness among the stakeholders, inadequacy of financial resources for CBC as well as lack of enabling policies and unharmonized policy for import and releases of natural enemies across the continent. A concerted effort among the various stakeholder is need to overcome these challenges.

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CLASSICAL AND FORTUITOUS BIOLOGICAL CONTROL

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With increasing global trade and travel, the introduction of harmful exotic insects is an increasingly pressing threat to agricultural production. If in newly colonised areas they find a wide availability of host plants and favourable environmental conditions, and in the absence of natural enemies capable of containing them, the new pests become established and give rise to huge infestations with serious economic and ecological repercussions. In invaded areas, the indigenous natural enemy complex is hardly able to adapt to the new insects, with the exception of generalist predators and parasitoids. Even, in the absence of co-evolved and specialist natural enemies, some of these generalist predators and parasitoids can be mass reared and used for augmentative biological control. Examples are the releases of the predatory bugs *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) against the tomato borer *Phthorimaea absoluta* Meyrick (Yao *et al.*, 2022), the egg parastoid *Anastatus bifasciatus* (Geoffroy) against the brown marmorated stink bug *Halyomorpha halys* (Stål) (Iacovone *et al.*, 2022), and the pupal parasitoid *Trichopria drosophilae* (Perkins) against the spotted wing drosophila *Drosophila suzukii* (Matsumura) (Rossi Stacconi *et al.*, 2019).

However, the activity of generalist predators and parasitoids is often not effective enough for satisfactory control of the exotic insects, and the new successful associations between indigenous natural enemies and exotic pests are few. Worthy of note in this context is the case of the eulophids *Diglyphus isaea* (Walker) and *Necremnus tutae* Ribes & Bernardo, ectoparasitoids of leaf miner larvae. The latter species, of Palaearctic origin, was only described following the introduction of *Pht. absoluta*, as it has shifted from its primary hosts, which are not yet fully known, to the exotic host, thanks to the numerous studies aimed at its control carried out in invaded areas (Gebiola *et al.*, 2015). As a result, intensive and repeated use of broad-spectrum insecticides is resorted to for the control of the new pests, which not only nullifies the integrated pest management programmes widely adopted today, but also have heavy side effects on human health, environment, and biodiversity. Alternatively, classical biological control, through the deliberate introduction of a natural enemy co-evolved with the exotic pest, is a powerful and lasting containment tool that can restore the natural balance present in the native range. Classical biological control is therefore a central component in the long-term suppression of pest species.

Classical biological control dates in the late 19th century with the introduction of the vedalia beetle *Rodolia cardinalis* (Mulsant) to control the cottony cushion scale *Icerya purchasi* Maskall in California. Since then, classical biological control has targeted hundreds of invasive insects, leading to the long-term suppression of several species (Cock *et al.*, 2016; Heimpel & Cock, 2018). In Italy, after the introduction of *R. cardinalis* by Antonio Berlese in 1901, several other natural enemies were successfully released prior to Presidential Decree (DPR) No. 120 of March 12, 2003. For example, programmes conducted by researchers at the University of Turin include the introduction of the two egg parasitoids *Polynema striaticorne* Girault against the buffalo treehopper *Stictocephala bisonia* Kopp & Yonke in 1968, and *Anaphes nitens* (Girault) against the eucalyptus weevil *Gonipterus scutellatus* Gyllenhal in 1978 (Arzone, 1994), up to the more recent introduction of *Torymus sinensis* Kamijo against the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu in 2003 (Fig. 1). Native to China, *D. kuriphilus* was first reported in Italy in 2002 and has rapidly spread throughout Europe, causing severe damage to chestnut production and generally compromising plant health. Although some indigenous parasitoids are capable of attacking the exotic host, their activity has proved inadequate to contain infestations, as has chemical control, which is not always feasible and, in any case, not decisive. Therefore, based on the experience already gained in other invasion areas, *T. sinensis* was introduced, which effectively reduced *D. kuriphilus* populations, re-establishing the natural balance, as shown by data collected years after the biological control programme was initiated (Avtzis *et al.*, 2019; Ferracini *et al.*, 2019) (Fig. 2).

Sometimes natural enemies can follow their prey or host, and adventive populations of such exotic natural enemies can be found in areas of new colonisation of the pest, resulting in fortuitous biological control. Again, there is no shortage of examples in our territory, from the discovery of the tachinid fly *Trichopoda pennipes* (F.) against the green stink bug *Nezara viridula* (L.) in 1988 (Salerno *et al.*, 2002) to the discovery of the encyrtid wasp *Psyllaephagus bliteus* Riek against the redgum lerp psyllid *Glycaspis brimblecombei* Moore in

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Fig. 1 - Release of *Torymus sinensis* on galls of *Dryocosmus kuriphilus* (left), and a parasitoid female laying the egg into the cynipid gall (right).

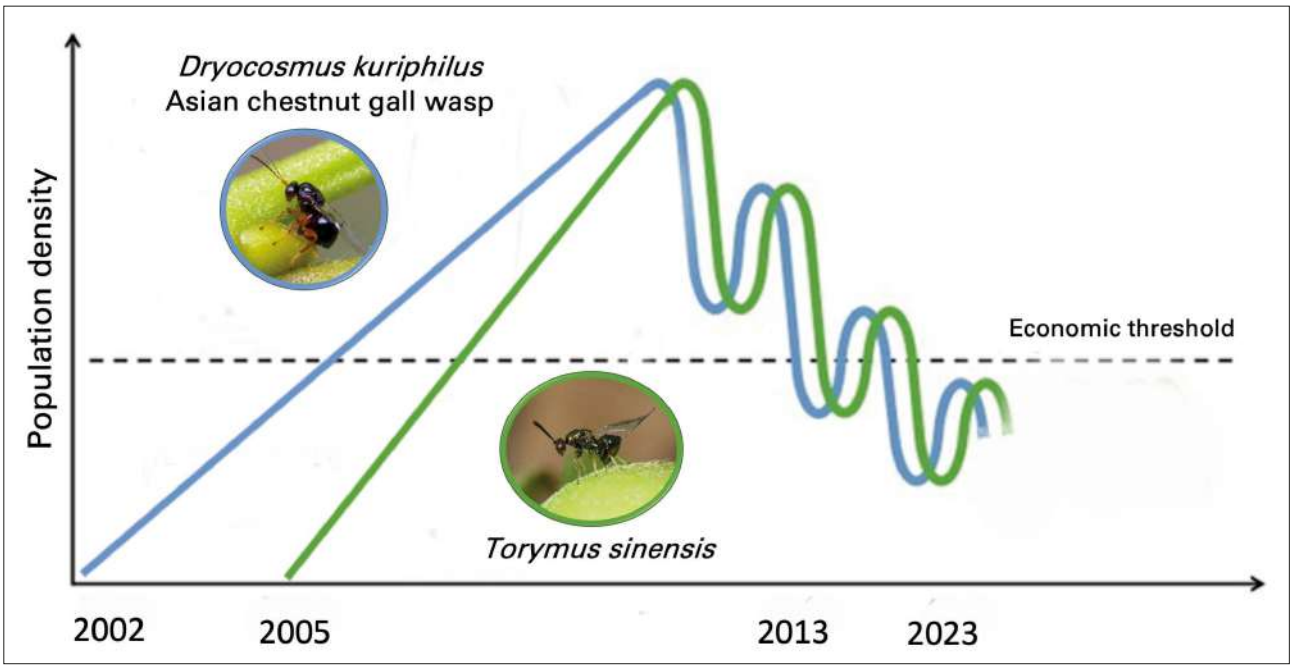

Fig. 2 - Population dynamics of the chestnut gall wasp *Dryocosmus kuriphilus* and its parasitoid *Torymus sinensis* following classical biological control programme in the invaded areas.

2011 (Caleca *et al.*, 2011). Paradigmatic is the case of two Neartic insect species infesting black locust trees, accidentally introduced into Europe, whose parasitoids from the same area were described for the first time in the newly colonised area, namely *Achrysocharoides robiniae* sp. nov. against the leaf miner *Phyllonorycter robiniellus* (Clemens) (Hansson & Shevtsova, 2010) and *Platygaster robiniae* sp. nov. against the gall midge *Obolodiplosis robiniae* (Haldeman) (Buhl & Duso, 2008). Of recent interest is the rapid spread in Europe of the scelionid wasps *Trissolcus japonicus* (Ashmead) (Fig. 3) and *T. mitsukurii* (Ashmead), effective egg parasitoids of *H. halys* in the native range, starting with the first reports in Switzerland in 2017 and Italy in 2018, respectively (Sabbatini Peverieri *et al.*, 2018; Stahl *et al.*, 2019; Moraglio *et al.*, 2020). Since then, findings of the two species have been rapidly increasing in several European countries (Fig. 4). Also, in the case of *D. suzukii*, during research aimed at identifying indigenous parasitoids capable of adapting to the exotic host, from which only generalist pupal parasitoids initially emerged (Mazzetto *et al.*, 2016), recently an exotic larval parasitoid, *Leptopilina japonica* Novković & Kimura, was obtained and is rapidly spreading in Italy (Puppato *et al.*, 2020).

Since the late 19th century, several classical biological control programmes have been implemented worldwide, and more than 6,000 introductions of more than 2,000 biological control agents have been carried out to

Fig. 3 - Adults of *Trissolcus japonicus*, *T. mitsukurii*, and *Acroclisoides sinicus* emerging from an egg mass of *Halyomorpha halys* (left), and *T. japonicus* female laying the egg into *H. halys* eggs (right).

contain insect pests, achieving a permanent reduction of over 170 noxious species (Cock *et al.*, 2016). Despite many successes, however, not all introductions have been equally successful, especially in the past. In some cases, natural enemies did not become established or became established but did not effectively contain the target pest, or even in a small percentage proved ecologically damaging, leading to significant side effects (van Lenteren *et al.*, 2006). Thus, classical biological control has produced important environmental benefits, but it has also introduced ecological risks that, over the past two decades, have prompted researchers to conduct thorough analyses and develop risk assessment protocols in an effort to make introductions ecologically safer (Barratt *et al.*, 2010; Heimpel & Cock, 2018). In many countries, regulatory requirements have become more restrictive, and approval for the release of an exotic predator or parasitoid is based on a thorough risk assessment, which provides information on its biology, ecology, and range of distribution, and estimates the likelihood and magnitude of adverse effects, such as reduction in biodiversity, reduction in non-target populations, interference with the

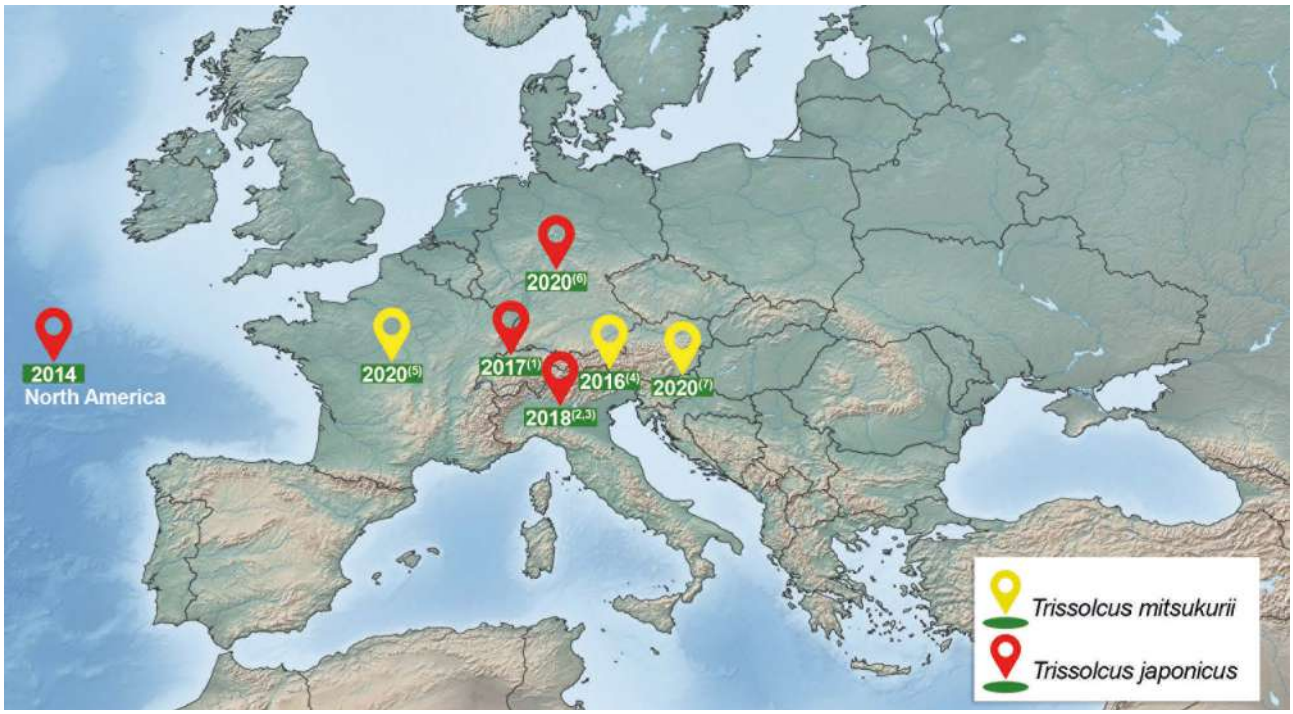

Fig. 4 - Record of adventive populations of *Trissolcus japonicus* and *T. mitsukurii* in Europe; (1) Stahl *et al.* (2019), (2) Sabbatini Peverieri *et al.* (2019), (3) Moraglio *et al.* (2020), (4) Scaccini *et al.* (2020), (5) Bout *et al.* (2021), (6) Dieckhoff *et al.* (2021), (7) Rot *et al.* (2021).

activity of indigenous natural enemies, that could occur as a result of release (Haye *et al.*, 2020).

In Italy, classical biological control has been suspended for more than 15 years. In fact, DPR No. 120 of March 12, 2003 banned any introduction of non-native species and populations, effectively preventing any biological control projects against exotic pests. As a result of the growing threat posed by some exotic pests, and in particular by *H. halys*, in recent years the situation has been unblocked, the current legislation has been amended (DPR No. 102 of July 5, 2019), bringing it moreover in line with what was already provided for in the relevant EU legislation, which covered the specific case of the introduction of allochthonous organisms for biological control. Moreover, the recent issuance of the Ministerial Decree of April 2, 2020 "Criteria for the reintroduction and repopulation of native species and for the introduction of non-native species and populations" allows an official application for the release of a biological control agent against an exotic pest.

Therefore, classical biological control has again become a viable option for the containment of exotic pest populations, obviously after thorough risk assessment studies to avoid adverse consequences. In Italy, national biological control projects are currently being implemented against *H. halys* using the egg parasitoid *T. japonicus* and against *D. suzukii* using the larval parasitoid *Ganaspis brasiliensis* (Ihering), initiated after extensive risk assessment studies on non-target organisms (Haye *et al.*, 2020; Biondi *et al.*, 2021; Sabbatini Peverieri *et al.*, 2021). Further investigations should be conducted in the coming years to verify the impact of the introduction of these biological control agents, comparing it with the impact of repeated broad-spectrum insecticide treatments applied against invasive exotic pests. In conclusion, because the benefit-risk analysis of classical biological control has shown that there were very few negative cases, and because, due to increased awareness, the safety of biological control releases has been greatly improved, based on past experience, a new era should be opened in which the benefits and risks are clearly and explicitly balanced to maximize the benefits and minimize the risks (Heimpel & Cock, 2018).

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Atti Accademia Nazionale
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LANDSCAPE-SCALE MANAGEMENT OF NATURAL ENEMIES OF AGRICULTURAL PESTS

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Landscape-scale management of natural enemies of agricultural pests

Pest natural enemies are key functional components of managed and natural ecosystems. In the last decades, managing insects, landscapes, and their interactions, to ensure the sustainability of ecosystem services and minimize the induction of disservices has been the focus of applied entomological research. However, this task is particularly challenging, as arthropods are often mobile organisms that depend on the availability of multiple resources occurring across multiple habitats. Their ability to locate these resources depends on their mobility and on the landscape composition, i.e. the relative proportion of habitat types, and configuration, i.e. the spatial arrangement of these habitats. As applied entomologists, to fully understand the processes that govern populations and communities of natural enemies across heterogeneous landscapes, we need to embrace this spatial complexity. As pest managers, we need to know how crop and green infrastructures influence pest population dynamics and their interactions with natural enemies. Starting from the pioneering work on fragmentation and pests, landscape ecology has made significant inroads towards understanding natural enemies' responses to land-use. Here, I will present a novel empirical approach to study species-habitat relationships using network ecology tools. From a policy perspective, a deeper understanding on how habitat-species networks work can open the door to effective landscape management for both beneficial insects and pests.

Key Words: Agro-ecology, Conservation biocontrol, Landscape ecology, Network ecology, Pests

Natural enemies and pests across agricultural landscapes

Understanding how natural enemies use resources across agricultural landscapes is essential for the design of effective management strategies to support biocontrol. Historically, patch-matrix models rooted in meta-population models (Levins, 1969) have largely focused on population responses to the amount and configuration of remnant suitable habitats within a hostile matrix (Tscharntke *et al.*, 2012). Central tenets of these models are that species dispersal occurs mainly between patches and that the focal community mostly relies on resources occurring within the patches. As it is becoming increasingly clear that many natural enemy and pest species utilize a range of different habitats and resources (Schellhorn *et al.,* 2014), landscape ecology has moved beyond the dichotomy of hostile vs. suitable patches to

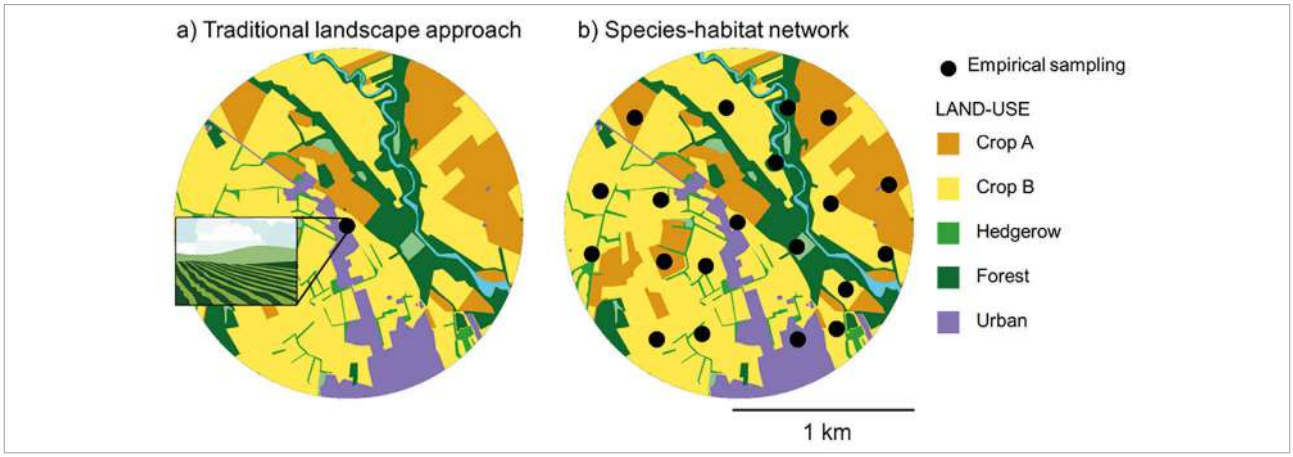

Fig. 1 - a) Traditional landscape approach where pests and natural enemies are sampled only in a focal field, b) Species-habitat network approach where multiple habitats are sampled across the same landscape.

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explicitly incorporate the heterogeneity typical of agricultural landscapes (Fahrig *et al*., 2011). The field of landscape ecology applied to biocontrol has made significant inroads in understanding natural enemies' responses to landscape processes (Tscharntke *et al.,* 2005; Tamburini *et al.,* 2016). In these studies, the species of interest is usually sampled in one focal crop and then related to the surrounding landscape by using the proportion of suitable or unsuitable habitats (Fig. 1a). One downside of this approach, however, is the lack of a mechanistic understanding of the links between multiple habitats and community-level processes, indicating the need for broader conceptual frameworks of spatial patterns in studying conservation biocontrol (Lami *et al.,* 2021).

Beyond the focal habitat: the species-habitat network

Recently, we proposed to consider the whole landscape as a unit to quantify and analyse natural enemy community response to landscape processes and then to use bipartite networks to analyse the resulting empirical data (Marini *et al.,* 2019; Lami *et al.,* 2021). In bipartite networks, two types of nodes exist, and interactions are analysed only between nodes of different types. In particular, we adapted existing bipartite networks to create species-habitat networks where the two types of nodes are the habitat types and the species occurring in each habitat. The links between species and habitats are represented by the number of individuals occurring in a certain habitat. The focal species community would usually belong to the same trophic level sharing a similar functional role such as ground-dwelling predators, parasitoids, insectivorous mammals etc..

Several studies have shown that individual species and community responses to landscape processes depend on the spatial scale over which the landscape metrics are quantified (Dainese *et al*., 2015). For ground-dwelling predators such as spiders and carabids the scale can range from a few hundred meters to one kilometre, while for parasitoids the scale can go up to a few kilometres. This problem is similar to the selection of buffer radii when adopting a traditional approach to quantify landscape composition or configuration (Steffan-Dewenter *et al.*, 2002). Once the spatial extent is defined, the species need to be sampled across the landscape using an 'habitat-centric' approach where the number of sampled sites within one habitat is proportional to its area. In agricultural landscapes, different habitats are often organized in patches including crop fields and semi-natural habitats such as hedgerows or forests. The focal species community may be used to guide the identification of habitat types that are functionally relevant (Fahrig *et al.*, 2011). Finally, understanding how and why the topology of the networks changes over time, and how these changes affect species resource use across the landscape can help to predict the consequences of human impacts upon natural enemies' dynamics (Schellhorn *et al.,* 2015).

To make the concept clear, I will provide a working example of species-habitat network, considering the tachinid species occurring across an agricultural landscape (size: 1 km^2). Any heterogeneous landscape and the species using its resources can be visualized as a bipartite network. In this example, we sampled the tachinid species occurring at 15 sites belonging to five habitats (hedgerow, soybean field, oilseed rape field, urban, wheat field) within a landscape mosaic (Fig. 2a). The nodes in the network are represented by the tachinid species and the sampling sites. The numbers indicate the link strength (number of individuals in each site) (Fig. 2b). The number of sampling sites is selected to be representative of the resources and habitat diversity. In the example, the tachinid-habitat network is built using the cumulative abundance from three rounds of sampling (spring, early summer and summer) using a transect walk method. In a transect walk, tachinids are recorded in a fixed width band (typically 5 m wide) within each site. Particular attention should be paid to the functional interpretation of the links. If we consider one crop field in this network, a tachinid species is recorded in that site because individuals can use multiple resources (e.g. host for reproduction, nectar for adult feeding or plants for roosting or shelter) or simply

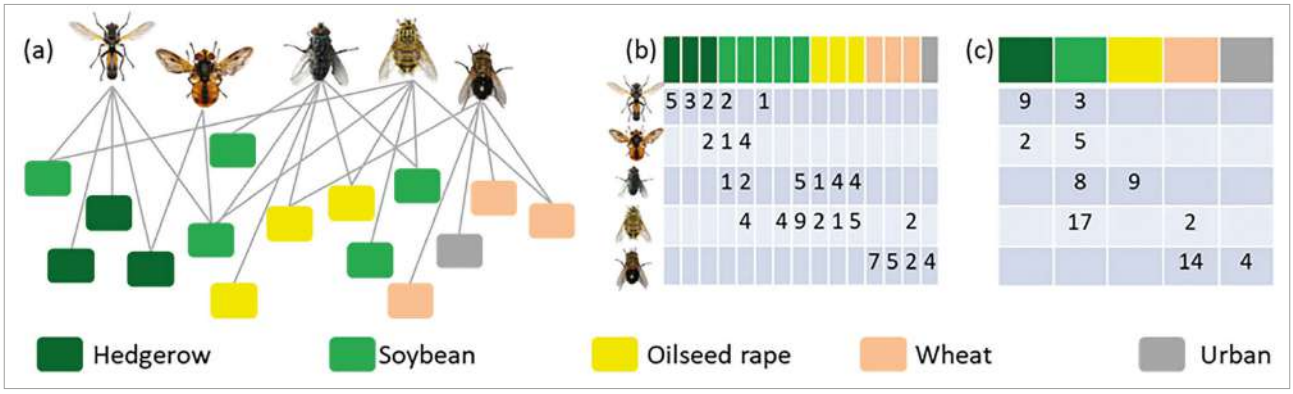

Fig. 2 - a) Example of a species-habitat network composed of 15 sites of varying size and quality belonging to five habitats in which a tachinid community is sampled, b) data matrix that can be derived from the sampling, describing the number of individuals recorded for each species in all sites; c) data matrix that can be derived from the sampling, describing the pooled the number of individuals recorded for each species per habitat category.

because individuals are using that site as a stepping stone for dispersal. Hence, the choice of the sampling method will dictate the interpretation of the ecological data.

Analytical tools to describe species-habitat networks

The appeal of network metrics is that they enable very powerful visualizations via a common language that defines the processes under investigation in terms of nodes and links. The existing metrics used to study properties of bipartite networks in both ecology and social sciences can easily be adapted to the study of species-habitat networks. These metrics can be broadly divided in two groups: properties of the whole network and node-level metrics that measure the role of single habitat sites or species in the landscapes. On the one hand, metrics at the network level synthesises information over the entire set of nodes and links. For example, the number of nodes (*e.g*. the species richness), the density of connections or the overall specialization are statistics used to describe the overall complexity of a network with a single number. On the other hand, metrics at the individual node level quantify differences in relative importance of either site or species. We can be interested in an individual or species that transmits disease, or identify species whose removal will result in secondary extinctions, or key habitat patches that connect other habitats (Lau *et al*., 2017). As metric choice will depend on the question, we advocate a hypothesis-driven approach whereby users decide *a priori* which metrics will address which research question. Extensive reviews of these metrics can be found in several studies (*e.g*. Dormann *et al.,* 2009).

Implications for biocontrol enhancement

Land-use change is massively reshaping agricultural landscapes worldwide, and is recognized as a key driver of biocontrol deterioration (Laliberté and Tylianakis 2010; Dainese *et al.,* 2019). Considering the upcoming EU targets to reduce by 50% the use and the risk of chemical pesticides by 2030 (Schebesta and Candel 2020), an urgent question is to understand how to manage whole landscapes to maximize functional biodiversity and the associated biocontrol services. One of the key challenges in conservation biocontrol is to identify landscape-wide interventions to support natural enemies and simultaneously to reduce pest pressures on crops (Schellhorn *et al.,* 2014). These interventions often require the introduction of novel habitats (e.g. hedgerows, flower strips) or changes in local field management (e.g. introducing rotation, conservation agriculture or organic farming etc.) (Tamburini *et al.,* 2016). One of the central assumption of many interventions to boost biocontrol is that many pest control agents are enhanced by the proximity to semi-natural areas (Schellhorn *et al.,* 2015) and that the spill-over of beneficial organisms from these habitats to agricultural fields is often fundamental for pest control enhancement (Blitzer *et al.*, 2012). However, we do not know yet how frequent is the movement of natural enemies from semi-natural habitats to crops and *vice versa*. Recently, we have tried to answer this important question by applying the species-habitat network approach to spiders and carabids inhabiting intensive agricultural landscapes in North Italy. In a first study, Nardi *et al.* (2019) showed that ground-dwelling spiders possess a high habitat specialization that constrains the ability to move between crop and non-crop habitats. In particular, encouraging the spill-over of spiders from semi-natural habitats to crops to enhance biological control might work for permanent crops, while for annual crops it would be more effective to improve local field quality for spiders that are crop specialists or to introduce open semi-natural habitats such as meadows or diverse grass margins. In a second study, Lami *et al.* (2021) showed that several carabid species are super-generalist and become even more generalist when landscapes are very simplified suggesting high chances of spill-over between different habitats. Another interesting potential application of this framework is to study pests' occurrence across different habitat types at the landscape scale. For instance, Cappellari *et al.* (2022) applied the species-habitat framework to elucidate which habitats are suitable for the vectors of *Xylella fastidiosa*, and how the vectors could potentially spread from non-crop habitats to olive groves. With this study, it was possible to identify key habitat patches that support vector populations, increasing the risks for pathogen transmission to olive trees. Another emerging topic in conservation biocontrol is whether increasing crop field size consistently exacerbate insect pest problems by disrupting trophic interactions between pests and natural enemies (Marini *et al.,* 2023). While the traditional landscape approach has failed to answer this very important question, species-habitat networks can help elucidating the spill-over dynamics and to help identifying optima landscape configuration to support an effective biocontrol service.

In conclusion, one key advantage of the application of the species-habitat network approach to conservation biocontrol is that the scale of the derived ecological information will match the scale of management interventions. Pest managers need to know how the different configurations of crop and green infrastructures influences pest population dynamics and their interactions with natural enemies. Only a deeper understanding of how natural enemies and pests use resources and move across heterogeneous landscapes can open the door to effectively manage the landscape to reduce pest pressures using natural biocontrol.

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MANIPULATING BELOWGROUND-ABOVEGROUND INTERACTIONS TO ENHANCE PLANT DEFENCE

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Lettura tenuta durante la Tavola Rotonda "Biological pest control: managing multitrophic interactions for sustainable agriculture". Seduta pubblica dell'Accademia – Firenze, 18 giugno 2022.

Manipulating belowground-aboveground interactions to enhance plant defence

In the last decade, a growing evidence built up on the role that belowground symbionts play in enhancing plant defence against aboveground insect pests. Arbuscular Mycorrhizal Fungi and *Trichoderma* species/strains have been deeply studied for their effect on plant direct and indirect defences. All these studies have demonstrated the species-specificity of these complex interactions particularly in respect to the symbiont strain and the feeding habit of targeted pest. Consequently, more data are needed to predict the output on plant defence against specific pests as elicited by specific root symbionts. The exploitation of belowground-aboveground interaction perfectly combines with other eco-friendly strategies of plant protection, including the use of plant-derived compounds.

Key Words: Arbuscular Mycorrhyzal Fungi, Trichoderma, Aphids, Parasitoids, direct and indirect defence

Introduction

Plant are sessile organisms that cannot move to escape biotic (and abiotic) stresses. However, far from being passing receivers, they have evolved sophisticated mechanisms of defence that can be roughly divided into constitutive and induced.

Constitutive defences are always active and represent the first barrier against biotic and abiotic stresses. They depend on intrinsic factors, including plant age and plant phenology, and extrinsic ones as constituted by the complex environmental conditions including temperature, humidity, light and by soil features (belowground) (Fig. 1).

Constitutive defences have a high metabolic cost and to save it plants evolved the so-called induced defences that mount only following a triggering stress. Herbivore-induced defences are among the most studied examples of induced defences. They include a complex system of metabolic responses that have proved to be specific in relation to the feeding habit of the invader. Simplifying, while insect chewers elicit the activation of the jasmonic acid pathway, piercing-sucking insects (and plant pathogens) trigger the activation of the salicylic acid one. In both cases, the metabolic cascade switches on specific defensive genes and the final release of specific blends of Volatile Organic Compounds (VOC).

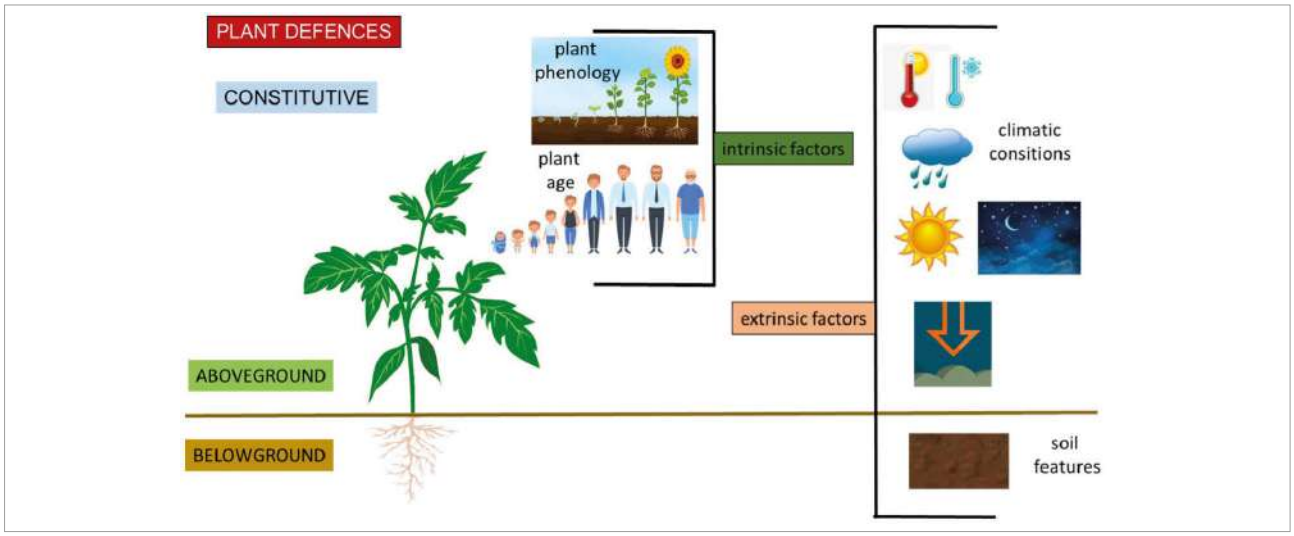

Fig. 1 - Schematic representation of factors affecting plant constitutive defences.

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The defensive output of induced responses could be direct or indirect. Direct effects on herbivore insect include the production of toxic compounds that alter its behaviour, development and reproduction. Indirect effects include the attraction of the natural antagonists of the herbivore mediated by the release of specific VOC. In both cases, the literature is particularly rich in examples covering a number of plant-pest-natural enemy systems.

Only recently, a new component has been considered in the interactions between plants and insects: the root symbionts (Fig. 2). By considering the incredible variety of soil microbiota, the fact that 80% of plant species are colonized by arbuscular mycorrhizal fungi (AMF) and that antagonistic fungi in the genus *Trichoderma* are ubiquitous, one could wonder whether "constitutive" plant defence do exist in nature. In fact, root colonization by AMF, Plant Growth Promoting Rhizobacteria (PGPR) or *Trichoderma* spp triggers plant responses that could impact on below and aboveground insect populations. Hence the question: could we profitably use these interactions for enhancing plant direct and indirect defence against insect pests?

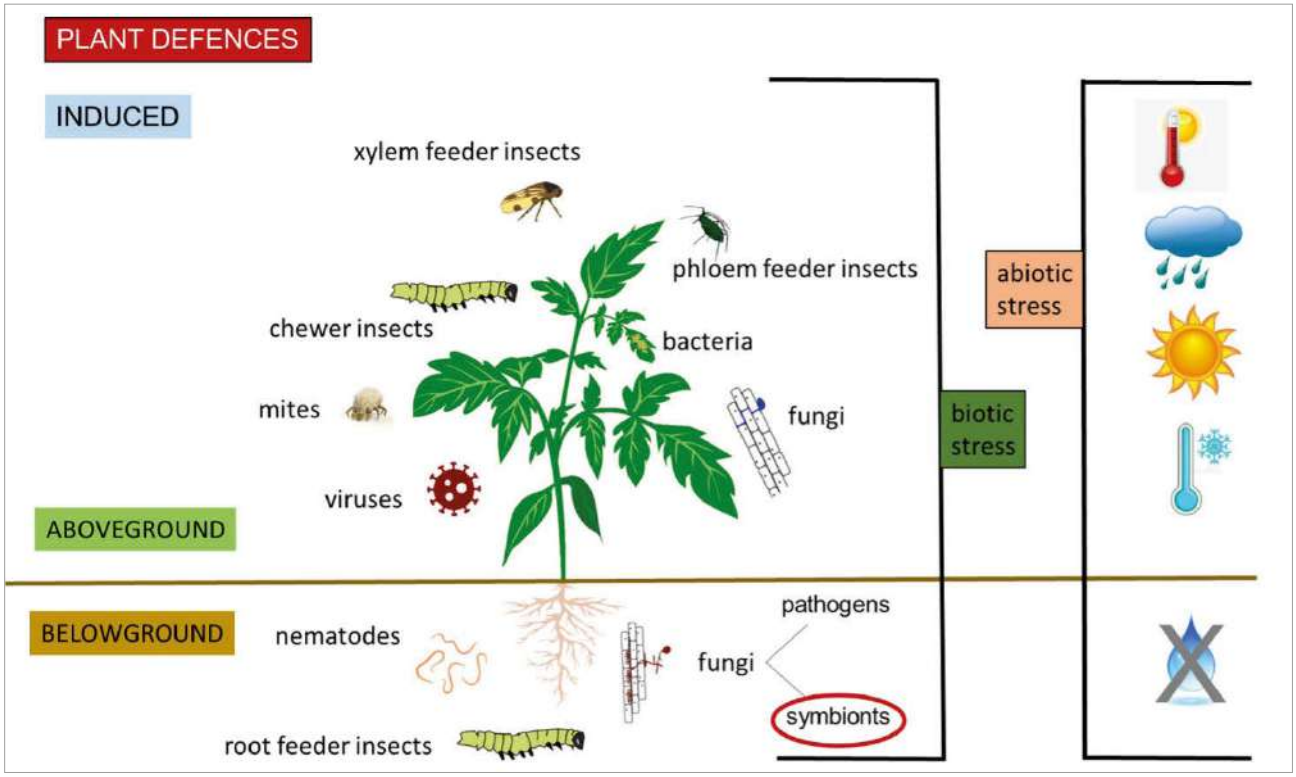

Fig. 2 - Schematic representation of factors affecting plant induced defences.

The role of root symbionts as elicitors of plant defences

Root symbionts involved in the elicitation of plant defences belong to 3 main groups: the mycorrhizal fungi, the antagonistic fungi, the Plant Growth Promoting Rhizobacteria (PGPR). The multiple effects induced by these organisms on plant direct and indirect defences are depicted in Fig. 3

Here, we will focus on arbuscular mycorrhizal fungi (AMF) and on *Trichoderma* spp and their effects on the activation of defences against different pests in tomato plant.

AMF and plant defence

Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) are common belowground plant mutualists and can influence the quantity and quality of resources available to herbivores. AMF associate with 80% of vascular land plants worldwide helping in phosphate and other nutrients supply especially in phosphorus deficient soils, enhancing plant resistance to drought (Volpe *et al.*, 2018) and lending a significant higher degree of bioprotection against various pathogens, including nematodes (Borowicz, 2006 and references therein) and fungi (Fritz *et al.*, 2006). A positive effect of AM fungi on soil structure has been indicated, making them a key component of sustainable agriculture (Johanson *et al.*, 2004). In some cases, plants growing with AM fungi can be 30% larger than plants without AM fungi (Gworgwor and Weber, 2003), and the increased quantity of resources caused by AM fungi has an impact on herbivore performance. For example, AM fungi may decrease herbivore performance by increasing their plant hosts' ability to produce nutritionally expensive chemical defences (Vannette and Hunter, 2013).

In tomato plants, root colonization by the AMF *Funneliformis mosseae* results in the induction of both direct and indirect mechanisms of defence against aphids (Fig. 4) (Guerrieri *et al.*, 2004).

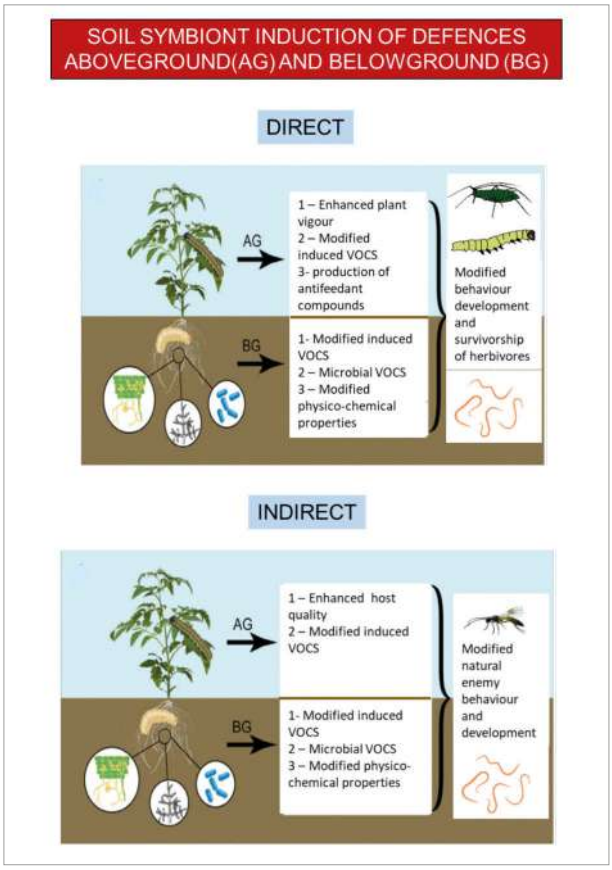

Fig. 3 - Schematic representation of the effects of root symbionts on plant direct and indirect defences (modified from Rasmann *et al.*, 2017).

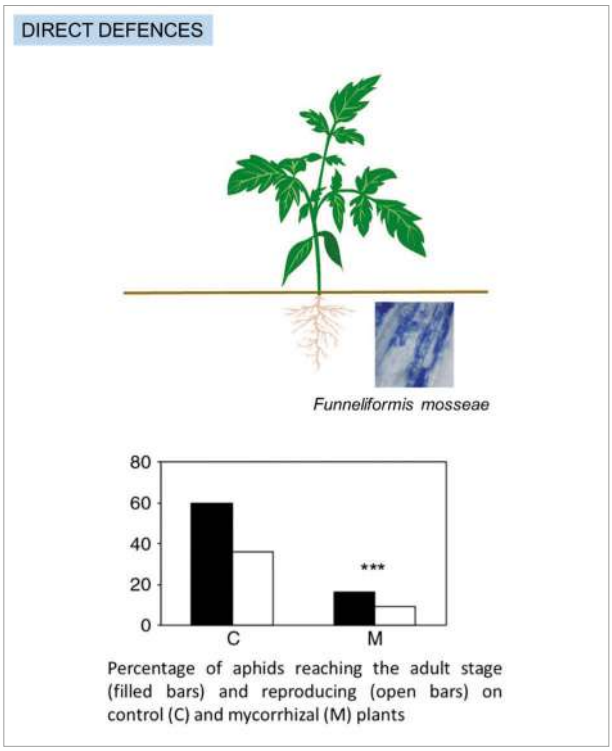

Fig. 4 - Direct defences against the tomato aphid *Macrosiphum euphorbiae* induced by AMF colonization (modified from Guerrieri *et al.*, 2004)

On mycorrhizal tomato less than 40% *Macrosiphum euphorbiae* aphids reached the adult stage and only about 10% reproduced. These effects are probably related to the production of antixenotic compounds that reduced the adsorption of plant nutrients coupled to a reduced palatability of the plant causing an alteration in the fixing behaviour of the aphids.

A significant increase of induced defences was also noted in tomato plants colonised by *F. mosseae*.

A significant increase of attraction towards the aphid parasitoid *Aphidius ervi* was recorded for uninfested mycorrhizal tomato (AMF) in respect to non-colonised uninfested control (CONTROL) (Fig. 5) and this was related to a change in the VOC released by plants upon root colonization by AMF. This difference in attractiveness was not noted when comparing uninfested mycorrhizal (AMF) and aphid-infested non-colonised (PHC) tomato

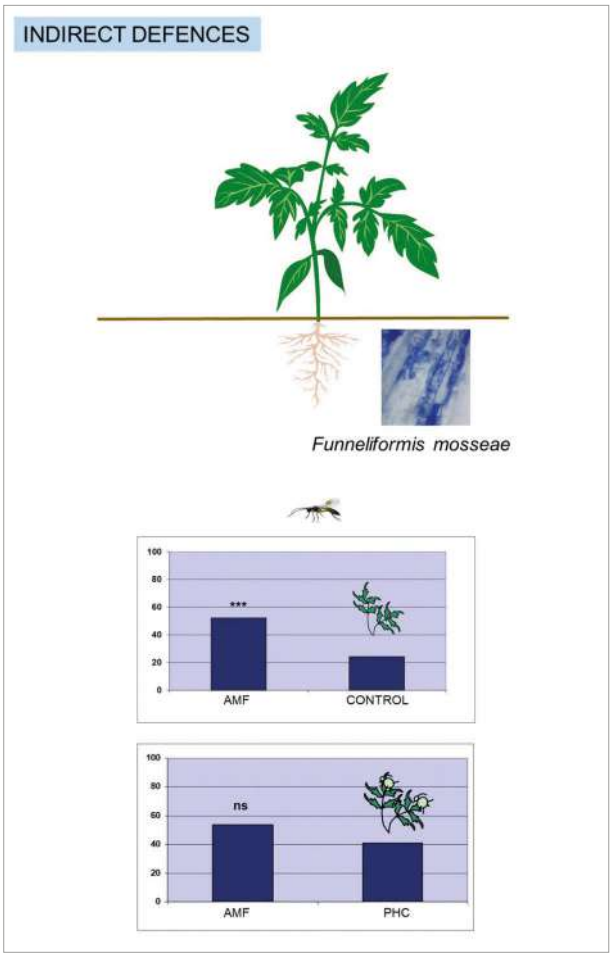

Fig. 5 - Indirect defences against tomato aphid *Macrosiphum euphorbiae* induced by AMF colonization (modified from Guerrieri *et al.*, 2004)

plants (Fig. 5). Hence, it was hypothesized that root colonization by *F. mosseae* induced the release of VOC that "mimic" aphid attack. It is known that a specific threshold of infestation in terms of number of aphids and duration of their feeding activity is needed to render a plant attractive towards an aphid parasitoid (Guerrieri *et al.*, 1999). In this context, mycorrhizal colonization could re-

duce the temporal gap between the ongoing aphid colonization and the arrival of the natural antagonist thus improving the biological control of aphid pests.

resulted in an enhanced defensive response by the plant as shown by a reduced survival rate of the tested aphid. Similarly, root colonization by *Trichoderma atroviride* result-

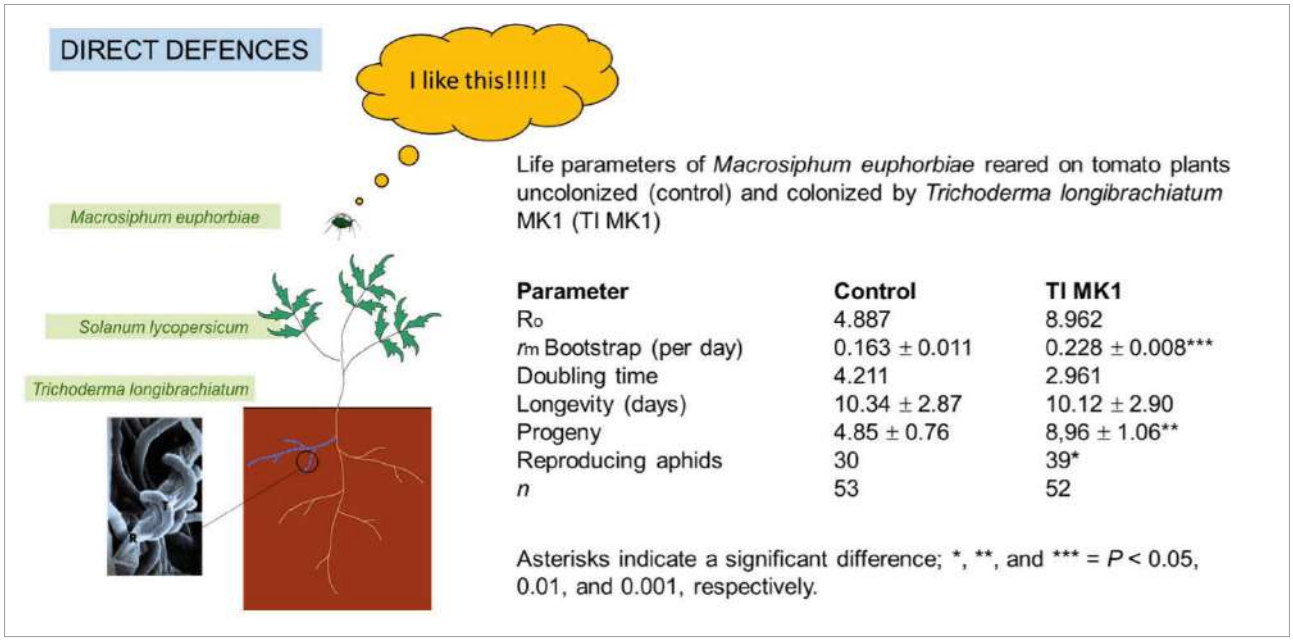

Fig. 6 - Reduction of direct defences against the tomato aphid *Macrosiphum euphorbiae* upon colonization by *Trichoderma longibrachiatum*.

Trichoderma and plant defence

Fungi belonging to the genus *Trichoderma* are distributed worldwide. They are known for their antagonistic activity against other microbes and their ability to colonize roots, establish chemical communication with the plant, and systemically alter the expression of many host genes. The success of this interaction may depend on the fungal strain–plant genotype combination (Tucci *et al.*, 2011). The relevant changes caused by *Trichoderma* spp. in the plant physiology often result in an improved abiotic stress resistance, nutrient uptake, resistance to pathogens, and photosynthetic efficiency (Hermosa *et al.*, 2012). Whilst a number of papers reported the efficacy of *Trichoderma* spp. or their metabolites in terms of induction of systemic resistance against plant pathogens (e.g. Vinale *et al.*, 2012), only in recent years it has been reported how these antagonist fungi can alter the performance of herbivores and their natural enemies aboveground.

In the system composed by tomato plant and the tomato aphid *Macrosiphum euphorbiae*, root colonization by *Trichoderma longibrachiatum* (strain MK1) resulted in an enhanced performance of the aphid as represented by a significant increase in both r_m index and progeny (Fig. 6)

This result was most probably due to the enhanced nutrition quality of the tomato as induced by root colonization by this specific strain of *Trichoderma*.

The specificity of plant responses in respect to different species/strains of root symbiont was confirmed in subsequent experiments including the same plant-aphid system and *Trichoderma atroviride* (strain P1) (Fig. 7)

In this case, the presence of the antagonistic symbiont

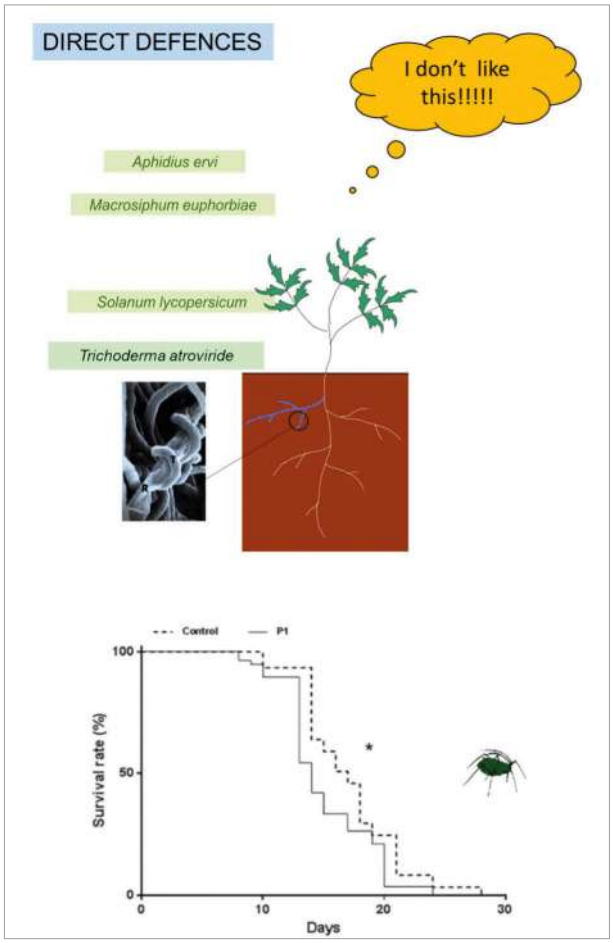

Fig. 7 - Induction of direct defences against the tomato aphid *Macrosiphum euphorbiae* upon colonization by *Trichoderma atroviride*.

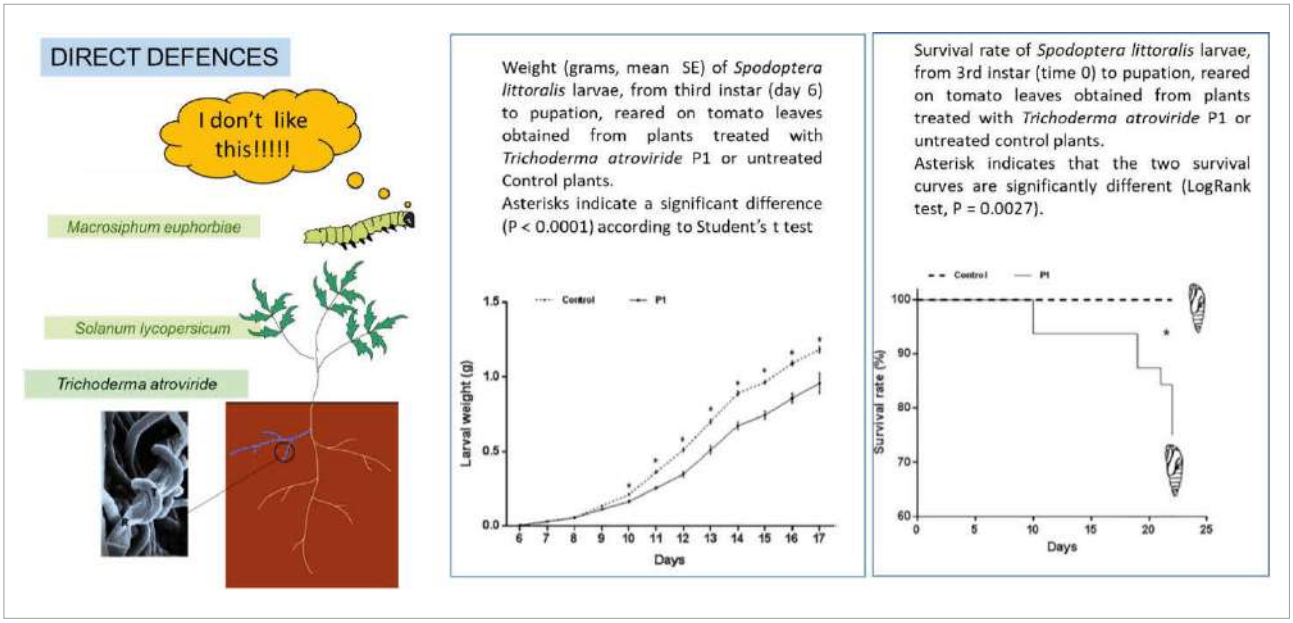

Fig. 8 - Induction of direct defences against *Spodoptera littoralis* upon colonization by *Trichoderma atroviride*.

ed in an enhanced defensive response against the chewer *Spodoptera littoralis* both in terms of larval weight and survival rate (to pupation) (Coppola *et al.*, 2019) (Fig. 8)

These differential responses induced by different species /strains of *Trichoderma* in terms of direct defences disappeared when considering plant indirect defences. Overall, regardless the species of *Trichoderma* colonizing tomato roots, we observed a significant increase of attractiveness towards the aphid parasitoid *A. ervi* (Figg. 9-11)

The increase of attractiveness towards *A. ervi* induced by *T. longibrachiatum* colonization was related to the release of specific VOC (Fig. 8) that have been proved important for the foraging behaviour of this parasitoid (Sasso *et al.*, 2007; 2009). The effect on indirect defences induced by *T. longibrachiatum* counter balance the abovementioned increased performance of the aphid pest *M. euphorbiae* elicited by the same fungal species.

Similarly, tomato colonization by *T. atroviride* (strain P1) resulted in the enhanced release of specific VOC and in turn to an increased attractiveness towards *A. ervi* (Coppola *et al.*, 2019)

Finally, a synergistic effect on the attractiveness towards *A. ervi* was recorded when tomato plants were colonized by *T. afrohartianum* (strain T22) and infested by the aphid *Macrosiphum euphorbiae* (Coppola *et al.*, 2017)

Summary and future directions

The species/specificity of plant responses to root colonization by different species and strains of symbionts requires a thorough effort of characterization if we want to exploit these interactions for the sustainable control of agricultural pests. These aspects are particularly important within the framework of EU Green Deal objectives

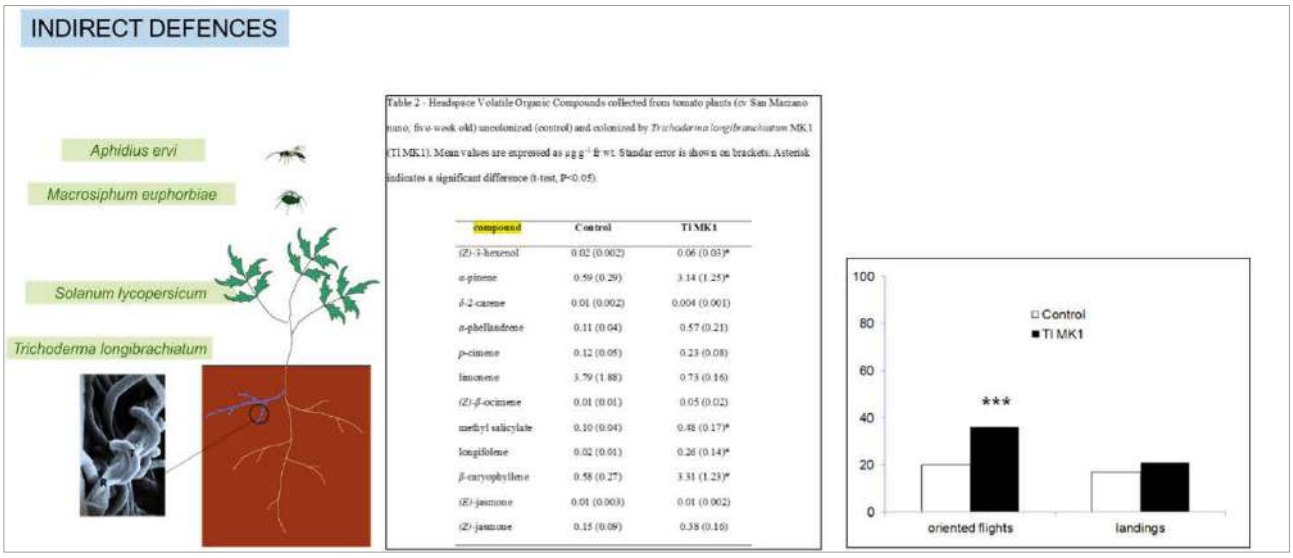

Fig. 9 - Indirect defences against tomato aphid *Macrosiphum euphorbiae* induced by *Trichoderma longibrachiatum*

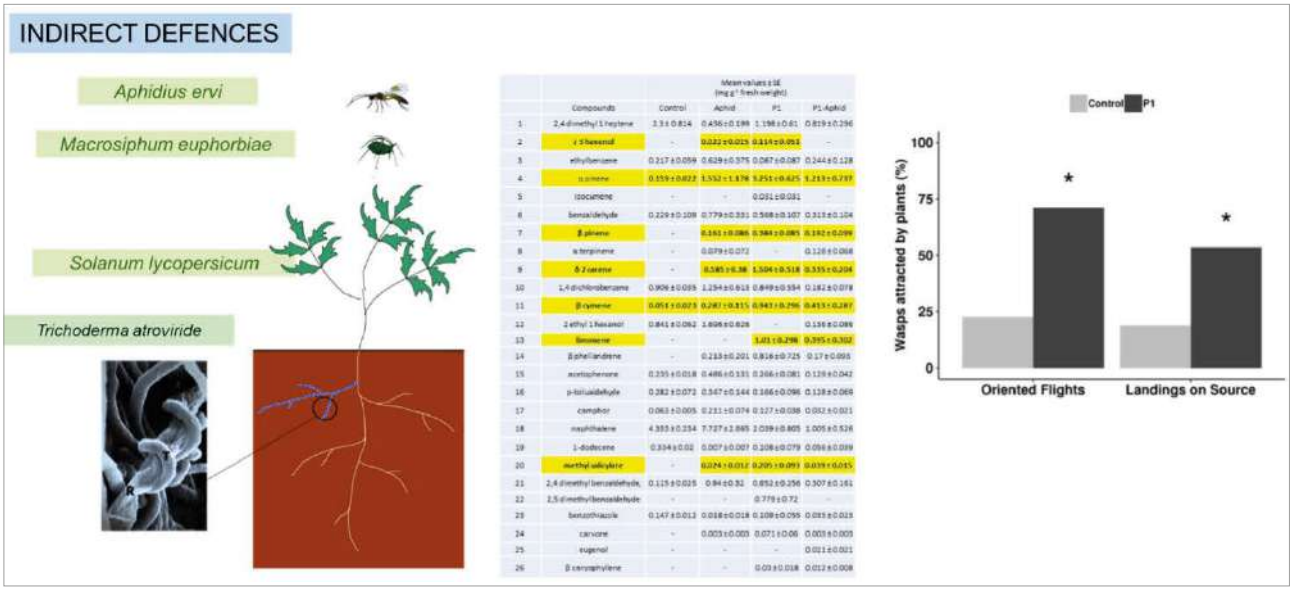

Fig. 10 - Indirect defences against tomato aphid *Macrosiphum euphorbiae* induced by *Trichoderma atroviride*

that fixed a 50% reduction in the use of synthetic pesticides by 2030. The use of root symbionts can be also combined with modern control strategies, including the use of natural derived compounds.

For example, the combined use of a plant derived hormone (systemin) aboveground and a *Trichoderma* strain (*T. afrohartianum*) belowground resulted in an efficen control of one of the most destructive pest of tomato worldwide, the tomato pinworm *Phthorimaea (=Tuta) absoluta* (Aprile *et al.*, 2022) (Fig 12).

The combined application resulted in the activation of specific defensive genes hampering the development and the longevity of the pest (Fig. 11)

Only by integrating multidisciplinary expertise it will be possible to progressively reduce the use of synthetic pesticides against pests that are becoming increasingly aggressive due to the climate change. The aim is to have an agriculture more and more sustainable that guarantees the quality and the safety of its products.

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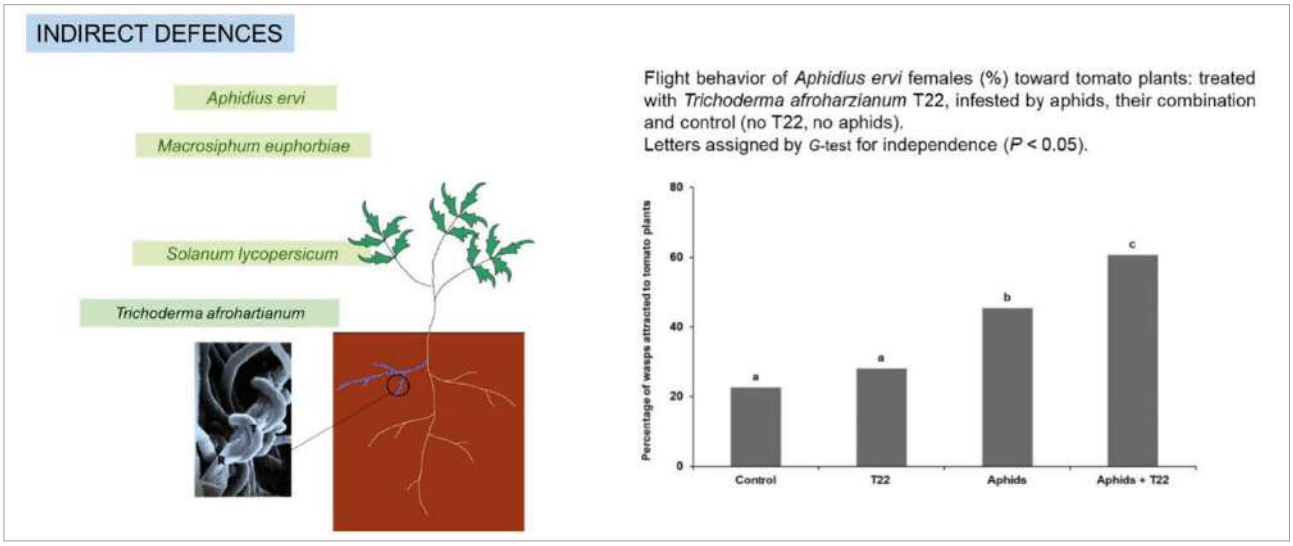

Fig. 11 - Indirect defences against tomato aphid *Macrosiphum euphorbiae* induced by *Trichoderma afrohartianum*

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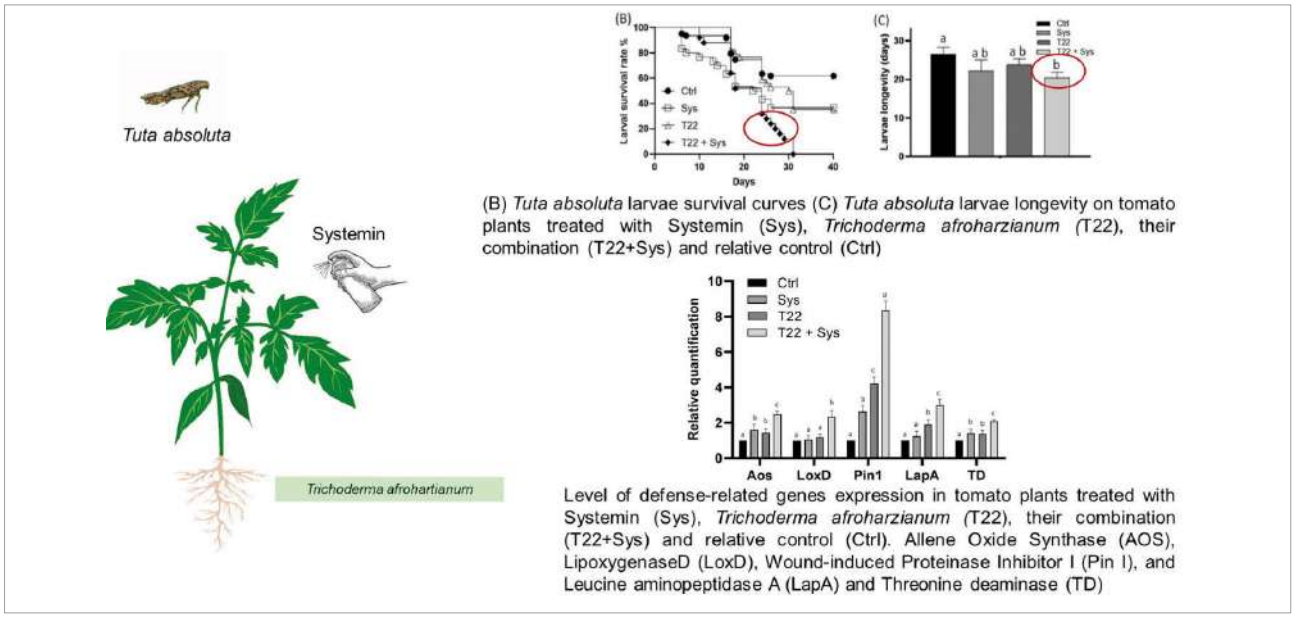

Fig. 12 - Effect of the combined use of a plant derived compound aboveground (systemin) and *Trichoderma* species (*T. afrohartianum,* T22) belowground for the control of the tomato pinworm.

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THE NON-TARGET EFFECTS OF INSECTICIDES ON INSECT NATURAL ENEMIES

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Sintesi della lettura tenuta durante la Tavola Rotonda "Biological pest control: managing multitrophic interactions for sustainable agriculture". Seduta pubblica dell'Accademia, Firenze, 10 giugno 2022.

The widespread use of synthetic insecticides is one of the main consequences of the *Green revolution* (Newman, 2015). In that era, insecticides were only considered substances used to kill insect pests with the aim of increasing crop yield (Cooper & Dobson, 2007). For several decades, the potential ecotoxicological outcomes of such practice have been neglected by the agroindustry and by policy makers (Rattner, 2009). Only after the warnings by various environmentalists worldwide, the most renowned being Rachel Carson with the book *Silent spring*, the awareness of the potential risks posed by pesticides on wildlife and on their ecological services increased (Carson, 1962, Newman, 2015). Biological control is, together with pollination, the agroecological service more at risk for side effects of insecticides. The toxicological impact toward insect natural enemies needs thus to be carefully evaluated prior introducing any toxicant into crop protection protocols. However, such an assessment is a high challenging task due to the high variety of insecticide mode of actions and, more importantly, because of the extreme biological and ecological diversity of non-target organisms (Biondi *et al*., 2012). Old generation insecticides were characterized by strong acute toxicity toward a broad range of exposed organisms. The assessment of their target and non-target toxicity is traditionally expressed with concentration-mortality response indexes, such as the estimation of median lethal concentrations (Desneux *et al*., 2007). By contrast, novel insecticides are developed with the main aim of being as more selective as possible. For this, novel insecticide mode of actions and applications are continuously discovered and implemented into the crop protection practice. Such substances are usually considered as *slower acting* because inducing a high degree of chronic sublethal effects rather than lethal ones (Guedes *et al*., 2016). Sublethal effects are those physiological and behavioral modifications occurring in organisms survived to the exposure to sublethal concentrations of insecticides (Desneux *et al*., 2007). In pests, insecticide sublethal effects translate into the prompt reduction of crop damage and in a longer-term reduction in pest population levels. However, for insect natural enemies the consequences of sublethal exposure are less obvious and are very often crucial for the ecotoxicological assessment and finally for the sustainability of crop protection programs involving novel insecticides (Desneux *et al*., 2007; Biondi *et al*., 2013). Indeed, sublethal effects of insecticides on insect predator and parasitoid are the consequence of the diverse types of exposure, i.e., topical, residual and for ingestion via the plant or via the prey/host, of the intrinsic physiological and biological features of the natural enemies, and of the extraordinary diversity of prey/predator and host/parasitoid interaction. Such scenario calls for a *case by case* evaluation of the potential side effects of novel insecticides toward natural enemies, before any novel insecticide get widespread used. This aspect is crucial for the current need of implementing resilient and sustainable copping system, as required by the modern agricultural models.

Key Words: Biological control, Biopesticides, Ecotoxicology, Integrated Pest Management, Sublethal effects.

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