Semiochemicals and Entomopathogenic Microbials (Fungi, Nematodes, Bacteria, Viruses)

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Biological control agents and semiochemicals have become essential parts of the integrated pest management of insect pests over the last several years, as the incorporation of semiochemicals with natural enemies and entomopathogenic microbials has been gaining significance. Semiochemicals can enable the successful dispersal of entomopathogenic microbials. Using semiochemicals to disseminate microbial pathogens is still at the initial stage. For dispersal of entomopathogenic fungus semiochemicals have been successfully used in field conditions, however same can not be said about the other microbials such as specially for bacterial and viral entomopathogens.

**Fungi**

Pathogens may be dispersed naturally by parasitoids, predators, and the feces of insects, birds, and mammals, and surface contamination \[1\]. However, for entomopathogenic fungi, natural dispersal, in additional to the aerial movement of spores, is also known to occur through the movement of the targeted insect pests and pollinators, as shown in honey bees in canola production, where honey bees disperse *B. bassiana*, increasing the mortality of *Lygus* sp. (Hemiptera: Miridae) \[2,3\]. A selective and assisted dissemination technique called auto-dissemination is also extremely helpful in spreading entomopathogens \[1\]. Auto-dissemination can be used to target both adults and larvae of some insect pests \[4,5,6\]. Semiochemicals are being used to increase the rates of fungal infection in several insects. Successful examples of the use of combinations of semiochemicals and entomopathogenic fungi include bark beetles (*I. typographus*), weevils (*Cylas formicarius* Fabricius [Coleoptera: Brentidae], *Cosmopolites sordidus* Germar [Coleoptera: Curculionidae]), moths (*Plutella xylostella* L. [Lepidoptera: Plutellidae]), stink bugs (*Plautia crossota* stali Scot [Hemiptera: Pentatomidae]), thrips (*Megalurothrips sjostedti* Trybom [Thysanoptera: Thripidae]), and aphids (*Phorodon humuli* Schrank [Hemiptera: Aphididae]) \[7\]. To make this method successful, an appropriate physical separation (including the distance) between semiochemicals and entomopathogenic fungus is needed to achieve the maximum output of autoinoculation \[8\].

Plants also host entomopathogenic fungi naturally \[9\], that remain as endophytic fungi after the conidia of an entomopathogenic fungus germinate and enter the plant cuticle \[10\]. The presence of these endophytic entomopathogenic fungi in plants causes mycosis in different insect pests \[11\]. Epiphytic fungi on plants are also reported to attract insects. Western yellowjacket [*Vespula pensylvanica* Saussure (Hymenoptera: Vespidae)] and the German yellowjacket [*V. germanica* Fabricius (Hymenoptera: Vespidae)] vector the fungus *Aureobasidium pullulans* ([de Bary] Arnaud) (Dothideales: Dothioraceae). A study done in orchards in Washington, USA found that the volatile compounds emitted by this fungus can attract eusocial wasps and that wasps and fungi appear to have a symbiotic relationship \[12\]. In a laboratory experiment also done in the USA, it was found that the hymenopteran parasitoids *Roptrocerus xylophagorum* (Ratzeburg) (Hymenoptera: Pteromalidae) and *Spathius pallidus* (Ashmead) (Hymenoptera: Braconidae) are attracted to bluestain fungi (genus *Ophiostoma* [Syd. and P. Syd.]), which are associated with bark beetles (Coleoptera: Scolytidae) feeding in pine trees. This study also found that such fungus-based attraction might not function for short-range host location \[13\].

The ‘lure and kill’ method has been highly effective for controlling some insect pests by using semiochemicals (especially pheromones) in conjunction with entomopathogenic fungi. Successful examples include the management of sap-sucking insects such as aphids (*P. humuli*), thrips (*M. sjostedti*), green bugs (*P. crossota*), and chewing and biting insect pests such as bark beetles (*I. typographus*), weevils (*C. formicarius and C. sordidus*), and moths (*P. xylostella*) \[7\]. Nevertheless, in most cases, such as sex-specific semiochemicals, which attract only one sex, the method is less effective. In addition, the ‘lure
and kill’ method is still not well developed for soil-dwelling insects, although Agriculture and Agri-Food Canada (AAFC) (Agassiz, BC, Canada) has created prototype granules of *Metarhizium brunneum* (Petch) (Hypocreales: Clavicipitaceae) combined with pheromone compounds that have showed some promising results for attracting species of *Agriotes* cutworms (Coleoptera: Elaterideae) to bait sources [14]. The use of pheromones in granulated or in pellet form could work well for soil-dwelling insects [15].

**Nematodes**

The efficacy of entomopathogenic nematodes (EPNs) mainly depends on the strain, formulation, and method of application [15]. However, in recent studies, several HIPVs from the roots of host plants that attract EPNs were examined as formulation additives. These HIPVs are secreted at damaged sites when their production is triggered by compounds in the saliva of phytophagous insects during feeding. Plants also release defense-related volatiles that can attract EPNs [16], [17], [18]. Furthermore, volatiles secreted by such nematodes also attract EPNs; for instance, the application of infected cadavers with EPNs proved to be more effective than the direct spraying of infective juveniles. When an extract of the infected cadavers was applied along with the aqueous suspension of *Heterorhabditis bacteriophora* (Poinar) Hb strain (Rhabditida: Heterorhabditidae), it was also found to be more infective than direct spraying to *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) [19]. In another study, macerated hosts infected with *Steinernema carpocapsae* (Weiser) All strain, *Steinernema feltiae* (Filipjev) SN strain (both Rhabditida: Steinernematidae) and *H. bacteriophora*, increased the dispersal of these EPNs in soil columns [20]. Ascarosides (a group of glycolipids which regulate mating and development) secreted by several EPN species result in a greater dispersal of various EPNs, in both natural and synthetic form [16]. Pheromone extracts from *S. feltiae* (SN strain) or *S. carpocapsae* (All strain), when tested on *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) larvae, showed an improved dispersal and efficacy, which suggests that pheromone-mediated enhancement of EPN efficacy could be achieved by exposing EPNs to specific pheromones [21].

**Viruses**

Most known entomopathogenic viruses are baculoviruses (four genera: Alpha-, Beta-, Gamma-, and Deltabaculoviruses), Reoviridae, Parvoviridae, or Nudiviruses [22]. The use of semiochemicals for the dispersal of entomopathogenic viruses has not been studied extensively. However, a combination of apple-associated yeasts and codling moth granulovirus (CpGV) increased the mortality of the codling moth (*Cydia pomonella* (L.) (Lepidoptera: Tortricidae) under both laboratory and field conditions [23]. The pheromone is known to increase the efficiency of the Granuloviruses in the insect pests *C. pomonella* and *Adoxophyes orana* (Fischer von Röslerstamm) (Lepidoptera: Tortricidae) [24]. In 1992, the potential of sex pheromone baited traps was first evaluated [48] in the USA, to auto-disseminate the *Baculoviruses* (nucleopolyhedrovirus (NPV)) against *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) [25], [26].

**Bacteria**

Among entomopathogenic bacteria, the best known is *Bacillus thuringiensis*. It has been known since 1901 and is used to manage several major insect pests in agriculture, forestry, and medicine [22]. Although the use of autoinoculator devices is reported to aid in dispersal of *Paenibacillus (=Bacillus)* popilliae (Dutky) (Eubacteriales: Bacillace) to manage *Popillia japonica* Newman (Coleoptera: Scarabaeidae) [18,87], the use of semiochemicals to improve the efficacy and dispersal of bacteria has not been explored.

**Protozoa**

The inclusion of semiochemicals in the dispersion of protozoans to manage insect pests is a scantily explored area and needs further exploration. Shapas et al. [27] evaluated generations of *Trogoderma glabrum* (Herbst) and indicated that they were reduced after the dispersal of protozoan pathogen spores, *Mattesia trogodermae* Canning. Pheromone-baited (synthetic sex pheromone, (E)-14-methyl-8-hexadecenal) spore-transfer sites were used to disperse the spores. In this study, it was also indicated that males became attracted to females and these males induced attempted copulation with the pheromone source, aiding in spore transfer to males [27].
Keywords

entomopathogenic fungus; attract and kill; autodissemination; entomopathogenic nematodes

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