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A Brief Review on *Lobesia botrana* Mating Disruption by Mechanically Distributing and Releasing Sex Pheromones from Biodegradable Mesofiber Dispensers

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Introduction

Within some 125 years in the development of the natural sciences, insect management passed through a number of distinguishable phases of increasing complexity and sophistication., Lobesia botrana management can serve as an example. Biochemical physiologists, classical entomologists, toxicologists, analytical and chemical ecologists, as well as engineers both at the molecular and field level, had about equal merits in achieving today's high state of knowledge and perfection. Although the golden age of all-round pest management is by no means in our hands, we collectively reached at least a status where we could claim considerable progress towards environmentally sound pest management unthinkable only 50 years ago [1-4]. In detail, we might briefly distinguish 7 major phases of accomplishments, leading to today's technical level:

(1) Fabre provided first evidence for sex specific attraction of giant Saturniid moth males to their conspecific females [5].

(2) Götz used solvent extracts of completely undefined composition for quantitatively monitoring the presence and distribution of Lobesia and Eupoecilia in South west German vineyards, in spite of nobody'ies knowledge of the chemical nature of these attractants [6,7].

(3) Butenandt et al. identified the first attractants in silkmoth females (Bombyx mori) and synthesized them chemically [8,9]. Karlson and Lüscher named them collectively "pheromones" [10]. Schneider and Kaissling provided the first quantitative evidence for electrophysiological action on the Bombyx antenna [11].

(4) Roelofs chemically identified the Lobesia botrana (Lep.: Tortricidae) pheromone as E,Z-9,11-dodecadienyl acetate [12].

(5) Neumann summed up his results on mating disruption in Palatinian vineyards and provided evidence that communication and mating disruption works not only in small fields but even better at the regional scale [13].

(6) Greiner and Wendorff reviewed the knowledge gained on nanofibers available then mainly in the fields of pharmaceuticals and innovative filter materials [14].

(7) Agricultural applications in the fields of pheromone disruption studies were pioneered by Hummel and awarded with a series of eight patents certified in Germany, Europe, and the US [15-19]. Relevant papers began to appear in rapid succession[20-24], the latest ones emphasizing additional progress towards mechanical fiber distribution in vineyards, thereby achieving cost reduction and environmental protection realized by fully biodegradable pheromone-mesofiber combinations. Pleasingly, colleagues from Brazil independently reported investigations on a related nanofiber test system in Brazilian peach orchards, again working with a related Tortricid pest species, the Oriental fruit moth Grapholita molesta [25] and B. Czarnobai de Jorges, pers. communication). This latter part was designed for monitoring purposes, not for mating disruption, Bbut it probably could be extended to fit and maeetch this capacity as well.

Accomplishments of the Approach

In a nutshell, our approach of integrating pheromone disruption with the new application technology via electrospun mesofibers makes sense, because pheromone dispensers of the RAC type by BASF and the "spaghetti" type by Shin Etsu are rather clumsy by today's standards and must be applied manually. In contrast, mesofibers can be dispersed mechanically and can be tailored to release precisely predetermined concentrations of synthetic pheromone into the air. Thus, male insects will be unable to trace a trivial whiff of natural pheromone concentration against the large background of the artificially dispersed pheromone. As a result, the males will be disoriented and will be unable to find and mate with virgin females. Thus, no viable eggs are deposited, and the propagation of the pest insect species is quite limited. Ideally, the resulting populationit is far significantly reduced below the economic threshold. This principle holds for several hundred pest insect species investigated so far by many labs all over the globe [3,26-28]. Monitoring insects and disrupting mating in insect populations thus can be considered proven beyond any doubt.

Since pheromones are very highly species specific, they may be used for equally specific insect management in the presence of many other arthropods, including beneficial insects. The pheromone (E,Z)-7,9-dodecadienyl acetate and indeed many other related insect pheromones are natural products of the intermediary lipid metabolism common to all animal, plant and microbial cells. If applied artificially, they do not form any residues but are completely biodegraded and converted to H_2O ad CO_2 within the "biochemical machinery" of the organisms and the environment.

Thus, the products, table grapes and high quality wine grapes, are free of pesticide residues. Such residues in the past, some of which caused off-flavors both in non-fermented and in fermented juices. Taste panels recognized the superior sensory qualities of both products thus generated under IPM (integrated pest management) via mating disruption. Meeting environmental concerns, Ppheromones and polyester based mesofibers can both be produced from non-petroleum based natural materials such as oils and fats as fully renewable and sustainable resources [29].

Materials and Methods

These have been amply introduced in our past publications and shall not be repeated here [2-3,20-24,27,30-32] Pheromones supported by mesofibers and by Isonet LE treatments were compared to untreated controls (experimental design according to Doye [31]. Male recapture rates are inversely proportional to the mating disruption effect. Negative controls are arbitrarily set as 100% for easier comparison with the positive effects depicted in the rows "mesofibers" and "Isonet LE" as positive control in the range a few percent recapture rate of males in pheromone treated vineyards.

(B) Experiments of mating disruption in Lobesia botrana males for seven weeks with modified mesofibers with long-lasting release kinetics manufactured according to [33,34] extend the usable time of the mesofibers to seven weeks, which is enough to cover one of the three flight periods of *L. botrana*. Included in the graph, there is

(I) a micrograph of a electrospun mesofiber bundle.

(II) the Isonet "spaghetti" dispenser, and (III) an EM micrograph of an individual mesofiber, counterstained with the heavy metal oxidant RuO_4, to visualize pheromone inclusions within the Ecoflex® fiber[20]. The resulting male recapture rates are, as expectable, only a few percent of those of the controls. This is in full agreement with numerous reports in the literature where disruption effects are described [2-4,13,21-24,26-28,30-31,35-36] **(Figure 1)** taken from Hummel et al. [35].

(Figure 2) provides evidence for a considerable step made forward towards precision viticulture. Manual application of dispensers is too expensive. It has, as dictated by economic pressures, to be replaced by mechanical or even automatic deployment of dispenser sources.

The ERO shootbinder **(Figure 2),** can be considered as a mechanical platform. Mounted on it are:

(A) "Shoot reduction cutters".

(B) Reels for deploying and fixing preformed ropes with electrospun mesofiber/pheromone inclusions, see [21,30,35,36].

(C) Weeding equipment (mounted at the bottom of the machine). All three mechanical components are to be operated simultaneously by a highly trained technician sitting in the driver seat. This integration of job components leads to a considerable quantitative reduction in labor costs, while creating job opportunities for higher qualified new specialists in this segment of viticulture.

Outlook

In the future, we will be investigating other mesofiber variants with longer disruptive effects lasting for up to half a year. Thus, only one treatment per growing season will would be sufficient. Moreover, the fiber material, the polyester Ecoflex®, is a biodegradable polymer variety [37]. Spent pheromone plastic dispensers of the 3rd generation, usually a nuisance in the orchard, will be a matter of the past. Moreover, there is commercial machinery available, depicted in **(Figure 2),** capable of triple capabilities:

(A) Deploying of pheromone loaded mesofibers, while at the same time.

(B) Performing pruning.

(C) Weeding tasks in the vineyard.

Taken together, we do not shy away from claiming an internationally and collectively achieved quantum step towards realizing precision agriculture in this specific segment of insect management within the course of the last 30-40 years. Pheromones, during that period, became widely known and appreciated for hundreds of different insect pest species (see reviews [3,27-28,32,38-40].

The effects demonstrated can best be summarized in **(Figures 1 and 2).**

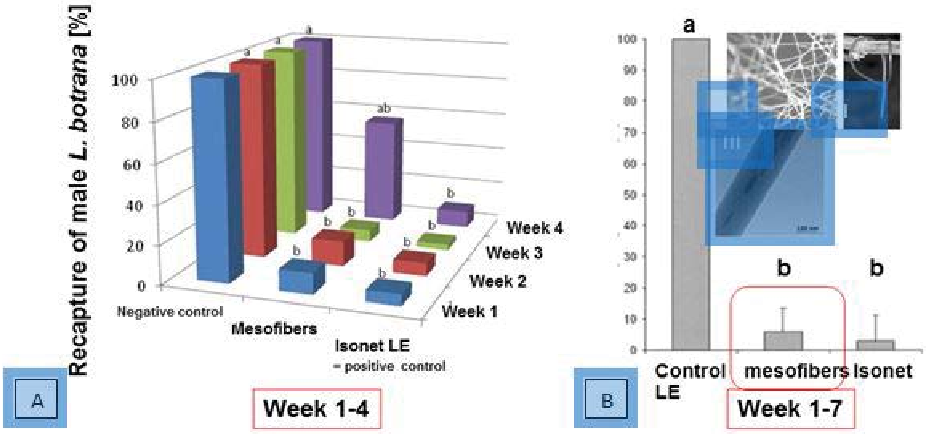

Figure 1 Results of mating disruption in *Lobesia botrana* males, achieved in field experiments.

Figure 2 Commercially available vineyard cultivation machinery (ERO shoot binder) simultaneously modifiable for mechanical deployment of pheromone loaded meso-fibers as well as weed control in South German vineyard of WBI Freiburg, Germany (photograph by M. Breuer).

Conclusion

First quite slowly, but then with accelerating rate, accomplishments in this area can be documented. Ultimately they will result in IPM programs of increasing complexity and penetration power for the control of many arthropod species currently of major importance in agri-culture, viti- and horticulture. Today we can, without undue prowess, conclude to have witnessed major achievements virtually unthinkable to our forefathers and teachers in 189086-1915, 1939, and even in 1959. Butenandt and Metcalf, both careful and critical scientists, had clear visions of an alternative future in plant protection [4,8,41,42]. But they were careful enough to disguise them with their ability for writing step by step progress reports [36] After all, they were well aware that they and their schools were ahead of their time by several decades. Dreams of the past, howeverconvincingly, materialized. After all, utopias are anticipated truths. By carefully designed steps in microanalytics, synthesis, entomology, polymer- and biochemistry, ecology, mechanical engineering and finally data handling, they paved the way towards precision agriculture achieved today. An interdisciplinary mosaic of intelligently linked steps is the visible result of 125 years of achievements in precision agriculture. Should political will and ample funding persist, standardized procedures could soon be available for a selection of a hundred of the most stubborn and persistent arthropod pests. It is not any longer a matter of missing technology but rather a matter of public will power to realize already available opportunities.

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