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SEMIOCHEMICAL STRATEGIES FOR TORTRICID MOTH CONTROL IN APPLE ORCHARDS AND VINEYARDS IN ITALY.

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Abstract

This review summarizes the work done in Italy in taking semiochemical-based management of orchard and vineyard pests from the research and development stage to successful commercial deployment. Mating disruption (MD) of codling moth *Cydia pomonella* was originally introduced in the Trentino-South Tyrol areas in order to address the development of CM resistance to insect growth regulators (IGR) and to mitigate the conflict at the rural/urban interface related to the extensive use of insecticides. Although the mountainous terrain of the area was not optimal for the efficacy of MD, commitment and determination led to the rapid adoption of MD technology throughout the region. Grower cooperatives and their field consultants were strongly influential in convincing growers to accept MD technology. Public research institutions conducted extensive research and education and provided credible assessments of a various MD technologies. By 2016, the deployment of MD in effective area-wide strategies in apple (21,200 has) and grapes (10,450 has), has resulted in better control of Tortricid pests and a substantial decrease in insecticide use. Collaboration between the research community and the pheromone industry has led to the development of increasingly effective single-species dispensers, as well as multi-species dispensers for the control of both target and secondary pests. Over the last 20 years hand-applied reservoir dispensers have shown excellent efficacy in both apple and grapes. Recently, aerosol dispensing systems have been shown to be effective in apple systems. Further research is needed on the efficacy of aerosols in grapes before wide spread adoption occurs. The successful implementation of MD in apple and grape production in Trentino-South Tyrol is helping to enhance the adoption of the technology in other Italian fruit production regions.

Keywords: *Cydia pomonella*; *Lobesia botrana*; mating disruption; area-wide pest management; Trentino – South Tyrol.

Introduction

With 2.3 million tons of apples produced on 52,000 hectares (ha) (Dalpiaz 2014) and 8 million tons of grapes produced on 700,000 ha (OIV 2015), Italy is the fifth and the fourth producer of apples and grapes in the world, respectively. While the bulk of apple cultivation takes place in the central and northern regions, mainly in Trentino-South Tyrol and secondarily in Veneto, Emilia Romagna and Piedmont, all Italian regions are involved in grape growing. The expanse of apple orchards and vineyards have been a visually appealing feature of the agricultural landscape and an attraction for tourists.

In general, such a level of production would entail a very high use of pesticides (http://agri.istat.it), which potentially could have toxicological and environmental implications and result in residues on the fruits and in the wine (Clementi et al. 2007; Lorenzin 2011; Pivato et al. 2015). Diseases such as apple scab, powdery mildew, and downy mildew require a high amount of fungicides. Tortricid moths (Lepidoptera, Tortricidae) are the most important insect pests in apples and grapes and historically have been controlled with multiple insecticide applications.

Apple. Economic losses associated with the incidence of the codling moth *Cydia pomonella* L. (CM) on apple crops are significant in Italy as indeed they are worldwide (Barnes 1991). CM can also damage other fruit crops such as pear, quince, apricot, plum, and walnut. Originating in Eurasia, CM has achieved nearly global distribution due to its ability to adapt to different habitats. In Italy, it represents the major insect pest in all apple production regions. There are two generations in Trentino-South Tyrol and three generations in more southern regions such as Emilia-Romagna (Pasqualini and Ioriatti 2009). Females lay eggs on the fruit or the leaves closest to the fruit, and the larvae bore into the fruit immediately upon hatching. Degree-days (DDs) and phenological forecasting models have been developed and widely used to predict CM flight periods, oviposition, hatching time and generation time for spring and summer moth broods (Butturini et al. 1992; Mattedi et al. 2008). Integrated pest management (IPM) programs generally rely on one to three insecticide applications with insect growth regulators (IGRs) applied during the first generation because of the efficacy against both eggs and young CM larvae. The application of these IGR sprays for CM also have efficacy against the overwintering larvae of leafrollers (Ioriatti et al. 2006). During the second and third generations, curative insecticides are generally applied and timed using traps and economic injury thresholds (Ioriatti et al. 2009a).

Despite extensive insecticide use, the economic impact of CM in pome fruits has been increasing. Factors such as climate changes, (Forti et al. 2003), less effective insecticides, and the emergence of insecticide resistance (Riedl and Zelger 1994; Ioriatti et al. 2007) has made CM control below economic thresholds more difficult.

Considerable research has focused on the development of more effective and environmentally safe strategies. Insecticide resistance has been addressed by integrating traditional chemical tools with other more selective tactics, including exclusion netting (Baiamonte et al. 2015), granulovirus (CpGV) (Pasqualini et al. 1994), and entomopathogenic nematodes (Curto et al. 2010) as well as various paraffin and plant oils (Caruso et al. 2012). Although these approaches minimize toxicity to beneficial organisms, they are either not sufficiently effective to gain widespread acceptance as stand-alone control measures.

In addition to the ubiquitous CM, other species of tortricids appear occasionally and need specific control measures in the apple-growing areas in Italy: the leafrollers (LR) *Adoxophyes orana* (Fischer v. Röslerstamm), *Archips podana* (Scopoli), *Pandemis heparana* (Denis & Schiffermüller), *P. cerasana* (Hübner), *Argyrotaenia ljungiana (pulchellana)* (Thunberg), and the oriental fruit moth (OFM) *Grapholita molesta* Busck (Pasqualini et al. 1983; Pasqualini 2000; Rama et al. 2001).

<u>Grapes</u>**.** Four species of Tortricidae feed on grape in Italy. *Lobesia botrana* (Denis & Schiffermüller) and *Eupoecilia ambiguella* (Hübner) are key pests that require specific annual control measures. *Argyrotaenia ljungiana* (Thunberg) and *Sparganothis pilleriana* (Denis &

Schiffermüller) cause occasional harm to grapes (Ioriatti et al. 2012). The European grapevine moth (EGVM), is historically present in Europe, Asia, and Africa (CAB 1974). Although widespread in all grape-growing areas, its economic importance is highest in southern Europe (Ioriatti et al. 2011b). Recently, EGVM has been found in Chile in 2008, California in 2009, and Argentina in 2010 (Gonzales 2010; Varela et al. 2010).

The European grape berry moth (EGBM), *E. ambiguella* is a significant insect pest in the vineyards of central Europe, where it has been gradually replaced in many areas by EGVM as the major pest. The shift started in the Mediterranean Basin and has now extended to Switzerland, Austria, France and southern Germany, where populations of the two species overlap. These moths are very polyphagous and share several host plants. When infesting grapes, EGVM larvae feed on flowers and berries (Ioriatti et al. 2011b). The anthophagous generation does not generally cause yield losses. The carpophagous generations are the most destructive due to larval feeding on green and ripe berries, which results in yield reduction. The presence of larvae, webbing and rotten berries, clearly affects the quality and salability of table grapes. Moreover, secondary infections of gray mold, *Botrytis cinerea* Pers., develop rapidly on damaged berries causing bunch rot that degrades the wine quality. EGVM and EGBM must thus be managed yearly to maintain their damage at an acceptable level.

Insecticides applied in the past against the two moths have been gradually replaced by more selective and less hazardous compounds for human health, such as spinosyns, avermectins anthranilic diamides and *Bacillus thuringiensis,* have been introduced into IPM strategies (Ioriatti et al. 2009b).

In Italian apple and grape orchards, the development of the sustainable management techniques for the Tortricidae has benefitted from the experience gained in Trentino-South Tyrol with the implementation of pheromone mating disruption (MD). The success in Trentino-South Tyrol was an important factor in the adoption of MD in Italian and European apple crops and vineyards. As mating disruption became a viable technology for growers, research led to the development of new MD formulations and new monitoring systems, IPM principles became the cornerstone in the management of non-target pests. Growers, advisors, researchers and industries were all involved in the research and development and the successful collaboration led to the widespread implementation of MD in the region.

From the pioneers to the industrial age

In Europe in the years immediately following the identification and the synthesis of CM and EGVM sexual pheromones (Roelofs et al. 1971, 1973), several field experiments were carried out in the development of pheromone traps to monitor adult flights (Charmillot et al. 1975; Roehrich et al. 1976) and to control the moths with mass annihilation techniques (Wildbolz et al. 1973; Charmillot and Baggiolini 1975). At the same time, the first applications of MD against CM and EGVM were carried out in France with promising results (Audemard et al. 1977; Roehrich et al. 1977). In this case the synthetic pheromone was impregnated and released through hand-made rubber tubing dispensers.

The first field trials on MD for apple crops in Italy were performed in 1980, in a 0.6 ha apple orchard, using the sprayable pheromone WP (Farmoplant – Novara – Italy) during the first and second flights of CM. In the following two years, MD was applied in two regions characterized by different CM population levels, using rubber tubing (Gummi Maag, Dűbendorf, CH) (Fig. 1A) impregnated with the CM pheromone (codlemone, E8,E10:12 Ac) (Maini et al. 1982; Sacco and Pellizzari-Scaltriti 1983). The active ingredient (a.i), with 1% BHT added as an antioxidant, was dissolved in hexane and pumped inside the tube in order to obtain a pheromone load of 1 g/m. After hexane evaporation, the tube was cut into 8-9 cm long pieces, which were applied in the orchard to obtain a dosage of 28-30 g/ha.

The results of these pioneering research trials were encouraging. Rubber dispensers were more effective than the aforementioned sprayable application in the inhibition of male catches in pheromone traps, although the pheromone release of a single application did not cover the entire moth flight. Infestation was not reduced by MD application in highly infested and non-isolated orchards, where additional chemical treatments were required (Maini et al. 1982). Conversely, the percentage of infested fruits was kept below an economic threshold (1-1.5%) and was significantly lower than the untreated control (32-43%), without supplemental curative insecticides, in a more isolated orchard and in a region where the climatic conditions were less favorable for CM development (Sacco and Pellizzari-Scaltriti 1983).

Mating disruption against EGVM was first tested in central Italy by Vita et al. (1985), covering a 2-hectare vineyard, with small rubber tubes impregnated with synthetic pheromones provided by IRCHA (Paris). Similar tests were conducted several years later with handmade experimental dispensers in Trentino-South Tyrol (Ioriatti and Vita 1990).

For both the apple crop and vineyard, the a.i contained in the dispenser was released for a limited time, covering only one flight of the moth and requiring multiple applications throughout the year.

<u>Reliable long-release dispensers.</u> In both agroecosystems, the development of reliable delivery systems accelerated when the industry decided to become involved. In the late 1980s, the first "industrial" MD dispensers - called ECOPOM - were developed by the Istituto Donegani (Novara, Italy). The efficacy of these biodegradable, resin-treated filter paper dispensers (Rama, 1997) to control CM and *A*. *ljungiana* was tested in comparison to the black BASF ampulla (Fig. 1B) (Michelatti et al. 1990). At the same time the first release of Shin-Etsu technology, the polyethylene tube dispensers for the control of *G*. *molesta* (Domenichini et al. 1990) and *L*. *botrana* (Ioriatti, unpublished data) appeared in Italy. Various formulations proposed by industry were then tested by local research stations (Edmund Mach Foundation and Laimburg experimental

station) in collaboration with the advisory services, in order to assess the MD efficacy and promote the development of the most reliable devices.

*Apple***.** The early prototype of ISAGRO's "flakes" was made with resin–treated filter paper equipped with a plastic hook to hang them on the apple branches (Arsura et al. 1992) (Fig. 1C). Isagro flakes were a matrix kind of dispenser impregnated with the a.i. mixed with stabilizers; the a.i. was either E8,E10-12OH for CM control or Z11-14Ac which is the main pheromone component for LR management. In some experiments the Z11-14Ac was either pure or mixed with a percentage of Z9-14Ac, another common component of the LR pheromone bouquet (Ioriatti and Dalla Serra 1992). The size and thickness of the flakes were modified in order to meet the required release rate of pheromone and avoid the plastic hook, as well as to make the dispenser entirely biodegradable (Rama 1997). The final version of these dispensers was distributed under the trade name Ecopom Combi®. The dispensers were impregnated with 250 mg of E8,E10-12OH and 200 mg of Z11-14Ac together with an antioxidant, a UV screener, and a release modulator. When applied at the recommended density of 300 dispensers/ha, they released codlemone constantly for about 100 days, and Z11-14Ac for the entire season (Rama 1997). With this codlemone release rate, two applications of dispensers were needed to cover the entire mating activity of CM (Ioriatti et al. 1997). This aspect and the consequent cost of the labor were probably critical for the widespread use of these dispensers, and despite their efficacy in controlling both CM and LR, they were not commercially successful (Rama 1997; Ioriatti et al. 1997). The same company subsequently developed a new mating disruption dispenser, Ecodian® CP (Fig. 1D), to control several species of Lepidoptera, including codling moth (Rama et al. 2002, Charmillot et al. 2005, Tasin et al. 2005,). Ecodian® CP dispensers, made of low-cost biodegradable material molded into a hook shape, are loaded with 10 mg pheromone and applied twice per season at a high density (1,400–2,000 dispensers/ha). The development of this high-density approach met the need for effective pheromone-based strategies regardless of the size, shape and isolation of the treated area by emphasizing the competition between natural and synthetic sources (the false trail following mechanism) (Angeli et al. 2007).

The EcoTape FTF technology (Certis, Saronno, Italy) was based on the same principle (Fig. 1E). This device consisted of a continuous adhesive tape carrying a pheromone point source located every 60 cm, loaded with 2.5 mg of codlemone. The tape was strung through the orchard canopy twice per season, in order to obtain a high density of pheromone point sources (2,000 - 5,000 /ha) (Trona et al. 2009).

The Check Mate CM dispenser (Consep Membranes Inc., Oregon USA) (Fig. 1F) was a membrane system designed to release codling moth pheromones. Since the early 1990s, its pheromone release rate over time and its efficacy in controlling CM infestation have been tested both in Trentino-South Tyrol as well as in other Italian regions (Trematerra et al. 1993). The first dispensers tested were loaded with 104 mg of codlemone and applied twice a season. The amount of pheromone per dispenser was later increased up to 270 mg, however the consequent release was still not enough to cover the entire flight activity of CM with one single application per year (Rizzi et al. 2008).

The first reservoir dispensers were developed by BASF (Limburgerhof, Germany) and were first tested on apple crops in Trentino-South Tyrol in 1988 (Boscheri et al. 1992). The BASF dispenser was made up of two ampulla whose plastic characteristics and color (black, brown and red) varied over the years. One of the ampulla was filled with codlemone, the other with Z11-14Ac, which were released linearly in relation to the temperature sum (Neumann 1992). While the application rate remained constant at 500 dispensers/ha from the first trials to the final set up, the amount of pheromone varied over the years and among the field trials from 500 to 200 mg/ampulla, in order to ascertain the optimal dosage covering the entire flight activity of the moths throughout the season (Boscheri et al. 1992; Waldner 1997b; Neumann 1997; Varner et al. 1997). When the highest loads were used, a large amount of both pheromones were not released and remained inside the dispenser at harvest (Waldner 1997b). The current product (RAK® 3), loaded with 140 mg of codlemone, ensures the release of the a.i. for the entire season (Rizzi et al. 2008).

A second reservoir dispenser applied at 1000 units/ha was tested in Trentino-South Tyrol in 1993 as Isomate C-white (ShinEtsu, Japan), containing 112 mg of codlemone and small quantities of secondary compounds of the natural blend, 12OH and 14OH. Isomate® C-white was replaced in 1994 by Isomate® C-brown, loaded with 126 mg of a.i. Currently, Isomate® C plus and Isomate® C TT (twin tube) are available on the market and should be applied at 1000 and 500 units/ha, respectively. In 1996, Shin Etsu began developing multispecies dispensers for the combined control of CM and the secondary leafroller *A. orana, P. heparana, A. ljungiana*. The first prototype was called “Isomate® C special” and contained codlemone and Z11-14Ac.

The need to simultaneously control several tortricid apple pests led to the formulation of dispensers that are still available on the market as “Isomate® C LR” for the combined control of CM and the above-mentioned leafrollers, and “Isomate® C OFM” for the control of CM and the oriental fruit moth, *G. molesta*. Both reservoir dispensers, Isomate® and RAK®, release the pheromone throughout the entire season with only one application and are thus currently the most widely applied dispensers.

In 2010 the effectiveness of an aerosol technology (Check mate Puffer® CM - Suterra LLC - Bend, OR, USA) for the control of CM was tested in Trentino-South Tyrol (Baldessari et al. 2013). Puffers are battery-powered devices containing about 70 g of a.i., which release pheromones from pressurized aerosol cans every 15 minutes for 12 hours from 6 p.m. to 6 a.m. or every 30 min for 24 hours. During each puff, a quantity of 6.95 mg a.i. is emitted. The high release rate of pheromone per puff from aerosol dispensers is thought to compensate for their low application densities (2 puffers/hectare).

Although the aerosol dispensers, Puffer-CM, have been used successfully in mating disruption of CM for over 15 years both in the US and EU (Shorey and Gerber 1996; Marti et al. 2007; Stelinski et al. 2007), implementation protocols have been modified empirically and optimized to match the amount of pheromone released per day and hectare by hand-applied dispensers (Casado et al.

2014). Puffer CM+OFM was used for the combined control of codling moth and oriental fruit moth (Angeli et al. 2013).

Two similar formulations, Isomate Mist (Mister) CM (Pacific Biocontrol, Vancouver BC, CAN) and Semios CM (semiosBIO Technologies Inc – Vancouver, BC, CAN) have been developed very recently and are used in the region. All these aerosol systems seem particularly suitable when MD is applied as an area-wide strategy in apple orchards.

Grapevine. The first commercial dispensers used in Trentino-South Tyrol for the control of vine moths were RAK® 1 + 2 (BASF). Each dispenser contained 360 mg of a.i. for EGVM and 400 mg for EGBM in 1991 and 240 mg and 350 mg respectively in 1992. Although loaded with different quantities of a.i., these two dispensers showed a similar emission rate and were able to cover the entire period of adult activity. In the following three years growers applied RAK® 2 dispensers, specific for EGVM.

Since 1996, ShinEtsu "Isonet® L" dispensers have been generally preferred over RAK® technology, because of their better performance in terms of efficacy, release rate and lower cost (Ioriatti et al. 2004; Caruso et al. 2014). The application of these dispensers, which are highly specific for EGVM, and the total abandonment of insecticides, has led after a few years to the random resurgence of residual populations of EGBM. Thus double dispensers have started to be used again , which are similar to those used between 1991-1992, but adopting the "Isonet® LE" (ShinEtsu) formulation, containing approximately 200 mg a.i. for EGVM and 200 mg a.i. for EGBM. The substantial reduction in residual populations of EGBM as a result of using these dispensers, has led since 2003 to the use of a new formulation, , "Isonet® L plus", containing 200 mg of a.i. for EGVM and 20 mg a.i for EGBM, which was designed by ShinEtsu for the particular situation in Trentino-South Tyrol. This dispenser combined the effectiveness in containing small populations of EGBM, with a lower cost (35% less) compared to Isonet® LE and only 5% higher than Isonet® L. Isonet® L Plus has therefore replaced Isonet® L in most vineyards, except for some hilly areas where Isonet® LE is still applied to control the residential populations of the two moths. This technical solution has been adopted in other regions of central and northern Italy where EGBM represents a potential problem.

Checkmate Puffer® LB aerosols, containing 28-34 g of E7, Z9 12AC and used at 2.5-4 units per hectare, have been recently legally registered and were first used in vineyards in 2016.

ther MD formulations such as Ecodian LB (ISAGRO), Splat Lobesia (Isca Technologies) (Fig. 1G), No Mate Lb – (Syngenta) (Fig 1H), Hercon® disrupt EGVM – (Hercon Environmental), Exosect autoconfusion (Exosect Limited) have been tested in vineyards at an experimental level but they have not achieved registration in Italy.

Monitoring in MD

The strict relationship between population density and MD efficacy has led to adaptations of the threshold concept (Thomson et al. 1999). Commercially acceptable levels of control, in which pheromones are used without supplemental spraying of insecticides, are generally achieved only when the initial densities of moths are relatively low. Consequently, from the outset, advisors and applied entomologists in Trentino-South Tyrol aimed to provide growers with empirical threshold values, in terms of adults or eggs or larvae, which could help them in the application of MD. As for the monitoring of adults in MD vineyards and apple orchards, the absence of catches in pheromone traps is not in itself an index of effectiveness. Indeed, total trap shut down does not mean the absence of matings by feral females (Charmillot 1992; Bagnoli et al. 1993; Carlos et al. 2005; Thomson et al. 1999), while catching even a few units, may constitute a useful index of alert. Although a close relationship between the infestation recorded in a generation with the next one or between the overwintering population and the spring population does not exist, empirical alert thresholds both for larval infestation and catches in pheromone traps have been proposed.

<u>Alert thresholds based on adult captures</u>**.** For apple crops, the need to spray was decided on the basis of the catches recorded in the standard monitoring trap charged with 1 mg a.i. If the traps captured more than three males during the first flight, an insecticide treatment was immediately

recommended. The attempt to improve the meaning of the adult captures in MD fields led to the adoption of overloaded pheromone lures which were able to monitor male flight more effectively than the standard lures (Charmillot and Bloesch 1987; Waldner 1997; Tasin et al. 1998). In this case, the alert threshold corresponded to 7-8 cumulated captures per trap (Ioriatti et al. 1997). An increase in total moth catches in MD apple orchards was also obtained in traps baited with a blend of codlemone and pear ester (Schmidt et al. 2006), and a commercial lure is now available. Terra-cotta flower pots (1.5 litres) filled with a 50% mixture of red wine and water are extensively used in mating-disrupted vineyards to monitor the presence of males and females, and to check the mating ratio of females (Bagnoli et al. 2013).

Thresholds based on larval infestation. Based on previous experiences (Mani and Schwaller 1992; Neumann 1992), the extension services in Trentino-South Tyrol suggested avoiding spring preventive treatments when the previous year's harvest damage by CM on apple had not exceeded 1% (Waldner 1997b). If the harvest damage was between 1% and 3%, the application of an additional insecticide to control the larval infestation of the first generation was recommended, as soon as the first fresh penetrations were detected. If the wormy fruits exceeded 3% at harvest, insecticide treatments were suggested as soon as CM oviposition took place according to the forecasting model. Insecticide treatments were repeated in relation to the infestation rate detected in the following fruit check. During the vegetative season, growers were invited to regularly sample fruits and check them for fresh entry holes. Supplemental insecticide treatments were recommended when an action threshold of 0.3% infested fruits in June was exceeded, 0.5% in July-August, and 0.8% from mid-August onwards (Waldner 2005; Mattedi et al. 2008).

The number of CM overwintering larvae collected in the corrugated cardboard applied to the apple trunk in fall was used as an estimation of the overwintering population density of CM in the orchard (Audemard 1980; Charmillot 1980) in relation to the potential success of MD applied the following year. If the population density exceeded 1,000 overwintering larvae per hectare, mating disruption would probably not be effective in the following year, owing to low damage tolerance (Witzgall et al. 2008). Although the number of larvae collected in the cardboard varied considerably with the changing size of the plants, the amount of fruit and the characteristics of the bark, this empirical estimation was useful in the early stages of MD implementation in order to have an approximate reference value, which would enable the control technique to be assessed. An indicative threshold of 0.5 overwintering larvae per carton was thus suggested, beyond which, MD as a stand-alone technique would not be able to control the next spring's population (Ioriatti et al. 1997).

With regards to the vineyard, as in France and Switzerland (Neumann 1992; Charmillot and Pasquier 2000), in Italy sampling for infested bunches in the first generation was considered critical to decide on supplemental insecticide treatments in the following generation. In Trentino-South Tyrol this threshold was around 5% of infested bunches depending on the varieties, and around 10% for the Sangiovese variety in Tuscany (Ioriatti et al. 2004).

Area-wide application of mating disruption

From the first local experimental applications in the late 1980s, both on apple and grapevine, MD was applied on a large scale within a few years. In Trentino-South Tyrol, MD was applied on about 400 ha (300 on apple and 100 on grape) in 1993, increased up to 23,500 ha (14,000 on apple and 9,500 on grape) in 2004, reaching 31,650 ha today, i.e. 21,200 ha on apple which accounts for 75% of the total apple orchard area and 10,450 ha of vineyards, which corresponds to 66% of the total grapevine growing area. Where MD is not applied, the moth populations are too small or the orchards are unsuitable, due to their size, shape, or topography (Waldner 2005). In vineyards the hand applied dispensers are still used in 100% of the treated area, whereas in apple orchards they now cover only 56% of the total surface and the remaining part is treated with aerosol technology (Puffers®, Mister®).

Trentino-South Tyrol is an alpine region with agriculture characterized by small farms whose average surface ranges between 1.1 and 2.5 ha (smaller in vineyards, larger in apple orchards), very often fragmented into several plots and sloping lands. These conditions would not be suitable for the application of a method such as MD, which exploits wide homogeneous and flat surfaces but is hindered by farm fragmentation. However, these potential structural limitations have been overcome by the widespread dissemination of cooperatives. The cooperative systems manage 84% of the apple surface, owned by 13,000 growers grouped into 53 cooperatives and three producer organizations. A total of 65% of the vineyards are owned by about 10,000 wine growers grouped into 26 winery cooperatives.

The strong motivation of the various actors who were promoting IPM at the field level was critical for the implementation of the area-wide suppression program. They have worked together to replace the most hazardous pesticides with non-pesticidal systems, primarily MD. The first outcomes were the improvement in worker safety, the reinforcement of the biological control of mites, and the mitigation of bystander's exposure to the potential pesticide drift. These actors worked together to improve the perception that apple and grapes were produced with high consumer-safety standards, reducing residues on the final product, and thus opening up new market opportunities (Drogué and DeMaria 2012; Damos et al. 2015).

Given that Trentino-South Tyrol attracts many tourists, the promotion of a 'clean' agriculture was the driving force behind the MD implementation. Another positive factor was the availability of public financial support for the growers adopting MD. In viticulture from 1998 to 2001 the contribution was about 50% of the cost of the dispensers, increasing to 100% for certified organic farms. In the following years the subsidy system was changed and wine growers received the difference in cost between the most expensive MD and the cheapest chemical control. At present, even though no financial help is offered to wine growers, they are still strongly motivated to adopt MD.

The financial support system has been different in the apple industry. Since 1996, through their producer organizations, apple growers have received 50% of the dispenser cost as a measure included in the Co-finance of Operational Programs of the common market organization for fruits and vegetables (CMO) (EU Reg. 2200/96). From an operational point of view, for both crops the application of MD was initially promoted and mediated by the cooperatives, who were involved in buying and distributing the dispensers. Consultants provided by public advisory services or the growers' organizations trained farmers regarding MD application, monitoring and efficacy assessment in the field. (Varner and Ioriatti 1992; Ioriatti et al. 1997; Waldner 2005). This approach led to the early implementation of some hundred hectares of MD, soon revealing that the method would work better on wide areas, by solving many of the limitations (fragmented farms, patchy applications, sloping plots) associated with its application and quite soon achieving high levels of efficacy.

In the first three years of area wide application (240 ha) in the apple orchards in Trentino, the number of chemical treatments for the control of CM and LR was respectively of 50%, 80% and 90% lower than in similar orchards, where only a chemical control was applied (Ioriatti et al. 1997). In the following years, the constant increase in the MD treated area led to a substantial decrease in the percentage of apple samples carrying insecticide/acaricide residues (Fig. 4) and thus met the requirements of the supermarket chains regarding the limits on pesticide residues on fruit, whose amount had to be well below that permitted by the law (Waldner 2009; Baldessari et al 2013a). The application of area-wide MD, also significantly reduced the environmental impact of the tortricid pest control (Ioriatti et al., 2011b).

In South Tyrol, MD applied on 14,000 ha, on average required an additional 0.9 chemical treatment to control CM compared to 6 insecticide treatments usually applied without MD. The results of sampling on 223 MD treated orchards reported an average harvest damage of 1.1%, with 77.6% of the orchards with less than 1% damage (Waldner 2005).

Results were more striking when MD was applied to control EGVM in vineyards. Nearly the entire MD treated area did not require additional chemical treatments to control EGVM and EGBM. One insecticide a year was necessary on less than 100 ha of marginal and sloping vineyards bordering a forest, where residential populations of EGBM occurred. This outcome of MD implementation mitigated the conflict between citizens and farmers, generated by the proximity of the chemical treated fields to private houses and tourist facilities. These experiences are also a good example for other Italian and European regions where MD could benefit from area-wide applications.

Following the example of Trentino – South Tyrol, in various Italian regions, big farms and grower associations have recently been planning to apply the method with an area-wide approach, making the total surface of Italian vineyards covered with MD about 30,000 ha in 2016. An impressive area-wide project was launched recently in Spain, in the vineyards of the DO Carinena (Spain), where MD was applied on 200 ha in 2011 and on 13,400 ha in 2015 with excellent results (Lucchi et al. 2015).

Possible Constraints

The application of MD is generally not a viable solution for all vineyards and apple orchards and their pests. MD is less effective when applied in small and isolated plots, which are sloping with an irregular shape, neither is it effective against a number of pests whose mating is not mediated by olfactory cues. In fact, MD is a very selective preventive method, which controls the target pest and in most cases allows the establishment of 'conservation biological control' against target and non-target pests. However, orchards and vineyards can host many arthropod species having the potential to reach the pest status. When MD is applied successfully to large areas, the reduction or elimination of insecticides can foster the occurrence of secondary pests that require specific control measures, thus outweighing the benefits of MD and limiting its adoption. The presence of different phytophagous species to the target ones is a problem concerning both initial and ongoing phases in the MD decision-making process. Local outbreaks of the coccids *Parthenolecanium corni* (Bouchè) and *Pulvinaria vitis* (L.), the pseudococcid *Planococcus ficus* (Signoret) and the drosophilid *Drosophila suzukii* Matsumura on grape and the constant noxious presence of aphids *Dysaphis plantaginea* (Passerini) and *Eriosoma lanigerum* (Hausmann) on apple are critical issues for growers applying MD.

A different and more complicated problem is connected to the management of Hemiptera vectors of pathogenic microorganisms. *Cacopsylla melanoneura* (Förster) and *C*. *picta* (Förster) (Psyllidae) on apple and *Scaphoideus titanus* Ball (Cicadellidae) on grapevines are specific vectors respectively of the phytoplasma of apple proliferation *Candidatus* Phytoplasma mali and of flavescence dorée, *Candidatus* Phytoplasma vitis, two diseases that in Italy are subject to mandatory control. Novel challenging strategies to control these insect vector by exploiting vibrational communication are promising (Polajnar 2015).

In the last 20 years the management of these non-target insects carried out with the judicious use of insecticides has not decreased the innovative weight of MD and the interest in its adoption.

Future needs and trends

The extensive application of MD on apple and grapevine in Trentino-South Tyrol has achieved very promising results. However further research and investments are needed to improve the efficacy of available tools and to manage the accidentally introduced alien pests, without ruling out the use of pheromones for tortricid control.

It is extremely important continue the search for better performing formulations such as those that exploit aerosol technology, which now offers a viable alternative to hand-applied dispensers on apple (Baldessari et al. 2013b). Aerosol technology enables the timely, consistent and homogeneous release of a large amount of pheromones according to the behavior of the pest and enables the cooperatives to directly control the application of the devices.

Unfortunately, while the foliar phytotoxicity caused by the contact of codlemone with the canopy can be solved by the appropriate placement of emitter units (Angeli et al. 2013), the problem of the propellant (r134a and r152a fluorine compounds), used in most cases to disperse the a.i, is still significant. Given that the propellant is one of the substances responsible for the ozone hole, it is a critical weakness in the system.

The effectiveness of aerosol formulations in viticulture still needs to be demonstrated. Since the first flight of EGVM takes place largely with the complete absence of vegetation, this represents a weak point in the system because of the rapid loss of a.i. into the atmosphere of the vineyard.

Another solution is to develop other multi-species dispensers for pests whose sex pheromone blend is known. Field trials to assess the efficacy of multidispensers are in progress not only by mixing the pheromones of Lepidoptera (i.e *L. botrana* + *A. ljungiana*), but also combining the pheromone of species belonging to different taxonomic orders such as *L. botrana* and *P. ficus*.

In all cases, the registration process of mating-disruption products and dispensing systems should be made easier, faster and cheaper. This would encourage manufacturers to invest in new formulations, which are selectively used for only one species and do not allow large incomes.

Monitoring in MD fields is still an open issue. Pheromone traps are not as reliable as stand-alone monitoring tools, since the information they provide needs to be integrated with frequent field scouting of target species in order to plan possible control measures. The great expectations of pear ester for monitoring CM females in apple crops (Light and Knight 2001) were misplaced: female catches are episodic and unfortunately do not provide significant improvement in the quality of information.

The development of more reliable monitoring systems remains an important objective wherever MD is applied. Liquid alimentary traps may be suitable for catching females, but are not selective and not easy to handle (Bagnoli et al. 2013). The multicomponent headspace of fermenting substances as well as the complex of plant-derived kairomones could represent a source of volatiles potentially useful for monitoring females in MD treated fields. (Anfora et al. 2009; Tasin et al. 2011).

In the last 20 years, the process of MD implementation has several times had to face sudden and unexpected issues, potentially jeopardizing its use. Of these, the ongoing climate change and the accidental introduction of new invasive species play a major role.

Climate change will alter the outbreak patterns and geographical ranges of insects. The consequences are difficult to predict, however the increasingly frequent infestation of the Mediterranean fruit fly *Ceratitis capitata* Wiedeman (Diptera: Tephritidae) in apple orchards in Trentino-South Tyrol and of *P*. *ficus* in vineyards are probably effects of climate change (Rigamonti 2004; Christanell 2013; Ioriatti 2015; Varner et al. 2015).

Despite the international cooperation and regulatory systems put in place to inhibit the spread of plant pests, the international trade of fruits and plants is mainly responsible for facilitating their spread. Among the potentially most invasive species endangering the apple and grapevine orchards in northern Italy, are the brown marmorated sting bug *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) and the spotted wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae) (Cesari et al. 2015; Cini et al. 2012).

In addition to being a suitable tool for IPM, MD as a stand-alone practice or combined with insecticide use could be an effective tactic for eradicating accidentally introduced alien moths, as recently observed in California for EGVM (Cooper et al. 2014; Lance et al. 2015). In addition, a multi-tactic program combining MD and a sterile insect technique to replace the supplementary insecticide treatments (Judd and Gardiner 2005) are under evaluation in Trentino-South Tyrol.

The adoption and the development of the area-wide mating disruption project in Trentino-South Tyrol have been undoubtedly fostered by the co-existence of several favorable factors which have brought together researchers, advisors, cooperatives, growers, pheromone distributors and industries. The success obtained in Trentino-South Tyrol has not been replicated in the rest of Italy, where the separation between research institutes and growers has hindered the dissemination of ecological strategies. In fact, in Italy MD is currently applied to 41% of the total surface cultivated with apple crops, more than 64% of which is in Trentino-South Tyrol. Conversely, MD covers only 4% of the total vineyard surface, approximately 30% of which is in Trentino-South Tyrol.

We believe that there would not be insurmountable obstacles to a further significant increase in MD implementation in Italy if the interest among research scientists in promoting and transferring existing knowledge will be cultivated.

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Legends

Figure 1. Different types of pheromone dispensers: A) Rubber tube handmade dispenser; B) BASF ampulla; C) Ecopom Combi ; D) Ecodian CP Isagro; E) CheckMate Suterra membrane; F) Ecotape FTF Certis; G) ISCA SPLAT; H) No Mate Lb (Syngenta)

Figure 2. The most common hand applied dispensers in Italy: A) ShinEtsu Isonet L; B) BASF-RAK dispenser; C) Isonet LE ShinEtsu dispenser.

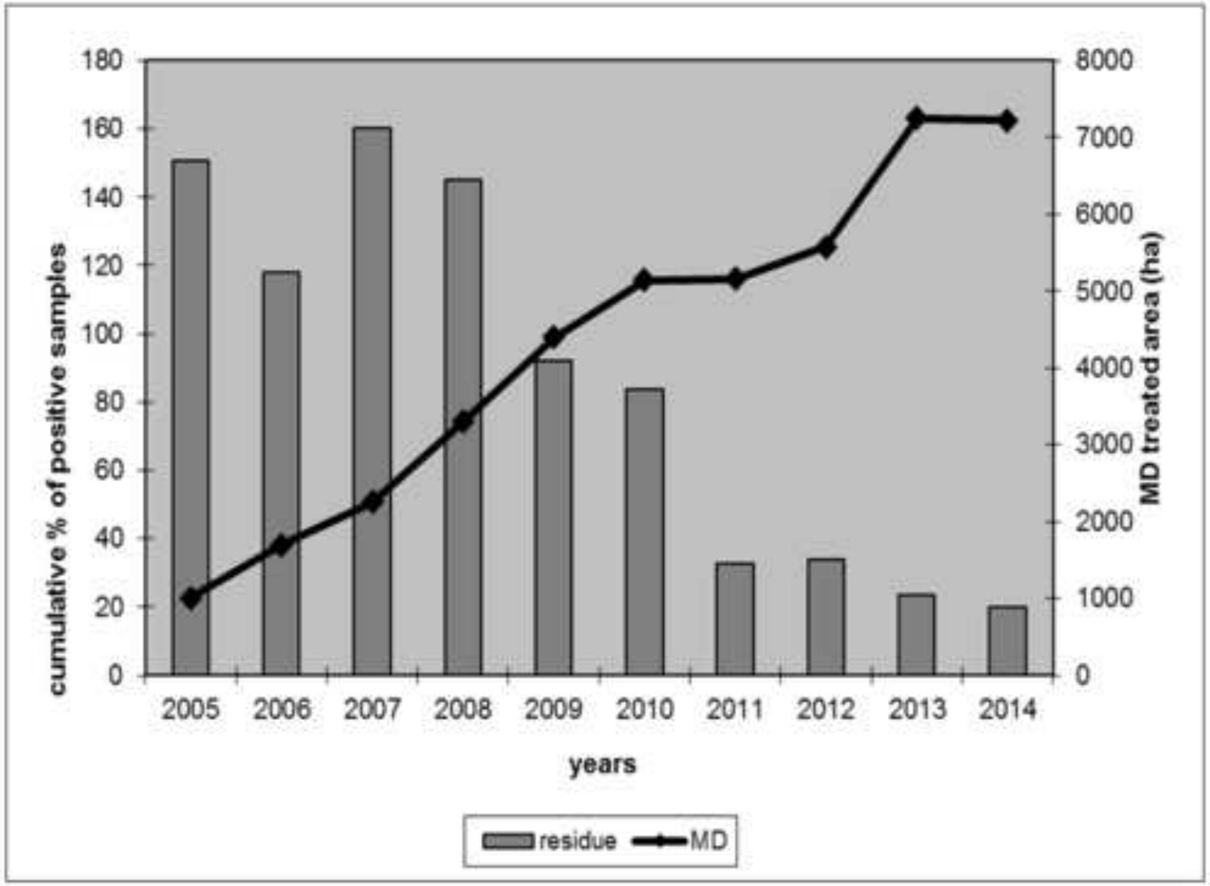
Figure 3. Aerosol dispensers: A) Puffer Suterra; B) Mister (Mist) ShinEtsu-Pacific Biocontrol; C) Semios CM (semiosBIO Technologies Inc)

Figure 4. Cumulative percentage of apple samples carrying insecticide/acaricide residues (gray bars) and area treated (ha) with mating disruption (black line) in Trentino. Samples of apple (N= 560 to 725 according to the year) are analyzed for insecticides and acaricide residues. Percentages of samples with single pesticide residues are cumulated and related to the MD treated area.


A
B
C
D
E
F
G
H


A
B
C


A
B
semiosBIO
C


180
160
140
120
100
80
60
40
20
0
cumulative % of positive samples
8000
7000
6000
5000
4000
3000
2000
1000
0
MD treated area (ha)
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
years
residue
MD