Bulletin of Insectology Supplemental Material

Title: Review on imidacloprid diffusion route and a case study: from apple orchard to the honey bee colony matrices

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Supplemental material S1. Location of the two apiaries (3 hives each) in "Non Valley" (Trentino, Italy). The two areas are separated by an airline distance of 9.3 km (Google Earth[®]). Low Imidacloprid Field (LIF), in the municipality of Tassullo on an altitude of 546 m a.s.l. (GPSS-coordinates: 46.333333°, 11.05°) and High Imidacloprid Field (HIF) in the municipality of Cloz on an altitude of 792 m a.s.l. (GPSS-coordinates: 46.421389°, 11.089444°), both in an agricultural landscape of Trentino-Alto Adige Region (Northern Italy). In the LIF municipality, most of the apple orchards were treated with imidacloprid (Confidor), but some were treated with spirotetramat (Movento), while in the HIF municipality only imidacloprid (Confidor) was used.

Supplemental material S2. Pesticides used in the apple orchard context of IPM in both experimental fields (LIF and HIF); in bold the treatment with Imidacloprid. For the treatment with imidacloprid (Confidor 200 SL) 15 hl/ha were used.

Treatment time	Commercial product	Category	Active ingredient	Dose used	Field	
	Grado 66 WG	Fungicide	Dithianon	50 g/hl	HIL/LIF	
	Tianon WG	Fungicide	goryActive ingredientDosejicideDithianon50 gjicideDithianon50 gjicideBupirimate50 gticideEtofenprox25 mticideFlonicamid8 gjicideDithianon50 gjicideDithianon50 gjicideDithianon50 gjicideDithianon50 gjicidePlufenoxuron60 mjicidePenconazole12.5 mjicideDithianon50 gjicideSulfur200 gjicideSulfur200 gjicideDithianon50 gticideImidacloprid40 mjicideDithianon50 gticideImidacloprid30 mticideImidacloprid30 mticideEmamectin benzoate220 gjicideSulfur200 gjicideSulfur200 gjicideDithianon50 gticideImidacloprid30 mticideEmamectin benzoate220 gjicideSulfur200 gjicideDithianon50 gjicideDithianon50 gjicideDithianon50 gjicideEmamectin benzoate220 gjicideDithianon50 gjicideDithianon50 gjicideDithianon50 gjicideDifenoconazole37 gjicideDifenoconazole37 g<	50 g/hl	HIL/LIF	
Late March to early April	Nimrod 250 EW	Fungicide	Bupirimate	50 g/hl	HIL/LIF	
	Trebon Up	Insecticide	Etofenprox	25 ml/hl	HIL/LIF	
	Teppeki	Insecticide	Flonicamid	Dose used 50 g/hl 50 g/hl 50 g/hl 25 ml/hl 8 g/hl 50 g/hl 37 g/hl 60 ml/hl 50 g/hl 37 g/hl 60 ml/hl 50 g/hl 12.5 ml/hl 40 g/hl 30 g/hl 200 g/hl 50 g/hl 200 g/hl 12 ml/hl 200 g/hl 60 ml/hl 100 g/hl 40 g/hl 100 g/hl 37 g/hl 230 g/hl 60 ml/hl 100 g/hl 37 g/hl 30 g/hl 50 g/hl </td <td>HIL/LIF</td>	HIL/LIF	
	Grado 66 WG	G Fungicide Dithianon		50 g/hl	HIF	
	Score 10 WG	Fungicide	Difenoconazole	37 g/hl	HIF	
	Colosseo	Insecticide	Flufenoxuron	60 ml/hl	HIF	
T	Delan WG	Fungicide	Dithianon	50 g/hl	LIF	
Late April to early May	Topas 200 EW	Fungicide	Penconazole	12.5 ml/hl	LIF	
	Delan WG	Fungicide	Dithianon	40 g/hl	LIF	
	Chorus	Fungicide	Pymetrozine	30 g/hl	LIF	
	Thiopron	Fungicide	Sulfur	Dose used 50 g/hl 50 g/hl 50 g/hl 50 g/hl 25 ml/hl 8 g/hl 50 g/hl 37 g/hl 60 ml/hl 50 g/hl 37 g/hl 60 ml/hl 50 g/hl 12.5 ml/hl 40 g/hl 30 g/hl 200 g/hl 50 g/hl 200 g/hl 12.5 ml/hl 50 g/hl 30 ml/hl 200 g/hl 12 ml/hl 200 g/hl 10 g/hl 10 g/hl 100 g/hl 100 g/hl 100 g/hl 30 g/hl 30 g/hl 30 g/hl 30 g/hl 30 g/hl </td <td>LIF</td>	LIF	
-	Grado 66 WG	Fungicide	Dithianon	50 g/hl	HIF	
	Dursban 75 WG	Insecticide	Chlorpyrifos	50 g/hl	HIF	
	Hubertex		Kaolin	200 g /hl	HIF	
	Confidor 200 SL	Insecticide	Imidacloprid	40 ml/hl	HIF	
	Topas 220 EW	Fungicide	Penconazole	12.5 ml/hl	HIF	
Mid May	Delan WG	Fungicide	Dithianon	50 g/hl	LIF	
	Confidor 200 SL	Insecticide	Imidacloprid	30 ml/hl	LIF	
	Affirm	Insecticide	Emamectin benzoate	220 g /hl	LIF	
	Delan WG	Fungicide	Dithianon	50 g/hl	LIF	
	Alisè 75 WG	Insecticide	Chlorpyrifos	50 g/hl	LIF	
	Thiopron	Fungicide	Sulfur	200 g/hl	LIF	
	Topas 220 EW	Fungicide	Penconazole	12 ml/hl	HIF	
	Thiopron	Fungicide	Sulfur	200 g/hl	LIF	
	Ohayo	Fungicide	Fluazinam	60 ml/hl	LIF	
Late May to early June	Hubertex		Kaolin	100 g/hl	LIF	
	Delan WG	Fungicide	Dithianon	40 g/hl	LIF	
	Hubertex	W Fungicide Bupirimate 30 g/ml F Insecticide Etofenprox 25 ml/hl F Insecticide Flonicamid 8 g/hl F G Fungicide Dithianon 50 g/hl F G Fungicide Difenoconazole 37 g/hl F Insecticide Flufenoxuron 60 ml/hl F F Fungicide Dithianon 50 g/hl F F W Fungicide Penconazole 12.5 ml/hl F Fungicide Pymetrozine 30 g/hl F F Fungicide Sulfur 200 g/hl S G F G Fungicide Dithianon 50 g/hl S G G F G	LIF			
-	Daithan	Fungicide	Dithianon	50 g/hl	HIF	
	Score 10 WG	Fungicide	Difenoconazole	37 g/hl	HIF	
	Affirm	Insecticide	Emamectin benzoate	ngredientDose usedianon50 g/hlianon50 g/hlianon50 g/hlimmate50 g/hlimprox25 ml/hlcamid8 g/hlianon50 g/hlconazole37 g/hloxuron60 ml/hlianon50 g/hloxuron60 ml/hlianon50 g/hlianon50 g/hlianon50 g/hlianon50 g/hlianon50 g/hlianon50 g/hlianon50 g/hlianon50 g/hlolin200 g/hlianon50 g/hlolin200 g/hlianon50 g/hlolin200 g/hlianon50 g/hlianon50 g/hlianon50 g/hlianon50 g/hlianon50 g/hlianon50 g/hlianon50 g/hlianon50 g/hlianon12 ml/hlianon50 g/hlianon50 g/hl <td>HIF</td>	HIF	
Mid-June	Ohayo	Fungicide	Fluazinam		LIF	
	Nimrod 250 EW	Fungicide	Bupirimate	50 g/hl	LIF	
	Prodigy	Insecticide	Methoxyfenozide	35 ml/hl	LIF	
	Score 10 WG	Fungicide	Difenoconazole	37.5 g/hl	LIF	

Supplemental material S3. The province of Trento is a mountainous district of Trentino-Alto Adige Region (Northeast of Italy) and an important apple producer (10,200 hectares) with an annual production of approximately 504,278 tons accounting for about 22.82% of Italian productions (Source: Istituto Nazionale di Statistica). Cultivated varieties include Golden Delicious (60% of production), Fuji, Red Delicious, Royal Gala, the local Renetta Canada and others. The most notable factor is the small average size of farms - about 2.5 hectares. The reduced size of farms has made it imperative for growers to band together into cooperatives to organize marketing. Today, almost 95% of the apples produced are assigned to the cooperatives. In 1989, the Public Administration of Trento approved a program for Integrated Production standards, so farmers could benefit from a market position with clearly defined quality standards.

Growers are obliged to sign agreements and the cooperatives are responsible for their members' activities. Since 1991, Integrated Fruit Production (IFP) guidelines have covered all aspects of production, including inspection for compliance and fines payable for infractions. The guidelines, which are updated every year, include the choice of varieties, pruning systems, grass cover, nutrition, thinning, irrigation, harvest time, farm records, and pesticide use. The list of approved chemicals is integrated with newly registered compounds judged consistent with IFP. Impacts on beneficial organisms and resistance management requirements are taken into consideration. Considering the high participation in cooperatives, the apple crop in Trentino is almost entirely managed by IFP standards. A minor portion of the orchards is organic (641 hectares) with a trend of increase over the years. Management of insects and pests according to IFP guidelines for apples, is achieved by combining sampling, thresholds and pest forecasts with biological and cultural control methods and the use of presumed selective pesticides. In the last twenty years, the use of more selective insecticides has facilitated the biological control of spider mites by predatory phytoseid mites and typically no miticide treatment is now applied.

Apple scab is managed with 12-18 treatments using preventative and curative fungicides. Codling moth has two generations per year and mating disruption is sometimes combined with insecticides (high-pressure orchards, 30%). The most common situation includes two aphicide applications at pre- and post-flowering stages and one additional treatment using insecticides with a different mode of action against leafrollers. Also, a spring insecticide treatment (pre-flowering stage) against psyllids is mandatory because they are vectors of Apple Proliferation (AP) disease. AP occurs in all countries of Central and Southern Europe, but its highest incidences are in Trentino and southwestern Germany.

The AP disease causes important economic losses due to small size of fruits and impoverished taste. In Trentino, uncontrolled aphids, codling moth, leafrollers and apple scab would likely damage 100% of the apples. By preventing damage from insects and pathogens, fungicides and insecticides play an essential role in defending productions, although nowadays efforts are directed towards a rationalization of their use in favour of more sustainable techniques and natural biological control.

Supplemental material S4. Absolute content of imidacloprid, olefin imidacloprid and 5hydroxy imidacloprid found in honey bee body (A), honey (B), bee bread (C), wax (D), pollen (E) and royal jelly (F), in both experimental fields (LIF and HIF). The limit of quantification (LOQ) is reported in brackets.

		Honey bee body (A)						
			LIF	-		HIF		
	Replicate	Imidacloprid (LOQ 2 ppb)	Olefin imidacloprid (LOQ 10 ppb)	5hydroxy imidacloprid (LOQ 5 ppb)	Imidacloprid (LOQ 2 ppb)	Olefin imidacloprid (LOQ 10 ppb)	5hydroxy imidacloprid (LOQ 5 ppb)	
	1	absent	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
April	2	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	3	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	1	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	
May	2	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	
-	3	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
June	2	absent	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
	3	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	1	< LOQ	< LOQ	< LOQ	absent	< LOQ	< LOQ	
July	2	< LOQ	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	3	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	

		Honey (B)						
			LIF		• • •	HIF		
	Replicate	Incide alamaid	Olefin	5hydroxy	Incide alamid	Olefin	5hydroxy	
		(I OO 2 mmh)	imidacloprid	imidacloprid	(LOO 2 mmb)	imidacloprid	imidacloprid	
		(LOQ 2 ppb)	(LOQ 10 ppb)	(LOQ 5 ppb)	(LOQ 2 ppb)	(LOQ 10 ppb)	(LOQ 5 ppb)	
	1	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	
April	1 2	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	3	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
	1	6.65	< LOQ	< LOQ	2.62	< LOQ	< LOQ	
May	2	4.00	< LOQ	< LOQ	3.00	< LOQ	< LOQ	
-	3	absent	< LOQ	< LOQ	4.00	< LOQ	< LOQ	
	1	3.00	< LOQ	< LOQ	4.00	< LOQ	< LOQ	
June	2	4.00	< LOQ	< LOQ	3.00	< LOQ	< LOQ	
	3	9.00	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	1	3.00	< LOQ	< LOQ	2.00	< LOQ	< LOQ	
July	2	< LOQ	< LOQ	< LOQ	3.00	< LOQ	< LOQ	
	3	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	

		Bee bread (C)						
			LIF			HIF		
	Replicate	Imidacloprid (LOQ 2 ppb)	Olefin imidacloprid (LOQ 10 ppb)	5hydroxy imidacloprid (LOQ 5 ppb)	Imidacloprid (LOQ 2 ppb)	Olefin imidacloprid (LOQ 10 ppb)	5hydroxy imidacloprid (LOQ 5 ppb)	
	1	9.450	< LOQ	< LOQ	25.500	< LOQ	< LOQ	
April	2	8.000	absent	absent	absent	absent	absent	
	3	16.000	absent	absent	7.000	absent	absent	
	1	27.700	< LOQ	< LOQ	54.100	< LOQ	absent	
May	2	6.000	absent	absent	37.000	absent	absent	
	3	7.000	absent	absent	64.000	absent	absent	
	1	17.000	absent	absent	24.000	absent	absent	
June	2	10.000	absent	absent	32.000	absent	absent	
	3	5.000	absent	absent	5.000	absent	absent	
	1	absent	absent	absent	absent	absent	absent	
July	2	2.000	absent	absent	absent	absent	absent	
	3	absent	absent	absent	absent	absent	absent	

		Wax (D)						
			LIF			HIF		
	Replicate	eplicate , , , , Olefin		5hydroxy	Imidaalannid	Olefin	5hydroxy	
		$(I \cap O \cap 2 \text{ mm})$	imidacloprid	imidacloprid	(I OO 2 mmh)	imidacloprid	imidacloprid	
		(LOQ 2 ppb)	(LOQ 10 ppb)	(LOQ 5 ppb)	(LOQ 2 ppb)	(LOQ 10 ppb)	(LOQ 5 ppb)	
	1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
April	2	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	3	absent	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	1	3.32	< LOQ					
May	2	5.00	< LOQ	< LOQ	3.00	< LOQ	< LOQ	
	3	absent	< LOQ	< LOQ	4.00	< LOQ	< LOQ	
	1	6.00	< LOQ	< LOQ	3.00	< LOQ	< LOQ	
June	2	2.00	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	3	10.00	< LOQ	< LOQ	absent	< LOQ	< LOQ	
	1	2.00	< LOQ	< LOQ	3.00	< LOQ	< LOQ	
July	2	absent	< LOQ	< LOQ	2.00	< LOQ	< LOQ	
•	3	4.17	< LOQ	< LOQ	2.17	< LOQ	< LOQ	

	Pollen (E)						
		LIF			HIF		
	Imidacloprid (LOQ 2 ppb)	Olefin imidacloprid (LOQ 10 ppb)	5hydroxy imidacloprid (LOQ 5 ppb)	Imidacloprid (LOQ 2 ppb)	Olefin imidacloprid (LOQ 10 ppb)	5hydroxy imidacloprid (LOQ 5 ppb)	
April	15.7	< LOQ	< LOQ	28.4	< LOQ	< LOQ	
May	66.5	< LOQ	< LOQ	91.1	< LOQ	< LOQ	

	Royal jelly (F)							
	Imidacloprid (LOQ 2 ppb)	LIF Olefin imidacloprid	5hydroxy imidacloprid	Imidacloprid (LOQ 2 ppb)	HIF Olefin imidacloprid	5hydroxy imidacloprid		
Julv	<l00< td=""><td>(LOQ 10 ppb) <loo< td=""><td>(LOQ 5 ppb) <loo< td=""><td><l00< td=""><td>(LOQ 10 ppb) <loo< td=""><td>(LOQ 3 ppb) <loo< td=""></loo<></td></loo<></td></l00<></td></loo<></td></loo<></td></l00<>	(LOQ 10 ppb) <loo< td=""><td>(LOQ 5 ppb) <loo< td=""><td><l00< td=""><td>(LOQ 10 ppb) <loo< td=""><td>(LOQ 3 ppb) <loo< td=""></loo<></td></loo<></td></l00<></td></loo<></td></loo<>	(LOQ 5 ppb) <loo< td=""><td><l00< td=""><td>(LOQ 10 ppb) <loo< td=""><td>(LOQ 3 ppb) <loo< td=""></loo<></td></loo<></td></l00<></td></loo<>	<l00< td=""><td>(LOQ 10 ppb) <loo< td=""><td>(LOQ 3 ppb) <loo< td=""></loo<></td></loo<></td></l00<>	(LOQ 10 ppb) <loo< td=""><td>(LOQ 3 ppb) <loo< td=""></loo<></td></loo<>	(LOQ 3 ppb) <loo< td=""></loo<>		