l'apis

DOSSIER

2017-2019 ENVIRONMENTAL BIO-MONITORING WITH BEES

Poste Italiane S.p.A. - Spedizione in A. P. - 70% - NO/ALESSANDRIA - Anno XXVII - Supplemento N. 2 - al N. 9/2019 di l'apis

SUMMARY

DOSSIER | 2017-2019 ENVIRONMENTAL BIO-MONITORING WITH BEES

by Luca Bosco, Marco Bergero, Francesco Panella, Sara Oldani, Chiara Concari

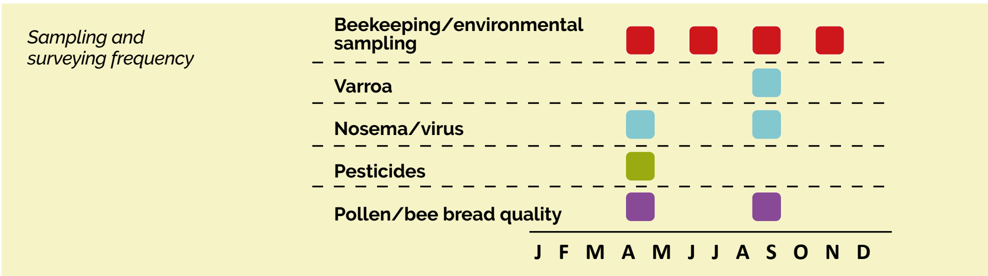
curated by **Sara Oldani |** Graphic design by **Graziana Garbeni**

On the cover *Bee on Helianthus annuus (Sunflower)*, Photo by **Carlo Gatti**

Beenet (story of an ambitious project)

Introduction by Claudio Porrini

The use of bees in environmental monitoring dates back to 1935 when J. Svoboda (1961) revealed the negative impact of industrial pollutants on bees collecting honey and pollen in Czechoslovakia highly populated and industrialized areas. In the following years, numerous studies were carried out to verify how effective this hymenopter could be as an indicator of the presence of contaminants in the environment. It is worth stressing the difference between two bio-monitoring methods right away:
a) the monitoring of bees' health whereby bees are the subjects of observation and where the surrounding environment is assessed and correlated with the evolution of the 'strength' of the family and the presence of various pathogens/contaminants (in this case pesticides) through observations, samples and laboratory analysis;
b) the bio-monitoring with bees whereby the environment is the subject of observation and bees are used as biological indicators.
Although the field protocols, laboratory analysis and data interpretation differ, in some cases the two methods can obviously overlap.
The first method was used in research programs such as ApeNet (2009-2010) and BeeNet (2011-2014), whereas the second was applied in projects such as the first great study carried out at a national level between 1983 and 1986, as well as in the following studies where environmental contamination from pesticides, heavy metals, radio-nuclides, polycyclic aromatic hydrocarbons, etc. were monitored in many agricultural, natural, urban and industrial areas in Italy.
However, as shown in the title, in this context, we will talk about the BeeNet project and its importance for apiculture. BeeNet and the earlier ApeNet were born from the need to create a national monitoring network in Italy so as to collect information on bees' health. The BeeNet network, which was managed by CREA-api, the University of Bologna, IZSVe and SIN, was organized in modules, each one made of five surveying control units (apiaries) in turn made of 10 hives each, located in geographic sites that are representative of the various agronomical and environmental contexts of the area. Each module was managed by a beekeeper who, 4 times a year, collected apicultural/environmental samples to assess the level of varroa infestation, virus and nosema infection in bees, as well as the presence of pesticides and raw proteins in bee bread.



The network was made of 300 control units approximately, that is, 60 modules, for a total of 3,000 monitored hives. The project included a 'Beekeeping First Responder Team' (SPIA) who, in collaboration with the local health veterinary services, gathered the episodes of bees' die-off reported by beekeepers - including those happened outside of the network. In special cases, the team promptly inspected the affected units. The BeeNet system was supported by a computer platform aimed at gathering and storing the information collected in the field as well as the results of laboratory analysis. The portal could count on interactive instruments to report anomalous events (bees' die-off) and allowed the thematic consultation of all the information related to the collected data as well as the compilation of statistics on a geographical basis.
Why BeeNet was an ambitious project? Mainly because it was innovative both in terms of research and for the relationship with beekeepers. From an experimentation perspective, the project facilitated the acquisition of important data on the diffusion and the quantity of the main pathogens (varroa, nosema, virus) and pesticides present in the hives. The following data, for example, were the most concerning. Between 2012 and 2014, about 50% of the stored bee bread samples were contaminated by at least one pesticide. Out of these, about 35% on average presented more than a residue: 48% were insecticides and 45% were fungicide. In addition, 29% of the active substances detected were no longer allowed. All the beekeeping organizations and associations contributed to the proper functioning of the project and, five years after the end of the project, many beekeepers still talk about BeeNet as a positive thing to recall.

Aspromiele project

Aspromiele actively participated in bio-monitoring studies with bees throughout the years, both on a local level (Valle Pellice, Turin, in 2001; Province of Genoa in 2003; Val di Susa, Turin, 2004-2005; Province of Cuneo, in 2011) and on a national level (2012-2014 BeeNet project and 2012-2014 Unaapi Pollen Project[1]). These experiences allowed the association to develop professional competence in the field. The need and the opportunity to examine the environmental issue in depth through the use of hive matrices is not the 'cultural' and technical preserve of Aspromiele and Unaapi or other beekeeping associations only. In fact, in the past years, public institutions as well as beekeeping companies showed their interest in issues related to the poisoning of bee colonies, being it punctual, chronicle or sub-lethal.

Although the institutions' interest strongly diminished, at least at a national level, after the closing of the BeeNet project in 2014, the quality of the data collected during the bio-monitoring activities proved to be valuable not only from a 'cultural' and technical perspective but from a political and practical perspective too. The data are indeed completely available so as to provide the world of beekeeping companies with a useful and efficient instrument, able to defend the survival of bees and protect the economic interests. The availability of reliable data on the environment health allows the development of efficient defence and counter-proposal strategies to oppose the frequent negligence of anthropic activities. In this perspective, environmental data gathering and discussion has become the main objective that made Aspromiele decide, in 2017, to start and set up a network of environmental bio-monitoring with the use of bees as indicators of the salubrity of Piedmont agro-environment.

The idea of the project took shape throughout time within the association thanks to its participation to the technical coordination activity of other government bodies' that deal with agricultural issues at a local level, in particular those belonging to Piedmont Plant Protection Division. At an institutional level, the principles behind the environmental bio-monitoring of cultivated areas perfectly match with the prescriptive purpose of the new industry regulation (NAP - National Action Plan to achieve the sustainable use of phytosanitary products) and its implementation nationwide. At the time this report is being written, the NAP is being revised and updated in view of the next RDP. The local bodies' availability to cooperate with the association strengthened Aspromiele's idea to activate a network of bio-monitoring control units whose data are shared with Piedmont Administration at all levels. The hope for an increased sensitivity towards the environmental safeguard issue and for a wiser protection of useful insects from the institutions can

Photo by Samuele Colotta

indeed only be achieved by sharing all the projects and the data gathered with them, so as to aim at a peaceful and enhanced cohabitation between beekeepers and farmers in all agricultural industries.

If on the one hand, the use of phytosanitary products keeps causing the contamination of the environment in the form of residues in food products able to cause both short-term and long-term issues to living beings, on the other hand, the legislation protects against this danger at a national level (art. 4 L. 313/2004). The national regulation then empowers the local governments to forbid, limit, penalize or grant exemptions. Within this context, the availability of shared data at an institutional level allows the officers in charge of bio-monitoring to verify the correct application of the legislative instruments and, in coordination with the Health Authorities, actively assess the impact of the phytosanitary choices.

If when talking about the spread of pesticides, the main

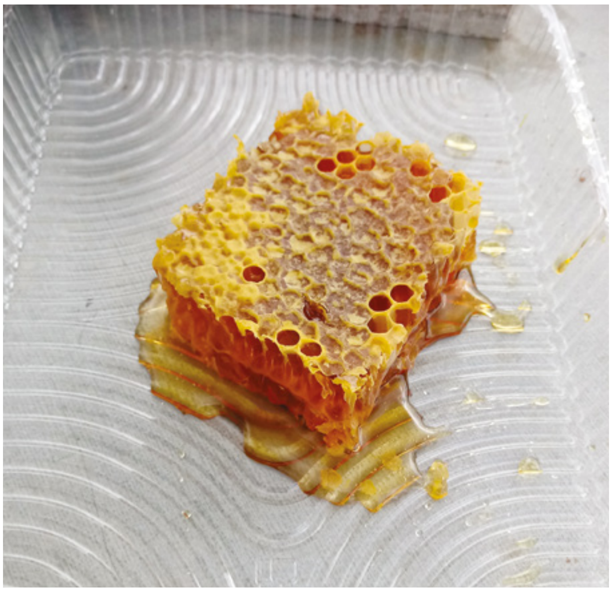


Photo by Samuele Colotta

[1] Data and results of this project are included in the following scientific publication: Simone Tosi, Cecilia Costa, Umberto Vesco, Giancarlo Quaglia, Giovanni Guido *A 3-year survey of Italian honey bee-collected pollen reveals widespread contamination by agricultural pesticides*, Science of the Total Environment 615 (2018) 208–218

Photo by Eleonora Bassi

issue is represented by the aerial drift towards the flowering spontaneous herbaceous species that, without precautionary mowing, spread the active ingredient used in the cultivation, then bees operate through their presence in the environment, a full monitoring activity able to measure the environmental contamination. The high mortality rate and the presence of chemical residues that can be found in bees' body and/or in the hive matrices (direct information), or the depopulation and/or the onset of diseases caused by chronic toxicity pesticides and/or by other pollutants (indirect information) totally represent the gauge of the health of the environment in an area that corresponds to the foraging area of each single hive monitored.
The main objectives of Aspromiele's bio-monitoring project with bees can be summarised as follows:

- investigate the presence of residues from anthropic activities in hive matrices and, therefore, in the environment where bees forage;
- investigate the relationship between the health of bee colonies and the residues observed;
- create a network of public and private agencies so that the beekeeping data can be acknowledged and used to measure the environmental sustainability of phytosanitary treatments;
- carry out an ongoing environmental monitoring activity with the aim of verifying the progress and highlighting the findings, by providing data to the agencies involved so as to allow them to revise and/or modify the norms that regulate the use of pesticides and the guidelines linked to the RDP's measures;
- manage to create solid instruments and structured activities within the RDP so as to assess, through the bees, the types of productive and phytosanitary defence choices as well as the incentives for the defence of pollinators and biodiversity;
- encourage a productive and, at the same time, environmentally friendly agriculture so as to preserve and boost biodiversity at all levels. In this perspective, Aspromiele aims at creating, supported by the institutions, an 'environmental certification' to show consumers the products harvested in the best environment, where bees' and pollinators' health is endangered by anthropic contamination to a lesser degree;
- provide beekeepers, farmers and the population with objective information on the level of environmental pollution monitored by bees.

In order to reach the objectives listed above, the bio-monitoring network is organized as follows within the project:

- monitoring of the Piedmont areas considered at a higher risk of environmental pollution;
- selection of apiaries (sites or units) that are representative of the area to be monitored;
- use of two or more hives per apiary, each one provided with a pollen-gathering trap, a scale, a bee counter, a thermometer, a hygrometer to measure climate variables and a net positioned in front of the hive with the purpose of collecting dead bees, called 'underbasket trap';
- monthly inspections and sampling, during the active beekeeping season to collect samples from the control units. In case of anomaly, immediate sampling of other matrices to be collected for analysis;
- glyphosate multi-residue and research analysis, a very specific and complex one, to be carried out separately, on all the samples gathered from nest honeycombs in each control unit.

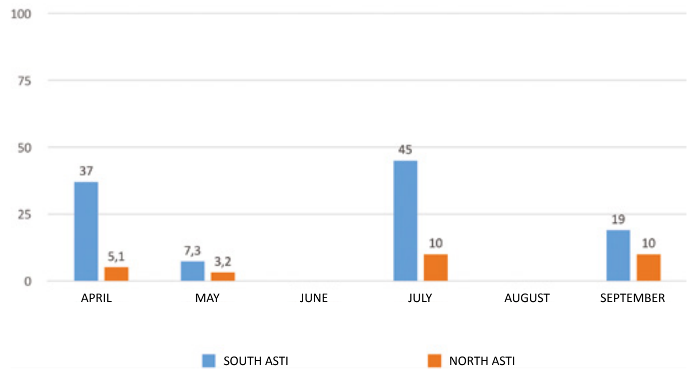
2017-2019 ENVIRONMENTAL BIO-MONITORING PROJECT

SITES GEOGRAPHICAL COORDINATES	*INSTALLATION*	*MANAGER*
CUNICO (NORTH ASTI)	2017	ASPROMIELE
MAGLIANO ALFIERI (SOUTH ALBA)	2018	ASPROMIELE
DEMONTE (BIANCO)	2019	ASPROMIELE
LEQUI BERRIA (ALBA NOCC)	2019	ASPROMIELE
MELLANA (PROVINCE OF CUNEO)	2018	AGRION
MANTA (PROVINCE OF CUNEO)	2018	AGRION
VILLAFRANCA P.TE (PROVINCE OF TORINO)	2018	AGRION
CARPENETO (PROVINCE OF ALESSANDRIA)	2018	AGRION
GAVI (PROVINCE OF ALESSANDRIA)	2018	CONSORZIO DOCG
GAVI (PROVINCE OF ALESSANDRIA)	2019	CONSORZIO DOCG

The table lists all the sites included in the environmental bio-monitoring project with bees and the associations the data belong to. The data of the 4 control units belonging to Aspromiele were used for the purpose of this study. The data belonging to other associations are confidential to date or still being processed.

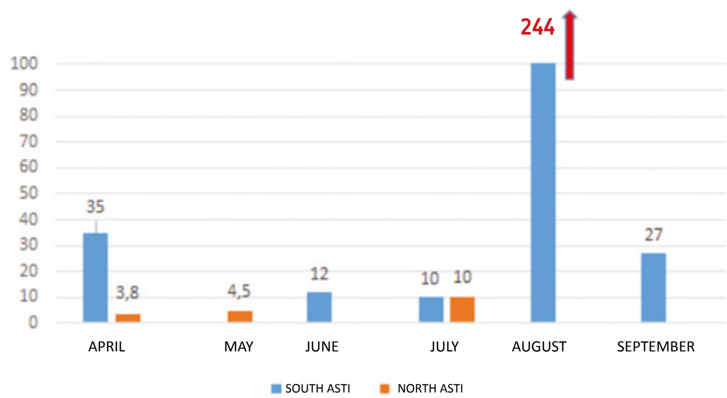
2017: an unexpected protagonist

Glyphosate in nest honeycomb honey



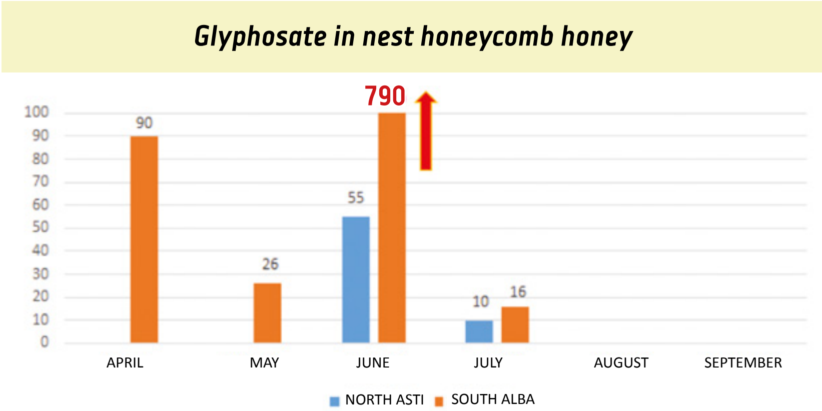
The first year of bio-monitoring activity with bees was carried out using the North Asti and South Asti control units. The two units are located in very similar environments in areas that are very interesting from a beekeeping perspective, characterized by wide wood areas alternating diverse and low intensity cultivations.
In terms of quantity, quality and concentration of molecules traditionally associated with the agricultural activity, the analysis showed the presence of traces (<10 ppb) of various pesticides such as chlorpyrifos, acetamiprid, cyprodinil, imidacloprid, mandipropamid and thiacloprid in April, May and July bee bread.
Considering the concentrations found, it is hard to establish whether the contamination could influence the health of bees synergically and meaningfully. The fact remains that, despite the monitored families did not present obvious symptoms and honey in honeycombs was absolutely free from contamination, molecules that are regularly registered for phytosanitary purposes and used in the indicated period, were gathered and stored by bees to enter the hive biological cycle.
During the entire monitoring period (April-September 2017) no severe mortalities were detected by observing the dead bees collected by the so called 'under-basket nets'. The meteorological data collected showed the heavy frost between 18th and 22nd April that jeopardised the whole acacia harvesting. These data were confirmed by observing a weight reduction of hives by at least two kilos between the beginning and the end of the flowering period. The number of flights recorded during the frost days declined drastically too.
Separate consideration must be given to the absolute and almost unexpected protagonist of the first year of bio-monitoring: glyphosate. In April 2017, a few months after the clamorous news about the presence of the herbicide in US and Argentinian honeys, Aspromiele decided to have specific analysis carried out for the detection of the molecule. The herbicide was not only present in overseas honeys, but concerning quantities and frequencies of the molecule were found in Piedmontese hives too. Glyphosate was found in 100% of bee bread and honey samples collected in April, May and July, though in quantities lower than 50 ppb (limit accepted by law for conventional honey consumption by humans). August bee bread from North Asti control unit showed a peak of 244 ppb, while 45 ppb in July honey from South Asti control unit confirmed the global trend: honeydews are more contaminated than the honey derived from nectar[2].

Glyphosate in nest bee bread



[2] For more information on 2017 bio-monitoring activities refer to Allais, Bergero, l'apis 2/2018 e *The Ambassadors*, our study, attached to l'apis 2/2019

2018: the bio-monitoring network grows



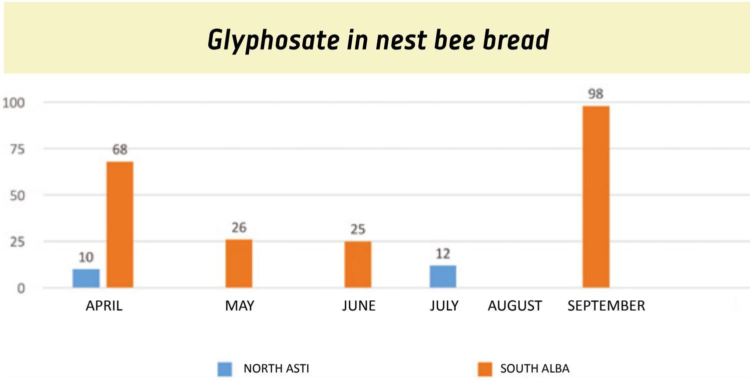
The data collected in 2017 and provided to the Authorities, the Agencies and the Piedmontese Institutions paid off since the beginning: 2018 bio-monitoring began with 4 new control units (added to the two existing Aspromiele's units) and located in 4 different agricultural areas in Piedmont. The new units were deployed thanks to the collaboration with Agrion (Foundation for research, innovation and technological development of Piedmontese agriculture) and the Piedmont Administration Phytosanitary Service, that provided a tangible contribution through its institutional support to the project and with the multi residues analysis carried out on Agrion's control units. To date, the data of these control units are still being processed and discussed with the Foundation and will be published with the 2019 season data, as soon as they become available. In 2018, another positive collaboration with the Consortium for the Protection of Gavi was started and the activation, at the consortium's expense, of a bio-monitoring control unit in the area where the prestigious and homonymous white wine is produced. This network of contacts laid the foundation for a model of collaboration with the productive agricultural world interested in improving their techniques and in proving the validity of the techniques already in use, characterized by a trend towards a more conscious and sustainable use of phytosanitary products.

The bio-monitoring pilot project proved to be a real success. In this respect, the Association was able to build a network of functioning collaborations across-the-board thanks to the warning of widespread contaminations from pesticides, particularly from the bio-monitoring undisputed protagonist: glyphosate. The data on its consistent, ubiquitous and persistent presence in hive matrices drew the attention of the Piedmontese parties concerned at all levels. The most debated herbicide of the moment succeeded where neonicotinoids failed, at least in Piedmont: it managed to create a solid though heterogeneous group committed to monitor and try to understand the complex matter.

In order to take advantage from the attention shown by the Piedmontese agricultural and institutional world, Aspromiele decided to enhance and expand its research by moving one of its control units to an area characterized by more intensive cultivations (though the South Alba apiary is located in a natural oasis) and by comparing the data from other hives to those derived from the surface water monitored by Arpa Piemonte. While moving the control unit proved to be a great strategy to obtain data deriving from meaningfully diverse areas, the comparison with the surface water data is to be seen as an 'investment' for the future, considering the huge amount of data needed over the years in order to draw the first conclusions. In the next chapter we will try to outline a suggestion (that is the only way it can be defined at the moment) derived by cross-reading the surface water and the hive data. This suggestion yet seems to provide a guideline for the continuation of the monitoring project and seems therefore to confirm the validity of the idea to include Arpa's measurements in a monitoring carried out with bees.

If on the one hand it takes time to understand the role of water in relation to the hive measurements, the information provided by the earth giving resources to the bees is easily comprehensible by mapping the territory that surrounds the control units within the range of 1,5 kilometers. Thanks to the collaboration with the Phytosanitary Department, it was possible to extrapolate the exact cartographic information on the cultivations nearby the control units. Knowing what is harvested near the hives and what are the specific treatments used for each cultivation definitely helps to promptly detect the possible source of environmental contamination.

By observing both the environmental data, derived from the technological control units, and the analytical reports related to 2018, we can claim that the environmental conditions of the first trimester, notably the temperatures, were slightly below the season average but did not have a negative impact on the spring harvest, validating a consistent though not extraordinary increase of weight in the whole period. Numerous rainy periods were recorded, especially in the month of May. In the months of April and June, the so called 'uderbasket traps' reported



bees' mortality in South Alba control unit. Glyphosate analysis resulted positive in hive bee bread and honey, especially in April and June, with exorbitant peaks in the honey collected from the nest honeycomb in June which showed a value of 790 ppb. Glyphosate is king in bee bread too, both in April and June. As for other residues, the North Asti unit reported 40 ppb of Fenazaquin in May bee bread, an acaricidal whose limit in food according to the European Union regulations is 10 ppb. In addition, 30 ppb of Piperonyl Butoxide were reported, a synergist used with pyrethroids.

In the second trimester, the environmental data recorded the heat wave in August and September, which prolonged the period of bees' activity. Bees' mortality was detected through the so called 'underbasket traps' in South Alba control unit yet in the month of July, whereas the situation improved in the months of August and September. As far as honey and bee bread analysis is concerned, residues were recorded in the months of July, with 16 ppb of glyphosate in the honey collected from nest honeycomb in South Alba and 10 ppb in the honey from nest honeycomb, in North Asti. In August we fortunately recorded a single glyphosate residue in South Alba station, though with the highest value of the trimester: 98 ppb. Positive reports during the second trimester recorded glyphosate residues only, while no other pesticides were detected by multi residue analysis which, during this season, received the financial support of Cooperativa Piemonte Miele too. Therefore, a lower frequency of contamination was generally observed in 2018, though with higher quantity of contaminants[3].

Analysis of the first two years of the project

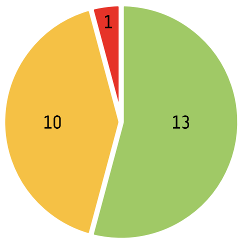
L'apis already published a study on glyphosate as an attachment of number 2 of the magazine, 2019. Therefore we will not discuss in detail again about its contamination dynamics and the hive symptomatology due to the residues found in the nests. For more information about these and other topics, the reader can refer to the February issue. However, it is impossible to talk about bio-monitoring without talking about glyphosate, as the herbicide is the molecule found systematically in any place, in any matrix and at any stage of the sampling. In addition, glyphosate seems to intensify its presence season by season and it seems to present contamination peaks more frequently within a single season. The findings show an upward trend for both frequency and intensity. While for insecticides and fungicides the records shows a slight decrease in the contaminations, glyphosate goes against the flow. A concerning data, especially in regards to the health of hives on which the herbicide, together with the 'normal' stressors such as varroa and food shortage, can have devastating consequences. What are the causes of this upward trend? An increased use of the pesticide? A greater precision in the analysis tools and methods that makes the herbicide easier to detect compared to the past? A lower ability of the soil to keep and metabolise the molecule, that translates into a greater 'participation' of glyphosate to the biological cycles of Nature? It is hard to provide a univocal answer to those questions: it is likely that all the factors sum up and contribute to the increased frequency and higher value of glyphosate presence in the analysis results. The analysis techniques, that obviously improved in the past 3 years, are now highlighting, more and more precisely, the extent of the environmental contamination caused by the molecule.

Bees are telling us that pollinators are affected by the impact of the various biocide molecules, notably of glyphosate. Could it be a biased reading, caused by some sort of peculiar tendency of bees to collect the molecule during their pollen gathering flights? The hypothesis is to be excluded because bees are not alone in showing us such a picture of the situation: the data on surface water contamination confirm bees' interpretation of the glyphosate phenomenon.

During the phases of cooperation and participation to the technical organization with Piedmont Administra-

[3] For more information on 2018 bio-monitoring, refer to Bergero, l'apis 2/2019 and *The Ambassadors*, our study, attached to the same number of the magazine

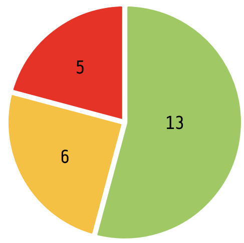
Glyphosate in 2017 samples



46% POSITIVE SAMPLES: 42% <> 10-50 ppb, 4% > 50 ppb

● >10ppb ● <>10 - 50 ppb ● > 50 ppb

Glyphosate in 2018 samples



46% POSITIVE SAMPLES: 25% <> 10-50 ppb, 21% > 50 ppb

● >10ppb ● <>10 - 50 ppb ● > 50 ppb

tion Phytosanitary Services, new collaborations with the Local Agency for the Environmental Protection (ARPA) were born. ARPA was appointed by Piedmont Environment Division to carry out environmental monitoring of surface and deep water of Piedmont water bodies. The Legislative Decree 152/06 (Environmental regulations), that implemented the European directive 2000/60/CE, establishes that the Member States reach 'environmental goals' that translates into the 'EQS' (Environmental Quality Standards): these are pollutant residues threshold values that are the same in all European Union countries. Exceeding these values in the monitoring points of surface and deep water is an infraction that a country commits at the expense of the whole community.

In 2016, ARPA recorded a decrease, compared to the previous trend, in the presence of imidacloprid[4] and other

	Varieties of substances found	Substances with more than 4 findings	Sampling points exceeding water quality standards	Banned substances
2015	61	31	12	//
2016	58	23	8	Alachlor Hexachlorobenzene Endosulfan
2017	68	43	10	Hexachlorobenzene
2018	**74**	**53**	11	Hexachlorobenzene

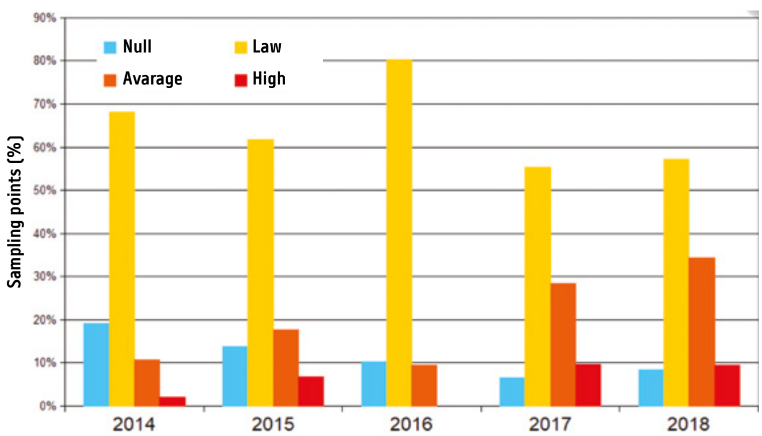
pesticide molecules in the contamination index of Piedmontese surface water. The warning seemed to be the beginning of a change in direction. Unfortunately, in the two following years, contamination started to get worse again, with an increase in the numbers of pesticides found in each site. If on the one hand, EU ban of neonicotinoids gives hope for a real change in direction in 2019 and might represent the first step towards the environment decontamination from systemic insecticides, on the other hand, Piedmontese surface water, at least until 2018, do not show an ideal situation and record an extremely concerning trend.

As for glyphosate and its metabolite AMPA - whose presence in water has been researched only since 2016, after the research itself was made mandatory by the Legislative Decree 152/06 - Arpa's data suggest a very serious situation in terms of herbicide environmental contamination. In three years of monitoring, positive results were more frequent and with higher peaks throughout time, with the worst data recorded in 2018: AMPA resulted present in 91% of monitored sites while glyphosate was present in 76,5% of

SURFACE WATER PESTICIDES – Active Ingredients details

ALACHLOR banned in EU and US
ESACLOROBENZENE banned worldwide by the Stockholm Convention (2001)
Organic compounds (including agrochemicals) by-product
ENDOSULFAN banned worldwide by the Stockholm Convention (2012)
Insecticide, acaricide

Contamination Index



[4] For more information on the role of neonicotinoids and imidacloprid in environmental contamination, refer to our study titled *'Neonicotinoids, the new DDT'*, attached to l'apis, number 2/2018 .

the monitored sites (in 2016, the percentage was below 30%). When talking about glyphosate, water, as well as hive matrices, record an increasing trend of residues in the environment.
In the already quoted study on glyphosate, we excluded a direct relationship between surface water hive matrices contamination as the concentrations in water were too low to account for the levels found in the hive. In addition, the almost complete absence of AMPA in hive matrices excluded that the contaminant was directly collected from water, where in fact the metabolite is way more present compared to the full molecule. However, if we talk about glyphosate, water and bees record the same phenomenon, without influencing one another. It is interesting to note the affinity between the results and the compatibility between the readings offered by parallel monitoring activities that contemplate the sampling of matrices and the use of survey techniques which may differ significantly between one another. By cross-reading water and bees data, we can therefore state without a

GLYPHOSATE 2016 *In 2016 Arpa Piemonte's laboratory based in Grugliasco developed a specific method to define the presence of* **glyphosate** *and its main metabolite* **AMPA (ampa aminomethylphosphonic acid)** *in water. The two parameters were therefore defined by analyzing about 150 samples of deep and surface water.*

SURFACE WATER MONITORING (84 samples collected in 73 different locations between August and October 2016)					
Active ingredient	Positive findings	% positive findings out of the total samples analyzed	Number of contaminated locations out of a total of 73	Range (min-max) µg/l	Average Value µg/l
AMPA	57	67.9%	50	0.1 – 15.9	1.80
Glyphosate	25	29.8%	24	0.1-1.8	0.31
DEEP WATER MONITORING (71 samples gathered from autumn countryside surface groundwater – September/November 2016)					
Active ingredient	Positive findings	% positive findings out of the total samples analyzed	Number of contaminated locations out of a total of 71	Range (min-max) µg/l	Average Value µg/l
AMPA	0	--	--	<0.1	--
Glyphosate	0	--	--	<0.1	--

GLYPHOSATE 2017 **Glyphosate** *and metabolite* **AMPA (ampa aminomethylphosphonic acid)** *in water. The two parameters were therefore defined by analyzing about 185 samples of deep water and 75 samples of surface water.*

SURFACE WATER MONITORING (185 samples collected in 36 different locations between April and December 2017)					
Active ingredient	Positive findings	% positive findings out of the total samples analyzed	Number of contaminated locations out of a total of 73	Range (min-max) µg/l	Average Value µg/l
AMPA	113	61%	28	0.1 – 7.7	1.32
Glyphosate	78	42%	24	0.1-2.2	0.35
DEEP WATER MONITORING (70 samples gathered from autumn countryside surface groundwater – September/November 2017)					
Active ingredient	Positive findings	% positive findings out of the total samples analyzed	Number of contaminated locations out of a total of 70	Range (min-max) µg/l	Average Value µg/l
AMPA	4	6%	4	0.1 – 0.5	0.2
Glyphosate	0	--	--	<0.1	--

doubt that the presence of glyphosate able to 'freely' circulate in the environment, without being withheld by soil minerals and not being degraded by soil bacteria, is increasing meaningfully. Although it is hard to estimate the extent of the use of the herbicide with precision and even though it is almost impossible to understand if the use of the molecule has increased in the past years - as the use of the molecule may vary significantly from season to season - the presence of the molecule in physical and environmental biological cycles is experiencing a constant and progressive increase. In other words, environmental contamination seem to have an increasing tendency that is more linear and progressive compared to the actual use of the molecule, as if a third factor was intervening

Surface water
PESTICIDES
Active ingredients details

2018
74 different substances detected (53 active principles with more than 4 positive findings)

SUBSTANCE	Number of locations with pesticide	% of locations with pesticide out of the total number of locations analyzed	Maximum Values
AMPA	31	91,18	13,2
QUINCLORAC	21	77,78	1,62
GLIFOSATE	26	76,47	2,7
ESACLOROBENZENE	13	76,47	0,12
PRETILACLOR	18	64,29	0,51
METOLACLOR	55	57,89	0,47
TERBUTILAZINA	50	52,63	0,6
TRICICLAZOLO	14	50,00	0,58
NICOSULFURON	46	48,42	0,72
BENTAZONE	41	43,16	6,35
BOSCALID	38	40,00	1,59
DESETILTERBUTILAZINA	35	36,84	0,21
BENSULFURONMETILE	10	35,71	0,31
IMIDACLOPRID	33	34,74	0,41
AZOXYSTROBINA	29	30,53	6,5
MCPA	28	29,47	0,34

Glyphosate and AMPA specific situation:

> specific analysis
> limited number of analysis
> they were searched for only in a few locations and in the summer months (highly focused sampling)
↓
The results cannot be used as a reference for water quality standards CLASSIFICATION of water bodies

to defer and amplify the persistency of the herbicide in the environment. This third factor could be identified in the increasing inability of soil to keep and metabolize the molecule. The hypothesis of a key role played by soil in such a mechanism of glyphosate distribution seems to take shape and become credible when reading the data available, even though unfortunately scientific literature is not helpful in proving this theory right or wrong.
However, this deduction seems to find confirmation in the study on the cultivations actually present in the proximity of the monitoring control units. By starting from the exact knowledge of cultivations present in the fields, we can hypothesize two main herbicide treatment periods, at least for the Piedmontese areas where the control units are placed: March/April for wheat, corn, hazelnut and vineyards, and June/July for hazelnut, vineyards and stubbles. The glyphosate found in hive matrices, as well as in surface water, does not accumulate during these periods or straight after the treatments. It is in fact present all along the season, as a background noise, even distant in time from the herbicide treatment periods. The environment seems to accumulate and release glyphosate in a way which is not linear and non consistent with the effective use of the molecule. Therefore, we have to go back and hypothesize, at least from a logical perspective, the soil inability to filter and metabolize the herbicide, which can freely circulate, at least in part. This free circulation, can indeed only be partially explained through the aerial drift which can perhaps account for the contamination peaks and the presence of the molecule on flowers of non-target plants. However, it cannot fully justify the background noise, which seems to depend on other factors.
The questions (and the consequent possible answers) on the persistence of the molecule in the environment and the background noise that 'spreads' the molecule all along the season were not the only difficult questions arisen during the first two years of bio-monitoring activity with bees. 2018 season posed other relevant questions, merely related to bees, for which finding an answer is just as difficult: what is the threshold beyond which a measurable biological damage to bees' families is caused? What is the mechanism that can explain the big difference between the contamination of honey collected from the nest and the one of honey collected from the honeycomb? For those who did not read our report on glyphosate, we remind that last year, particularly in South Alba apiary, while honey collected from the nest was highly contaminated (with a peak of 790 ppb), honey from honeycombs was always free from contamination. Doubts on what is the natural filter of honey spontaneously arose and the hypotheses that the bees could play the role of the filter seems to be rational and able to explain the remarkable weakening of bees' families following the contamination peak that reached almost 800 ppb. What happens in the hive after foraging bees collect glyphosate? And what are the consequences?
Basically, the first two years of bio-monitoring activity with bees left us with more questions than answers in addition to the verification of the various contaminations. Questions that originated hypothesis still to be verified. Arpa's data as well as the data on the intended use of the terrains around the control units did not give irrefutable answers but provide a direction for a more in depth-analysis in 2019 and in the years to come.

Therefore, 2019 season started with the following questions:

1. what persistency can glyphosate have in the environment after being sprayed?
2. what is the natural filter between nest and honeycomb?
3. is it possible to establish a damage threshold to bees' families by deeply researching their microbiology within the bio-monitoring context?

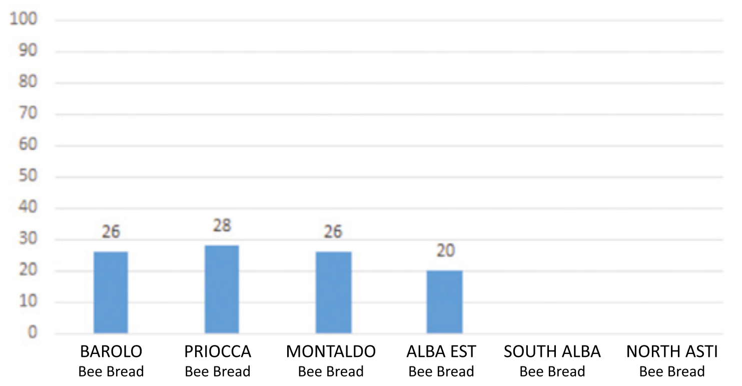
2019 extra sampling - hazelnut project

Photo by Carlo Gatti

In order to answer the first of the questions, Aspromiele decided to start 2019 season with an extra sampling, carried out in a period that preceded the beekeeping season and the herbicide treatment time. The beginning of February is the ideal time as bees' families have not yet started their development and no meaningful flowering has happened yet with the exception of hazelnut in Southern Piedmont. Hazelnut trees are not treated with herbicides in February (they will be at the end of March) and that is the period of further time distance from the last herbicide treatment, happened in July. Hazel pollen sampling, at least in the Alba region, has a highly relevant statistical value in terms of results reliability: it is the only and highly abundant pollen available to bees at the beginning of February.

Samples were collected in 6 different apiaries, all located in the Langhe, Roero and Mofferrato areas, in the hills considered UNESCO World Heritage Sites. One of these samples was collected from the North Asti bio-monitoring unit, located in an area that is not suited for intensive hazelnut cultivation; a second sample was collected from South Alba control unit, located in an area where hazel trees were planted a few years ago, are not yet characterized by abundant flowering and have not received meaningful herbicide treatments yet.

February Glyphosate



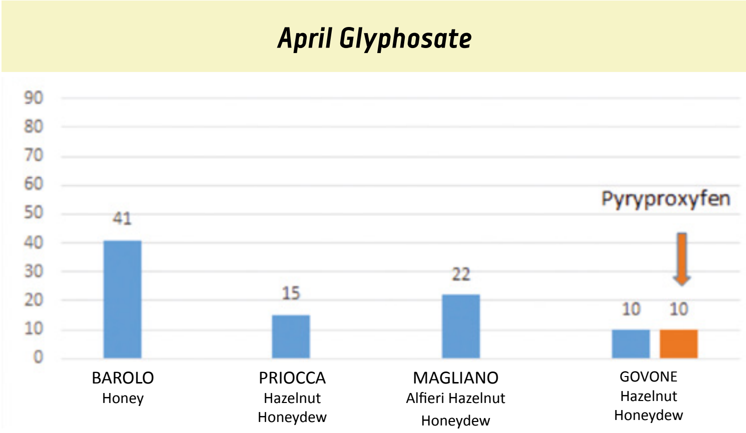
Hazelnut pollen in North Asti and South Alba did not contain glyphosate. In all other areas, characterized by hazel trees in production, the molecule was present in significant amounts between 20 and 30 ppb.

These data have two important implications:

1. hazelnut trees produce contaminated pollen 7 months after the last herbicide treatment. The herbicide circulates in the plant when vegetation starts growing abundantly again and this implies that the molecule is easily accessible to the hazelnut tree. Understanding if it is 'stored' in the soil or in the roots is not relevant at this stage: glyphosate persistence in a hazelnut field can be measured in at least 7 months!
2. In Southern Piedmont hazel flowering is of great importance for bees: up to a few years ago the abundant availability of pollen in February caused an early and substantial development of bees' families so that in March Alba bees had nothing to envy to those who spent their winter in Liguria, near the sea. The increase of hazel cultivation recorded in the past years could therefore be seen as a further potential advantage. However that was and is not the case: the sudden appreciation of hazelnuts caused an increased intensity of insecticide and herbicide treatments to hazelnut fields. While insecticide treatments have had a direct impact on hives, the presence of glyphosate in hazelnut pollen could explain why Alba bees now usually have false starts. In a few years, thanks to the hazelnut market, a territorial resource turned into a good reason to be forced to practice escape nomadism already in January.

In monoculture areas, hazelnuts do not only offer a huge amount of pollen to bees, they also offers great quantities of honeydew, thanks to the help of aphids and cochineal insects. In 2019 as well as in 2017, honeydew represented the only source of food for Alba bees for the whole month of April and until mid May. Honeydew

Photo by Carlo Gatti



is collected by bees after the first herbicide treatment of the season and therefore it is more exposed to contamination, at least on paper.

Hazelnut honeydew samples were gathered in the same apiaries where bee bread samples were collected in February, with the exception of the apiaries located in Montaldo Roero and North Asti, where bees did not collect honeydew from hazelnut.

Analysis reports state that glyphosate is present in hazelnut honeydew with values that are very similar to those found in bee bread from the same plants in February, despite an herbicide treatment was applied in the meantime. It is therefore quite likely that the plant does not draw directly from the 'source' of glyphosate sprayed in the field a few days before but to a 'tank' with a long-term storage, where it picks up in a more systematic and random way.

Hence, the hypothesis of contamination and the mechanism of soil failed metabolism returns to be the most plausible: glyphosate is preserved in soil and gets absorbed by plants in a gradual and consistent way, with a mechanism that dilutes and accumulates the effects of spraying at the same time. In short and with a metaphor, plants do not fish from the creek but from the sea where the creeks flow into. Provided that our hypothesis turns out to be trustworthy, of course.

In this view, the only result that exceeds 40 ppb appears meaningful: the sample collected in Barolo is the only one where hazelnut honeydew was not pure but contaminated by a very significant percentage of dandelion (at least 60%) collected in the vineyard rows. To be noted that these vineyards have not received any herbicide treatments in years. Although the area is no longer subject to systematic herbicide treatments, dandelion plants keep distributing glyphosate to bees. In front of this data, Aspromiele searched for a validation by consulting beekeepers in the area: they confirmed the systemic contamination of dandelion honey (always within the maximum limits allowed) produced in the Langhe area where the great wines are produced: the exact same area where chemical treatments were banned a few years ago. This is a very substantial data that leaves little doubt on the role of soil in the contamination dynamics of hive matrices while leaves more than a doubt on the ability of Piedmontese soils to metabolize glyphosate as well as their future ability to receive more.

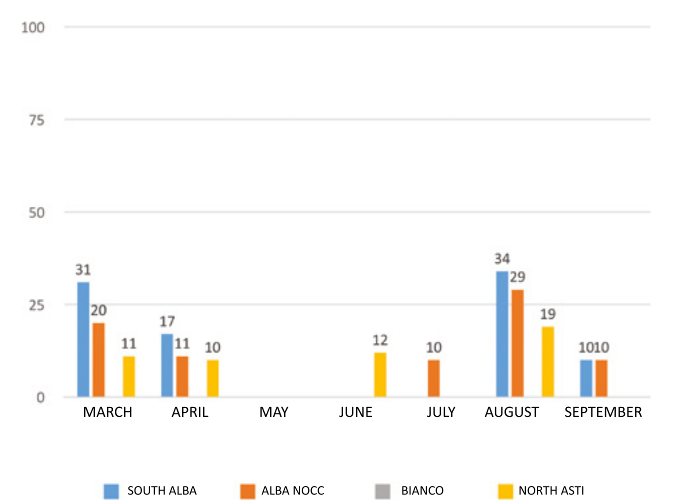
If the extra monitoring activity on hazelnut provided very precise indications on how to measure the persistence of the molecule in the soil, it likewise provided indications on the direct consequences of hazelnut farmers' attempt to control hazelnut parasites: in the chart a residue of Pyriproxyfen, an insecticide specifically used against cochineal insects, is shown in orange. This is a clear and direct consequence of a treatment applied out of time, when the acari were already active.

Photo by Carlo Gatti

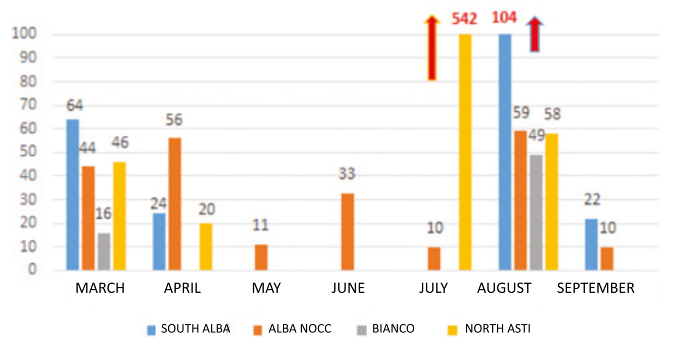
2019: further developments

The season that just finished witnessed an increase of Aspromiele's bio-monitoring control units, from 2 to 4. The increase was also accompanied by an in-depth analysis of the hive matrices. One of the control unit was activated in a mountain area (Bianco), located about 850 metres above sea level, outside from any intensive cultivation. The other unit is located in the Alta Langa region, in an area completely cultivated with hazelnuts (called Alba Nocc), most of which are organic.
The two 'historic' units were instead enhanced with more in-depth and specific analysis: North Asti control unit had its own bee bread sample analyzed to find 200 active ingredients from antropic activity (they use to be 100). In addition, wax samples were collected from the nest: one was collected from a honeycomb built during the season (new wax), the other was collected from an older honeycomb (old wax). Samples of propolis and bees were collected too. The following chapter will focus on North Asti control unit.
South Alba control unit, that recorded the most significant peaks during the last season, was the scene of a true experiment. In concomitance to the bee bread and honey samples collected from nest honeycombs, samples of young bees were gathered too to carry out microbiological analysis. The experiment aims at testing an instrument which is completely new to the bio-monitoring with bees: a microbiological marker of the hive health in relation to the glyphosate contamination recorded in the nest. The question we are trying to answer is the following: is it possible to monitor the health of a hive that is subject to food contamination through the monitoring of bees' intestinal bacteria population? A chapter will be entirely dedicated to this topic too.
The enhancement of the chemical-physical analysis on the two control units is not the only news in 2019. Aspromiele also decided to have palynological analysis done on samples of bee bread that resulted positive to one or more active ingredients, for all the monitoring control units. The palynological analysis, that involves the qualitative and quantitative microscope observation of pollen grains, is able to detect the exact amount, expressed as a percentage of the total and on the volume, of any species present in the sample (see report). It is extremely useful to understand with precision what is the origin of the pollen and therefore of the contamination, thus allowing to deduct how the active ingredients circulate and are spread in the environment. We will see the analysis and the presentation of the results later in details.
The collaboration with Agrion was confirmed for 2019 too. The availability of the four control units located in the research centres of the Provinces of Turin, Cuneo and Alessandria remains the same. The Consortium for the Protection of Gavi renewed its participation to the project and, after a meeting with Aspromiele, decided to enhance its monitoring activity by adding a control unit in a sensitive area of the territory where episodes of apiary poisoning were recorded in the past. We would like to commend the Consortium for investing further private resources for a common and collective purpose.
By observing the report on 2019 analytical data and the results shown in the carts, we can see how the frequency and the intensity of glyphosate residues found unfortunately remains the same for this season too.
Two striking data stand out from the report. The first refers to the finding of glyphosate in March and August bee bread in Bianco, a mountain area located 850 metres above sea level. Unfortunately March sample was too scant to carry out 2 analysis but August sample was submitted to palynological analysis too: this will reveal from which plants bees gathered pollen from. The second data refers to a new exorbitant peak of glyphosate (542 ppb) found in bee bread of North Asti control unit in the month of July. Was that due to aerial drift from herbicide treatments on stubbles in this case? Palynological analysis will help us shed light on the contaminations.
Another outstanding, and unfortunately 'recurring' data, refers to the 104 ppb of glyphosate found in South Alba

Glyphosate in nest honeycomb honey



Glyphosate in nest bee bread



control unit bee bread in the month of August. In the same period, a peak of 98 ppb was recorded in the same Going back to the monitoring data we can say that in addition to glyphosate, other active ingredients were found in bee bread, especially in South Alba control unit, though exclusively in the month of May: 20 ppb of mandipropamid, a concentrated suspension fungicide authorized on vines, potatoes and horticultural crops; 40 ppb of spiroxamine, another fungicide authorized on vines and traces of metalaxyl (4 ppb), a fungicide specifically used on vines against peronospora. Substances other than glyphosate were found in pollen in Alba Nocc control unit in April: 50 ppb of tau-fluvalinate, an insecticide authorized on fruit and horticultural crops, as well as active ingredient used in apiculture against varroa but never used in the colonies of Alba Nocc control unit.

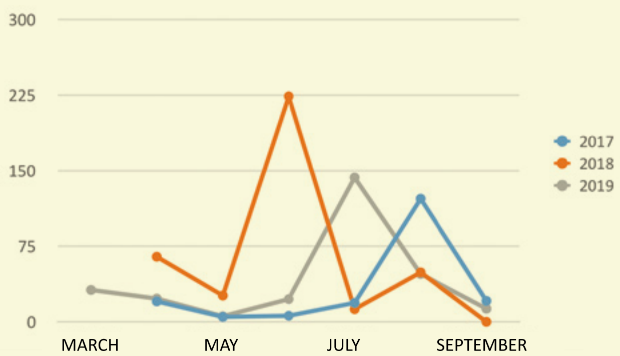
During the monthly samples collection, possible anomalous mortality episodes were monitored through the reading of the so called underbasket traps and the hives were visited to check the health of the families. No anomalous mortality episodes happened in the traps. However, during September sampling, one of the two families belonging to North Asti control unit died out while the other was really weakened. The death of a family cannot certainly be considered an exception in a season like 2019, which was definitely critical for Piedmontese apiculture. However, the fact that the family who perished was the one that recorded the highest peak in the season, almost 550 ppb of glyphosate, cannot be considered a coincidence. As for South Alba control unit, although no visible mortality episodes were recorded in front of the hives, the end of August experienced a depopula-

Hazardous hypothesis or well-founded reasoning?

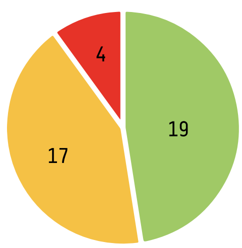
Overall, we can observe that the presence of glyphosate in honey and bee bread in 2019 is found mainly in spring and autumn and its presence is scarce or non-existent in the months of May and June. This is diametrically opposed to 2018 trend characterized by peaks concentrated in April and June and with low or null values recorded until the end of summer, with the exception of the peak recorded in the bee bread collected in South Alba unit in August. From this perspective, North Asti control unit with its 3-year data history, allows us to provide better structured and very interesting reasoning on the distribution of contamination. The distribution recorded in 2019 does not confirm 2018 distribution but it perfectly reflects the one in 2017. Season 2019 and 2017 are very similar from a beekeeping perspective too, with honey and bee bread shortage right between April and June. Conversely, the same period in 2018 was the best one from a beekeeping point of view, with decent acacia and wildflower honey harvesting, though contamination was the worst. One could think that a good nectar and pollen flow could be the cause of glyphosate presence with peaks concentrated when the season offers the best harvesting. However, the hypothesis seems to be proven wrong by the great second part of summer 2018 which was generous with nectar and pollen but almost free from contamination. Perhaps plants were able to 'drain' the herbicide earlier compared to the two other seasons, thus depleting their reserve already in July? This is definitely a questionable hypothesis, given the available data, but if we reconsider the idea of soil as a glyphosate tank where plants are 'forced' to draw from, then the reasoning could make sense: the plant absorbs a higher amount of glyphosate in periods of maximum vegetation activity such as flowering, and if climate allows that, the plant 'gives glyphosate back' to bees. If the plant cannot give glyphosate back then it accumulates to return it on another occasion; in case it manages to do it, it cleans itself as well as the portion of surrounding soil. The following herbicide treatment 'impregnates' the soil again and the cycle starts again to alternate contaminated with 'clean' flowering.

With this in mind, in 2017 and 2019 plants were able, for climate reasons, to clean the soil during spring and the second part of summer, whereas in 2018 they managed to do that in the middle period of the season in a more 'aggressive' but more durable way. Unfortunately, there are no scientific data to confirm or prove this hypothesis wrong, being the chemical cycle of glyphosate still to be explored. This still remains an appealing interpretation that will be put to the test in the next season, especially by Alba Nocc control unit that in 2019 recorded a contamination trend compatible with that of other seasons, though with a less consistent presence of glyphosate, especially in bee bread, during the whole monitored period. This data matches the beekeeping season in the Langhe region that witnessed numerous and quick micro-flowering activities alternating with just as quick periods of absolute shortage. Perhaps the environment emptied its glyphosate tank in a non continuous nor resolute way during the whole season? It is hard to say but we will return to the matter in the paragraph dedicated to palynological analysis.

AVERAGE GLYPHOSATE RESIDUES



Glyphosate in 2019 samples



53% POSITIVE SAMPLES: 42% <> 10-50, ppb 11% > 50 ppb

● >10ppb ● <>10 - 50 ppb ● > 50 ppb

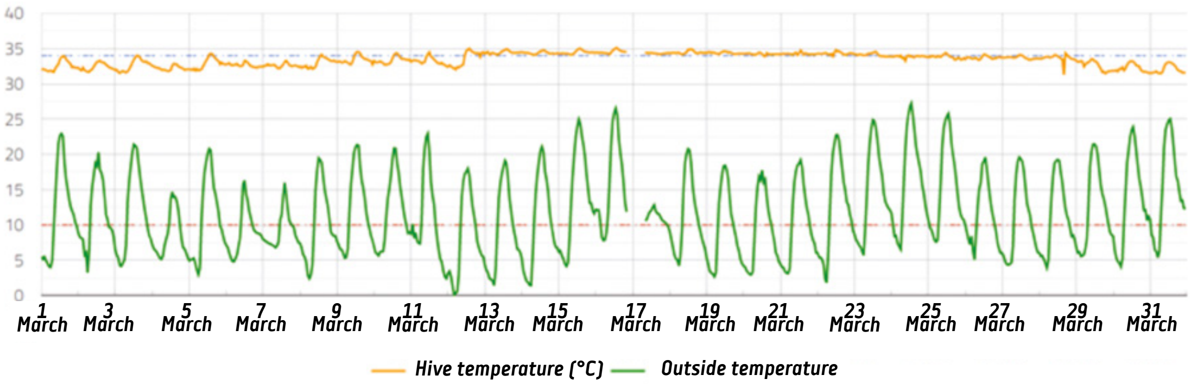
Photo Aspromiele

tion of the families, with young bees disappearing just after coming out of the cocoon after the summer brood blockage. The 104 ppb of glyphosate found in pollen in August do not seem a coincidence either.
At this stage it is important to understand if it is possible to outline a threshold of glyphosate damage, beyond which the consequences on bees become visible. From this perspective, it is interesting to understand if the idea of microbiological monitoring of bees could give exhaustive answers.
2019 meteorological data collected by Melixa technological systems allowed, as in the previous years, to precisely record the terrible climatic trend of the season that just concluded. The average temperatures during the spring months, between March and May, were rather low: 10 °C in March and only 15 °C in May; temperatures started to increase again in June to become burning hot in the mid summer months, with an average temperature of almost 30 °C in the month of July. September and October still recorded high temperatures around 18-20 °C on average. As for the rains, strong precipitations were recorded until the month of May to then leave room to drier periods in the summer months. A reduction in the flight flows was recorded not only in the rainy days but also in the hottest months and days with temperatures above 30 °C. This situation proves how intense heat has a negative impact on bees' activity. In addition, the trend of hive weight highlighted the sad and negative record achieved by 2019 beekeeping season, with the worst production result ever. From the analysis of environmental data we can deduce that climate anomalies, combined with the contamination from pollutants collected by bees in the environment, are causing an issue that is still widely underestimated.

Photo Aspromiele

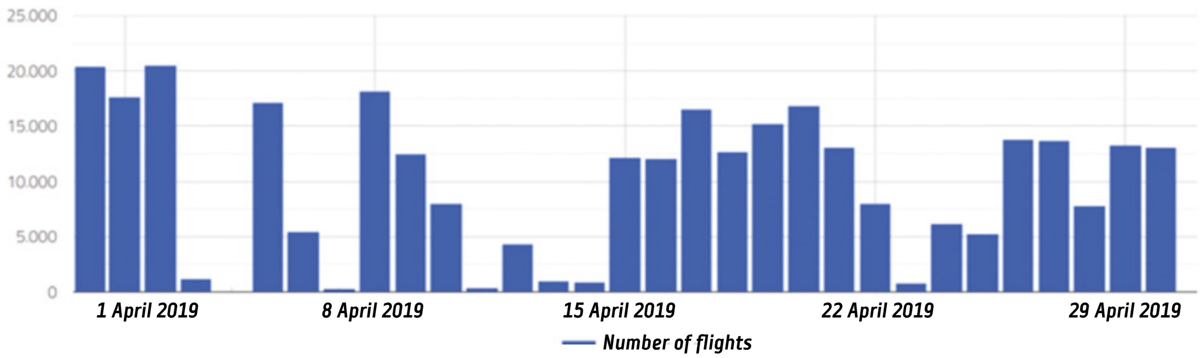
AT 15.132: temperatures in °C from 01/03/2019 to 01/04/2019

North Asti, March temperature



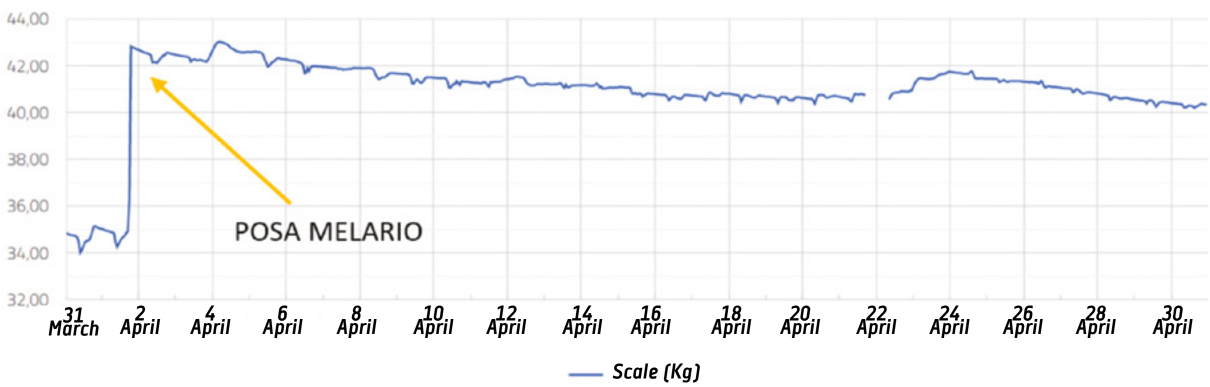
AT 15.132: flights from 31/03/2019 to 30/04/2019

North Asti, March flights



AT 15.132: weight in kilos from 31/03/2019 to 30/04/2019

North Asti, April weight



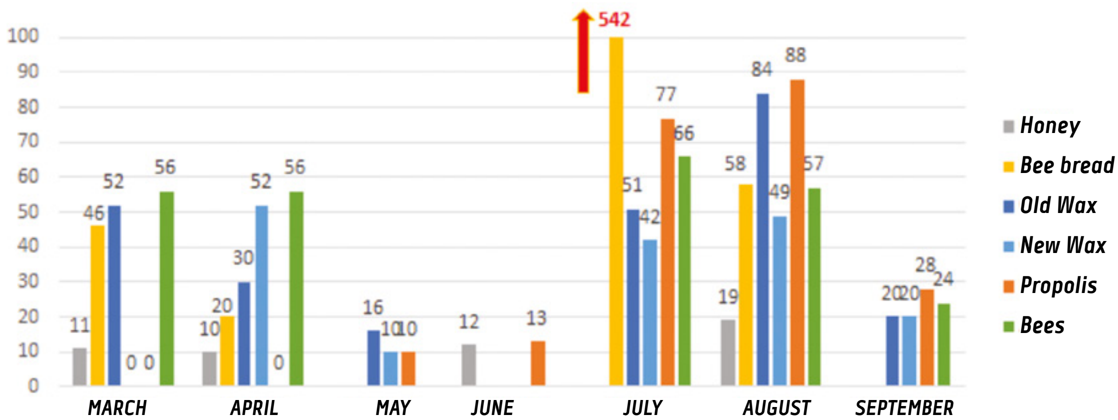
North Asti control unit: all hive matrices analyzed

With North Asti Control unit, Aspromiele decided to examine the contamination dynamics of the whole hive in depth. On top of the glyphosate specific analysis, multi residue analysis were conducted to investigate 200 active ingredients, instead of the standard 100, on all the hive matrices. In addition, the presence of heavy metals was researched as a source of environmental pollution: these data, still not available at the time this study is being written, will be discussed separately together with the bio-monitoring matter.

This chapter therefore entirely focuses on the results obtained from the complete analysis of all the hive matrices of the North Asti bio-monitoring control unit.

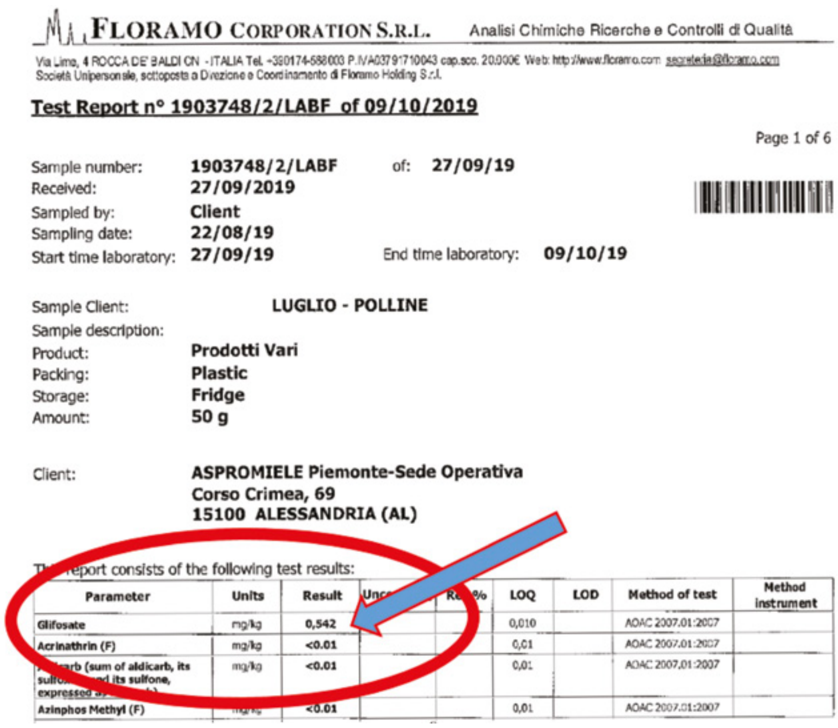
Glyphosate is everywhere, systematically present in all the matrices and with values that are absolutely significant. The most concerning data refers to the bees, but wax and propoli raise serious questions on the salubrity of the accommodation where bees found themselves living in. From this perspective, it is interesting to measure the parabolic increase that characterized, before reshaping in September, propolis contamination from the months of May. It is also interesting to note how wax contamination varied throughout time even though all the samples belonged to the same nest. If for propoli we can hypothesize a contamination dynamic strictly connected to the seasonal trend of herbicide treatments and resin production by plants, with the aerial drift playing an important role in the direct distribution of the molecule on the material

Glyphosate Matrices



gathered by bees, the matter appears to be way more complex in the case of wax. While with propolis, glyphosate seems to stratify in the nest, as propolis itself, with wax it seems to come and go and its values go up and down proportionally to honey, bee bread and bees values and drop to zero when glyphosate contamination experience a significant pause, like the one in May and June. In other words, the matrix does not seem to accumulate residues at all: wax seems to be systematically dirtied by bees more than actually contaminated and the phenomenon has quite likely to do with the little affinity of the herbicide for lipids. While propolis, once transported into the nest, can be considered as a potential active source of glyphosate in the hive and is ready to distribute its residues each time it gets handled by bees, wax seems to preserve a memory of glyphosate flow into the hive rather than actively participate to the internal cycle of the molecule. As usual, our interpretation would need to be supported by many more data than those available, but if we were right, wax could be used as an indicator of the presence of the herbicide in the nest during a wider time range compared to that of the punctual monthly sampling. In other words, if with the monthly sampling of honey, bee bread and bees we get snapshots of the situation, by analysing that specific portion of honeycomb instead of another, or those specific bees instead of others, with wax we could have a rough indication of how things went during a certain timeframe, preceding the sampling. Going back to the salubrity of bees' accommodation, propolis and wax contamination cannot certainly be considered a negligible factor in the health dynamics of the hive but we can definitely affirm with enough certainty that the most serious issue is perhaps another one. The residues found in bees definitely constitutes a more concerning data: in the months of March and April, bees resulted to be the most contaminated matrix, more than honey and bee bread; although they lost their record in the months of July, August and September, they still present very significant values. In September, the residues found are present in the bees but not in bee bread or honey, as if the insects had collected the herbicide from other environmental matrices, or if they maintained a record from the previous months. It is interesting to observe that there are no obvious and direct relationships between the bees and the other matrices of the hive: if bee bread is contaminated that does not mean that bees are too, and the same can be said for honey. In May, wax and propolis were contaminated, while bees resulted clean; in June, while honey and propolis were contami-

Floramo Corporation S.r.l. – Chemical Analysis and Research, and Quality Check



FLORAMO CORPORATION S.R.L. Analisi Chimiche Ricerche e Controlli di Qualità

Via Lime, 4 ROCCA DE' BALDI CN - ITALIA Tel. +390174-588003 P.IVA03791710043 cap.soc. 20.000€ Web: http://www.floramo.com segreteria@floramo.com
Società Unipersonale, sottoposta a Direzione e Coordinamento di Floramo Holding S.r.l.

Test Report n° 1903748/2/LABF of 09/10/2019

Page 1 of 6

Sample number: **1903748/2/LABF** of: **27/09/19**
Received: **27/09/2019**
Sampled by: **Client**
Sampling date: **22/08/19**
Start time laboratory: **27/09/19** End time laboratory: **09/10/19**

Sample Client: **LUGLIO - POLLINE**
Sample description:
Product: **Prodotti Vari**
Packing: **Plastic**
Storage: **Fridge**
Amount: **50 g**

Client: **ASPROMIELE Piemonte-Sede Operativa**
Corso Crimea, 69
15100 ALESSANDRIA (AL)

T[illegible] report consists of the following test results:

Parameter	Units	Result	Unc[illegible]	Re[illegible]%	LOQ	LOD	Method of test	Method instrument
Glifosate	mg/kg	0,542			0,010		AOAC 2007.01:2007	
Acrinathrin (F)	mg/kg	<0.01			0,01		AOAC 2007.01:2007	
[illegible]arb (sum of aldicarb, its sulfo[illegible] [illegible]d its sulfone, expressed [illegible]	mg/kg	<0.01			0,01		AOAC 2007.01:2007	
Azinphos Methyl (F)	[illegible]	<0.01			0,01		AOAC 2007.01:2007	

Photo by Carlo Gatti

Picture taken from the magazine 'L'Informatore Agrario'

nated, bees were still clean. In this case, data are still not enough even to dare a hypothesis: there are no elements to track down a randomness in these relationships. Other seasons are necessary to establish whether a real correlation exists and if that correlation could indicate an important contamination dynamics.
What is definitely not random is the key role played by bees. They get in touch with glyphosate in the external environment both through their body surface and by swallowing nectar and pollen; when they come back to the hive, they first spread and then gather glyphosate again, perhaps the one that their sisters scattered on the wax and, through propolis, on the hive wood. When they concentrate nectar to transform it into honey, they can swallow the molecule again, maybe keeping a part of the contamination within their bodies; then larvae are fed with bee bread, therefore bees start to absorb glyphosate since their early days after the eggs hatch. Bees are definitely the most exposed elements within the hive. Therefore, their contamination was taken for granted even before bees were analysed.
However, the recorded data were surprising: always above 50 ppb and higher than those referring to honey. If honey is an optimal accumulation matrix for glyphosate because the contaminated nectar of millions of flowers is concentrated in a few hundred grams, bees are the primary bioaccumulator[5], as they gather from various sources and concentrate contamination within themselves before they do it in honey. The accumulation in honey is therefore secondary, or we could say residual, and by itself it could not witness the entire quantity of glyphosate that enters the hive. Residues in honey are 'permanent' and can witness the contact between the bees and the molecule even a long time after the harvesting. On the contrary, the residues in the primary bio-accumulator run out with bees' death or depopulation. Unlike other matrices, bees get out of the hive and, above all, they evacuate and die: therefore they represent the only way out of the hive for glyphosate, and the only element able to actively reduce the contamination within the nest.
Bees gather the molecule, they deposit it into the nest, swallow it again, collect it with their own legs, absorb, 'digest' and convey it outside of the hive through their faeces and body surface. Basically, the bees are glyphosate vehicle and filter within the hive: they clean the wax up by collecting the molecule with their body surface and they (partially) clean the nectar up while transforming it into honey.
For example, bees' role might explain the difference in contamination between the honey from the nest and the honey from the honeycomb, for example (provided that there is enough space in the nest to allow the nectar to ripen), and it can explain the observations produced by Berg et al. 2018[6], claiming that bees are a glyphosate contamination vehicle for the environment surrounding the nest. This does not mean that bees are the direct source of environmental pollution. It means that bees are not just victims of the contamination but they are also the direct and incontrovertible evidence of the extreme mobility of the molecule. What the American research portrays is indeed the glyphosate cycle: the molecule is sprayed, it spreads in the environment, it lands on flowers or reaches them through the plant sap, it contaminates the bees, through their bodies, it goes back to the environment expanding and making its scope uniform.
In other words, the biological dynamics that might save the honey in the honeycomb from the risk of more serious and penalizing (for the beekeepers) contaminations, condemn the bees to ongoing and daily contacts with the molecule. The results of the bio-monitoring analysis tell us that this contacts are quantitatively important and this data, as confirmed by Motta et al. 2018[7], could represent a serious problem for the hive.

[5] *From wikipedia: In toxicology, bioaccumulation is the process through which polluting and toxic substances, such as DDT, dioxins, furans or Fluorides, accumulate within an organism, in higher concentration than those found in the surrounding environment. This accumulation can occur through breathing, ingestion or simple contact, depending on the characteristics of the substances. The term 'bioaccumulation' was introduced between the 50s and 60s by a group of US naturalists who found high concentration of DDT in the organism of some bird species. Following this discovery, DDT was banned in the US and in many other countries in 1973.*

[6] Carl J. Berg, H. Peter King, Glenda Delenstarr, Ritikaa Kumar, Fernando Rubio, Tom Glaze, *Glyphosate residue concentrations in honey attributed through geospatial analysis to proximity of large-scale agriculture and transfer off-site by bees*, Plos One, 2018

[7] Erick V. S. Motta, Kasie Raymann, and Nancy A. Moran, *Glyphosate perturbs the gut microbiota of honey bees*, PNAS October 9, 2018

2019 PALYNOLOGICAL ANALYSIS

Analysing contaminated bee bread samples and establish their exact origin allowed to reliably connect the glyphosate in the matrix to its environmental source. The connection allows us to establish the plants where bees gathered the molecules from and speculate therefore on the contamination modality of vegetables. We are revealing right away that we can openly assume a connection with the aerial drift on flowered plants only on a few occasions. In most cases, pollens come indeed from plants that have nothing to do with the cultivations. As for August samples, the contaminated plant was one of the most spontaneous: ivy.

CALCULATIONS RESULTS

Pollen Types	Calculations	% Granules	% Volume
Salix	490	90	59
Compositae T <35 micron	20	4	15
Prunus < 40 micron	12	2	13
Quercus robur	6	1	4
Crataegus	1	p	1
Indeterminati/Unidentified	2	p	1
Malus/Pyrus/Sorbus	2	p	1
Papaver	5	1	1
Fraxinus ornus	1	p	p
Lotus	2	p	p
Melilotus/Trigonella	2	p	p
Veronica	p	p	p
Totale conteggio	543	100	100
Spore e ife fungine (come % granuli pollinici)	non osservati		
Granuli di amido (come % granuli pollinici)	non osservati		
Altro -			

MARCH - SOUTH ALBA UNIT

This report refers to a sample in which 64 ppb of glyphosate were found. Results are clear: 90% of the observed pollen belongs to plants from the genus Salix, the willows. In this case, willows are present in bare terrains along the Tanaro riverbed, in an area contiguous to the arable lands that already underwent herbicide treatments in March 2019, especially the wheat and rapeseeds. Was it aerial drift on flowered willows then? This cannot be excluded, as it cannot be excluded that the molecule was directly absorbed by the soil that had been recently sprayed with the herbicide.

MARCH - ALBA NOCC UNIT

This report refers to a sample with 44 ppb of glyphosate. The species with the highest percentage is the chestnut, despite the fact that its recovery, together with ivy, in a sample collected in a honeycomb in March is definitely out of season. The honeycomb certainly contained pollen gathered by bees in the past season and it is therefore impossible to see as significant the presence of chestnut and ivy as March source of contamination. The situation is different for Prunus (wild cherry), mainly present in high volumes and really significant for the period. Glyphosate from wild cherry then? It is impossible to state with certainty the connection between the plant and the molecule being the contamination, at least in theory, a possible residue from 2018. The area is characterized by hills with steep slopes and the presence of real cliffs: while hazelnut fields occupy the crest and the mildest and best exposed slopes, the bush housing the cherry trees occupy the least accessible sides and the least sunny gorges. The environment is ideal for aerial drift phenomena if it was not for the fact that in March the herbicide had not been sprayed yet in hazelnut fields.

CALCULATIONS RESULTS

Pollen Types	Calculations	% Granules	% Volume
Prunus < 40 micron	151	26	51
Hedera	119	21	34
Artemisia	63	11	3
Castanea	166	29	3
Compositae T <35 micron	8	1	1
Laurus	1	p	1
Salix	41	7	1
Clematis	3	1	p
Compositae A	2	p	p
Compositae H	4	1	p
Cruciferae	2	p	p
Indeterminati/Unidentified	3	1	p
Plantago	1	p	p
Rubus	4	1	p
Spore di ruggine	2	p	p
Trifolium repens	2	p	p

MARCH - NORTH ASTI UNIT

This report refers to a sample containing 46 ppb of glyphosate. The most represented vegetable species are, in descending order, Prunus, Quercus and Salix. In this case there are no doubts: we are facing a contamination clearly derived from pollen gathered from non cultivated species. Although Asti hills are milder than Alba hills, they are an ideal place for aerial drift, with woods that even if located on a different hill, are a few hundreds, if not a few dozens of meters away from the cultivated fields, as the crow flies. For North Asti as well as for Alba Nocc unit, March was not the disinfestation month, even though 2019 season was characterised by weed vigour and luxuriance. If in this case contamination modality is supposed to remain a hypothesis, the fact that pollen derives from spontaneous plants - in particular from wild cherry - implies the widespread and invasive presence of glyphosate on the flowerings of the entire woodland area located in bees' operating range. In March wood seemed to have gathered from a wide herbicide tank that it is hard to believe it was caused by aerial drift, given the low number of arable lands in the area.

CALCULATIONS RESULTS

Pollen Types	Calculations	% Granules	% Volume
Prunus < 40 micron	262	50	76
Quercus robur	77	15	14
Salix	149	28	4
Acer negundo	2	p	p
Betulaceae/Corylaceae	3	1	p
Castanea	7	1	p
Compositae T <35 micron	4	1	p

APRIL - SOUTH ALBA UNIT

This report refers to a sample containing 24 ppb of glyphosate. The samples collected from South Alba unit are more composite than those considered so far as they include locust, cruciferous, brassica and grasses. In this case all the plants flowering at the time of sampling are cultivated or installed in terrains that are very close to plots that underwent herbicide treatments. However, it is interesting to observe how the contamination of this sample that we could define as very 'agricultural' and that was collected during a period that was one of the most highly affected by herbicide treatments, presents a lower contamination than those found in samples of more 'spontaneous' pollen. Basically, the closer to the area sprayed with glyphosate, the lower the contamination values. A real nonsense that can be interpreted in various ways, none of which can however shed light on the data. According to the beekeeper in charge of the unit, bees were very much interested in the rapeseed, as the locust had just flowered. Can the main contamination be ascribable to rapeseed, then diluted with cleaner pollens? The hypothesis cannot be excluded but palynological analysis does not help us in this respect. The presence of chestnut pollen is to be reported: the beekeeper inserted honeycombs that still had chestnut traces from the previous season.

CALCULATIONS RESULTS

Pollen Types	Calculations	% Granules	% Volume
Prunus < 40 micron	262	50	76
Quercus robur	77	15	14
Salix	149	28	4
Acer negundo	2	p	p
Betulaceae/Corylaceae	3	1	p
Castanea	7	1	p
Compositae T <35 micron	4	1	p

APRIL - ALBA NOCC UNIT

This report refers to a sample with 56 ppb of glyphosate. The species indicated in the analysis are, in decreasing order, oak, walnut, cherry, horse chestnut and broom. The only species that could be cultivated is the one belonging to the cruciferous family, represented by 3% of the sample volume and by 6 hectares of arable lands for rapeseed as shown by cartographies. Even in this case, agriculture does not seem to be involved, unless we consider rapeseed as a strong contaminant and the other species as diluents. Pollen percentages weaken the validity of this hypothesis: spontaneous flowerings are the source of contamination, there are little doubts about this. The sampling time coincides with the herbicide treatments in hazelnut fields, therefore aerial drift cannot be excluded; the fact remains though that even in April, as well as in March, woods in the Alba Langhe area distribute significant amounts of glyphosate. Was it aerial drift again? Or the presence of the most widespread molecule, perhaps in the terrains that feed the woods? It is hard to say.

Pollen Types	Calculations	% Granules	% Volume
Quercus robur	125	24	31
Juglans	53	10	22
Prunus < 40 micron	47	9	17
Aesculus	101	19	6
Genista	36	7	4
Cruciferae	21	4	3
Fraxinus ornus	57	11	3
Compositae T <35 micron	8	2	2
Crataegus	2	p	1
Indeterminati/Unidentified	7	1	1
Pinaceae	2	p	1
Sambucus nigra	25	5	1

CALCULATIONS RESULTS

Pollen Types	Calculations	% Granules	% Volume
Cornus sanguinea	89	17	63
Cruciferae	202	39	15
Juglans	40	8	8
Chamaerops	107	21	4
Robinia	25	5	2
Prunus < 40 micron	5	1	1
Salvia	5	1	1
Aesculus	18	3	p

APRIL - NORTH ASTI UNIT

This report refers to a sample with 20 ppb of glyphosate. By analysing the pollen, we can observe a prevalence of cruciferous that represents almost 40% of the total, followed by the presence of an unusual pollen, that of Chamaerops - a decorative plant, the classic garden palm - and that of crabgrass. Agricultural areas, home gardens and the borders of woods are therefore represented by this sample. If we exclude that herbicide treatments can be applied to gardens, cruciferous and crabgrass are located in an area that is definitely affected by glyphosate spraying or aerial drift. However, once again, the 'agricultural' sample records a contamination of 'only' 20 ppb, with spontaneous flowerings of the previous samples always between 40 and 60 ppb. Another case of 'diluted' cruciferous?

MAY - ALBA NOCC UNIT

This report refers to a sample of 11 ppb of glyphosate. It is one of the most composite samples of the season with pollen typical of polyphytic meadows and woods (oak, black bryony and walnut). It is impossible to find the source of contamination, even though neither of the represented environments can be considered as being affected by direct herbicide treatments. The presence of glyphosate is not significant, therefore any interpretation would be a wild guess in this case. Chestnut pollen shows up again: the beekeeper substituted the honeycomb.

CALCULATIONS RESULTS

Pollen Types	*Calculations*	*% Granules*	*% Volume*
Juglans	44	8	15
Trifolium repens	99	19	13
Trifolium pratense	29	6	11
Cornus sanguinea	9	2	10
Tamus	58	11	10
Quercus robur	31	6	6
Prunus < 40 micron	16	3	5
Robinia	25	5	4
Chamaerops	33	6	2
Crataegus	5	1	2
Pinaceae	3	1	2
Castanea	64	12	1

CALCULATIONS RESULTS

Pollen Types	*Calculations*	*% Granules*	*% Volume*
Graminaceae > 35 micron	81	16	35
Compositae H	140	28	13
Medicago	38	7	11
Plantago	79	16	9
Zea	1	p	4
Indeterminati/Unidentified	16	3	3
Thymus	12	2	3
Trifolium repens	23	5	3
Amaranthaceae/Chenopodiaceae	38	7	2
Centaurea jacea/solstitialis	11	2	2
Trifolium hybridum	12	2	1

JULY - NORTH ASTI UNIT

This report refers to a sample with 542 ppb of glyphosate. The highest peak of the season, recorded during the herbicide treatment of stubbles, shows a palynological profile that is absolutely compatible with the one coming from the complex of flowerings normally present in a stubble in the month of July. The presence of graminaceous, plantains and amaranth leaves little room for doubt: bees had gathered pollen from a recently treated stubble, in an uncultivated area located in close proximity. The only "intruders" were alfalfa and clover pollens, present in significant quantities, but we have no evidence to claim whether these plants were contaminated or not. It remains a fact that an activity that is considered ecological (because of the lower fuel consumption and the consequent lower greenhouse gas emissions) such as stubbles herbicide treatment can turn out to be a real danger for the environment and the bees. Bees can no longer rely on the flowers that up to not many years ago bloomed in the stubbles in the month of July and, on top of that, they literally risk their lives collecting significant amounts of glyphosate. Definitely not an ecological activity! The bio-monitoring highlights how this practice is efficient in reducing pollinators' biodiversity: it deprives them of 90% of their food in the toughest months of summer and seriously contaminates the remaining 10%.

AUGUST - NORTH ASTI UNIT

This report refers to a sample with 58 ppb of glyphosate. The quantity of ivy pollen in the sample stands out right away, which puzzles us because the percentage implies an almost certain correlation with the plant. However, ivy grows and flowers in slopes and gorges that are less exposed to the sun, far from cultivated areas; it is hard to think about a significant ivy flowering in the proximity of a corn field, a hazelnut field or a vineyard. It is particularly hard to think about an herbicide treatment occurred at the end of August/beginning of September, at the time the samples were collected: the aerial drift phenomenon is therefore to be excluded. The other pollens found in the samples fall, as in July, in the spontaneous flowerings present in the stubbles. Is it therefore possible that contamination derives from other pollens, perhaps diluted by ivy? It is possible, though not likely, as we will see hereinafter.

CALCULATIONS RESULTS

Pollen Types	*Calculations*	*% Granules*	*% Volume*
Hedera	275	54	75
Compositae H	71	14	5
Artemisia	68	13	4
Plantago	30	6	3
Graminaceae > 35 micron	6	1	2
Centaurea jacea/solstitialis	5	1	1
Compositae T >35 micron	2	p	1
Thymus	5	1	1
Amaranthaceae/Chenopodiaceae	7	1	p
Betulaceae/Corylaceae	1	p	p
Bignoniaceae	1	p	p

AUGUST - SOUTH ALBA

This report refers to a sample with 104 ppb of glyphosate. In this case there are no doubts, contamination derives from ivy. Besides, as previously mentioned, the 104 ppb found in 2019 match the 98 ppb found in 2018 and recorded in the same period always in South Alba unit. There is no palynological analysis available for the past season but it is likely that the 2018 sample had the same vegetable origin as the sample of this year. In this area, ivy is much more exposed to aerial drift phenomena compared to North Asti area, being woods located on hills slopes that overlook the Tanaro valley directly: woods basically act as banks for the cultivated flat area and glyphosate droplets dispersed by the wind inevitably lay on the "walls" of this natural hollow that is the Alba narrow alluvial plain. The fact remains that the last herbicide treatments in the area occurred in mid July: how could ivy still be contaminated almost two months later? At this stage it is inevitable to single out the soil and the absorption and release dynamics of the molecule: the aerial drift could have laid on woods terrains becoming available to plants only after some time. However, why in ivy? What made the molecule 'move' only at the end of August? The only plausible hypothesis is rain. Theoretically, August heavy storms, by moisturising the soil, could have brought the molecule, 'ready' to be absorbed, to the plants roots; and the only plant in full swing in August is ivy, that right in this period is about to flower. The hypothesis implies the inability of soil to metabolise glyphosate because the molecule would preserve itself almost unaltered for two whole months ready to enter the ivy. Is that a long shot? Imagination? Or the only plausible explanation. Let's move on.

CALCULATIONS RESULTS

Pollen Types	*Calculations*	*% Granules*	*% Volume*
Hedera	468	91	92
Graminaceae > 35 micron	16	3	4
Amaranthaceae/Chenopodiaceae	14	3	p
Ambrosia	1	p	p
Citrullus	1	p	p
Compositae H	4	1	p
Compositae T >35 micron	1	p	p

AUGUST - ALBA NOCC UNIT

The report refers to a sample with 59 ppb of glyphosate. There are no doubts in this case too: the source of contamination is the Langhe ivy. In this area the plant is way less exposed to aerial drift compared to South Alba unit but the plausible hypothesis on the contamination dynamics cannot be the same as the ones proposed for the control units located in the Tanaro valley. In this case too, as in the other two bio-monitoring control units examined above, pollen in August is not the only contaminated matrix: nest honey, that was probably ivy honey, presents significant contamination values: 19 ppb in North Asti, 34 ppb in South Alba and 29 ppb in Alba Nocc Unit. This data makes the situation even more concerning: the ivy was literally impregnated with glyphosate, as if it had absorbed and given back to bees all the herbicide accumulated in its habitat during the season. Peaks recorded at the same time in August in all the control units suggest that summer rains really released glyphosate that had been 'locked' in the terrain since June and July drought.

CALCULATIONS RESULTS

Pollen Types	*Calculations*	*% Granules*	*% Volume*
Hedera	498	93	95
Compositae T >35 micron	5	1	1
Ambrosia	10	2	p
Castanea	2	p	p
Compositae A	2	p	p
Compositae H	7	1	p

AUGUST - BIANCO UNIT

This report refers to a sample with 49 ppb of glyphosate. No doubts in this case either: ivy is at fault. We should not be surprised at this stage unless for the fact that the plant flourished at 850-900 metres above sea level. This data, even more than the one recorded in March in the same location, is the real enigma of the season, a mystery that disturbed ANAS too (the National Autonomous Roads Corporation) in trying to provide a logical explanation to the presence of the molecule in an Alpine valley. ANAS confirmed that the roads edges in the area have not undergone herbicide treatments in 3 years at least and the beekeeper in charge of the unit informed us that the only cultivation within the range of 3 kilometres from the control unit is a corn field... Where is the molecule coming from then? What mechanisms caused it to concentrate into the ivy flowering? Was it accumulated into the soil and then 'released' by the rains? Did it fall out from the sky? It is really impossible to explain this data.

CALCULATIONS RESULTS

Pollen Types	*Calculations*	*% Granules*	*% Volume*
Hedera	437	87	93
Plantago	48	10	4
Trifolium repens	12	2	1
Calluna	p	p	p
Caryophyllaceae	p	p	p
Castanea	p	p	p
Compositae H	4	1	p

Palynological analysis proved to be a crucial tool for a better interpretation of the environment bio-monitoring data as they allowed us to connect the contamination to the plant species where bees gathered pollen from. It was indeed crucial as it enabled us to outline a very different and much more concerning scenario that what the other two seasons suggested. With the exception of North Asti unit in July, where the link between contamination and agricultural activities is clear and direct, in the other cases the most significant contaminations throughout the whole season occurred on spontaneous plants flowerings, in most cases on timber trees that grow exclusively in woodlands. The higher presence of glyphosate in woods, recorded in periods preceding and following the herbicide treatments, compared to that found in cultivations seriously questioned the hypothesis of a widespread and systematic aerial drift on flowers. It was not about the aerial drift, at least in 2019.
Pollen analysis drew our attention towards another contamination dynamics, where soils, perhaps assisted by rains, are the key players. Unfortunately, as we have already mentioned, there is no scientific evidence to support or discredit this theory but logic and common sense tell us that, after excluding the other hypotheses, the one that remains is quite likely the right one. It is therefore legitimate, at least in theory, to talk about a molecule that, spread over big surfaces by the aerial drifts, accumulates in terrain, becoming available to plants only when the humidity levels in the soil facilitate plant growth. From this perspective, plants act as a filter for the soils, absorbing and exuding glyphosate by cleaning the terrain and leaving the molecule to insects. After a new herbicide treatment, the molecule accumulates again and with the rain a new cycle begins. Is that just imagination? Possibly, but as we said above, that is the only plausible hypothesis left. It would be interesting and desirable to verify this hypothesis with a specific study.
It is worth mentioning that some of the samples were too scant (in some periods pollen was really scarce) and they run out during the chemical and physical analysis therefore it was not possible to carry out palynological analyses. At the time this study is being written, we do not have the report on September samples yet.

Microbiological monitoring

As previously mentioned, South Alba control unit witnessed a real experiment, that involved the use of extra samples of young bees, aimed at carrying out microbiological analysis on bees' intestines. The purpose of the test was that of verifying the potentiality of an instrument theoretically able to measure 'in real time', the correlation between the presence of glyphosate in the nest and the alteration of bees' intestine microbiota. A remark is necessary before starting to describe the instrument. The study carried out by Motta et al. in 2018 titled *Glyphosate alters bees' gastrointestinal microbiota*, previously quoted, proves the detrimental effects of glyphosate on bees' intestinal bacteria and indicates dysbiosis, which causes the exposure to pathogens attacks, as the real problem created by the molecule. One of the main bacteria in bees' intestine has a gene called EPSP that can normally be linked to plant genome. And *Snodgrassella alvi*, this is the name of the bacterium, from a genetic perspective, has the same characteristics and the same vulnerability to glyphosate as plants. The EPSP gene is indeed the only objective of glyphosate. The herbicide inhibits the cellular biochemical process derived from the gene, blocking an essential and vital process in all plants... and in *Snodgrassella*. As glyphosate target is highly selective, it is considered not harmful for animals, just because animals do not have this gene; and this thesis is the bedrock of the herbicide risk assessment. Because of *Snodgrassella alvi*, bees represent an exception to the rule.
But what are the practical consequences of the sensitivity

of *Snodgrassella* to the molecule? Bees exposure to pathogenic bacteria such as *Serratia marcescens*, highly present in nature and permanent guests of their intestine. These are pathogens that normally cannot find a space where to take root, however in the absence of the protective biofilm produced by *Snodgrassella* on the intestine walls, they find fertile ground for their development. Bees, particularly the young ones whose intestinal microbiota is still taking shape, die from septicaemia, even days after getting exposed to glyphosate.
However, *Serratia marcescens* is not the only opportunist microorganism, ready to exploit the advantages offered by an intestinal dysbiosis: also the microsporidium *Nosema ceranae* takes advantage of the ecological niche cleared by bacteria such as *Snodgrassella*. *Serratia* and *Nosema* are the-

[8] For more information on the matter of glyphosate aerial drift refer to Lucio Lucadamo, Anna Corapi, Luana Gallo, *Evaluation of glyphosate drift and anthropogenic atmospheric trace elements contamination by means of lichen transplants in a southern Italian agricultural district*, Air Quality, Atmosphere & Health, April 2018, Volume 11, Issue 3, pp 325–339

refore two optimal indicators of bees intestinal dysbiosis: if their populations grow, then the population of 'useful' bacteria has undergone an alteration. On the contrary, if their presence is marginal, that quite likely means that the bee is in good health. What we just explained is actually an extreme simplification of the microbiological mechanisms that regulate the hive health. However, we have reason to consider the two pathogens as a good thermometer to measure bees' health wellbeing.

On this basis, the idea to collect 30 young bees each month (young bees are the most highly exposed to glyphosate effects) in order to monitor the populations of *Serratia marcescens* and *Nosema ceranae*, seemed a good one to test an absolutely new monitoring system though potentially very efficient. It is worth saying that monthly sampling is not sufficient to affirm with scientific certainty that the glyphosate present in the nest is the real cause of a measurable damage to the hive. Dozens of sampling in the same number of environmental scenarios would be required only to outline the microbiological profile of an individual hive. It would then be necessary to start from that hive to measure the actual impact of glyphosate. Aspromiele's project does not aim at becoming a scientific study, at least for the moment. Therefore, monthly samples, whose data will be cross checked with those on pollen contamination, are sufficient to test the instrument. In other words, the question we are trying to answer is the following: are the contaminations detected with the bio-monitoring able to favour, in the short term and in a measurable way, a dysbiosis and therefore the growth in the population of the two pathogens? If the answer is yes, then the microbiological analysis will be able to snapshot the dysbiosis, otherwise, a more in depth, well-structured and expensive analysis will be required.

Unfortunately, at the moment we still do not have the reports of the microbiological analysis, therefore the reader will have to refer to a future issue of l'apis for the results of this experiment. For the moment, we can only say that if the microbiological instrument was efficient, then it would be the only instrument able to establish the glyphosate damage threshold for hives. At the moment, we know that it is harmful for bees but we do not know what doses would make the damage to the hive visible.

Of course the depopulation of South Alba unit following the 790 ppb found in honey in 2018, the depopulation of the same unit in September 2019 following the 2014 ppb found in bee bread (together with the 34 ppb of honey in the same sampling) and the extinction of North Asti unit in September 2019, again following the 542 ppb found in bee bread seem far more than mere coincidences. However, we do not have the elements to unequivocally link these episodes to glyphosate, also because bees do not die for glyphosate poisoning but they die for septicaemia or for *nosema* disease. It is therefore important to link the presence of glyphosate to the increase in the populations of *Serratia* and *Nosema*, if we want to untangle the knot of the effects of the herbicide molecule on hives.

In addition, being able to connect glyphosate to the onset of these pathogens could provide an interpretation for the numerous and inexplicable phenomena of depopulation in hives that now regularly occur in front of Italian (but not only) beekeepers' eyes, in each season. What if glyphosate was really the root cause of these phenomena?

Conclusions

2019 bio-monitoring season leaves us with more questions than answers, questions that we will try to investigate further in the coming seasons. For the moment, we can affirm without a doubt that, as far as glyphosate is concerned, the more one investigates the more the situation is disquieting, not to say alarming. The herbicide molecule present in pollen at 850 metres above sea level, after the frustration of Aspromiele's intention to own a control unit in Bianco, seriously questions the conviction that the glyphosate present in hive matrices could be attributed to the proximity of flowerings to agricultural areas. Contaminated pollen derived from spontaneous flowerings in other control units, besides reinforcing the doubts on the direct connection between agricultural areas and contamination, particularly questions the role of aerial drift too, which is taken for granted and considered 'exhaustive'. This aerial drift does not seem to be involved in the most concerned phenomenon of the season at all: the contamination of ivy in all four control units, from Asti to Valle Stura, a long time from the herbicide treatments period. Not only one of the most precious and important pollens of the whole season proved to be a problem for bees instead of a resource but its contamination from glyphosate leaves an only hypothesis as plausible: Piedmont soils play a key role in the spread of glyphosate, which is definitely more important and crucial than the role of air. This hypothesis has very serious implications, more serious than those connected to the contamination peak recorded on North Asti stubbles, which 'condemned' the unit hive, as it provides for a vision of the future that is far from being rosy. Even if glyphosate was banned in this moment, it is quite likely

Picture taken from the magazine Agrinotizie: Stubbles herbicide treatment

Photo by Carlo Gatti: Bee on ivy

that bees would still keep collecting glyphosate for a long time, way longer than the 7 months recorded as the molecule persistence in hazelnut fields, with bee bread monitoring in February. Perhaps they would no longer incur in the 542 or 790 ppb, clear evidence of the direct contact between the herbicide and the flowers, but they would still be subject to glyphosate flows able to create chronic dysbiosis within the hives. From this perspective, the microbiological analysis of bees that 'witnessed' the 104 ppb on South Alba ivy is going to be an interesting one: if a variation in bees' microbiota was recorded, the data would not only match the peak recorded but also the depopulation of hives at the end of August, reported by the beekeeper in charge. If the three phenomena could be linked to one another, we would have strong evidence of the effects that the environment has, nowadays, on the hive.

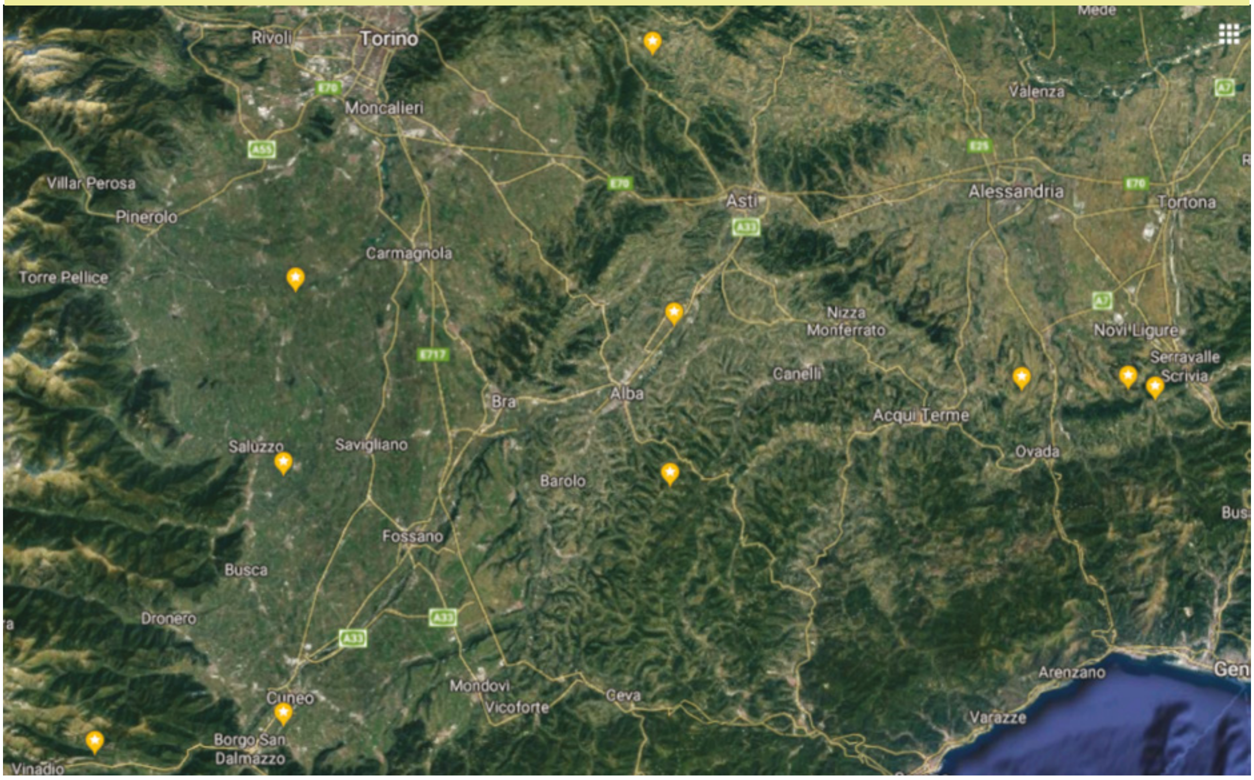
In the meantime, we can only report the picture that emerged from this season bio-monitoring to the local Authorities: it is a darker picture than the one reported in the previous seasons and much more concerning if we look at the future.

After listing the negative and concerning aspects, there is some good news, which comes from the Gavi area. In spite of the fact that data are still confidential and in the process of being analysed, we can report a better picture than the one recorded in Aspromiele's units, particularly as far as glyphosate pollution is concerned. This proves that improving is possible and this can be done by practicing a high quality sustainable agriculture. Good news comes from the 'external' collaborations which are increasing to prove the growing interest in Aspromiele's project. It is worth mentioning new and important collaborations with the Piedmont Administration Environment Department and the Biodiversity Department, with Ipla (Institute for the wood plants and the environment), with the University of Turin through the 'Progetto vite 4.0' and with the biomonitoring collaboration with CARE, the regional reference centre for apiculture belonging to Asti Zooprophylactic Institute.

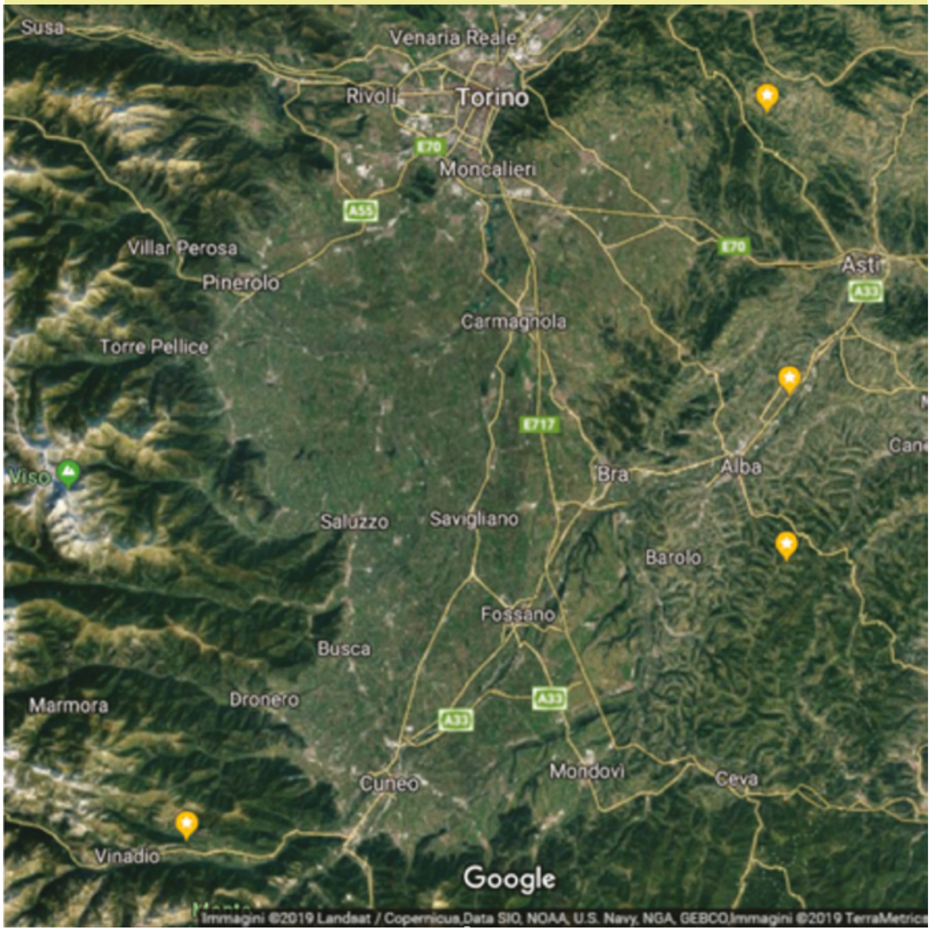
Among the good news, we want to highlight the conviction, the professionalism and the commitment made available by the people who work at the Aspromiele's project. The effort made by the Association, that pays for the monitoring expenses with their own funds (25,000 € spent in for analysis only in 2019), is widely rewarded by the interest shown by the Institutions, Private and Government agencies linked to the agricultural world, by beekeepers and by the world of beekeeping associations. The acknowledgement of the value of the project is without a doubt the best incentive for the seasons to come and we like to think that it is great news for the future of bees and the environment. This perspective proves to be an increasingly useful (and cost effective) instrument, suitable for a better understanding of the environmental impact of anthropic activities and, as a consequence, to direct the government policies. In our opinion, it is therefore obvious that the project could be officially included among the activities of the next Cap, as a measuring tool of the results obtained and the improvements made throughout time.

To conclude, we would like to draw the attention of the world of scientific research: the environmental bio-monitoring with bees brought the Association to begin to discover the chemical and biological cycle of glyphosate which requires to be analysed in depth because bees, the sentinels of the environment, with their aches and pains, are reporting an issue that directly affects all of us, more than we can imagine. To those who can listen to them, bees raise questions and mysteries, and suggest hypotheses that surround us and could perhaps... kill us. See you in the next episode.

CONTROL UNITS OVERVIEW



ASPROMIELE'S CONTROL UNITS



ALBA NOCC CONTROL UNIT

USE OF ALBA NOCC SOIL > 2.5 ha	*AREA (ha)*	*% out of the total area*
Hazelnut	241,6	49,2
Wood	126,6	25,8
Polyphyte meadow	30,1	6,1
Non-agricultural use	22,5	4,6
Hazelnut II	19,0	3,9
Wood	13,2	2,7
Non-agricultural use - constructions	6,8	1,4
Non-agricultural and non-residential constructions	6,3	1,3
Arable lands	6,1	1,2
Non-agricultural use and uncultivated	4,3	0,9
Ryegrass	3,1	0,6
Vine	3,1	0,6
Specialized arboreal cultivations	2,9	0,6
Non-agricultural use - other	2,6	0,5
Polyphyte meadow (forage)	2,5	0,5

SOUTH ALBA CONTROL UNIT

USE OF SOUTH ALBA SOIL > 5 ha	*AREA (ha)*	*% out of the total area*
Hazelnut	356	19,2
Soft wheat	300	16,1
Vine	280	15,0
Corn	232	12,5
Polyphyte meadow	126	6,8
Non-agricultural use	80	4,3
Inactive agricultural terrains	71	3,8
Wood	58	3,1
Barley	36	1,9
Non-agricultural use - constructions	27	1,5
Oat	22	1,2
Arable lands	22	1,2
Pear	20	1,1
Specialized arboreal cultivations	17	0,9
Non-agricultural and non-residential constructions	17	0,9
Arboriculture	15	0,8
Ryegrass	10	0,5
Cardoon	10	0,5
Greenhouse	9	0,5
Apricot	9	0,5
Plum	9	0,5
Peas	8	0,4
Alfalfa	7	0,4
Soy	6	0,3
Vegetable gardens	6	0,3
Peach	6	0,3
Flax	5	0,3
Millet	5	0,3
Non-agricultural use - other	5	0,3

BIANCO CONTROL UNIT

USE OF NORTH ASTI SOIL > 5 ha	*AREA ha*	*% out of the total area*
Polyphyte meadow	280	25,4
Wood	173	15,7
Soft wheat	125	11,3
Corn	110	10,0
Hazelnut	69	6,3
Barley	62	5,7
Vine	43	3,9
Inactive agricultural terrains	32	2,9
Non-agricultural use	30	2,7
Soy	25	2,3
Alfalfa	20	1,8
Pea	18	1,6
Triticale	17	1,5
Arboriculture	14	1,3
Non-agricultural and non-residential constructions	12	1,1
Non-agricultural use - other	9	0,8
Non-agricultural use - constructions	9	0,8
Ryegrass	7	0,6
Specialized arboreal cultivations	5	0,4
Vegetable gardens	4	0,3

NORTH ASTI CONTROL UNIT

USE OF BIANCO SOIL > 2.5 ha	*AREA ha*	*% out of the total area*
Wood	226,9	28,8
Polyphyte meadow	207,4	26,3
Polyphyte pasture	96,8	12,3
Pasture	70,6	9,0
Wood	44,2	5,6
Mixed Pasture	38,5	4,9
Wooded pasture	30,7	3,9
Wooded pasture (bush)	22,9	2,9
Polyphyte meadow (forage)	19,9	2,5
Non-agricultural use	15,6	2,0
Non-agricultural use - constructions	6,1	0,8
Chestnut	3,7	0,5
Non-agricultural use - other	2,6	0,3
Specialized arboreal cultivations	2,5	0,3

Associazione Produttori Miele Piemonte AsProMiele

MINISTERO DELLE POLITICHE AGRICOLE ALIMENTARI E FORESTALI

UNIONE EUROPEA

Realizzato con il contributo del Reg. Ue 1308/13