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ABSTRACT

Three viral diseases transmitted by aphids can cause significant yield losses in beet, straw cereal and oilseed rape production. For a long time, these crops were protected against yellows by targeting aphids using plant protection products that have recently been banned (neonicotinoid family) or are still authorised, but whose effectiveness may be inadequate following the appearance of aphid populations resistant to certain insecticide active substances.

Academic researchers, technical institutes and companies have worked together as part of the ABCD_B research project, with the aim of evaluating new solutions for protecting these crops against viruses. Work was carried out on the three species, both in natural field conditions and under controlled conditions.

The results highlighted and quantified differences in varietal sensitivity within each of the crop species (wheat, oilseed rape, sugar beet). At the same time, a major effort to evaluate biocontrol products has produced encouraging results for two active substances (azadirachtin and paraffin oil), even though their efficacy remains significantly lower than that of reference solutions.

KEY WORDS: Aphids, viruses, straw cereals, oilseed rape, sugar beet, biocontrol, varieties

INTRODUCTION

Three viral diseases transmitted by aphids in the persistent mode, barley yellow dwarf diseases (BYD due to B/CYDV transmitted by *Rhopalosiphum padi* and *Sitobion avenae*), oilseed rape yellows (TuYV transmitted by *Myzus persicae*) and beet mild yellowing (BMYV transmitted by *Myzus persicae*) cause significant yield losses. Over the last few decades, crop protection against these diseases has mainly been based on controlling the populations of insects carrying the viruses, using chemical solutions based on neonicotinoids. However, the ban on this family of active ingredient from 2018 in France and the identification of insects resistant to other plant protection products have considerably reduced the number of molecules of interest for controlling insect vectors of BYDV, TuYV and BMYV. As a result, alternative solutions need to be identified to limit the health risks associated with these viruses.

To achieve this, academic researchers, technical institutes and companies worked together as part of the ABCD_B research project, the objectives of which were to evaluate new solutions likely to be of technical and agronomic interest, and then to specify the conditions for implementing the most appropriate solutions in order to optimise their effectiveness. Two types of solution were evaluated:

-Varietal, with a comparison of the behaviour of different genotypes of three cultivated species to identify possible varietal tolerance/resistance and understand the mechanisms involved,

-Biocontrol products, with a comparison of different solutions aimed at controlling aphids or modifying their behaviour and indirectly limiting the transmission of viruses to plants.

The value of the experimental methods was assessed by measuring various indicators relating to aphids (survival, multiplication), viruses (plant infection and viral accumulation) and crops (symptomatology and impact of the disease on yield). The data collected for a tested variety (a variety with an unknown resistant/tolerant/susceptible phenotype or a variety treated with a biocontrol product) was compared with data acquired with a variety receiving insecticide protection using a reference product.

Field work was carried out under conditions of natural infestation to assess the agronomic interest (in particular crop yield) of the solutions under evaluation. At the same time, studies have also been carried out under controlled conditions in the laboratory. In these conditions the biological process of viral infection has been subdivided into various elementary stages in order to gain a better understanding of the mechanisms involved, with a view to optimising the conditions under which the solutions are implemented in the field.

The solutions were voluntarily evaluated in analytical experiments, an essential step before being able to propose the use of the most relevant solutions in a combined and integrated way in a technical itinerary.

The methods used and results obtained within the ABCD_B project are presented in the following order: varietal leverage for the three crops (soft wheat, oilseed rape, sugar beet), then evaluation of biocontrol products (all crops).

VARIETAL LEVER - WHEAT

In-field evaluation:

Fifteen trials have been set up for the 2019, 2020 and 2021 seasons to assess possible differences in susceptibility to BYDV between soft wheat varieties. The trials were carried out in regions with recurrent problems of BYDV (Northern Nouvelle-Aquitaine, Pays de la Loire, Centre Val de Loire, Rhône-Alpes, Occitanie) and sown at early dates (sowing up to 15 days earlier than the recommended sowing dates) to increase the chances of infestation by aphids. All trials were conducted up to harvest.

Sixteen varieties with different characteristics (earliness at bolting and heading, yield components) were explored, ten of which were present in all the trials, including three reference varieties (UNIK, NEMO, RUBISKO) which were monitored more closely for the frequency of plants infested by aphids during the autumn.

In each trial, the varieties were compared without (NT) and with (T) insecticide treatment (Karaté Zéon, 0.075 l/ha, a.i: lambda-cyhalothrin, 1 or 2 applications), according to a criss-cross design with 4 (or 3) replications per modality of microplots of 15 to 20 m², spaced 30 to 60 cm apart. With the exception of the insecticide treatment in the autumn, all the methods followed the same technical cultivation itinerary.

After monitoring aphid infestations on the three reference varieties in the autumn, the main measurements concerned BYDV symptoms (% area affected and characterisation of symptoms using a scoring grid) and yield.

- *Aphid infestations in autumn*

Aphids taken from plants were analysed in 13 trials (2 trials without aphids). Identifications showed that the species *Rhopalosiphum padi* (9 trials out of 13) was present in the majority of cases. The species *Sitobion avenae* was present in 11 trials and was in the majority in 4 trials. Aphid infestations remained below 2% of infested plants in 5 trials. Among the 10 other trials with a rate of infested plants equal to or greater than 2%, 6 had infestations exceeding a rate of 10% of infested plants.

The nature, intensity and dynamics of infestations varied between trials. However, there was no difference in the percentage of plants infested by aphids between the three reference varieties monitored.

- *Symptoms of BYDV and yield losses*

Analysis of the results obtained on the three reference varieties present in each trial enables the fifteen trials to be classified into 2 groups according to the level of harmfulness of BYDV calculated on the basis of the differences measured between treated and untreated varieties. Nearly half of the trials (8 trials) resulted in low to moderate yield losses (<10%), while the harmfulness was higher (> 15% difference between treated and untreated varieties) in the other 7 trials.

We applied the method recommended by the CEB ("Commission des essais biologiques") for the validation of trials evaluating direct control solutions (which requires a significant difference between the untreated control and the control treated with a reference product). This led to the selection of the 7 trials where the harmfulness of BYDV was high. The statistical analysis was first carried out at the level of each trial, then the data was analysed by grouping the 7 trials together, integrating the residual variance and the degrees of freedom of each trial, followed by an analysis of variance (Newman & Keuls test). This analysis of the data revealed differences between the varieties studied ($p\text{-value}<0.05$) in terms of symptoms (Figure 1) and yields (Figure 2). The area affected by BYDV symptoms was close to 40% for 5 varieties (CHEVIGNON, LG ABSALON, NEMO, RGT LIBRAVO, SOLINDO CS) whereas it was significantly higher, at almost 60%, for 3 others (HYKING, RGT CESARIO and UNIK). Symptom intensity (leaf discolouration, shortened plants) was assessed visually. This scoring of symptom characteristics did not reveal any differences between soft wheat varieties.

An analysis of yields enables us to classify varieties into 3 sensitivity groups (Figure 2):
- Less susceptible varieties: 16 to 19% yield difference (SOLINDO CS, RGT LIBRAVO, NEMO and LG ABSALON),
- Intermediate varieties: 20 to 23% difference in yield (CHEVIGNON, HYKING),
- the more sensitive varieties: 24 to 29% difference in yield (RGT CESARIO, RUBISKO, UNIK and KWS EXTASE).

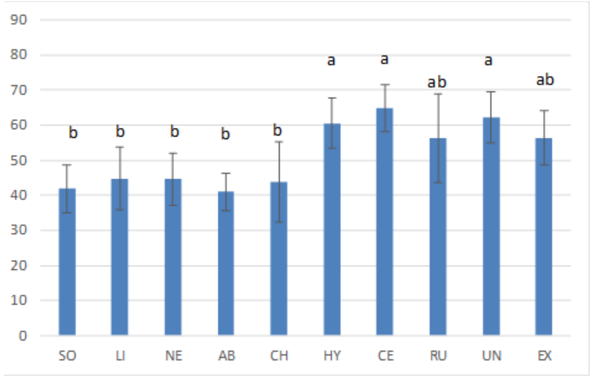

Figure 1: Frequency of BYDV symptoms in 10 varieties (average of 7 trials) ranked by increasing yield loss (p-value<0.05).

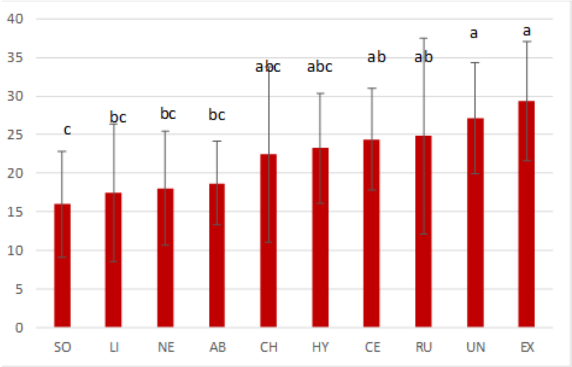

Figure 2: Percentage yield loss compared with the situation with insecticide treatment for 10 varieties (average of 7 trials) (p-value<0.05)

The experimental network, carried out on several sites and over several seasons, has revealed significant differences in varietal susceptibility to BYDV, with yield losses ranging from one to two times between the most and least susceptible varieties.

Within this range of varieties, the differences measured between yield losses are not explained by differences in earliness (bolting, heading), yield potential or by specific characteristics of the yield components. For these different criteria, more marked differences were observed between varieties in the same class (e.g. earliness) than between the classes studied. The differences in sensitivity thus appear to be linked to the intrinsic characteristics of the varieties. No variety showed complete tolerance/resistance. The mechanisms involved have yet to be identified and characterised.

Results obtained under controlled conditions:

Work carried out under controlled conditions has made it possible to assess a range of 12 wheat varieties - all of which were evaluated in the field in parallel - with regard to several parameters involved in the epidemiology of BYDV, concerning either aphid-host interactions (aphid multiplication, colonisation rate) or virus-host interactions (infection rate, viral accumulation and latency period) (Pichon et *al.* 2022).

The data generated to monitor the infection rate of the different wheat varieties (reflecting the level of host susceptibility, i.e. the success of the primary infection taking place when the disease is introduced into the plot) does cannot differentiate the 12 genotypes (Figure 3-a). However, analysis of viral accumulation measured using immunological tests showed that in three varieties (NEMO, TARASCON and HYKING, Figure 3-b) the maximum level of accumulation of Barley yellow dwarf virus-PAV (BYDV-PAV) was significantly lower than that observed in infected plants of the RUBISKO variety. Conversely, the viral accumulation described for the KWS EXTASE variety was significantly higher, suggesting more favourable virus-plant interactions for this variety.

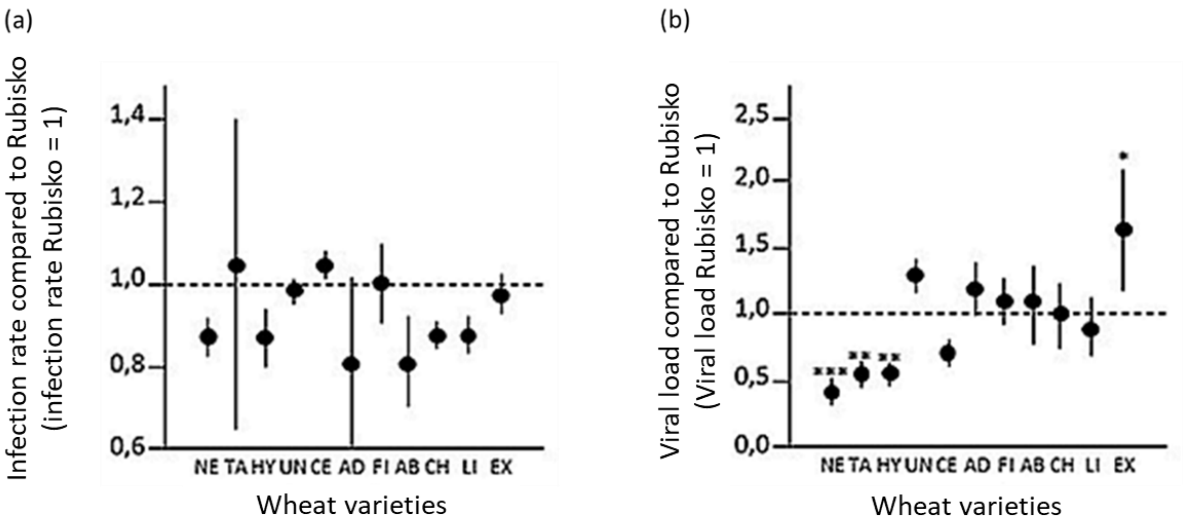

Figure 3: Ratios of infection rates (BYDV-PAV4) of 11 genotypes compared with the RUBISKO variety (a), and respective virus load ratios (b) three weeks after inoculation. Asterisks illustrate a significant difference (obtained with a quasibinomial generalised linear model (GLM)) between a tested variety and the reference variety RUBISKO. * :>0.05 ; **>0.01, ***>0.001.

In order to study all the virus-vector-plant interactions simultaneously, a multi-plant system ('Arena test' type) was set up and applied to 6 of the 12 varieties previously studied. The parameters collected during this experiment showed that i) the rate of colonisation of the plants in the system by aphids showed no significant difference between the varieties evaluated and the reference variety (RUBISKO), ii) the total number of aphids produced was significantly lower for the ADVISOR variety, iii) the average number of aphids present on colonised plants was equivalent for all the varieties tested and iv) whatever the method used to calculate the rate of viral transmission within the multi-plant system, no significant difference between the reference RUBISKO and the different varieties tested could be demonstrated.

The last parameter involves analysing the latency period within infected plants. This period is particularly important for understanding the disease's dispersal dynamics within the plot, as it describes the time after which an infected plant in turn becomes infectious, thereby helping to spread the virus. In the study carried out on the reference variety RUBISKO, infected plants turned out to be sources of virus for transmission as early as $3^{\text{ème}}$ days after inoculation, with a viral transmission success rate of around 15%. By $7^{\text{ème}}$ days after infection, the source plants had reached their maximum potential (around 65% transmission rate). Thus, the emergence from latency observed for the RUBISKO variety began as early as the first few days after infection and seems to have ended before $7^{\text{ème}}$ days after infection. When this study was carried out on other genotypes, several differences in the dynamics of latency emergence were observed, in particular for the HYKING, TARASCON and UNIK genotypes. For these three varieties, the transmission rate associated with source plants that had been infected for 7 days was significantly lower than that of the RUBISKO variety. These three varieties also emerged from latency more slowly.

All the descriptive elements of the infectious process provided by this study highlight the behaviour of these wheat varieties in the face of the arrival of *R. padi* and the BYDV-PAV that aphids can carry. Although none of the varieties studied was able to limit the effectiveness of the introduction of the disease into wheat plots (equivalent infection rates), several of the stages in the process of spreading infectious outbreaks (aphid reproduction and latency period) proved to be less effective for certain genotypes, which can be seen as encouraging for further work aimed at developing new protection strategies against dwarf yellows in barley in soft wheat plots. In addition, a relationship tends to emerge between viral accumulation analysed under controlled conditions and yield loss assessed in the field. As a result, it would be appropriate to continue joint investigations in the field and under controlled conditions, focusing on the few genotypes that showed contrasting behaviour in one or other of these situations. These results also confirm the need for more precise means to identify and quantify the accumulation of viral particles in plants.

VARIETAL LEVER - OILSEED RAPE

In-field evaluation:

Eight trials were set up in the Haut-de-France and Grand-Est regions during the 2017-2018 and 2018-2019 seasons. These trials compared the behaviour of a partially resistant TuYV variety (LG ARCHITECT) and a susceptible variety (reference DK EXCEPTION). Another variety (RGT QUIZZ) was added to two trials in 2018-19 in order to test the hypothesis of reduced susceptibility attributable to a distinct mechanism compared with LG ARCHITECT. Samples from six trials in the 2016-2017 season were used and analysed to provide consolidated results from a total of 11 usable trials over three seasons (Ruck et *al.* 2021-a).

In the conditions of the 2018-2019 season favourable to significant virus pressure, the partially resistant variety LG ARCHITECT showed a higher virus load than in previous experiments (2016-2017 and 2017-2018). However, even under this high viral pressure, the prevalence of TuYV and its viral load within infected plants remained significantly lower than the sensitive reference DK EXCEPTION (Figure 4), underlining its partial resistance status**.**

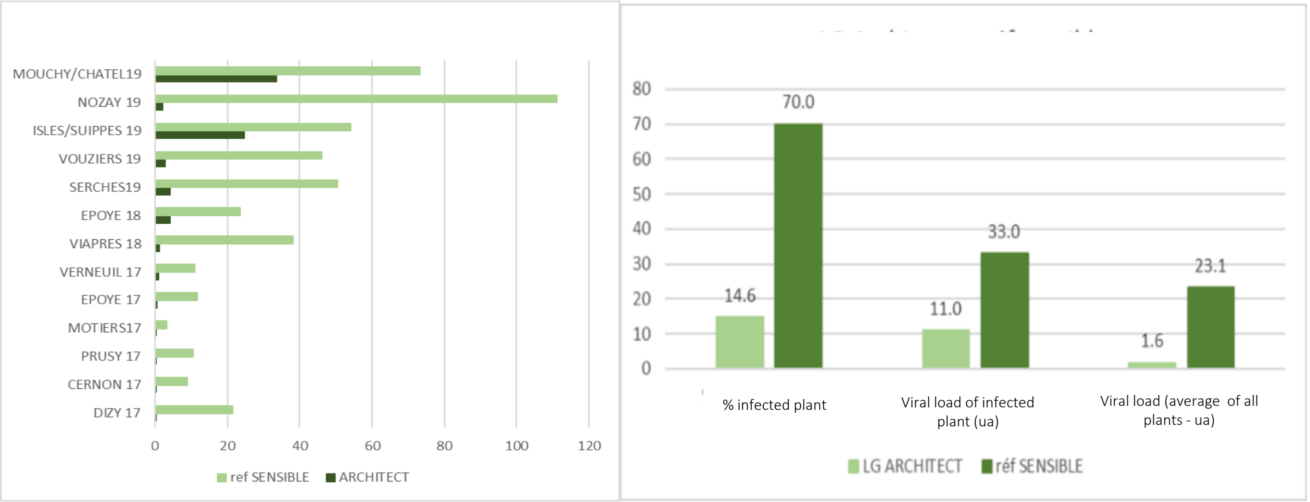

Figure 4: Viral infection of LG ARCHITECT vs sensitive reference, summary and details per assay
(The presence of TuYV in samples was validated by spectrophotometric measurements (Elisa test)

au: Arbitrary unit defined from a reference sample)

Trials carried out in different locations and during different campaigns have made it possible to evaluate the behaviour of LG ARCHITECT under different viral pressures ranging from low to very high (Ruck et *al.*, 2018, 2019b). These contrasting situations showed that (Table 1):

- under low pressure, LG ARCHITECT does not offer any yield gain over the susceptible variety because of the moderate incidence of the virus in this type of scenario,
- under medium to heavy pressure, the TuYV resistance of LG ARCHITECT results in an average yield gain of around 6% compared with an untreated susceptible variety. In these situations, where the

resistant variety shows a benefit compared to an untreated susceptible variety, aphicide protection provides a yield gain only on the susceptible variety,

- under very high viral pressure, the data do not show any yield gain for LG ARCHITECT. In this situation of very high viral pressure, aphicidal protection does not provide any yield gain, whether the variety is resistant or susceptible.

Table 1: Percentage of infected plants and yield for different levels of virus pressure at the beginning of winter.

	Low infection 1 trial				Medium infection 3 tests				Strong infection 3 trials				Very strong infection 4 tests			
	LG ARCHITECT		Sensitive ref.		LG ARCHITECT		Sensitive ref.		LG ARCHITECT		Sensitive ref.		LG ARCHITECT		Sensitive ref.	
	untreated	treaty	untreated	treaty	untreated	treaty	untreated	treaty	untreated	treaty	untreated	Treaty	untreated	Treaty	untreated	Treaty
% infected plants	2.5	0	22.5	17.5	9	7	84	80	20.1	13.3	95	90.6	32	23.4	90	73.3
	b	b	a	a	b	b	a	a	b	b	a	A	b	b	a	a
Yield q/ha	44.1	45.2	44.6	43.8	47	47.6	43.8	44.8	49.9	51.8	46.8	49.7	45.5	45.1	45	46.7
	a	a	a	a	ab	a	c	bc	a	a	b	a	a	a	a	a

Results obtained under controlled conditions:

The field work was supplemented by an experimental approach under controlled conditions aimed at characterising the resistance phenotype observed in the LG ARCHITECT and RGT QUIZZ genotypes by comparing them with the sensitive reference DK EXCEPTION.

The methods used and the results obtained (presented in the article Souquet et *al*., 2021a, 2021b) demonstrated the partial resistance status of the LG ARCHITECT genotype thanks to a reduction in the infection rate. To complete the characterisation of the partial resistance carried by LG ARCHITECT, the experimental set-up was adapted to assess the effect of the age of the plant at the time of inoculation. The results showed partial resistance behaviour for plants inoculated at the 2-4 leaf stage and mature resistance behaviour for plants inoculated at a more advanced growth stage. In addition, analysis of infected plants revealed (i) a poor quality of infected LG ARCHITECT plants as a source of virus for transmission and (ii) a prolonged latent period for infected plants. Thus, the dynamics of virus spread in the field should be slower for LG ARCHITECT compared with susceptible oilseed rape genotypes, which should lead to a reduction in the dynamics of expansion of infectious foci and the maintenance of a higher proportion of healthy plants in the field.

The data generated showed that, compared with the susceptible reference, plant-vector interactions were not affected for the LG ARCHITECT variety, suggesting that the partial resistance carried by LG ARCHITECT is expressed only with respect to the virus. The data associated with RGT QUIZZ do not allow it to differentiate itself from the susceptible reference under the experimental conditions of our study.

VARIETAL LEVER – SUGAR BEET

In-field evaluation:

In 2019, 2 trials of varietal resistance to the beet mild yellowing virus were set up in Normandy and Aisne. A total of 14 hybrids under selection were supplied by the main beet breeders, in comparison with 2 commercial varieties assumed to be susceptible, EPERVIER and PLATINA KWS. Each genotype was sown in microplots of 3 rows by 6m, with 4 randomised replications.

In the Coucy-Lès-Eppes trial in the Aisne department, an artificial inoculation of *Myzus persicae* carrying the BMYV virus was carried out on one half of the trial. This made it possible to measure the yield losses caused by the virus inoculation. While the control variety PLATINA KWS showed a susceptible profile (Figure 5), the EPERVIER control variety proved to be intermediate among all the genotypes tested. The MARIBO VY2, SV1 and KWS J4 varieties had a significantly lower level of severity (% of surface area with symptoms) than the best of the control varieties (EPERVIER). The MARIBO VY2, SV1 and KWS J4 varieties had a maximum of 7% symptomatic leaves and a yield loss of less than 10%.

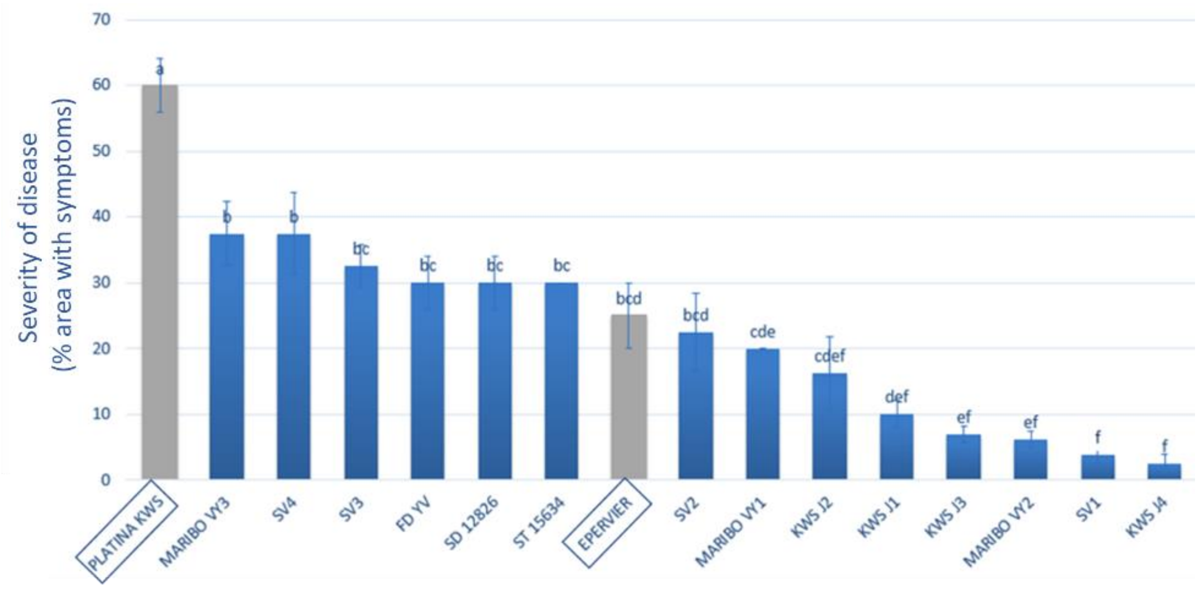

Figure 5: Severity of yellows on all beet genotypes. Scoring carried out on 12 August 2019, Coucy les Eppes

In 2020, 14 genotypes were again tested in addition to the 2 control varieties PLATINA KWS and EPERVIER. Only 4 common genotypes were kept out of the 14 genotypes from 2019, due to a lack of seed availability and the decision to look for material that was likely to perform better. The Luneray trial (76) under natural conditions showed greater variability in yellows scores. The common genotypes between 2019 and 2020 are identified in orange in figure 6 below. The results confirm those observed in Aisne in 2019, with MARIBO VY2 and SV1 showing statistically significantly fewer symptoms than the control variety EPERVIER. Nevertheless, their yields are 78% and 65% of the controls respectively. Other genotypes tested only in 2020 showed good behaviour against yellows and illustrate the progress made by breeders. Their yields vary from 76% to 93% of the controls, with an average of 88%, which again highlights the impact of the breeding effort.

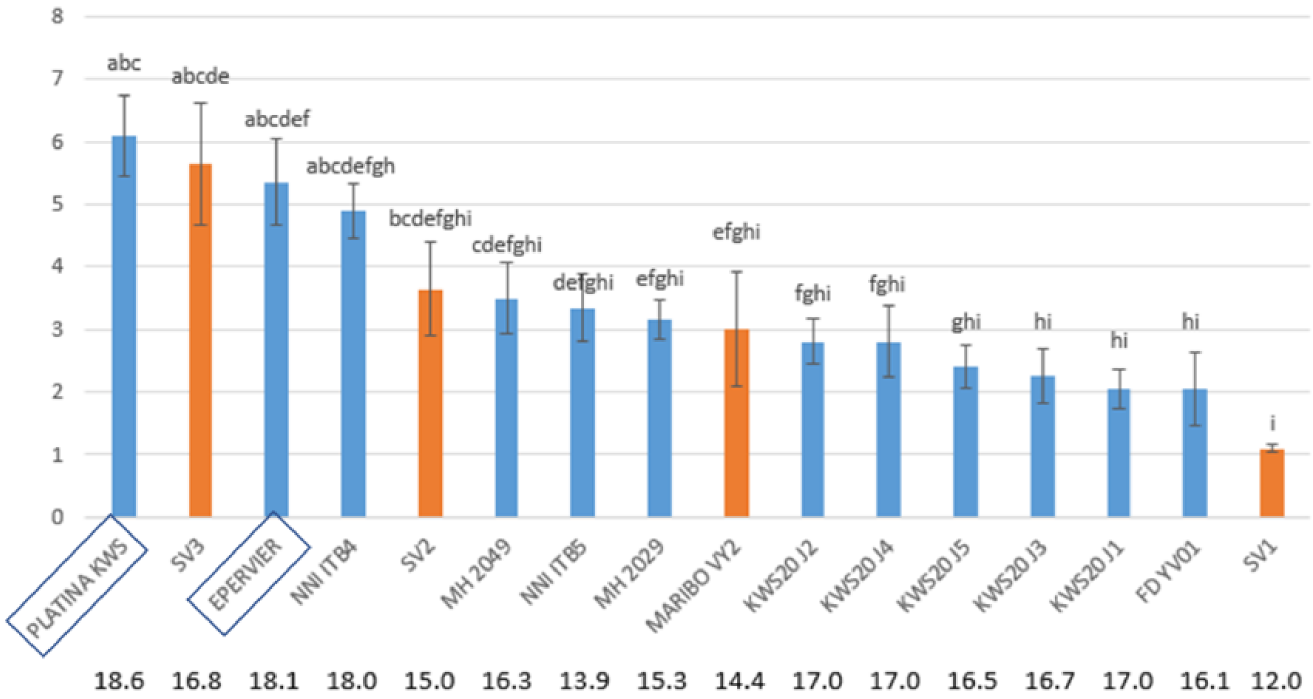

Figure 6: Average severity of yellows measured on 22/07/2020 and 21/08/2021 in the Luneray trial (76) in 2020. Below the graph: sugar yield (t/ha)

Results obtained under controlled conditions:

In the case of sugar beet, setting up experimental systems under controlled conditions required optimisation of the seedling production system used in the laboratory and calibration of the viral diagnostic procedures (Souquet et *al.*, 2021b). The work did not make it possible to identify genotypes that were less sensitive than the sensitive reference used in the study (VY0). In addition, the quantitative approach used to describe the viral load of infected plants showed that all the genotypes accumulated the virus with an efficiency equivalent to, or even greater (in the case of VY10) than, the susceptible reference. Work on vector-plant interactions revealed several genotypes (VY2, VY6 and VY10) with favourable "host" behaviour for *Myzus persicae*. These results indicate that the pool of genotypes introduced into the study does not include any resources unfavourable to infectious dynamics. This finding led to the decision not to pursue work on characterising latency in the sugar beet pathosystem.

BIO-CONTROL PRODUCTS LEVER [SUGAR BEET, RAPESEED, STRAW CEREALS]

In-field evaluation:

- **Experimental systems**

Biocontrol products were evaluated in different fields during the 2018-2019 and 2019-2020 seasons with various trials on oilseed rape, winter barley (a species favoured over common wheat because the severity of the disease is more severe on winter barley) and sugar beet in situations exposed to natural aphid infestations (Robin et *al., 2021a, 2021b)*. On barley, additional trials were carried out in 2020-2021. A total of 17 trials were carried out: 3 on oilseed rape, 10 on winter barley and 4 on sugar beet.

The aim of each trial was to compare several solutions under evaluation (around 6 solutions per trial) with an untreated control method (UNTR) and a method protected with a reference product based on lambda-cyhalothrin on barley (Karaté Zéon, 0.075 l/ha), based on flonicamid on oilseed rape (Teppeki, 0.1 kg/ha) and on sugar beet (Teppeki 0.14 kg/ha applied with an adjuvant oil for insecticide sprays).
On oilseed rape and barley, the trials were conducted using a block design (4 replicates); on sugar beet, 2 replicates of 60 m² were carried out on a large plot, and one trial (2019 - site 76) was conducted using a block design (3 replicates).

The solutions studied (Table 2) are essentially composed of natural substances and micro-organisms. Some solutions were evaluated on all three crops. The methods that produced encouraging results in 2019 have been maintained for the 2020 trials and supplemented with new experimental methods to be evaluated.

Table 2: Breakdown of solutions evaluated (dose/ha per application) by crop, year and trial site.

	Crops	**oilseed rape**		**winter barley**			**sugar beet**	
	Years	**2019**	**2020**	**2019**	**2020**	**2021**	**2019**	**2020**
	Number of tests	2	1	2	4	4	2	2
Active ingredient (a.i.)	**French department codes**	78, 80	80	17, 81	17, 81, 18, 49	17, 81, 18, 49	62, 76	62, 76
Azadirachtin 1		36 g a.i.	36 g a.i.	36 g a.i.	36 g a.i.	44 g a.i.	36 g a.i.	36 g a.i.
Azadirachtin 2			29 g a.i.		20 g a.i. (17, 81)			
Paraffin oil A (10 l formulated product)		X	X	X	X	10 OR 5 L	X	
Paraffin oil B (10 l formulated product)			X		X	(17, 81)		X
Paraffin oil C (10 l formulated product)						(17, 81)		
Fatty acids (7.68 kg a.i.)					X			
Sweet orange oil (1.6 l product)				X				
Maltodextrin (5.98 kg a.i.)		X		X	(81)		X	X
Sucrose (50 g)		X (78)						
Lecanicillium muscarium Ve6 $2x10^{13}$ *CFU*		X (80)					X	X
Beauvaria bassiana GHA $2.5x10^{12}$ *CFU*								X
Sulphur (4.2 kg a.i.)		X		X			X	
Garlic extract (3 or 6 l formulated product)			3 l		6 l (17, 81)			
Kaolinite clay (10 kg formulated product)		X					X	

a.i.: active ingredient; CFU: Colony forming unit, X = Modality present in the trial.

Each product was applied several times, generally 4 applications (T1, T2, T3 and T4) approximately one week apart with a 200 l/ha spray, in order to increase the chances of demonstrating the efficacy of the solution being evaluated. The reference was applied according to the usual recommendations, often at T2, with the exception of the beet trials in 2020 (T1 and T3).

The value of the solutions was assessed using various indicators relating to aphids (frequency of infested plants, average number of aphids per plant), infection (visual estimation of the percentage of plot surface affected on barley and sugar beet, virological analysis on oilseed rape) and final yield.

- **Results**

Repeated applications of azadirachtin produced interesting results, particularly on oilseed rape in 2019 (Figure 7) and again in 2020, both in terms of the frequency of infested plants and the number of *Myzus persicae* per plant: 4 applications of azadirachtin provided better protection against aphids than the reference applied once. However, viral infection was not significantly reduced compared with the untreated control, either with azadirachtin or the insecticide reference. On barley, repeated application of azadirachtin also showed some effectiveness, with an average yield gain of almost a third of that achieved with the reference (+10 q/ha *compared with* 37 q/ha for the reference applied on a single date). Different doses of azadirachtin were evaluated, but no dose effect was observed in our trials. However, in autumn 2019, when conditions were particularly rainy, the performance achieved with this modality was lower than that achieved in the other 2 campaigns. On sugar beet, the repeated application of azadirachtin helped to reduce the numbers of A. *fabae* aphids, with a slight drop in yellows symptoms in one trial. In the 2020 trials, with only 2 or 3 applications, no effect was observed on *Myzus persicae* infestations. Overall, the results of azadirachtin applications to oilseed rape are encouraging with regard to *Myzus persicae* in the autumn. Further studies will be needed to determine the frequency of application and the doses needed to provide satisfactory protection.

Repeated applications of paraffin oil provided a certain level of protection, with variations depending on the target crop. On oilseed rape, interesting results obtained in 2019 were not confirmed in 2020. Similarly, on beetroot, gains were only observed in 2019. On barley, 9 trials were carried out over the 3 campaigns. Repeated application of paraffin oil reduced aphid infestations and increased yield, with a gain of half that achieved with the reference: +17 q/ha with paraffin oil compared with +33 q/ha for the reference (Figure 8). This solution offered the best protection on average, with a more pronounced effect in the 2020 campaign (high pressure of yellows disease). These results obtained as part of the ABCD_B project confirm earlier results obtained with the same paraffin oil (Arvalis data). The work carried out in the field in 2021 highlighted the impact of the positioning of applications: Two late applications (T3, T4) resulted in a yield gain of 9 q/ha compared with 11 q/ha with 4 applications. The other combinations gave only a small yield gain (3 q/ha) whether the rate applied was 5 or 10 l/ha. Tests with other paraffin oils also showed an increase in yield compared with the control, but never more than with the reference paraffin oil. It should be noted that symptoms of phytotoxicity were observed in a few trials - with low aphid infestations - when applied in conjunction with a herbicide treatment (post-seeding). These symptoms proved to be relatively fleeting. Further work needs to be carried out in the laboratory and in the field to define the optimum conditions of use (efficacy, selectivity).

Concerning the other solutions under evaluation:

- Applications of the fatty acid-based solution or sweet orange oil were not very effective and yield gains were not significant (average gain of 1 to 3 q/ha).
- Maltodextrin applications, which are not effective against *Myzus persicae* on oilseed rape in 2019, have shown a favourable effect on aphid infestations on sugar beet. On barley, the benefits of this solution were more limited (average gain of 5 q/ha).
- Repeated applications of a strain of the entomopathogenic fungus *Lecanicillium muscarium* resulted in a significant reduction in yellows symptoms, comparable to that of the reference (1 trial [relatively inaccurate], Figure 9); this effect was not observed on oilseed rape (not shown). In 2020, the results obtained on sugar beet with a strain of another entomopathogenic fungus, *Beauvaria bassiana,* showed a lower level of efficacy;

this result will need to be validated. Given the mode of action of these fungi, the optimum conditions of use (application conditions, weather conditions) need to be specified.

- In 2019, applications of sulphur were effective against *Aphis fabae* aphids on sugar beet, but had no effect on target aphids on oilseed rape or barley.
- The garlic extract-based solution tested in 2020 did not prove effectiveness on oilseed rape (1 trial). On barley, the initial results were a little more encouraging in terms of aphid infestation, but the yield gains observed in two trials were not significant.
- Repeated applications of kaolinite clay on oilseed rape and sugar beet were not effective. These results confirm those obtained previously on barley (Arvalis data, 2016).

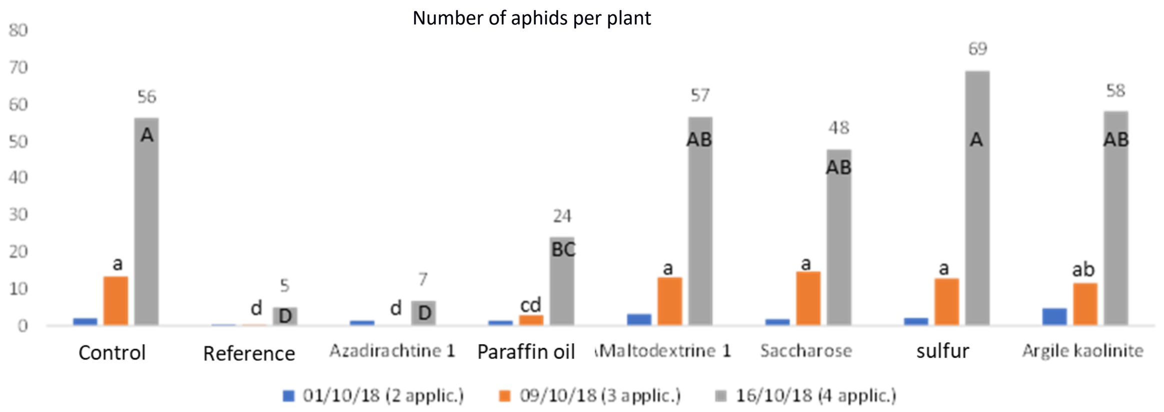

Figure 7: Changes in aphid numbers per plant on oilseed rape - one site (2019 -80). *p-value* < *0.05*

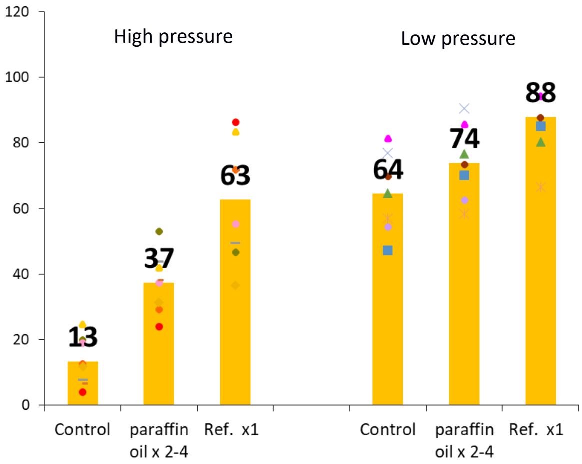

Figure 8: Winter barley yield (q/ha) according to different protection strategies and grouped according to the yield potential of the control (<25 q/ha, > 45 q/ha) - 15 trials

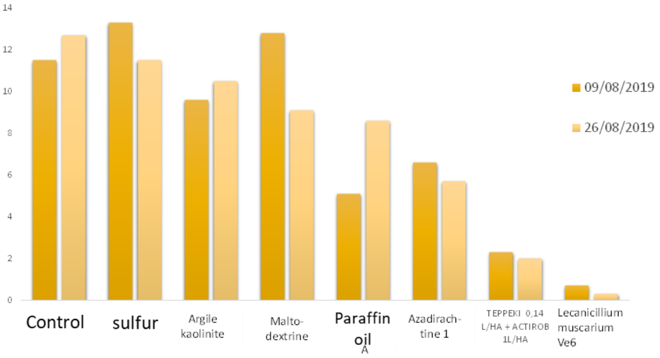

Figure 9: Evaluation of different beet protection solutions (one trial, 2019 - 62). Visual rating of severity of yellows
Translation : Témoin = control – Souffre = sulfur – maltodextrine = maltodextrin - Huile paraffine = paraffin Oli – Souffre = sulfur

Results obtained under controlled conditions:

The work carried out under controlled conditions focused on two modalities that had previously demonstrated their value in field trials:

- Paraffin oil (Finavestan Ema, 10 l/ha for a spray volume of 200 l/ha)
- Azadirachtin (Azatin, 1.4 l/ha, spray volume 200 l/ha).

Work was carried out simultaneously on the three pathosystems: *Myzus persicae* on oilseed rape and sugar beet, and *Rhopalosiphum padi* on winter wheat. The following lessons were learned from the results:
- Applications of paraffin oil lead to an increase in the mortality of *M. persicae* on sugar beet. However, the fecundity of both aphid species (*R. padi* and *M. persicae*) is not affected by this treatment. It is worth noting that treated sugar beet and oilseed rape plants show a lower infection rate than untreated plants.
- The application of azadirachtin leads i) to increased mortality of *R. padi* and *M. persicae* and ii) to lower fecundity of *R. padi* on barley and *M. persicae* on sugar beet. However, the health data associated with the treated plants show that the infection rates observed is not altered by the application of this biocontrol product.

The two biocontrol products evaluated under controlled conditions have the potential to impact aphid multiplication and viral transmission. Efficacy appears to vary according to the aphid species and plant species. Finally, the data show that their efficacy can be curative and preventive in controlling dwarf yellows in barley by increasing mortality or lowering the fecundity of *R. padi*.

DISCUSSION

The solutions evaluated as part of the ABCD_B project are usually only partially effective, with the level of protection depending on the level of viral disease.

For soft wheat, the differences in susceptibility measured between varieties are proportional to the pressure of the disease. This means that protection against aphids is still necessary, whatever the sensitivity of the variety, as soon as there is a risk of BYDV. With the aim of activating other levers, a number of biocontrol solutions were evaluated. Only azadirachtin and paraffin oil showed any efficacy in our trials. Paraffin oil has an average efficacy of around 50% in both low and high disease pressure situations (Robin et *al.*, 2022). The combination of these two levers - the choice of a tolerant variety protected by a biocontrol product - seems appropriate for soft wheat in order to limit losses. However, since the two levers are only partially effective, their combination will not restore the level of yield previously obtained with imidacloprid or currently with the application of products from the pyrethroid family.

For oilseed rape, azadirachtin showed interesting results in controlling aphid populations. However, this efficacy did not significantly reduce the viral load on protected plants, either with azadirachtin or the insecticide reference, and no significant difference was observed in yield under our experimental conditions. Varietal resistance is the priority lever for protecting oilseed rape against the risk of TuYV disease (Ruck et *al.,* 2021). The value of biocontrol solutions, and azadirachtin in particular, should be assessed for protection against the transmission of viral mosaics by aphids. The two known mosaics in oilseed rape are less common viruses, but potentially highly damaging, and for which TuYV resistance does not appear to be effective. Combining a TuYV-resistant variety with a biocontrol product could therefore be a good way of combating both yellows and mosaics. The application of biocontrol products on varieties resistant to Turnip yellows diseases would also make it possible to reduce the selection pressure on resistance to Turnip yellows disease and maintain the durability of the genetic lever for protection against viruses.

For sugar beet, the results obtained show both a wide variability in behaviour between varieties and an encouraging outlook given the evolution of symptoms observed between the varieties assessed in 2019 and those assessed in 2020. However, the yield loss of the least symptomatic hybrids remains high, and the two genotypes with fewer symptoms in the field showed no interest in the indicators assessed under controlled conditions. This raises the question of the relevance of judging varieties on the basis of their symptoms.

CONCLUSION

The ABCD_B project was carried out by a large and diverse consortium. The work concerned three main crops (sugar beet, straw cereals and rapeseed) and was carried out in the field and under controlled conditions by partners from academic research, applied research and companies.

As far as genetic leverage is concerned, the operational consequences vary from one crop to another. For oilseed rape, the results have made it possible to support the positioning and development of varieties with partial resistance to the TuYV virus in the field. For soft wheat, the results confirm the value of the genetic lever for protection against BYDV and open the way to the selection of more resilient genotypes. The methods used and results produced within the framework of ABCD_B should help private research to propose soft wheat varieties that are less susceptible to BYDV. As for sugar beet, the results obtained in the field are encouraging, even if they have not yet been confirmed under controlled conditions. This work is a precursor to other more ambitious and collaborative projects being undertaken as part of the PNRI project.

At the same time, the evaluation of numerous biocontrol products has produced encouraging results for two active ingredients: azadirachtin and paraffin oil. However, the efficacy of these solutions is significantly lower than that of the reference products. The work carried out under controlled conditions makes it possible to explain - at least partially - the modes of action, which should help to optimise the use of these products in the field. These results will enable technical institutes, companies and other development actors to support biocontrol solution(s) for uses for which authorisations are being considered.

The extensive work carried out as part of the ABCD_B project to evaluate and select the most appropriate solutions means that these solutions can continue to be evaluated with farmers. The combination of the two levers - the choice of a tolerant variety protected by a biocontrol product - will be of varying interest depending on the crop: while it seems potentially appropriate in the near future for soft wheat, it is currently more limited for protecting oilseed rape against Turnip yellows disease, given the effectiveness of the genetic lever, although the potential benefits of increasing the durability of the genetic lever against yellows or limiting the incidence of mosaics should be explored. For sugar beet, the combination of genetic levers and biocontrol products could be studied once varieties that are less susceptible to yellows have been identified.

Ethics

The authors declare that the experiments were carried out in compliance with the applicable national regulations.

Declaration on the availability of data and models

The data supporting the results presented in this article are available on request from the author of the article.

Declaration on Generative Artificial Intelligence and Artificial Intelligence Assisted Technologies in the Drafting Process.

The authors have used artificial intelligence-assisted technologies to translate from French to English.

Declaration of interest

The authors declare that they do not work for, advise, own shares in, or receive funds from any organisation that could benefit from this article, and declare no affiliation other than those listed at the beginning of the article.

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