OCL 2015, 22(3) D302
© S. Cadoux et al., Published by EDP Sciences 2015
DOI: 10.1051/ocl/2015014

OCL
Oilseeds & fats Crops and Lipids
Available online at:
www.ocl-journal.org

Research Article – Dossier
Open Access

Rapeseed: some examples of current French research
Colza : quelques exemples de recherche en France

Dossier

Intercropping frost-sensitive legume crops with winter oilseed rape reduces weed competition, insect damage, and improves nitrogen use efficiency

Stéphane Cadoux[1,★], Gilles Sauzet[1], Muriel Valantin-Morison[2], Célia Pontet[1], Luc Champolivier[1], Céline Robert[1], Jean Lieven[1], Francis Flénet[1], Olivier Mangenot[1], Pascal Fauvin[1] and Nathalie Landé[1]

[1] CETIOM, Avenue Lucien Brétignières, 78850 Thiverval-Grignon, France
[2] UMR 211 Agronomie, INRA-Agro Paris Tech, Avenue Lucien Brétignières, 78850 Thiverval-Grignon, France

Received 13 February 2015 – Accepted 31 March 2015

Abstract – Mixing plant species in agroecosystems is highlighted as an agroecological solution to reduce pesticides and fertilizers while maintaining profitability. In the French context, intercropping frost-sensitive legume crops with winter oilseed rape is potentially interesting and began to be implemented by farmers. In this study we aimed at measuring the services and disservices of this intercrop with three different legume mixtures, in terms of growth and yield for rapeseed, ground cover of weeds in autumn and damage caused by rape winter stem weevil. The experiment was carried out at four sites from 2011 to 2014. We showed higher total aerial dry weights and total aerial nitrogen contents in the intercrops compared to sole winter oilseed rape in November. The companion plants contributed to the control of weeds and the mitigation of rape winter stem weevil damage, notably through the increase in the total aerial weight. In spring, after destruction of the companion plants, the intercrops had partially compensated a reduction in the N fertilization rate (–30 kg per hectare) in terms of aerial nitrogen content in rapeseed, with no consequences on the yield which was maintained or even increased. There were probably other interactions such as an improvement in rapeseed root exploration. The consequences were an increase in the nitrogen use efficiency in intercrops. The intercrop with faba bean and lentil showed the best results in terms of autumn growth, weed control, reduction in rape winter stem weevil damage, and rapeseed N content in spring and yield. Intercropping frost-sensitive legume crops with winter oilseed rape is thus a promising way to reconcile yield and reduction in pesticides and fertilizer use and perhaps to benefit more widely to the cropping system.

Keywords: Intercropping / winter oilseed rape / companion plants / legume crops / agroecological services

Résumé – Associer des légumineuses gélives au colza d'hiver contribue à réduire la compétition des adventices, les dégâts d'insectes, et à améliorer l'efficience d'utilisation de l'azote. Associer des plantes au sein d'un agroécosystème est mis en avant comme un levier agroécologique permettant de réduire les intrants tout en maintenant la rentabilité. En France, le mélange de légumineuses gélives en association avec le colza d'hiver est une pratique qui présente des intérêts potentiels et commence à se développer chez les agriculteurs. L'objectif de ce travail est d'étudier les intérêts et limites de trois associations en termes de croissance et de rendement du colza, de maîtrise des adventices et de dégâts de charançon du bourgeon terminal. Des expérimentations ont été conduites des 2001 à 2014 dans 4 lieux. Les couverts associés à base de légumineuses gélives permettent d'augmenter la biomasse totale produite et la quantité d'azote aérien en entrée hiver, comparé au colza seul. À l'automne ils contribuent à la réduction de la couverture du sol par les adventices et des dégâts occasionnés par le charançon du bourgeon terminal, notamment par cet effet de supplément de biomasse produite. Au printemps, les couverts associés permettent de compenser, en partie seulement, une réduction de fertilisation azotée de 30 kgN/ha, sans incidence sur le rendement qui est maintenu, voire amélioré. D'autres phénomènes positifs compensent donc cet écart d'azote contenu dans les parties aériennes. En conséquence, l'efficience d'utilisation de l'azote est améliorée avec les associations. En termes d'efficacité des couverts, le mélange

★ Correspondence: cadoux@cetiom.fr

féverole-lentille présente les meilleurs résultats en termes de compétition vis-à-vis des adventices, de réduction des dégâts de charançon du bourgeon terminal, d'azote accumulé au stade G4 et de rendement du colza. L'association d'un couvert gélif de légumineuses au colza d'hiver est donc une piste pour réduire les intrants, sans impacter le rendement et peut-être apporter des bénéfices plus largement au système de culture.

Mots clés : Cultures associées / colza d'hiver / plantes compagnes / légumineuses / services agroécologiques

1 Introduction

Despite significant gains in productivity, intensive agriculture is criticized for its harmful effects on the environment (Tilman *et al.*, 2002). In today's environment of ongoing rapid growth in world population, agriculture must succeed in reconciling the production of food, feed and biomass, and the conservation of the environment (Robertson and Swinton, 2005). New agricultural systems are required, and one way is to base them on ecological intensification *i.e.* favoring services provided by biotic interactions in terms of soil fertility and pest control (Doré *et al.*, 2011; Gaba *et al.*, 2014). Plant species diversity in agroecosystems is often presented as a pillar of agroecological approach (Vandermeed *et al.*, 1998; Ratnadas *et al.*, 2012; Gaba *et al.*, 2014). Mixing plant species can result in improving productivity, pest regulation, carbon and nutrient sequestration (Malézieux *et al.*, 2009).

The use of living mulches, defined as "a cover crop sown either before or with a main crop and maintained as a living ground cover throughout the growing season" (Hartwing and Ammon, 2002), is a promising solution. Many authors demonstrated that the introduction of living mulch provides control of weeds or, at least, a low chemical weed control (Abdin *et al.*, 1997; Agegnehu *et al.*, 2008; Hiltbrunner *et al.*, 2007; Ilnicki and Enache, 1992), to decrease insect damage (Costello, 1994; Hooks and Johnson, 2004; Theunissen, 1994), and to increase N availability (Thériault *et al.*, 2009; Thorsted *et al.*, 2006). However, the introduction of living mulch could induce competition between the cash crop and the living mulch (Teasdale, 1996). Most studies reported a yield reduction of the cash crop (Bergkvist, 2003; Carof *et al.*, 2007; Picard *et al.*, 2010; White and Schott, 1991), even if a few ones reported no yield loss (Ilnicki and Enache, 1992). Nevertheless, the yield reduction mentioned before occurred for systems where the living mulch was still growing during the main phases of yield components establishment particularly in the spring (Carof *et al.*, 2007; Hiltbrunner *et al.*, 2007).

In France, winter oilseed rape (WOR) is the second arable crop in terms of cultivated areas. It is also the second arable crop in terms of treatment frequency index (5.5 on average) and nitrogen applied per hectare (162 kg/ha on average) (AGRESTE, 2014). This leads to environmental impacts, notably pesticide losses in water (Sausse *et al.*, 2012). Indeed, in the French context (soil, climate, cropping systems, etc.), rapeseed is particularly subject to weed infestation (Primot *et al.*, 2006) and insect pressure (Valantin-Morison, 2012). The specificity of winter oilseed rape (*e.g.* taproots, breaking cereal diseases cycles, nitrogen catchment, etc.) makes it an important crop in French rotations dominated by straw cereals (AGRESTE, 2014) and justifies the design of innovative cultural practices (Valantin-Morison, 2012). Studies of brassica species based on mixed cropping are rare (Banik *et al.*, 2000; Schröder and Köpke, 2012), especially with WOR (Bergkvist, 2003). Jamont *et al.* (2013) demonstrated the niche complementarity of winter oilseed rape intercropped with faba bean in the early growth but this study only focused on nitrogen use and was carried out under controlled conditions. The particular interest in mixing winter oilseed rape with frost-sensitive legume crops is due to different potential positive interactions: (i) the early sowing of WOR seems favorable to a significant growth of legume crops; (ii) the competitive effect of WOR to N acquisition in the soil should favor, the N fixation of legume crops, and thus its potential competition; (iii) the likely destruction of the legume crop during the winter should favor its N release, with no competition against WOR and no additional herbicide application. Therefore, the benefit for WOR could be better than "permanent" living mulch.

The aim of this study was to assess the effect of three mixtures of frost-sensitive legume crops – faba bean + lentil (BL); grass pea + fenugreek + lentil (GFL); purple vetche + common vetch + berseem clover (VVT) – intercropped with winter oilseed rape on the growth of the intercrop, the weed cover, the damage caused by rape winter stem weevil in autumn, and on the aerial nitrogen content in spring and the yield of winter oilseed rape.

2 Materials and methods

2.1 Site characteristics and experimental design

The experiments were performed at two sites (Murs and Villedieu-sur-Indre, 36, central France) in 2011 and at four sites (Murs and Villedieu-sur-Indre, 36, central France; Chambon, 17, western France; Morville-sur-Seille, 54, north-eastern France) in 2012, 2013 and 2014. The main characteristics of each site are presented in Table 1.

Winter oilseed rape (*Brassica napus*) – subsequently referred to as "WOR" – was grown as sole crop and intercrop with three different legume mixtures: (i) faba bean (*Vicia faba*) + lentil (*Lens culinaris*) – subsequently referred to as "BL" –; (ii) grass pea (*Lathyrus sativus*) + fenugreek (*Trigonella foenum-graecum*) + lentil – subsequently referred to as "GFL" –; and (iii) purple vetche (*Vicia benghalensis*) + common vetch (*Vicia sativa*) + berseem clover (*Trifolium alexandrinum*) – subsequently referred to as 'VVT'. In terms of sowing densities, the intercrop was based on the additive principle. The sowing density of the WOR varied according to the trial (Tab. 2). The sowing density of BL, GFL and VVT was 55 kg ha^{-1}, 35 kg ha^{-1} and 20 kg ha^{-1}, respectively.

The experimental plots (120 to 200 m^2) were set up in a randomized block design with WOR, WOR + BL,

Table 1. Main site characteristics.

	Villedieu-Sur-Indre	Murs	Chambon	Morville-Sur-Seille
Location	central France	central France	western France	northeastern France
Latitude	46.846 °N	46.914 °N	46.125 °N	48.917 °N
Longitude	1.538 °E	1.161 °E	–0.849 °O	6.157 °E
Soil type	clay-limestone	sandy loam	clay-limestone	clay loam
Soil depth	30 cm	70 cm	90 cm	90 cm
Climate	oceanic modified	oceanic modified	oceanic	semi-continental
Average temperature				
2010/08–2011/07	11.5 °C		/	/
2011/08–2012/07	10.8 °C		13.2 °C	10.8 °C
2012/08–2013/07	10.6 °C		12.6 °C	10.1 °C
2013/08–2014/07	11.8 °C		13.4 °C	11.6 °C
Average precipitation				
2010/08–2011/07	489 mm		/	/
2011/08–2012/07	679 mm		753 mm	630 mm
2012/08–2013/07	769 mm		1215 mm	674 mm
2013/08–2014/07	652 mm		972 mm	660 mm

WOR + GFL, WOR + VVT as treatments with three replicates. Other intercrops were studied in each trial but varied between sites and years and were thus not included in that analysis.

2.2 Management practices

In each trial, sole WOR was managed according to local agriculture guidelines and conditions. Soil tillage was based either on plowing (P), reduced tillage (RT) or direct seeding (DS). The nitrogen (N) fertilization rate was calculated according to the balance sheet method, which determines the *a priori* optimal fertilization rate (subsequently referred to as "*X*" rate), with the CETIOM method "*réglette azote*" (Lagarde and Champolivier, 2006). The management of intercrops was the same as for sole WOR except in terms of broad leaf herbicide applications – reduced when the doses to be applied were considered potentially phytotoxic for legumes- and nitrogen fertilization rates – systematic reduction of 30 kg ha^{-1} (subsequently referred to as "*X*-30" rate) –, assuming a contribution of legumes to WOR N nutrition and seeking to offset the extra cost of legume seeds. The main characteristics of the management practices are presented in Table 2.

2.3 Measurements and calculations

Four types of measurements were carried out: aerial dry weights and nitrogen contents at two dates (November and G4 stage); weed competition, estimated by the percentage of ground cover by weeds in Autumn; insect damage; and yield. All these measurements are detailed thereafter. They were not carried out on all the sites described before; the distribution of the measurements is detailed in Table 3.

The aerial biomass was collected manually in three areas of 0.4 m^2 per plot, and then weighed, at two dates: November (before the first significant frost) and early G4 stage (BBCH 73), considered as the stage of maximum N accumulation in the aerial parts of the WOR. For the first measurement, in November, the collected biomass was separated into two fractions, *i.e.* winter oilseed rape and companion plants. The dry matter content was obtained after drying a representative subsample at 70 °C during 48 h. The aerial dry weight (*ADW*) was calculated as the product of fresh weight and dry matter content. The total nitrogen concentration in aerial biomass (r_N) was determined using the Dumas method. The aerial N content (ANC) was calculated as the product of aerial dry weight and N concentration.

These measurements were also carried out at F2 stage (BBCH 61) in order to calculate the nitrogen nutrition index (NNI), used as an indicator of underestimation or overestimation of the *a priori* optimal fertilization rate '*X*' ($NNI < 1$ or $NNI > 1$, respectively), with the equation (1) defined by Colnenne *et al.* (1998):

$$NNI = \frac{r_N}{4.48 \times ADW^{-0.25}}, \tag{1}$$

where NNI is the nitrogen nutrition index, r_N is the nitrogen concentration in aerial biomass and ADW is the aerial dry weight.

The percentage of ground cover by weeds was estimated in subplots, representing approximatively half of the plots (60 to 100 m^2), receiving no broad leaf herbicide. These time-consuming measurements were only carried out in four trials: Chambon 2012/2013 (in October); Villedieu-Sur-Indre 2012/2013, 2013/2014; and Murs 2013/2014 (in November). The relationship between the percentage of ground cover by weeds and the aerial dry matter in November has only been evaluated in the three trials where both measurements were carried out in November.

Damage caused by rape winter stem weevil (*Ceutorhynchus picitarsis*) was used to estimate the effect of intercropping WOR with companion plants on insects. Indeed, rape winter stem weevil is considered as one of the most harmful pests of WOR in its main cropping area in France (central and eastern France). After laying in autumn, larvae can migrate in the spring, toward the terminal bud and destroy it, leading to bushy plants with reduced fertility. We thus used the percentage of bushy plants at F2 stage (BBCH 61) as indicator of insect damage. It was estimated at both sites where

Table 2. Main characteristics of the management practices at each trial (DS = direct seeding; RT = reduced tillage; P = plowing).

	2010/2011		2011/2012				2012/2013				2013/2014			
	Villedieu-Sur-Indre	Murs	Villedieu-Sur-Indre	Murs	Chambon	Morville-Sur-Seille	Villedieu-Sur-Indre	Murs	Chambon	Morville-Sur-Seille	Villedieu-Sur-Indre	Murs	Chambon	Morville-Sur-Seille
WOR variety	Neptune	DK Esquisite	DK Exstorm	DK Esquisite	Cash	DK exquisite	DK Exstorm	DK exquisite	DK Exstorm	DK Exquisite	DK Exstorm	DK Exquisite	DK Exstorm	DK Exquisite
WOR sowing density (grains m-²)	52	52	52	52	39	38.3	52	52	39	42.7	50	50	39	33
Tillage	DS	RT	DS	RT	P	P	DS	RT	P	P	DS	RT	P	P
N Fertilization rate (Sole WOR, kg ha-1)	185	185	170	130	190	142.5	168	160	190	140	172	172	180	160
N Fertilization rate (Intercrop, kg ha-1)	155	155	140	100	160	112.5	135	130	160	110	141	141	150	130
Broad leaf herbicides (Sole WOR, ha-1)	0	Chrono 1l	Lontrel 1.25l	Lontrel 1.25l	Novall 1.5l	Noval 1l	0	0	Noval 1.5l	Noval 1l	Callisto 0.15l Lontrel 75g	Callisto 0.15l Lontrel 0.75g	Noval 1l Vivendi 1l	Acrux 1l Colzor trio 3.6l
Broad leaf herbicides (Intercrop, ha-1)	0	Chrono 1l	0	0	0	Noval 1l	0	0	Noval 1.5l	Noval 1l	Callisto 0.15l Lontrel 75g	Callisto 0.15l Lontrel 0.75g	Noval 1l Vivendi 1l	Acrux 1l Colzor trio 3.6l Lontrel 75g
Companion plant destruction date (and mode)	December (frost)		January/February (frost)		Ferbuary (frost)	January (frost)	February (frost)		/	February (frost)	January (herbicide)		February (frost)	March (herbicide)

Table 3. Measurements carried out at each site (WOR = winter oilseed rape; CP = companion plants; ADW = aerial dry weight; ANC = aerial N content).

	2010/2011		2011/2012				2012/2013				2013/2014			
	Villedieu-Sur-Indre	Murs	Villedieu-Sur-Indre	Murs	Chambon	Morville-Sur-Seille	Villedieu-Sur-Indre	Murs	Chambon	Morville-Sur-Seille	Villedieu-Sur-Indre	Murs	Chambon	Morville-Sur-Seille
WOR ADW and ANC	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CP ADW and ANC	x	x	x	x	x	x	x	x	x	x	x	x	x	x
% Bushy plants			x	x			x	x			x	x		
% weed cover							x		x		x	x		
WOR ADW and ANC			x	x	x	x				x	x	x	x	x
Grain Yield	x	x	x	x	x	x	x	x		x	x	x		x

Table 4. Number of observations per treatment, p-values and levels of significance of the statistical analysis for the four factors (WOR = winter oilseed rape; CP = companion plants; ADW = aerial dry weight; ANC = aerial N content, [N] = aerial N concentration; NUE = nitrogen use efficiency).

	n/	*p*-value			
	Treatment	Treatment	Site	Year	Site*year
WOR ADW November	42	0.05	4.98e-10***	< 2.2e-16***	1.98e-15***
CP ADW November	42	2.14e-05***	3.63e-11***	< 2.2e-16***	0.11
WOR + CP ADW November	42	3.17e-09***	< 2.2e-16***	< 2.2e-16***	1.31e-10***
WOR ANC November	42	0.33	2.36e-09***	< 2.2e-16***	7.25e-15***
CP ANC November	42	9.95e-07***	4.71e-10***	< 2.2e-16***	0.09
WOR + CP ANC November	42	5.06e-13***	2.55e-10***	< 2.2e-16***	4.26e-06***
WOR [N] November	42	2.95e-05***	< 2.2e-16***	< 2.2e-16***	7.21e-16***
% Bushy plants	18	0.10	0.02e-02***	8.73e-11***	7.41e-06***
% weed cover	12	0.01***	2.39-0.9***	< 2.2e-16***	/
WOR ADW G4 (all trials)	27	0.01**	< 2.2e-16***	0.05e-1**	1.26e-07***
WOR ANC G4 (all trials)	27	0.19	< 2.2e-16***	0.02^e-2***	0.21
WOR ANC G4 ($NNI < 1$)	12	0.28	2.73e-05***	3.15e-05***	/
WOR ANC G4 ($NNI > 1$)	15	0.15	2.16e-12***	0.56	/
Grain Yield (all trials)	36	0.02*	< 2.2e-16***	< 2.2e-16***	< 2.2e-16***
Grain Yield ($NNI < 1$)	15	0.21	< 2.2e-16***	4.69e-05***	3.01e-08***
Grain Yield ($NNI > 1$)	21	0.04e-01**	0.07e-02***	6.16e-16***	0.07
Grain yield deviation from sole WOR	36	0.06e-01**	6.18e-05***	3.18e-09***	0.35
Grain NUE	36	< 2.2e-16***	< 2.2e-16***	< 2.2e-16***	< 2.2e-16***

* Significant effect ($0.05 > p \geqslant 0.01$). ** Very significant effect ($0.01 > p \geqslant 0.001$). *** Highly significant effect ($p < 0.001$).

the pressure was the highest (Villedieu-Sur-Indre and Murs), in 2011/2012, 2012/2013 and 2013/2014, on 100 plants per plot. In the six trials with measurements, no autumn insecticide had been applied, except in Villedieu-Sur-Indre in 2012 where a "Karate zéon" (λ-cyhalothrine) was applied on all plots at a rate of 0.075 l ha^{-1}.

At maturity of the WOR, 20 m^2 were harvested. The standard yield was calculated after determination of the actual dry matter content (h) and the actual impurity content (i) according to the equation (2):

$$\textit{Standard yield} = \textit{fresh yield} \times \frac{100-h}{100-9} \times \frac{100-i}{100-2}, \qquad (2)$$

where h is the actual dry matter content and i is the actual impurity content.

The grain nitrogen use efficiency (NUE) was calculated according to Good *et al.* (2004) as:

$$NUE = \frac{\textit{Standard yield}}{\textit{Fertilizer}\ \mathrm{N}}, \qquad (3)$$

where NUE is the nitrogen use efficiency, and '*Fertilizer N*' is the amount of fertilizer N applied per hectare.

In addition to the absolute value, three variables of the intercrops: ADW in November, ANC at G4 stage and grain yield were also calculated, in each trial and for each block, as the deviation from the value of the sole WOR. These calculations in relative value were used to assess the competition/facilitation effect of the companion plants.

All measurements were not carried out in all trials. In particular, no measurements were carried out in Chambon in 2013 due to unsuccessful destruction of the companion plants and a consecutive high competition of the WOR in the spring. All details are given in Table 3.

2.4 Statistical analysis

Statistical analyses by ANOVA were performed, after verifying that distributions were normal and variances homogeneous, using R software ("aov" function) to assess the effects of the four treatments, year, site and site*year interaction, on different variables (Tab. 4), according to the equation (4):

$$\gamma_{ijkl} = \mu + T_i + S_j + Y_k + S_j \times Y_k\,(B_l) + \varepsilon_i, \qquad (4)$$

where Y_{ijkl} is the criterion variable (see Tab. 4), μ is the mean, T_i stands for the treatment condition i, S_j for the site j, Y_k for the year k, B_l for the replicate l and ε_{ijkl} is the residual. All terms are considered as fixed effect.

The significance of the differences between treatments were estimated using the Student, Newman & Keuls ("SNK.test" function) range test with $\alpha = 0.05$.

The link between variables was assessed with simple and multiple regressions of R software ("lm" function), according to the general equation (5):

$$\gamma_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \ldots + \beta_k x_{ki} + \varepsilon_i, \qquad (5)$$

where Y_i is the response variable value for measure i, x_{1i}, x_{2i}, …, x_{ki} are the regressors, $\beta_0, \beta_1, \beta_2, \ldots, \beta_k$ are intercepts and ε_i is the residual.

DOSSIER

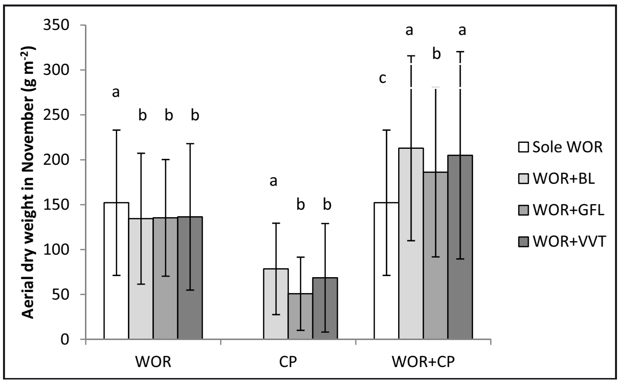

Fig. 1. Aerial dry weight of the winter oilseed rape (WOR), the companion plants (CP) and the total WOR + CP, in November and for the four experimental treatments.

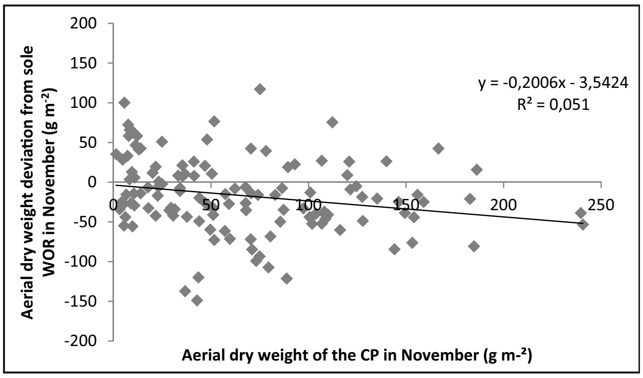

Fig. 2. Relationship between the aerial dry weight deviation from sole winter oilseed rape (WOR) and the aerial dry weight of the companion plants (CP) in November.

3 Results

3.1 Growth in autumn

In November, the aerial dry weight of the sole WOR averaged 152 g m^{-2} (Fig. 1). The effects of site and year were significant (Tab. 4) with Villedieu-Sur-Indre = Murs > Chambon = Morville-Sur-Seille and 2011 2012 = 2014 > 2013, respectively. The aerial dry weight of the intercropped WOR was slightly lower (135 g m^{-2} on average), whatever the companion crop (Fig. 1; Tab. 4). The aerial dry weight of the companion plants was approximately half of that of the WOR, and was significantly higher for BL compared to GFL and VVT (Fig. 1; Tab. 4). The effects of site and year were significant (Tab. 4) with Murs > Chambon > Villedieu-Sur-Indre > Morville-Sur-Seille and 2012 > 2014 > 2011 > 2013, respectively. Whatever the companion plant, the total aerial dry weight was significantly higher for the intercrops (WOR + companion plants) compared to sole WOR and ranked as follows: WOR + BL = WOR + VVT > WOR + GFL > Sole WOR (Fig. 1; Tab. 4).

The competition/facilitation due to the companion plants in autumn was estimated through the deviation of aerial dry weight from sole WOR. A competition effect (decrease in WOR ADW with the increase in CP ADW) should be due to the share of water, nutrient or radiation. A facilitation effect (increase in WOR ADW with the increase in CP ADW) should be due to an improvement of WOR root exploration. The deviation of aerial dry weight from sole WOR decreased very slightly but significantly ($p = 0.01103$) with the increase in the aerial dry weight of the companion plants (Fig. 2). However, in absolute terms, the aerial dry weight of the sole WOR and the companion plants were positively and significantly (p = 1.438e-05) correlated (Fig. 3).

In November, the aerial N content of the sole WOR averaged 42.6 kg ha^{-1} (Fig. 4). The aerial N content of the intercropped WOR did not differ significantly from the sole WOR, whatever the companion crop (Fig. 4; Tab. 4). The N concentration of the WOR was significantly higher for the WOR + BL and WOR + VVT treatments compared to sole WOR and WOR + GFL treatments (Tab. 4). The aerial N content of the companion plants was significantly higher for BL compared to GFL and VVT (Fig. 4; Tab. 4). The total aerial N content was significantly higher for the intercrops (WOR + companion plants) compared to sole WOR and ranked as follows: WOR + BL = WOR + VVT > WOR + GFL > Sole WOR (Fig. 4; Tab. 4).

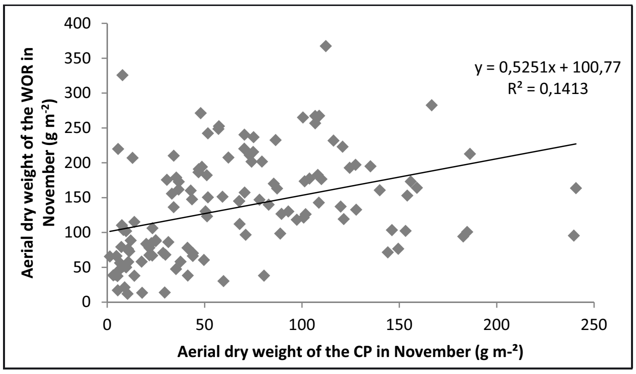

Fig. 3. Relationship between the aerial dry weight from sole winter oilseed rape (WOR) and the aerial dry weight of the companion plants (CP) in November.

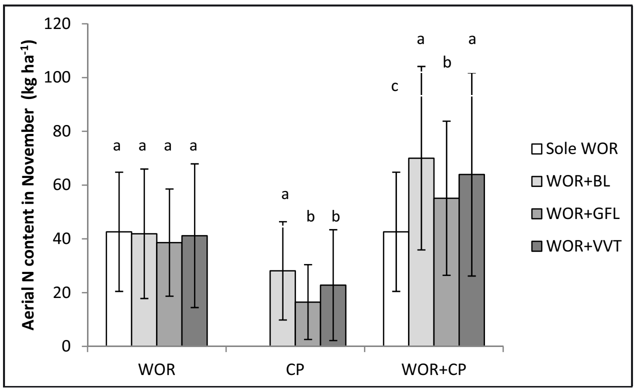

Fig. 4. Aerial N content of the winter oilseed rape (WOR), the companion plants (CP) and the total WOR + CP, in November and for the four experimental treatments.

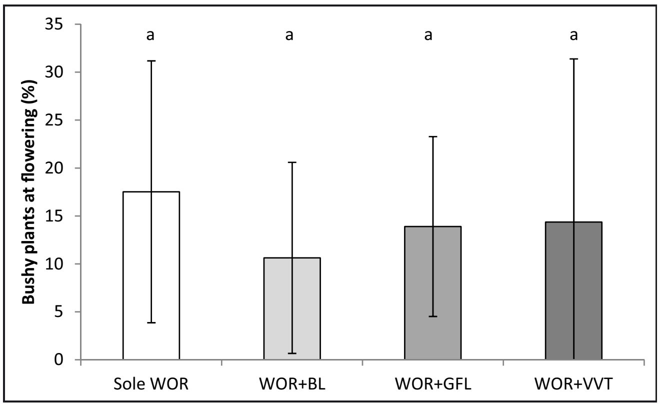

Fig. 5. Percentage of bushy plants at flowering for the four experimental treatments.

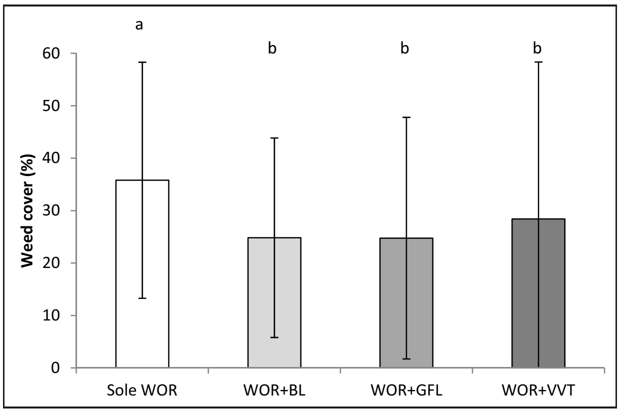

Fig. 7. Percentage of weed cover in Autumn for the four experimental treatments.

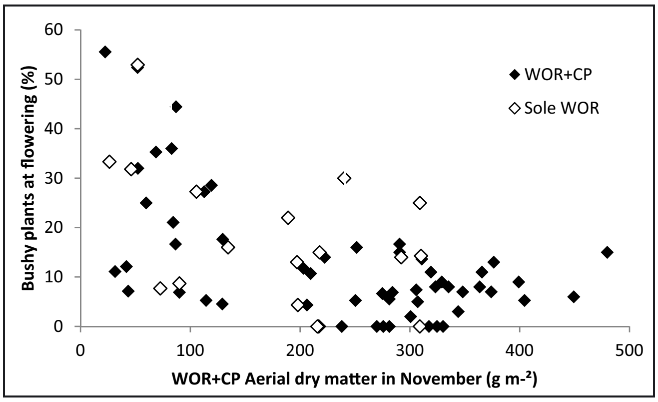

Fig. 6. Relationship between the percentage of bushy plants at flowering and the total aerial dry weight of sole winter oilseed rape (Sole WOR) or winter oilseed rape + companion plants (WOR + CP) in November.

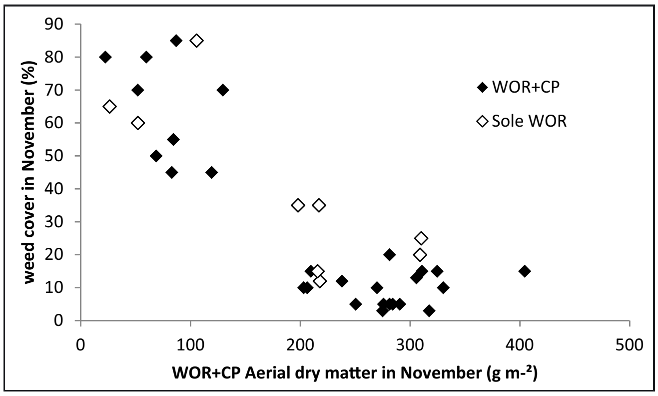

Fig. 8. Relationship between the percentage of weed cover in November and the total aerial dry weight of sole winter oilseed rape (Sole WOR) or winter oilseed rape + companion plants (WOR + CP) in November.

3.2 Autumn insect damage

The damage caused by the rape winter stem weevil in autumn was estimated through the percentage of bushy plants at flowering. It was lower for intercrop treatments than for sole WOR treatments (Fig. 5). The difference was not significant, but the p-value was low (0.095; Tab. 4).

The percentage of bushy plants decreased with the increase in total aerial dry weight of plants, whatever its source (Sole WOR or WOR + companion plants). When considering intercrops, it was below 20% from 200 g m^{-2} of total aerial dry weight (Fig. 6).

3.3 Autumn weed cover

The percentage of weed cover in Autumn was significantly lower for intercrop treatments than for sole WOR treatments (Fig. 7). It decreased with the increase in total aerial dry weight of plants whatever its source (Sole WOR or WOR + companion plants). When considering intercrops, it was below 20% from 200 g m^{-2} of total aerial dry weight (Fig. 8).

3.4 WOR Aerial dry weight and N content at G4 stage

At the early G4 stage, the treatment effect on the aerial dry weight was significant (Tab. 4). The values were higher for the intercrop WOR + BL than for the other treatments (not shown). The differences of aerial N contents between the other treatments were not significant, despite the reduction of the N fertilization rate (–30 kg per hectare) for the intercrops (Fig. 9; Tab. 4). In order to analyze the effect of a possible overestimation of the *a priori* optimal N fertilization rate 'X' which could explain this absence of difference, the trials were divided into two categories: (i) trials in which the NNI at flowering of the sole WOR was below 1 (*i.e.* 'X' was not considered as overestimated) and (ii) trials in which the NNI at flowering of the sole WOR was above 1 (*i.e.* 'X' was considered potentially overestimated). In both cases, no significant differences were found between treatments on the aerial N content (Fig. 9; Tab. 4). More surprisingly, the trend was an increase in the aerial N content of the intercrops compared to sole WOR in the trials with $NNI < 1$, and a decrease in the aerial N content of the intercrops compared to sole WOR in the trials with $NNI > 1$.

The main hypothesis to explain the contribution of companion plant to WOR N nutrition is its mineralization which

Table 5. *p*-values, levels of significance (and estimated regression coefficients) and multiple *R*-squared of the regressions (WOR = winter oilseed rape; CP = companion plants; ADW = aerial dry weight; ANC = aerial N content, [N] = aerial N concentration).

Variables to explain	Explanatory variables			R^2
	CP ADW	CP [N]	CP ADW* [N]	
WOR ANC deviation from sole WOR at G4	0.42367	0.83613	0.07515	0.04873
Grain yield deviation from sole WOR	0.02436* (–0.005697)	0.02116* (14.602003)	0.55770	0.096

* Significant effect (0.05 > *p* ⩾ 0.01). ** Very significant effect (0.01 > *p* ⩾ 0.001). *** Highly significant effect (*p* < 0.001).

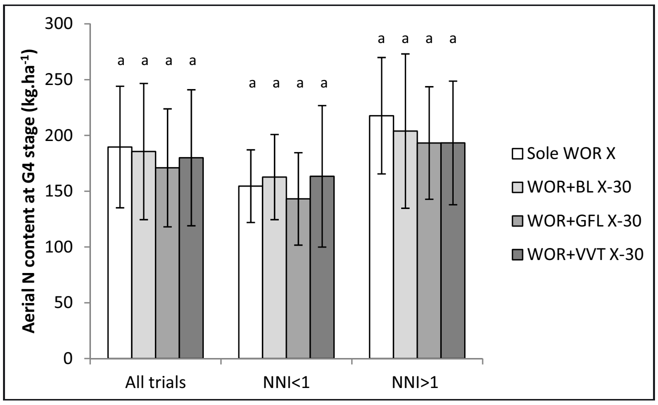

Fig. 9. Aerial N content of rapeseed at early G4 stage in all trials, in the trials in which the NNI of the sole WOR was < 1 and in the trials in which the NNI of sole WOR was > 1, for the four experimental treatments.

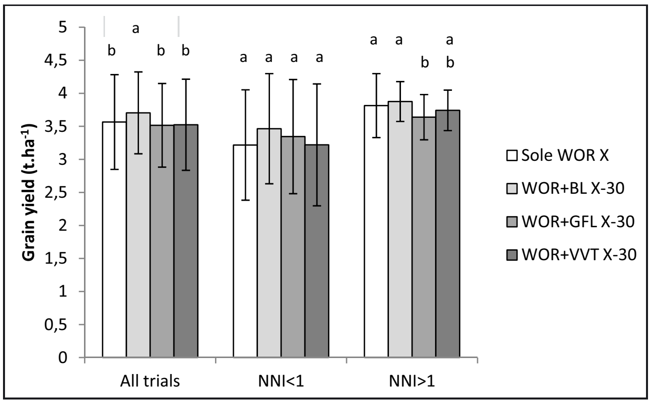

Fig. 11. Standard grain yield for oilseed rape in all trials, in the trials in which the NNI of the sole WOR was < 1, and in the trials in which the NNI of sole WOR was > 1, for the four experimental treatments.

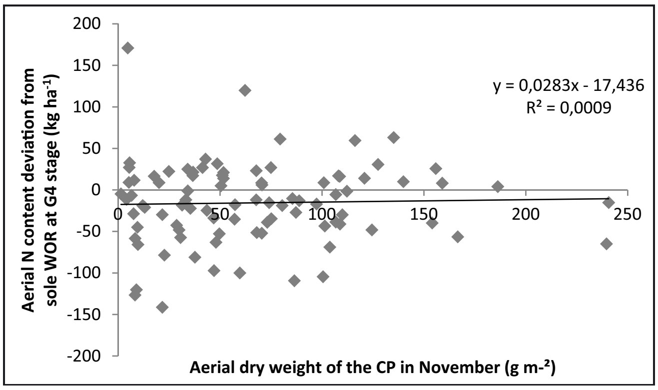

Fig. 10. Relationship between the aerial N content deviation from sole winter oilseed rape (WOR) at early G4 stage and the aerial dry weight of the companion plants (CP) in November.

should increase with the increase in the CP N content and the CP N concentration. In out experiment, the aerial N content deviation from sole WOR was not explained by the aerial dry weight of the companion plant in November (Fig. 10) neither by its N concentration nor the interaction between both variables (Tab. 5).

3.5 WOR yield

Despite the reduction of the N fertilization rate (–30 kg per hectare) for the intercrops, the grain yield was significantly higher for WOR + BL than for the sole WOR (+0.14 t ha^{-1}). No significant differences were found between the two other intercrops and the sole WOR (Fig. 11, Tab. 4). The effects of site and year were significant (Tab. 4), with Chambon > Murs > Villedieu-Sur-Indre = Morville-Sur-Seille and 2014 = 2012 > 2011 > 2013, respectively. Due to similar or even higher yield with reduced N fertilization rate, the grain NUE was significantly improved with intercropped WOR compared to sole WOR (Tab. 4), and ranked as follows: WOR + BL > WOR + VVT = WOR + GFL > Sole WOR.

In order to analyze the effect of a possible overestimation of the *a priori* optimal N fertilization rate '*X*' which could explain the absence of difference in yields, the trials were divided into two categories: (i) trials in which the NNI of the sole WOR at flowering was below 1 (*i.e.* '*X*' was not considered as overestimated) and (ii) trials in which the NNI of the sole WOR at flowering was above 1 (*i.e.* '*X*' was considered potentially overestimated). Surprisingly, the grain yield was systematically higher for the intercrops than for the sole WOR in the trials in which the NNI of the sole WOR was below 1 (insignificant difference). The grain yield deviation of the three intercrops from sole WOR decreased in the trials in which the NNI of the sole WOR was above 1 (Fig. 11, Tab. 4).

Considering all trials, the grain yield deviation from the sole WOR was significantly affected by site and year and ranked as follows: Chambon (+0.428 t ha^{-1}) > Villedieu-Sur-Indre (+0.040 t ha^{-1}) = Murs (–0.032 t ha^{-1}) = Morville-Sur-Seille (–0.089 t ha^{-1}) and 2013 > 2011 = 2012 > 2014, respectively (Tab. 4). It decreased significantly (*p* = 1.814e-06) with the increase in the grain yield of the sole WOR (Fig. 12). The multiple regression showed that it was also

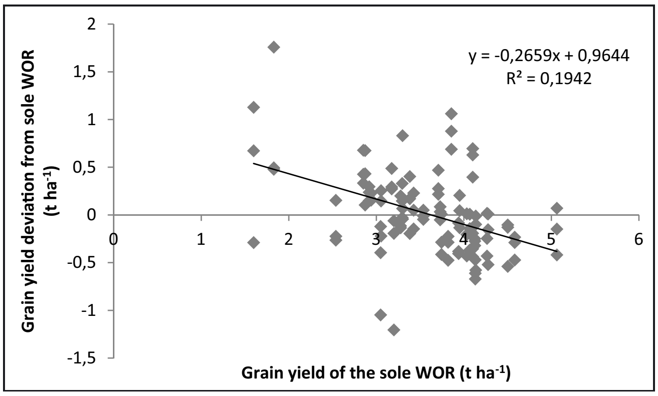

Fig. 12. Relationship between the grain yield deviation from sole winter oilseed rape (WOR) and the grain yield of the sole WOR.

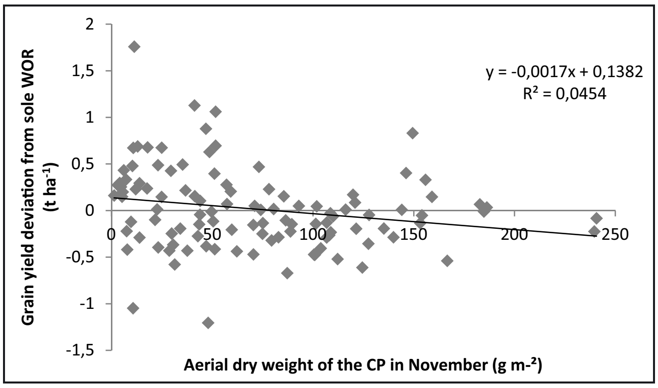

Fig. 13. Relationship between the grain yield deviation from sole winter oilseed rape (WOR) and the aerial dry weight of the companion plants (CP) in November.

affected by both the aerial dry weight (negatively) and the aerial N concentration (positively) of the companion plants in November (Fig. 13, Tab. 5).

4 Discussion

4.1 Competition/facilitation effect of the companion plants

In autumn, the slight competitive effect of the companion plants on the aerial dry weight of winter oilseed rape had no consequence on its aerial N content. This was due to an increase in the aerial N concentration of the WOR in the intercrop treatments (except for WOR + GFL). These opposite effects could be explained by a likely competition of the companion plants for light on the one hand and facilitation for root exploration on the other hand. Indeed, net N transfer from the legume mixtures to the WOR in autumn is unlikely to be significant (Jamont *et al.*, 2013). The aerial dry weight of the companion plants largely offset the slight reduction in the aerial dry weight for winter oilseed rape; intercropping was thus a way to maximize the total aerial dry weight and the total aerial N content (winter oilseed rape + companion plants) in autumn.

The companion plants were destroyed during winter in all trials (except Chambon in 2013), either by freezing or by herbicides. The aerial dry weight deviation from the sole WOR treatments was not observed any longer at early G4 stage, considered as the stage of maximum aerial N content in the WOR. The reduction of the N fertilization rate of 30 kg per hectare for the intercrops was partially or even completely compensated in the intercropped WOR. The first hypothesis explaining this effect is the mineralization of the companion plant residues, and the absorption of this N by the WOR. Indeed, Redin *et al.* (2014) showed a fast mineralization of crop residues decomposing at the soil surface, in particular with Fabaceae crops. The absence of relationship between the deviation of the aerial N content from the sole WOR treatments and the companion plants characteristics (aerial dry weight, aerial N concentration and interaction between both variables) could be due to the importance of the multiple factors controlling the mineralization of mulches: biochemical residue quality, residue placement, soil water content (Coppens *et al.* 2007), or climatic conditions during mineralization. However the mineralization of companion plant residues is not likely to be the only factor explaining the compensation in N acquisition in the WOR. Indeed, in the experiment of Redin *et al.* (2014) the C mineralization was not complete after 120 days under subtropical conditions. Moreover, the aerial N content of the companion plants in our experiment was lower than 30 kg per hectare on average. A possible facilitation for P extraction is unlikely to explain any difference since our experiments were all carried out in well supplied soils. An indirect effect through an improvement of root exploration is thus a possible hypothesis. Indeed, Shroder and Kopke (2012) showed that intercropping faba bean and oil crops resulted in more regular horizontal root distribution and enhanced the root length density in the subsoil. This phenomenon could also explain the equivalent or even higher grain yields in intercropped treatments compared with sole WOR treatments, despite a slightly lower aerial N content at the stage of maximum aerial N content. Intercropping winter oilseed rape with frost-sensitive legume crops leads to improve its grain nitrogen use efficiency.

However it is interesting to highlight that the aerial N content deviation from sole WOR decreased with the increase in sole WOR NNI, and that the yield deviation from sole WOR decreased with the increase in sole WOR NNI and yield. This suggests that the benefit of intercropping WOR with legume crops increases with the decrease in soil fertility or nitrogen availability. This could be explained again by an improvement of WOR root exploration due to companion plants, which could be beneficial or have almost no effect when soil N and water content availability are respectively limiting or not limiting. This is consistent with Hauggaard-Nielsen (2005) who showed, in intercrops, that critical interspecific competition for plant growth factors in nutrient poor soils and in low input systems explained the greater importance of facilitative root interactions in such conditions.

4.2 Effect of companion plants on the weed control in autumn

Our results showed the contribution of the companion plants in the control of weed cover. This had yet been demonstrated with living mulches in no-till cropping systems

(Ilnicki and Enarche, 1992; Hiltbrunner *et al.* 2007), and with intercropping (Agegnehu *et al.*, 2008; Bulson *et al.*, 1997; Hauggaard-Nielsen *et al.*, 2001). Lorin *et al.* (2014) showed that, in the case of winter oilseed rape intercropped with frost-sensitive legume crops, the main factor explaining such effects was the increased competition for light, itself linked with the biomass production of the companion plants and the complementarity of its shape with the one of WOR. Our results suggest a possible substitution of the biomass of both intercropped plants. This is particularly interesting since the threshold of 200 g of aerial dry weight per square meter for an efficient competition *vs.* weeds was reached on average by intercropped treatments but not by sole WOR treatments. This contribution of the companion plants to weed control could compensate a reduction in herbicide applications, necessary to avoid phytotoxicity against legumes (Sauzet, pers. comm.).

4.3 Effect of companion plants on damage caused by rape winter stem weevil

Our results show a likely effect of the companion plants on damage caused by the rape winter stem weevil. This positive effect of the companion plants had been reported for different brassicas and different insects (Theunissen, 1994; Costello, 1994; Hooks and Johnson, 2004), and explained by a variety of phenomena: visual and olfactory confusion, mechanical barrier, increased presence of natural enemies, *etc*. (Theunissen, 1994). In our experiment, there was a clear effect of the total aerial dry weight (Sole WOR or WOR + CP) on the reduction of the percentage of bushy plants. The superposition of the observations related to both sole WOR and WOR + companion plants suggests a substitutive effect of both the companion plants and the WOR biomass, when the total weight exceeds 200 g m^{-2}. The biomass effect of the WOR is likely due to the increase in the difficulty for the larvae to reach the terminal bud with the increase in stem size (C. Robert, pers. comm.). The biomass effect of the companion plants should be due to an increase in the phenomena described by Theunissen (1994) with the increase in aerial weight. Through an increase in the total aerial dry weight in November, intercropping legume crops with winter oilseed rape appears thus as a way to mitigate damage caused by rape winter stem weevil. However, this effect, alone, seems too limited and variable to advise reduce insecticide applications. It could rather be a contribution to an integrated pest management strategy.

5 Conclusion

In this study, we demonstrated the various benefits of mixing frost-sensitive legume crops with winter oilseed rape. We showed the higher total aerial dry weight and total aerial nitrogen content in the intercrops compared to sole winter oilseed rape in November. The companion plants contributed to the control of weeds and the mitigation of the rape winter stem weevil damage, notably through the increase in the total aerial weight. In spring, after destruction of the companion plants, the intercrops had partially compensated a reduction of the N fertilization rate (–30 kg per ha^{-1}) in terms of aerial nitrogen content in rapeseed, with no consequences on the yield which was maintained or even increased. The grain nitrogen use efficiency was consequently improved in intercrops.

Further research is needed to better understand the mechanisms involved in these effects and to better analyze the variability observed and the suitability in different contexts of soils and climate. The effect of legume species has to be analyzed in order to clarify which plant traits have to be chosen in several combinations of companion plants. Finally, since the mineralization of the living mulch during the rapeseed growth seems incomplete, an evaluation of the fate of N after harvest would be necessary.

Acknowledgements. This work was carried out under the project "Phyto-sol", which is part of the French "Ecophyto plan" led by the Ministry of Agriculture, with the financial support of the National Agency for Water and Aquatic Environments by credits from the fee for diffuse pollution. The authors are grateful to N. Urvoit, D. Poisson, R. Lange, D. Forget, D. Collin for their technical assistance, and to L. Lot for English revision.

References

Abdin OA, Coulman BE, Cloutier DC, Faris MA, Smith DL. 1997. Establishment, development and yield of forage legumes and grasses as cover crops in grain corn in Eastern Canada. *J. Agron. Crop Sci.* 179: 19–27.

Agegnehu G, Ghizaw A, Sinebo W. 2008. Yield potential and land-use efficiency of wheat and faba bean mixed intercropping. *Agron. Sustain. Dev.* 28: 257–263.

AGRESTE. Enquête pratiques culturales 2011. Principaux résultats. AGRESTE les dossiers 21, juillet 2014.

Banik P, Sasmal T, Ghosal PK, Bagchi DK. 2000. Evaluation of Mustard (Brassica compestris Var. Toria) and Legume Intercropping under 1:1 and 2:1 Row-Replacement Series Systems. *J. Agron. Crop Sci.* 185: 9–14.

Bergkvist G. 2003. Influence of white clover traits on biomass and yield in winter wheat-or winter oilseed rape-clover intercrops.*Biol. Agric. Hortic.* 21: 151–164.

Bulson HAJ, Snaydon RW, Stopes CE. 1997. Effects of plant density on intercropped wheat and field beans in an organic farming system. *J. Agric. Sci.* 128: 59–71.

Carof M, De Tourdonnet S, Saulas P, Le Floch D, Roger-Estrade J. 2007. Undersowing wheat with different living mulches in a no-till system. I. Yield analysis. *Agron. Sustain. Dev.* 27: 347–356.

Colnenne C, Meynard JM, Reau R, Justes E, Merrien A. 1998. Determination of a critical nitrogen dilution curve for winter oilseed rape. *Ann. Bot.* 81: 311–317.

Coppens F, Garnier P, Findeling A, Merckx R, Recous S. 2007. Decomposition of mulched versus incorporated crop residues: modelling with PASTIS clarifies interactions between residue quality and location. *Soil Biol. Biochem.* 39: 2339–2350.

Costello MJ. 1994. Broccoli growth, yield and level of aphid infestation in leguminous living mulches. *Biol. Agric. Hortic.* 10: 207–222.

Doré T, Makowski D, Malézieux E, Munier-Jolain N, Tchamitchian M, Tittonell P. 2011. Facing up to the paradigm of ecological intensification in agronomy: revisiting methods, concepts and knowledge. *Eur. J. Agron.* 34: 197–210.

Gaba S, Lescourret F, Boudsocq S, *et al.* 2014. Multiple cropping systems as drivers for providing multiple ecosystem services: from concepts to design. *Agron. Sustain. Dev.* DOI 10.1007/s13593-014-0272-z.

Good AG, Shrawat AK, Muench DG. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends Plant Sci.* 9: 597–605.

Hartwig NL, Ammon HU. 2002. Cover crops and living mulches. *Weed Sci.* 50: 688–699.

Hauggaard-Nielsen H, Ambus P, Jensen ES. 2001. Interspecific competition, N use and interference with weeds in pea-barley intercropping. *Field Crops Res.* 70: 101–109.

Hauggaard-Nielsen H, Jensen ES. 2005. Facilitative root interactions in intercrops. *Plant Soil* 274: 237–250.

Hiltbrunner J, Jeanneret P, Liedgens M, Stamp P, Streit B. 2007. Response of weed communities to legume living mulches in winter wheat. *J. Agron. Crop Sci*. 93: 93–102.

Hooks CR, Johnson MW. 2004. Using undersown clovers as living mulches: effects on yields, lepidopterous pest infestations, and spider densities in a Hawaiian broccoli agroecosystem. *Int. J. Pest Manage.* 50: 115–120.

Ilnicki RD, Enache AJ. 1992. Subterranean clover living mulch: an alternative method of weed control. *Agric. Ecosyst. Environ.* 40: 249–264.

Jamont M, Piva G, Fustec J. 2013. Sharing N resources in the early growth of rapeseed intercropped with faba bean: does N transfer matter? *Plant Soil* 371: 641–653.

Lagarde F, Champolivier L. 2006. Le raisonnement et les avancées techniques permettent de réduire la fertilisation azotée ? le cas de Farmstar-colza®. *OCL* 13: 384–387.

Lorin M, Valantin-Morison M, Jeuffroy MH. 2014. Undersowing winter oilseed rape with frost-sensitive living mulches in order to improve weed control and biological N supply. In XIIIth congress of the European Society for Agronomy. Debrecen, Hungary.

Malézieux E, Crozat Y, Dupraz C, *et al.* 2009. Mixing plant species in cropping systems: concepts, tools and models. A review. *Agron. Sustain. Dev.* 29: 43–62.

Picard D, Ghiloufi M, Saulas P, de Tourdonnet S. 2010. Does undersowing winter wheat with a cover crop increase competition for resources and is it compatible with high yield? *Field Crops Res.* 115: 9–18.

Primot S, Valantin-Morison M, Makowski D. 2006. Predicting the risk of weed infestation in winter oilseed rape crops. *Weed Res.* 46: 22–33.

Ratnadass A, Fernandes P, Avelino J, Habib R. 2012. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. *Agron. Sustain. Dev.* 32: 273–303.

Redin M, Recous S, Aita C, *et al.* 2014. How the chemical composition and heterogeneity of crop residue mixtures decomposing at the soil surface affects C and N mineralization. *Soil Biol. Biochem.* 78: 65–75.

Robertson GP, Swinton SM. 2005. Reconciling agricultural productivity and environmental integrity: a grand challenge for agriculture. *Frontiers Ecol. Environ.* 3: 38–46.

Sausse C, Cerrutti N, Hebinger H. 2012. Impact du colza sur l'environnement: évaluation et limites méthodologiques. *OCL* 19: 155–163.

Schröder D, Köpke U. 2012. Faba bean (*Vicia faba* L.) intercropped with oil crops – a strategy to enhance rooting density and to optimize nitrogen use and grain production? *Field Crops Res.* 135: 74–81.

Teasdale JR. 1996. Contribution of cover crops to weed management in sustainable agricultural systems. *J. Prod. Agric.* 9: 475–479.

Thériault F, Stewart KA, Seguin P. 2009. Use of perennial legumes living mulches and green manures for the fertilization of organic broccoli. *Int. J. Vegetable Sci.* 15: 142–157.

Theunissen J. 1994. Intercropping in field vegetable crops: pest management by agrosystem diversification – an overview. *Pesticide Sci.* 42: 65–68.

Thorsted MD, Weiner J, Olesen JE. 2006. Above-and below-ground competition between intercropped winter wheat Triticum aestivum and white clover *Trifolium repens*. *J. Appl. Ecol.* 43: 237–45.

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. 2002. Agricultural sustainability and intensive production practices. *Nature* 418: 671–7.

Valantin-Morison M. 2012. Comment favoriser la régulation biologique des insectes de l'échelle de la parcelle à celle du paysage agricole, pour aboutir à des stratégies de protection intégrée sur le colza d'hiver ? *OCL* 19: 169–83.

Vandermeer J, van Noordwijk M, Anderson J, Ong C, Perfecto I. 1998. Global change and multi-species agroecosystems: concepts and issues. *Agric. Ecosyst. Environ.* 67: 1–22.

White JG, Scott TW. 1991. Effects of perennial forage-legume living mulches on no-till winter wheat and rye. *Field Crops Res.* 28: 135–48.

Dossier

Cite this article as: Stéphane Cadoux, Gilles Sauzet, Muriel Valantin-Morison, Célia Pontet, Luc Champolivier, Céline Robert, Jean Lieven, Francis Flénet, Olivier Mangenot, Pascal Fauvin, Nathalie Landé. Intercropping frost-sensitive legume crops with winter oilseed rape reduces weed competition, insect damage, and improves nitrogen use efficiency. OCL 2015, 22(3) D302.